ON THE LARVAL AND PUPAL STAGES OF ANOPHELES MACULIPENNIS, MEIGEN.

(Plates IV—V, 1 Text-Fig.)

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PART I. THE LARVA.

The great importance of an exact knowledge of the etiology of malaria has given a remarkable impetus to the study of mosquitoes. It has resulted in the growth, during the last few years, of a very extensive literature relating to these insects, and especially to those of the genus *Anopheles,* which serve as the alternative and definitive hosts of human malarial parasites. Comparatively few writings, however, deal with their internal anatomy.

In the present instance an attempt is made to describe the internal structure of the larva and pupa of *Anopheles maculipennis,* concerning which no adequate account yet exists. I am indebted to Mr A. E. Shipley for suggesting to me this line of investigation, and for placing at my disposal a large number of microscopical sections. Prof. G. H. F. Nuttall has also generously assisted me during its progress. The work has been carried out in the Morphological Laboratory at Cambridge during a tenure of an 1851 Exhibition Scholarship.

The Larva.

For the purposes of study larvae were collected in the neighbourhood of Cambridge during the year 1906. They were met with on various dates, ranging from May 9th up to August 10th, and were obtained from the following four localities. (1) A small bricked excavation in the middle of a field; it measured one and a half feet in cubical dimensions. In addition to larvae of *Anopheles,* the water contained *Cyclops*, green larvae of *Chironomus*, a few small Coleoptera and confervoid Algae. (2) A roadside pond partly covered with *Lemna*, and containing great numbers of Ostracoda, together with red larvae of *Chironomus*. (3) Still places in the backwaters of the Cam. (4) Two ponds situated in a field. There were no trees near them, and their banks were fringed with a good deal of miscellaneous herbage. In one of the ponds they were met with in company with larvae of *Dixa*, *Chironomus*, and of an undeterminal species of Psychodidae. The pond also contained numerous dragon-fly larvae belonging to several species, together with may-fly larvae and a number of small fish (measuring up to about eight inches in length). In the other pond there were no fish but great numbers of dragon-fly and may-fly larvae, and also larvae of the Stratiomyid fly *Odontomyia*. Both ponds contained an abundance of various Hemiptera and small Coleoptera.

The *Anopheles* larvae were scarce during the whole period, and although they were sought for elsewhere none were discovered in any other localities around Cambridge.

The external morphology of the larva is now comparatively well known. Good descriptions and figures have been given by Meinert (1886, p. 373), Grassi (1900), Howard (1901), and Nuttall and Shipley (1901, p. 45). The account given by the two latter authors is the fullest and most satisfactory and, moreover, is accompanied by the best and most detailed figures yet published. In the present paper only a brief description of the general external features of the *Anopheles* larva will be given.

External Form.

The larva may be divided into three distinct regions: (1) the *Head*, (2) the *Thorax*, (3) the *Abdomen* (Fig. 1)[1].

The *Head* is invested by a well-defined brown chitinous capsule. Its diameter from above downwards very nearly equals that taken from side to side (Fig. 2). At its anterior end it carries a pair of bunches of dark brown hairs; they are termed by Nuttall and Shipley the "brushes"[2]. The movements of these organs in the vertical plane set up a current in the water and by this means particles of food are wafted towards the mouth. These brushes are borne on a median anterior area termed by Meinert the "clypeus." It seems probable that this sclerite should be regarded as an undifferentiated clypeo-labrum.

[1] For Figs. 1—20 see Plate IV, for Figs. 21—33 see Plate V.

[2] By Blanchard and other writers they are termed the rotatory organs.

Stretching transversely across the middle of the head is a dark band of pigment, and over this area is disposed a row of six feathered hairs. A stout branched hair is placed immediately above, and overhanging each brush. Between these two hairs, and lying very close together on either side of the median line, are a pair of simple bristle-like hairs, slightly branched at their extremities (*fr.h.* in Fig. 1). This row of four frontal hairs has been shown by Grassi to have significance as a specific character[1], and he figures their differences in *Anopheles claviger* (*maculipennis*), *A. bifurcatus*, *A.* (*Myzomyia*) *superpictus* and *A.* (*Myzorhynchus*) *pseudopictus*.

The paired appendages of the head consist of the antennae, the mandibles, and the first maxillae. Between the two maxillae there lies a pointed toothed plate. It is termed by Meinert the "under lip" and by Felt (1904) the "labial plate"; the latter writer figures it for the larvae of a large number of N. American Culicidae. This sclerite is most probably to be regarded as the representative of the second maxillae which have united to form a single organ. A similar plate is often found among Dipterous larvae, and usually possesses a second sclerite lying immediately above it. This is the case in *Chironomus*, *Dicranota*, *Phalacrocera*, and *Pericoma*. The lower plate is regarded by Miall and Hammond (1900) as the "submentum" and the upper one as the "mentum." They believe that the mentum has gradually slipped behind the submentum so that the latter completely hides it when viewed from below. In *Anopheles*, the "labial plate" corresponds with the "submentum" of the *Chironomus* larva; it bears on its upper, or pharyngeal surface a considerable array of strongly chitinised teeth and ridges, but I have not been able to homologise any of them with tolerable certainty with a "mentum." A strongly-defined median area is also present and carries the orifice of the salivary duct; it, therefore, is to be regarded as the hypopharynx (Fig. 5).

The larval eyes are situated one on either side of the head, a short distance behind the antennae. Slightly anterior and dorsad of the larval eyes lie the primordia of the compound eyes of the imago. They consist of a variable number of isolated elements (ommatidia), which increase as the larva approaches the pupal period.

The *Thorax* shows externally only very slight indications of a separation into the three characteristic segments. Over its surface are

[1] Theobald (*Monograph of the Culicidae*, Vol. III. p. 15) states that this character has proved of great value.

distributed several groups of feathered hairs (Plate IV, Fig. 1). Lying immediately behind the most anterior row of these hairs on either side, and overhanging their bases, is a curious flattened notched process (*prc.* in Fig. 1) described by Nuttall and Shipley. As to the significance of these organs little can be said. I would suggest that possibly they have been derived from a pair of prothoracic spiracles which, although now lost in the Culicidae, are present in the larvae of the allied family of the Psychodidae.

The *Abdomen* consists of nine segments. The first two segments carry a pair of feathered hairs on each side. The third segment carries a single one on either side, and in the succeeding segments these hairs lose their pinnations, become smaller in size, and reduced to naked bristles. Situated on the dorsal aspect of each segment from the third to the seventh, inclusive, is a pair of small conically branched hairs, the palmate hairs of Nuttall and Shipley (*A.* in Figs. 1 and 2). Each palmate hair consists of a stalk carrying at its apex a whorl of flattened lanceolate leaflike branches. Other hairs are present on these segments but these two types are the most important. Both Stephens and Christophers (1902) and James and Liston (1904), in their accounts of the Indian species of *Anopheles*, show that the number of the palmate hairs, and the shape of each leaflet, afford useful specific characters. The eighth segment carries on its dorsal side the two spiracles, and has undergone modification for this purpose, a rather complex skeleton being developed for the support of those structures. The ninth abdominal segment contains the anus (*an.* in Fig. 3), and around that aperture are arranged two pairs of tracheal gills (*a.g.*). At the hinder margin of the segment, and overhanging the anus, are placed four very long and curved feathered hairs. Ventrally, the segment carries on each side a row of nine long hairs, also of the feathered type, the two rows arising from a common chitinous base.

Note on its Habits.

None of the numerous figures which occur in literature are strictly accurate as regards the method by means of which the larva attaches itself to the surface film. Nuttall and Shipley remark (1901, p. 57) that "the larvae lie with the long axis of their bodies parallel with the surface of the water.... Viewed from the side, the respiratory apparatus, as also the palmate hairs upon the dorsal surface of the abdominal segments, are seen to indent the surface film. The palmate hairs just referred to produce a series of minute bilateral indentations in the film, making it appear, on superficial observation, as if the dorsal surface of the larva actually protruded above the surface." After an examination of living larvae, I am able to confirm this observation. On referring to Fig. 2 it will be further noted that while the larva remains just beneath the surface film, the ventral side of its head lies uppermost, the head having been rotated through an angle of 180°. In this attitude it can readily use its brushes to set up a current in the water. This has the effect of sweeping towards the mouth whatever small organisms and débris may be floating immediately under the surface film. The movement of the brushes can be readily observed when a larva is transferred, with a drop of water, on to a microscopical slide, and a cover-glass placed very lightly over it. In its efforts to free itself the brushes can be seen to be in active movement, and the head will occasionally be seen rotating on its neck. This latter movement is performed with remarkable rapidity and ease.

The Integument.

The integument consists of a chitinous cuticle and, underlying it, the hypodermis or chitogenous layer.

The *Cuticle* is smooth and transparent. In the region of the head it is thicker than elsewhere and is seen to be bright yellow in thin sections. Over other parts of the body, except in a few localised places, it is quite colourless. It consists of two layers; an outer and much thinner but highly refractive layer, and a relatively thick inner stratum, which is much softer, and apparently only partially chitinised (Fig. 9). The inner layer is much the more readily stainable of the two. In prepared sections the cuticle is sometimes seen to have split at the junction of these two layers during the process of cutting. In several larvae only the outer layer was found to be present, the inner stratum having been absorbed. This was found to be the case prior to ecdysis and in the narrow space once occupied by the inner layer of the cuticle the hypodermis was seen to be secreting a new layer of chitin on its outer surface.

The *Hypodermis* consists of a single layer of cells resting internally on a delicate basement membrane. Its cellular nature is best seen towards the middle of each of the segments, especially on their dorsal aspect (Fig. 8). In the head, and at the sides and junctions of the segments, the hypodermis appears as an undifferentiated stratum of protoplasm containing scattered flattened nuclei which are, as a rule, hard to detect (Fig. 7). Distributed through the hypodermis in various places are some enormously enlarged cells (Figs. 12 and 13). These are trichogenous cells, which secrete the feathered and palmate hairs already referred to. They are pyriform in shape and, on account of their size, bulge through the hypodermis into the body-cavity. They contain a large round nucleus with a very prominent nucleolus, and around the latter are arranged threads of chromatin. Those which secrete the palmate hairs are smaller than those which secrete the other type. The row of four hairs which overhang the anus have the largest cells at their bases, and in transverse sections taken through that part of the body these cells form very striking objects, each one nearly equalling the rectum in diameter! In favourable sections a fine thread has been observed to pass into the base of each palmate hair; most probably this is a nerve fibre (*n'.* in Fig. 12).

Excepting in the head region, there occur in many places immediately beneath the hypodermis, and adherent to its inner surface, a layer of flattened irregularly shaped cells (Fig. 8)—the *sub-hypodermal cells* of Viallanes (1882, p. 12). Very frequently several of these cells are united together, and in their protoplasm is distributed a quantity of greenish granular particles. A similar tissue to the above is present in the larvae of *Musca, Eristalis* and *Chironomus.* In the latter case, Miall and Hammond (1900, p. 38) conclude from the various stages of aggregation which these cells exhibit, and from their slow changes of figure, they can move from place to place and, that however they may be scattered, they retain the power of combining into an epithelium. They regard them as being the wandering cells (Wanderzellen) of Metschnikoff and Kowalevsky. Viallanes states that these cells in *Musca* and *Eristalis* undergo changes analogous to those of the fat-body during the transition from the larva to the pupa. Their protoplasm contains spherical granules which increase in size and number as the larva gets older.

The Digestive System.

The *mouth-parts* together enclose a space or chamber, at the posterior end of which is situated the mouth itself. The epipharynx forms the roof of this chamber, the mandibles form the sides, and the maxillae, together with the labial plate, the floor (Fig. 4). The side walls are further completed by the overlapping of the brushes on either side, and also by the prominent hairs on the mandibles which meet those of the brushes.

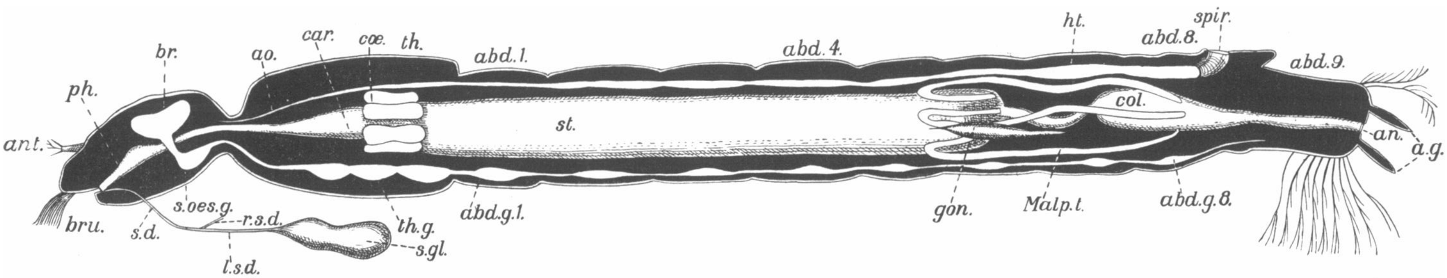
The epipharynx is provided with groups of setae (Fig. 5), and the hypodermis underlying them has a markedly columnar structure. Very possibly they are to be regarded as being of a sensory nature and perhaps gustatory in function. The hypopharynx and the anterior surface of the labial plate have a strong armature of chitinous tooth-like projections.

The Fore-Gut:—The fore-gut is divisible into pharynx and oesophagus.

The *Pharynx* is a capacious chamber situated in the anterior half of the head (Fig. 5). Its walls consist of a chitinous intima, continuous with the general cuticle of the body-wall, and resting on a layer of epithelium. Outside the epithelium is a strongly developed layer of muscles (Figs. 10 and 11).

At its commencement, the pharynx is much flattened in the dorso-ventral plane and has, moreover, a greater transverse diameter than in the succeeding portion. It is somewhat crescentic in shape when viewed in section owing to the development of a pair of lateral crests (Fig. 10). A little further backwards, its transverse diameter narrows but, on account of the increased development of the lateral crests, its cresentic shape becomes intensified. At the hinder part of the pharynx the crests become less prominent and, converging, eventually fuse with one another in the median dorsal line, and just in front of the commencement of the oesophagus. The chitinous intima of the roof of the pharynx, between the two crests, becomes considerably thickened on either side and forms a pair of rigid plates (*c.p.*): along the middle line between these plates the intima still remains flexible. Traced posteriorly the plates narrow very much, and terminate at a point just in front of the union of the two lateral crests of the pharynx. On the outside of each chitinous plate, and situated on the crest of its side, is a rod-like thickening of the intima (*rd.* in Figs. 10 and 11), which serves to give a firmer attachment to certain of the pharyngeal muscles. Situated in the crests of the pharynx is a dorsal and ventral longitudinal row of firm bristle-like setae (*s.* in Figs. 10 and 12).

The pharynx is provided with an elaborate musculature. These muscles have been found to agree in their general arrangement with the pharyngeal muscles of the larva of *Culex* as described by Thompson (1905, p. 145), and as most of the names given to them by that



Diagrammatic Figure showing the general organization of the larva of *Anopheles maculipennis*. × *circa* 20.

ant.	antenna.	*coe.*	caeca.
ao.	aorta.	*ht.*	heart.
bru.	brush.	*l.s.d.*	left salivary duct.
br.	brain.	*r.s.d.*	right do.
*abd.*1.	1st abdominal segment.	*s.d.*	main do.
*abd.*4.	4th do.	*s.gl.*	left salivary gland.
*abd.*8.	8th do.	*s.oes.g.*	sub-oesophageal ganglion.
*abd.*9.	9th do.	*ph.*	pharynx.
*abd.g.*1.	1st abdominal ganglion.	*Malp.t.*	Malpighian tube.
*abd.g.*8.	8th do.	*gon.*	gonad.
a.g.	anal gills.	*st.*	stomach.
an.	anus.	*spir.*	spiracle (right).
col.	colon.	*th.*	thorax
car.	cardia.	*th.g*	thoracic ganglia

observer are convenient and appropriate, they are adopted in the present description.

The principal pharyngeal muscles are as follows:—

A series of *dorsal pharyngeal muscles*; these are transversely placed fibres stretching between, and uniting the lateral crests of the pharynx (m_4 in Figs. 6, 10 and 11).

A pair of *lateral pharyngeal muscles*; they arise from the integument of the head, close to the point of origin of the antenna on either side, and slightly to the inside of that appendage. They cross the head-cavity obliquely, and become inserted on the summits of the pharyngeal crests (m_3 in Figs. 6 and 11).

A paired series of *anterior lateral pharyngeal muscles* (lesser lateral muscles of Thompson). These consist of four or five slender muscles on each side, each group arising from the antero-lateral border of the head just in front of the antenna of its side. They pass directly inwards in a horizontal fashion, and are inserted into the wall of the pharynx on either side, near to its commencement (m_2 in Fig. 6).

A pair of stout *epipharyngeal muscles*; these arise from the roof of the head a little anterior to the brain. They pass forwards, above the pharynx, at first nearly parallel with one another, but eventually converge to become inserted close together, about the point where the roof of the pharynx becomes continuous with the epipharynx (m_1 in Figs. 5 and 6).

A pair of *longitudinal pharyngeal muscles* (not described by Thompson in the *Culex* larva). These are a pair of slender muscles placed closely together, and lying in the dorsal trough of the pharynx between its lateral crests and beneath the dorsal pharyngeal muscles (m_5 in Fig. 11).

A pair of *elevator muscles* arise from the roof of the head a short distance in front of the brain. They pass almost directly downwards, and are inserted closely together on the roof of the pharynx near to the point where the two lateral crests become confluent (m_6 in Fig. 6).

A pair of *retractor pharyngeal muscles* arise from the hind wall of the head not far from the occipital foramen. They pass to the posterior margin of the pharynx to be inserted a little way behind the points of attachment of the elevator muscles (m_9 in Fig. 6).

A pair of *diagonal muscles*; these muscles arise from the posterior part of the head somewhat external to the retractor muscles. They pass very obliquely forwards and cross one another as they do so, and in this way they become attached to the posterior part of the pharynx a little in front of the retractor muscles. The point of attachment of each diagonal muscle is situated on that side of the pharynx opposite to its point of origin (m_8 in Fig. 6).

A pair of groups of *lateral dilator muscles*; these are attached to the side walls of the posterior portion of the pharynx, and the immediately adjacent portion of the oesophagus. Each group consists of a bundle of very slender muscles, and they take their origin from the integument of the ventro-lateral region of the head (m_7 in Figs. 6 and 11).

The walls of the pharynx contain a series of circular muscle fibres which are principally developed in relation to its posterior portion. Over the anterior part of the pharynx they are restricted to its ventral and lateral walls (Figs. 5 and 6).

The food is carried into the mouth in the current of water set up by the rapid motion of the brushes and, after being masticated to some extent by the action of the mandibles and maxillae, is passed into the pharynx. It is not possible, however, to make much out as regards the actual working of the pharyngeal muscles in the living larva on account of the want of transparency of the integument of the head. It appears to take place somewhat as follows. When the pharynx becomes filled with food a contraction of its dorsal muscles take place, resulting in the drawing together of the two pharyngeal crests, and causing the two dorsal chitinous plates to form an acute angle with each other, and to come very nearly in contact with its floor. The action of these muscles is assisted by that of the circular and diagonal muscles, and the result of their combined action is that the pharyngeal cavity becomes greatly reduced and much altered in its shape. The effect is the forcing of the food backwards into the oesophagus, and constituting the act of swallowing. The lateral, elevator, and retractor muscles most probably serve to assist in bringing about the divergence of the pharyngeal crests after swallowing, but very likely fulfil other functions also. At the junction of the pharynx with the oesophagus the food is propelled backwards by means of the lateral dilator and circular muscles, and afterwards the work is carried on entirely by the muscles of the oesophagus.

The *Oesophagus* is a narrow tube of simple structure (Figs. 3 and 16). Its innermost coat is a well-defined chitinous intima, the middle coat is an epithelium, and the outermost is muscular. The cellular nature of the epithelium is usually only to be discerned by the presence of nuclei. The muscular coat consists of a strongly developed layer of circular fibres which are markedly striated. Each muscle band is in contact with its fellows, and in this way the muscular coat forms a complete investment to the oesophagus (Fig. 25). They increase in thickness as they are traced backwards, and attain their maximum in the oesophageal valve described below. Near to the junction with the pharynx, several radial muscle fibres pass outwards between the circular bands, and are attached to the walls of the head; they function as the dilators of the oesophagus. Outside the muscular coat is a thin membrane of connective tissue. The lumen of the oesophagus alters in shape a good deal in different parts, and is much reduced by inwardly directed bulgings, which involve both the cuticular and epithelial layers.

Followed backwards into the middle of the thorax, the oesophagus joins the cardia, or first division of the mesenteron (Fig. 3). It is prolonged into the cavity of the latter for some distance as an inner tube and then becoming reflected on itself, passes forwards again to unite with the epithelium of the mid-gut (Fig. 25); in this way there is formed an oesophageal or cardiac valve. Both the chitinous intima and the epithelium of the fore-gut are thus reflected, but the circular muscle bands cease some distance before the bend is reached: some stout longitudinal fibres are also present. The space between the inner or downwardly directed wall and the outer or reflected wall of the oesophagus contains a good deal of blood, and there is also a circular sinus situated between the muscular layer and the inner wall (Fig. 25). This structure surrounds the gut in the form of a complete ring; it is covered by a layer of connective tissue, and its interior shows a spongy structure, its cavity being much intersected by strands of a fibrous nature. It has been found in several Dipterous larvae, and is regarded as being a blood sinus. A fibrous band passes from the oesophagus across to the shoulder or apex of the cardia (*lig.* in Fig. 25); it contains no muscle fibres, and in all probability functions as a ligament. It appears to be derived from the outer connective tissue coat of the oesophagus, and it passes into that of the cardia.

An oesophageal valve seems to be of very general occurrence among the larvae of Diptera, and it exhibits a fairly wide range of complexity. In the *Dicranota* larva it is very simple, and the oesophagus only protrudes very slightly into the mesenteron. In Culex it is in the same condition as in *Anopheles*. In *Simulium*, according to the figures given by Miall and Hammond (1900, p. 61), the circular muscles are more strongly developed, and extend close down to the apex of the valve, the blood sinus is much larger and, moreover, differs from that of *Anopheles* in being placed between the layer of circular muscles and the outer or reflected wall of the valve. In *Chironomus*, according to those observers, the valve has the same general arrangement as in *Simulium*, but is complicated by secondary foldings of the walls of the oesophagus, which involve both the intima and the epithelium, and are termed by them the upper and lower intermediate bands. In the *Ptychoptera* larva the valve attains a remarkable complexity of structure. According to van Gehuchten (1890, p. 185) there are secondary foldings as in *Chironomus*, and there is a special sphincter muscle situated at the commencement of the valve, in addition to the usual circular fibres. The blood sinus is very extensive, and its cavity is traversed by numerous strands, some of which are muscular and others elastic. There is further, a secondary folding in the wall of the cardia forming a circular "proventricular valvule" (van Gehuchten, Plate 1, Fig. 5 and Plate 3, Fig. 44).

The Salivary Glands:—The salivary glands are situated in the thorax one on either side of the digestive canal. Each gland is a hollow vesicle lined by a single layer of cells (Fig. 22), and passing at its proximal end into a narrow duct (Text-Fig. p. 298). The two ducts unite within the head near to the sub-oesophageal ganglion, and the unpaired channel thus formed opens into the mouth on the dorsal aspect of the labial plate in the median line (*ap.* in Fig. 5). In the very young larva the salivary glands are relatively of less bulk as compared with those of full-grown examples. The structure of the glands is extremely simple, each consisting of a layer of epithelial cells, bounded on the exterior by a thin coat of connective tissue. The cells are very large and are polygonal; they bulge inwards to some extent into the lumen of the gland. Their nuclei are extremely prominent (Figs. 22 and 23), and they each contain a large nucleolus, together with thick loops of chromatin. The ducts are very thin walled, and are lined with a delicate intima: the median duct just before opening on to the hypopharynx expands into a slightly bulbous enlargement. No "spiral thread" could be detected though it is found in the salivary ducts of the adult mosquito.

The Mid-gut or Mesenteron:—The mid-gut is a long cylindrical tube extending from the middle of the thorax nearly to the posterior border of the sixth abdominal segment, where it joins the hind-gut (Text-Fig.). Throughout the greater part of its course it is of a uniform calibre, but it narrows somewhat near its posterior end. It is separable into three divisions, viz. (1) the *Cardia*, (2) a circlet of *eight caecal pouches*, (3) the *Stomach*.

The *Cardia* is that portion of the mid-gut which encloses the oesophageal valve (Figs. 3 and 25). I have followed Miall and Hammond in using this term in preference to the name proventriculus which is employed by many authors. The word proventriculus has long been associated with the gizzard in Insecta which is derived from the stomodaeal ectoderm. Miall and Hammond have demonstrated by a study of the embryological development of *Chironomus* that the wall of the cardia is produced from the endoderm. There is every reason to believe that the same is the case in *Anopheles*, and many other Dipterous larvae. This is supported by the fact that the cells of the cardia have the same general character as those lining the other parts of the mid-gut. The break where the epithelium of the oesophagus terminates, and that of the cardia commences, is clearly marked (Fig. 25), and the cardiac chamber of the stomach may be regarded as extending from this point backwards to where the eight caeca are given off from the mid-gut. The epithelium of the cardia is, for the most part, composed of large and markedly columnar cells, which stain more deeply than those of any other part of the mesenteron. Their nuclei are large and very prominent, and contain a good deal of chromatin in the form of coiled threads (Fig. 25). Further backwards, however, the epithelium instead of being columnar becomes cubical, and then passes by a gradual transition into that lining the caeca. The cardia is invested exteriorly by a coat of connective tissue; there are no muscle fibres present in its walls.

The *Caeca* are eight sub-equal diverticula of the mesenteron arranged in the form of a ring or circlet (Figs. 3 and 26). Each caecum is constricted across its middle, as if it were bound by a circular ligament (Fig. 25). Nothing, however, of such a nature is present and, moreover, the caeca contain no muscle fibres in their walls. The epithelial lining consists of rather larger cells than those of the stomach, and they bulge somewhat into the central cavity (Fig. 18). Externally they rest on a membrane of connective tissue. The caeca are filled with a thin dark yellow fluid which is coagulable by alcohol (Figs. 18 and 25). At times the free portions of the cells bordering the cavities appear swollen by a transparent fluid secretion, but in no instance were they observed to give off protrusions as the cells of the stomach frequently do.

These caeca are of frequent occurrence among Dipterous larvae, and vary very considerably in size and number. In the larvae of both *Culex* (Raschke), and *Ptychoptera* (Gehuchten), eight caecal diverticula are present as in *Anopheles*. In those of *Tipula* (Hammond), *Piophila* (Dufour), *Simulium* (Miall), *Anthomyia* (Vaney), *Volucella* (Künckel d'Herculais) and *Calliphora* (Lowne), there are four caeca. In *Sciara* (Packard), *Ceroplatus* (Dufour) and *Sapromyzia* (Dufour), there are only two, but in these instances they are very long and tube-like. In *Chiro-*

nomus the caeca are small and very numerous, and arranged in three sets (Miall and Hammond). Caeca are wanting, however, in *Phalacrocera* (Miall and Shelford), *Dicranota* (Miall) and *Psychoda* (Dell).

With regard to the function of these organs, it may be mentioned that Hoppe-Seyler, Plateau, Krukenberg, and others maintain that the digestive properties of the already mentioned fluid contained within them are of a similar nature to that of the pancreas in vertebrates.

The *Stomach* comprises the whole of the remaining portion of the mesenteron (Fig. 3 and Text-Fig.). Its walls consist of the following, passing from within outwards:—

1. The peritrophic membrane (*p.m.* in Figs. 14 and 15). It commences on the cardiac epithelium close to the junction of the latter layer with that of the oesophagus. This membrane invests the whole of the inner surface of the mesenteron, excepting that it is not reflected into the cavities of the caeca.

2. The epithelial layer, which is composed of columnar polygonal cells with prominent rounded nuclei. Each nucleus contains a sharply defined nucleolus, together with a number of large chromatic granules (Fig. 14).

3. The basement membrane; it is a well-developed coat and stains readily (*b.m.* in Figs. 14 and 15).

4. The muscular coat in the form of an inner circular and an outer longitudinal series of fibres, both of which are striated (Figs. 14 and 15).

5. The connective tissue coat. It is an extremely thin membrane which invests the outer surface of the muscle fibres, and also lines the quadrangular spaces formed between them. In the latter case this coat is in contact with the basement membrane.

The epithelium lining the anterior third of the stomach is very uniform in character (Fig. 25). Towards its middle, however, they are seen to alter and give off protrusions into its cavity (Fig. 15). These protrusions are finely granular, and nearly transparent; they stain only extremely faintly. They are composed of accumulations of secretion which eventually become detached as spherical masses. In these cells the chromatin granules were observed to have a tendency to become concentrated into the centres of the nuclei. Van Gehuchten has specially studied the digestive system of the larva of *Ptychoptera contaminata*, and has described similar cells. He explains their function as follows. When secretion commences within the cells the clear fluid elaborated in their protoplasm increases the intra-cellular tension until, eventually, the fluid breaks through certain weak places in the membrane of the cell and bulges out into the lumen of the mid-gut in the form of a pear-shaped vesicle of a liquid rich in albumens, at first attached to the free face of the cell, but eventually becoming set free (1890, p. 238). In *Anopheles* these protrusions can be seen along the epithelium of the stomach nearly to where it joins that of the intestine.

Distributed at irregular intervals at the bases of the epithelial cells are small pear-shaped cells (Fig. 15). They lie between the larger cells and are disposed either singly or in groups of two or three. Similar cells to these are common in the mid-gut of various insects. It is probable that after the epithelial cells have burst and discharged their secretion, these small cells are the centres from which the epithelium is regenerated. These cells are extremely well shown in the stomach of the cockroach (*Periplaneta*), and Miall and Denny have pointed out their close resemblance to Watney's buds in Mammals. In this insect they have described all the stages in the development of new epithelium from these buds that have been noted by Watney in the case of Mammals.

The epithelial cells of the whole mesenteron occasionally exhibit a well-marked "striated margin" or "Härchensaum" along their free edge bordering the gut cavity. I have found it to be best and most frequently seen in the cells of the caecal diverticula; the only place where I have not observed it with any certainty is on the large columnar cells of the cardia, but I do not wish to infer that those cells never develop this structure. In those preparations where orange G has been used as a second stain, the striated border shows up very distinctly, and appears rich yellow in colour (Fig. 18). The presence of this border is eminently characteristic of cells in the resting condition. It is very often to be seen in preparations of the mid-gut of various insects, and it bears a close resemblance to the "striated hem" found in the intestinal epithelium of Vertebrata. A great deal of diversity of opinion exists as to its structure, origin, and function. Under a high magnification it *appears* to be made up of extremely delicate and closely-set vertical processes or filaments, which rest on a basal membrane. The best and most recent account of this structure is given by Vignon (1901, p. 371).

The peritrophic membrane may be regarded as a thin tube which completely encloses the food as it passes through the mesenteron. It is quite colourless and, on account of its resistance to the action of alkalies, it is inferred to be of a chitinous nature. It is only to be seen, as a rule, in actual contact with the mesenteric epithelium at its point of origin near to where the mid-gut joins the oesophagus. Over the rest of the gut it is separated from the epithelium by a space of variable width, and usually filled with a granular fluid most probably of a secretory nature. The membrane crosses the apertures of the caeca, but is not reflected into their cavities (Fig. 25), and it extends for a considerable distance into the hind-gut (Figs. 20 and 25). An examination of the exuviae of *Anopheles* larvae shows that during the periods of ecdysis the peritrophic membrane is shed and got rid of through the anus. In some instances it was seen in a crumpled condition in the hind-gut prior to being evacuated. In others, the membrane was seen to be double, one tube lying within the other. This occurrence is to be explained by the fact that the old membrane was still *in situ*, and the new one had already been formed around it.

A chitinous lining to the mid-gut is of frequent occurrence in most orders of Insects, as well as occurring in the Myriapoda, certain Crustacea, and a few Gasteropoda (*Helix*, *Limax*, and *Lymnaea*) (Schneider, 1890, p. 92). How far the membrane is strictly homologous in these various groups it seems at present impossible to say. With regard to its origin among Insecta, very diverse views are held and, moreover, it is extremely probable that its mode of development differs among various insects. Gehuchten (1890, p. 273) has studied it in the larva of *Ptychoptera*, Cuénot (1895, p. 293) in the Orthoptera, Vignon (1901, pp. 382, 396, and 537) in the larva of *Chironomus* and in the "silkworm," and Balbiani (1890, p. 1) and Plateau (1878, p. 85) in the Myriapoda. All these observers agree in regarding it as being a product of the cells of the mesenteron. Balbiani, Plateau and Vignon (in the silkworm) state that it is a secretion formed at the surface of the whole of the epithelial lining of the mesenteron. Gehuchten, Cuénot and Vignon (in the *Chironomus* larva) regard it as being secreted in a localised area of the cardia. Schneider (1890, p. 89) has studied this membrane very briefly in a number of Insecta, and terms it the funnel ("Trichter"). He states that it is a backward continuation of the cuticular lining of the oesophagus, but he did not study its process of formation. If his observations be substantiated by future enquiry, it would seem that there are two types of this membrane to be found among the Insecta which are analogous, and not homologous with one another. In the one case it would seem that it is a product of the ectoderm (Schneider), and in the other a product of the endoderm (the other authorities quoted). However it may be for some of the forms Schneider has studied, it is far from certain whether it is of the nature he claims it to be in the case of the *Chironomus* larva. The detailed researches of Vignon go a long way towards proving that it really arises as a chitinous secretion, at first of a fluid nature, but becoming coagulated a short time after its emission, and produced by a row of thickened epithelial cells situated in the anterior region of the cardia.

After a study of a number of prepared sections of the mid-gut of the *Anopheles* larva, I am inclined to believe, with Vignon and others, that the peritrophic membrane arises in the anterior part of the cardia. The large deeply-staining columnar cells which are characteristic of this region, and have already been referred to (p. 303), have all the characters of very active secretory cells, and I regard them as being the seat of origin of this membrane. The latter commences close to the point where these cells arise, and in some instances I have observed it in close contact with the inner face of this epithelium (Fig. 24); elsewhere it only *touches* the walls of the gut in places. It seems most likely that the membrane, as it is being secreted, is pushed backwards into the stomach by the food as it (the food) issues through the oesophageal valve.

With regard to the function of the peritrophic membrane, the most probable suggestion is that it seems to protect the mesenteric epithelium from abrasion by hard and resisting particles of food.

Schneider states that a chitinous membrane is present in the mid-gut of Thysanura, Orthoptera, in many Neuroptera and Coleoptera, in ants and wasps among Hymenoptera, and in larvae of Diptera and Lepidoptera. He mentions that it is wanting in *Carabus*, *Dytiscus*, *Coccinella* and *Bruchus* among Coleoptera, in some Hymenoptera, in the adults of Lepidoptera and in Hemiptera. He claims that all those insects (together with their larvae) which possess the membrane or "funnel," as he terms it, eat solid and indigestible substances for food, while those which do not possess it take their food in a fluid form.

Since the food is separated off from the mesenteric epithelium by means of this membrane, the question arises as to how the digestive secretions come into contact with the food and the method by which absorption takes place. Gehuchten (1890, p. 272) states that "sans aucun doute" the digestive secretions traverse the peritrophic membrane by means of osmosis, and by a similar means the elaborated products come in contact with the epithelium ready for absorption. Vignon (1901, p. 538) explains the process in a similar manner. Miall and Hammond (1900, p. 58) remark that a granular fluid is present in the narrow space between the epithelium and the membrane; it also contains granules of larger size which they believe to come from the food. They believe, however, that it is unnecessary to suppose that the secreted fluid *diffuses through* the peritrophic membrane; the granules just noted indicate that another communication exists. They believe it probable that the fluid squeezed out from the food in the oesophagus and oesophageal valve passes down the cylindrical tube formed by the peritrophic membrane, and that it is regurgitated into the outer space by the contractions of the powerful circular muscles situated at the commencement of the small intestine. Furthermore, Dell (1905, p. 293) states that he has observed reversed peristaltic contractions in the intestines of the living *Psychoda* larva. The digested food has been seen to be carried up into the space between the peritrophic membrane and the mesenteric epithelium by this means. In the case of the larva of *Anopheles* and other insects, however, the peritrophic membrane does not cease at the termination of the mid-gut, but is prolonged for some distance into the hind intestine. In such instances regurgitation would of necessity take place very far backwards, and if this took place the products of digestion would surely become liable to be contaminated with faecal matter. In other cases, the peritrophic membrane extends as far backwards as the anus, and under such conditions regurgitation could not take place at all.

The theory of diffusion seems to explain the process more adequately and does not present any very great difficulties. It is well known that animal membranes are permeable to salts and sugars but not to proteids, which are of the nature of colloids (non-diffusible substances). The proteids of lower molecular weight, namely the proteoses and peptones, however, are highly diffusible when compared with albumin (Schäfer, 1898, p. 45). The action of the digestive secretion would, however, most likely convert the albumins of the food into substances analogous to peptones, and in this way the difficulty would be obviated.

The Hind-gut:—The Hind-gut commences about the middle of the sixth abdominal segment (Text-Fig. p. 298). It may be divided into three regions, viz. *ileum*, *colon* and *rectum*.

The *Ileum* receives at its commencement five Malpighian tubes (*M.t.*), and extends to the beginning of the eighth segment of the abdomen. It is lined by a flattened epithelial layer, but it was not possible to distinguish any cell boundaries; an extremely delicate intima invests the cells where they border the cavity of the gut (Fig. 17). The epithelium rests on a basement membrane (*b.m.* in Fig. 17), and situated outside the latter is a very strongly developed coat of circularly disposed muscle fibres, and they are succeeded by a layer of longitudinal muscles. The whole is covered exteriorly by a thin connective tissue membrane. The circular muscles are extremely closely packed together, each band being in contact with its fellows. A short distance beyond the commencement of the gut they attain a great thickness (Fig. 17).

The *Malpighian tubes*, as already stated, are five in number, the odd tube being situated on the dorsal side (Fig. 3). This same number is present in the adult mosquito. They have the usual structure seen in insects. From the point where they arise from the gut, up to about where they form a loop to redouble and pass backwards, the limits between the individual cells of their epithelial lining are scarcely indicated, but over the rest of their course the cells are very distinct and bulge somewhat into the central cavities of the tubes (Figs. 3 and 21).

The protoplasm of the cells contains an abundance of dark granules, most probably of an excretory nature. Externally, the tubes are invested by a membrane of connective tissue. Five is a very unusual number for the Malpighian tubes, nevertheless it is present also in the larvae of *Culex, Psychoda, Ptychoptera*, and of the Blepharoceridae. For this reason Müller (1881, p. 499) has proposed to unite these forms together into a common group which he terms the "Pentanephria." According to Eysell (1902, p. 341) five Malpighian tubes apparently also occur in *Aedes cinereus* (imago).

The *Colon* is a much wider tube than the ileum, and it lies mostly within the eighth abdominal segment (Text-Fig. p. 298); it passes by a gradual transition into the rectum. Its epithelial lining is well developed, and consists of large polygonal cells with prominent nuclei, though the cell boundaries are not always to be distinguished. It is lined internally by a thin intima (Figs. 19 and 20). The colon is provided with a strongly-marked coat of circular muscles (Figs. 3, 19 and 20); these bands are placed at very regular intervals from one another. When the alimentary canal of the larva is dissected out these muscles show very prominently on the exterior (Fig. 3), but they do not, however, attain the thickness of those of the ileum. Externally, the colon is invested by connective tissue.

The terminal chamber of the hind-gut or *Rectum* (Fig. 3 and Text-Fig. p. 298) can be distinguished from the colon by its very much thinner epithelial lining, which resembles that of the ileum. It is lined by a delicate cuticle or intima, and its circular muscles are disposed in a similar fashion to those of the colon, only they are not so pronounced when viewed in section. There are no longitudinal muscles.

The Respiratory Organs.

The Respiratory Organs of the *Anopheles* larva consist of the tracheal system, and possibly the two pairs of anal processes situated around the hinder extremity of the body are of the nature of gills.

The *Tracheal System* (Fig. 27) communicates with the atmosphere by means of a single pair of spiracles located on the dorsal aspect of the eighth abdominal segment. It is, therefore, an example of the metapneustic arrangement.

There are two principal longitudinal trunks, lying one on either side of the mid-dorsal line of the body (*l.t.*). These vessels run from the spiracles directly forward into the thorax. In the latter region each trunk divides into a pair of branches which supply the various organs of the head. From the two main longitudinal trunks smaller tracheae arise which pass to the different organs in the body, and to a very large extent they maintain a segmental arrangement. In each abdominal segment (excepting the ninth) an outwardly directed vessel is given off on either side (*t.t.* in Figs. 27 and 28). It soon bifurcates into an anterior and a posterior branch; the anterior branch anastomoses with the posterior one of the segment in front, and the posterior branch with the anterior one of the segment behind. In this fashion a secondary longitudinal trunk is formed along each side of the body. Where this latter vessel meets the transverse vessel (*t.t.*) in each segment, a branch arises which chiefly supplies the gut and part of the musculature (*o.* in Fig. 28). A short distance behind it there is similarly repeated in each segment a second branch (*p.*) which, passing downwards, divides into numerous twigs supplying the nerve cord and ventral muscles. At the point of its bifurcation each transverse vessel is continued outwards to the integument in the form of a delicate strand (*stig.c.* in Fig. 28). The point where the latter meets the external cuticle is marked by a minute chitinous scar. These strands, or stigmatic cords as they are sometimes termed, are perhaps to be regarded as the vestiges of the primitive invaginations which form the initial tubes leading from the spiracles to each segmental system in holopneustic insects. In the case of *Anopheles*, however, since all the spiracles, with the exception of the posterior pair, have been closed up, the initial tubes, having no further use, have atrophied into chitinous cords or strands. This seems to be brought about through their lumina becoming obliterated by means of a chitinous deposit. The stigmatic cords in the present larva are difficult to make out on account of their small calibre and their transparency. They are to be seen in many Dipterous larvae, and can be studied with greater facility in the larva of *Eristalis*, among others, than in that of *Anopheles*.

The two longitudinal trunks are united with one another by means of segmentally repeated commissures. In the region of the eighth abdominal segment the two main trunks give off a great number of small branches along their inner and ventral aspects (Figs. 27, 30 and 31). It has not been possible to detect any traces of the spiral thickening in these branches, and I believe that they are without it. At their origins from the main trunk these branches have a tendency to be united together into small bundles opening into depressions or crypts in the walls of the former (Figs. 31 and 33). Traced further along their course they are seen to separate apart, and to pass to the walls of the terminal chamber of the heart (Fig. 30). On account of the thinness of the walls of the ultimate branches (or capillaries) of these tracheae, it seems very probable that the blood is brought into close contact with the oxygen contained in them, and in this way a kind of "lung" is formed.

In addition to *Anopheles* I find, after an examination of the larvae of *Dixa* and *Culex*, both by means of dissections and serial sections, that a similar arrangement is present in them. In the case of *Dixa*, the branches were seen to arise almost immediately beneath the spiracular apertures, and some of them went directly to the heart, while others appeared to float freely in the blood space contained within the segment. In *Culex* the arrangement is identical with that of *Anopheles* (Fig. 29), excepting that the branches are not quite so abundant. Raschke (1887, p. 133), however, in his description of the larva of *Culex nemorosus*, makes no mention of this feature, but he states that in association with the anterior end of the rectum are great numbers of fine tracheae which are devoid of the spiral thickening. He states that they pass through the wall of this portion of the gut and, subdividing, terminate in countless numbers of minute twigs lying in papilla-like folds situated within the rectum. This arrangement of the tracheae is not represented in any of his figures of the larva, and I have been quite unable to discover any traces of it after having examined a considerable number of *Culex* larvae. Furthermore, Vaney (1902, p. 138) in his studies of Dipterous larvae, mentions the relationship just described that exists between the tracheal system and the heart, but makes no reference to the presence of tracheae in the walls of the rectum. Thompson (1905, p. 145) in his detailed account of the alimentary canal of *Culex* likewise makes no mention of it. In the light of this evidence I would suggest the probability that the branches described by Raschke are the identical ones which are seen in Fig. 29, and, therefore, is it not probable that as these branches lie immediately over the rectum, and obscure most of the heart from view at the point where they are situated, Raschke has been deceived into believing that they supply the rectum?

A similar disposition of the tracheae in relation with the heart is mentioned by Vaney (1902, p. 138, and Plate IV, Fig. 59) in the larva of *Psychoda sexpunctata*, and he figures the two bunches of tracheae arising from the main longitudinal trunks. Dell (1905, p. 300) in his paper on this larva says that "In the last segment, immediately dorsal to the hinder part of the heart, there arise from each longitudinal trachea a number of small branches, which break up into branches of distribution in the neighbourhood of the pericardium." Neither of these observers, however, have devoted more than a passing reference to this relationship of the tracheae.

A somewhat similar system, by means of which the haemocoelic and tracheal systems are brought into close relationship with one another, is described by Viallanes (1882, p. 65) in the case of a larva living in mud, and which he believes to belong to the genus *Ctenophora*. In this instance he states that the dorsal vessel is in the form of a tube open at its anterior and posterior extremities, and there are no valves or ostia. He believes that this condition coincides with a peculiar arrangement of the tracheal system. The larva in question possesses a pair of spiracles situated at the hinder extremity of the body. From either spiracle there runs throughout the length of the animal a main tracheal trunk, and just in front of the middle of the last segment a transverse connection joins the two trunks together. It is at the level of this anastomosis that the dorsal vessel commences. Between the spiracles and this anastomosis the two main tracheal trunks give off in all directions a great number of small branches. These branches float freely in the cavity of the last segment, where they are bathed with the blood it contains, and they are present in such numbers as to occupy the greater part of it. In this instance the blood receives oxygen from the tracheae before entering the heart.

The above cases are the only instances that I have been able to come across where the tracheal and circulatory systems are in intimate connection with one another. The question arises as to why this arrangement should occur in the particular forms mentioned, and apparently be wanting in most other Dipterous larvae. As a possible solution, I would suggest that perhaps it is correlated with a reduction in the number of the spiracles. It will be observed that the larvae of *Anopheles*, *Culex*, and *Dixa* are all metapneustic forms, and similarly the larva regarded by Viallanes as being that of a species of *Ctenophora*.

In the case of the *Psychoda* larva, in addition to the posterior spiracles, there is also a minute pair situated on the first (or prothoracic) segment. In all probability this latter pair of spiracles are examples of vestigeal organs, for indeed Dell (1905, p. 299) remarks that "they are not open and probably not functional, since they are always immersed in water or mud." If this be true, although the larva is morphologically amphipneustic, physiologically it is metapneustic, as in the other instances quoted. It would be rash, in the light of these few instances, to regard this condition as being characteristic of metapneustic larvae, and nothing more than the possibility of its occurrence being correlated with that type of tracheal system is here suggested. Just as Prof. Miall (1891, p. 458) has remarked with regard to the distribution of haemoglobin, that we have a tolerably satisfactory reason for its occurrence in a number of aquatic animals whose respiration is limited, and whose surroundings make it a matter of difficulty to procure a sufficient supply of oxygen, we have, however, to admit that many similar animals, under the same conditions, manage perfectly well without it. In the same way, very probably, many metapneustic Dipterous larvae will be found where this relationship between the tracheal system and the heart is likewise dispensed with. Such an admission is not a logical refutation of the explanation. In those larvae, where there is but a single pair of functional spiracles, it is by no means unreasonable to believe that the passage of oxygen to the various organs of the body must be an extremely slow process. There is the possibility of this disadvantage being eliminated in the instances related above, for the blood as it is pumped forwards would absorb oxygen directly from the tracheae, and in this way the oxygenation of the tissues would be greatly expedited. I am fully aware, however, how difficult it is to give a correct interpretation of natural contrivances, and how easy it is to formulate an idea as to how a certain fact may be explained. As Semper (1899) remarks, there is very little trouble needed to imagine some process by which hypothetical fundamental causes may have led to the result which has been actually observed.

The spiracles are situated close to the middle line on the dorsal aspect of the eighth abdominal segment (Fig. 27). They are supported by a complex chitinous skeleton, which is a development of the tergum of that segment; it has been described in detail by Nuttall and Shipley (1901, p. 64). The spiracles lie fully exposed to the air, and are of an extremely simple structure, being little more than simple chitinous rings which lead directly into the main tracheal trunks. Almost immediately within their apertures the walls of the tracheae are seen to be covered with a layer of branched chitinous trabeculae (Figs. 31 and 32). These trabeculae extend along the ventral walls to the point where the tracheal branches are seen to take their origin; dorsally, however, they extend some distance further inwards (Fig. 31).

The *Anal* (tracheal) *Gills* consist of two pairs of delicate leaf-like outgrowths of the integument situated around the vent. The two gills of each pair are placed dorso- and ventro-laterally respectively in relation to that aperture. The cavities of the gills are in free communication with the general haemocoelic cavity of the animal (Text-Fig. p. 298) and, therefore, contain blood, and they are capable of a considerable amount of extension and retraction, which depend upon the quantity of blood contained within them at a given time. Each pair of gills is supplied by a tracheal branch arising from the main air-trunk close to the spiracle of its side (*g.t.* in Fig. 27). Just before reaching the gills it bifurcates into two branches, one passing to each gill. Within the gill the branch immediately sub-divides, and the capillaries thus formed do not undergo further sub-division, but pass straight to the apex of the organ. Here they redouble and pass backwards for some distance.

On account of the thinness of the integument investing these organs, and the fact that they are well supplied with blood and tracheae, there is good reason to believe that they function as accessory respiratory organs. They are very characteristic of Culicid larvae, but are not to be confounded with the anal gills of the Chironomidae which contain blood but no tracheae. It is noteworthy that, according to Christophers (1906), in the larva of a *Stegomyia* (*scutellaris?*) they are extremely large and, moreover, the latter has a habit of remaining for long periods at the bottom of the water.

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REFERENCE LETTERING.

...	(Fig. 31) Direction of the section in Fig. 32.
A, A.	Palmate hairs.
abd_8.	Eighth abdominal segment.
abd_9.	Ninth ditto.
a.g.	Anal gills.
an.	Anus.
ant.	Antenna.
ap.	Aperture of salivary duct.
B.	Stigmatic plate.
b.	Brush.
b.m.	Basement membrane.
br.	Tracheal branches passing to heart.
b'r'.	Ditto in transverse section.
b.s.	Blood space.
b.sin.	"Blood sinus."
c.	Cuticle.
car.	Cardia.
c.e.	External layer of the cuticle.
c.i.	Internal ,, ,,
c.p.	Chitinous plate.
c.m.	Circular muscles.
c'.m'.	Connective tissue membrane.
co.	Colon.
coe.	Caeca.
crp.	Tracheal crypt.
e.	Epipharynx.
ep.	Epithelium.
ex.w.	External surface of cardia.
fr.h.	Frontal hairs.
f.g.	Frontal ganglion.
g.t.	Tracheal trunk supplying anal gills.
h.	Hypodermis.
hs.	"Härchensaum."
ht.	Heart.
il.	Ileum.
in.	Intima.
i.w.	Inner wall of oesophageal valve.
l.	Labrum.
lb.	Labial plate.
lig.	Ligament of connective tissue.
l.m.	Longitudinal muscles.
l.t.	Principal (or lateral) tracheal trunk.
l'.t'.	Secondary lateral tracheal trunk.
m.	Mandible.

m'.	Greatly thickened circular muscles of oesophagus.
m_1.	Epipharyngeal muscles.
m_2.	Anterior lateral pharyngeal muscles.
m_3.	Lateral pharyngeal muscles.
m_4.	Dorsal pharyngeal muscles.
m_5.	Longitudinal pharyngeal muscles.
m_6.	Elevator muscles.
m_7.	Lateral dilator muscles.
m_8.	Diagonal muscles.
m_9.	Retractor pharyngeal muscles.
ms.	Mesenteron.
mx.	Maxilla.
mx.p.	Maxillary palp.
n.	Nucleus.
n'.	Nerve fibre (?).
nc.	Nucleolus.
o.	Tracheal branch to gut and muscles.
oes.	Oesophagus.
o.w.	Outer wall of oesophageal valve.
p.	Tracheal branch to nerve cord and ventral muscles.
ph.	Pharynx.
p.m.	Peritrophic membrane.
prc.	Dorsal notched process.
r.	Rectum.
rd.	Chitinous rod-like thickening.
s.	Groups of setae.
s.c.	Stigmatic cord.
s.d.	Salivary duct.
sec.	Fluid contents of caeca.
s.g.	Sub-oesophageal ganglion.
s.gl.	Salivary gland.
si.	Respiratory siphon.
sk.	Supporting skeleton (stigmatic plate) of spiracles.
s.o.	Probable sense organs.
sp.	Spiracle.
sub.hd.	Sub-hypodermal tissue.
t.	Tooth-like projections.
t.p.	Tergal plate.
tr.	Trachea.
trab.	Chitinous trabeculae.
t.t.	Transverse tracheal trunk.
x.	Bulging in inner wall of oesophageal valve caused by food in stomach.

EXPLANATION OF PLATES IV and V.

PLATE IV (Figs. 1—20).

Fig. 1. Fully grown larva, dorsal aspect × 16. [From Nuttall and Shipley.]

Fig. 2. Fully grown larva viewed laterally to show method of attachment to surface film of the water × 12.

Fig. 3. The digestive canal of a fully grown larva.

Fig. 4. Diagram to show the relations of the mouth-parts to one another.

Fig. 5. Longitudinal and slightly oblique section taken through the mouth and pharynx together with the salivary duct (very young larva).

Fig. 6. Dorsal aspect of the pharynx showing its musculature; reconstruction from serial sections. (Diagrammatic.)

Fig. 7. Section through the integument.

Fig. 8. Section through the integument from the median dorsal region of the abdomen shewing sub-hypodermal tissue.

Fig. 9. Section through the cuticle.

Fig. 10. Transverse section through the commencement of the pharynx.

Fig. 11. Transverse section of the pharynx, taken a short distance beyond the middle.

Fig. 12. Section through palmate hair and trichogenous cell at its base.

Fig. 13. Section through the bases of two feathered hairs of the abdomen together with their trichogenous cells.

Fig. 14. Section through the wall of the stomach.

Fig. 15. Ditto, showing protrusions.

Fig. 16. Transverse section of the oesophagus near its commencement.

Fig. 17. Transverse section through the commencement of the ileum.

Fig. 18. Portion of the wall of one of the caeca of the mesenteron; the cells show a marked "härchensaum" along their inner faces.

Fig. 19. Transverse section through the colon.

Fig. 20. Longitudinal section through the colon.

PLATE V (Figs. 21—33).

Fig. 21. Longitudinal section taken through the junction of the mid-gut and hind-guts and passing through the commencement of one of the Malpighian tubes.

Fig. 22. Transverse section across a salivary gland at its widest part.

Fig. 23. Nucleus of secretory cell of salivary gland.

Fig. 24. Section through the glandular part of the epithelium of the cardia showing the peritrophic membrane in close contact with the inner surface of the cells.

Fig. 25. Longitudinal section through the oesophageal valve, the cardia and two of the caeca.

Fig. 26. Transverse section through the line *a...b* in the preceding figure: partly diagrammatic.

Fig. 27. General view of the tracheal system.

Fig. 28. Figure showing the segmental tracheal systems in two of the abdominal segments: the arrow is pointing towards the head of the larva.

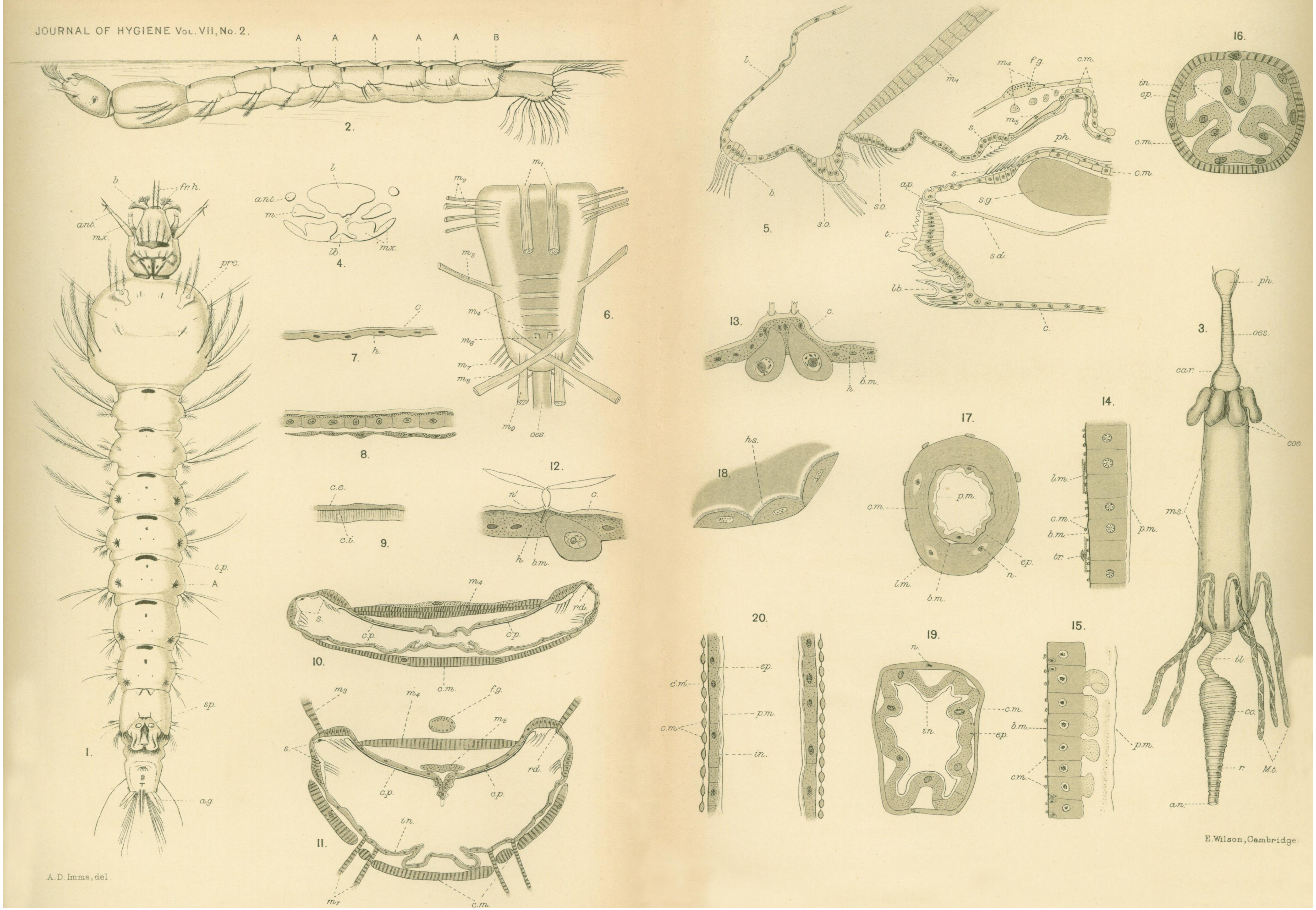
Fig. 29. Hinder portion of the abdomen of a *Culex* larva showing numerous branches, passing from the two main tracheal trunks to the heart.

Fig. 30. Horizontal and longitudinal section through the posterior extremity of the heart showing numerous tracheal branches passing to its walls.

Fig. 31. Longitudinal section through the left spiracle and the commencement of the main trachea.

Fig. 32. Transverse section through the line *···* of Fig. 31, showing the trabeculae arising from the walls of the trachea.

Fig. 33. View of the inner surface of one of the main tracheal trunks showing the "crypts," and the numerous apertures of the branches which pass to the heart.



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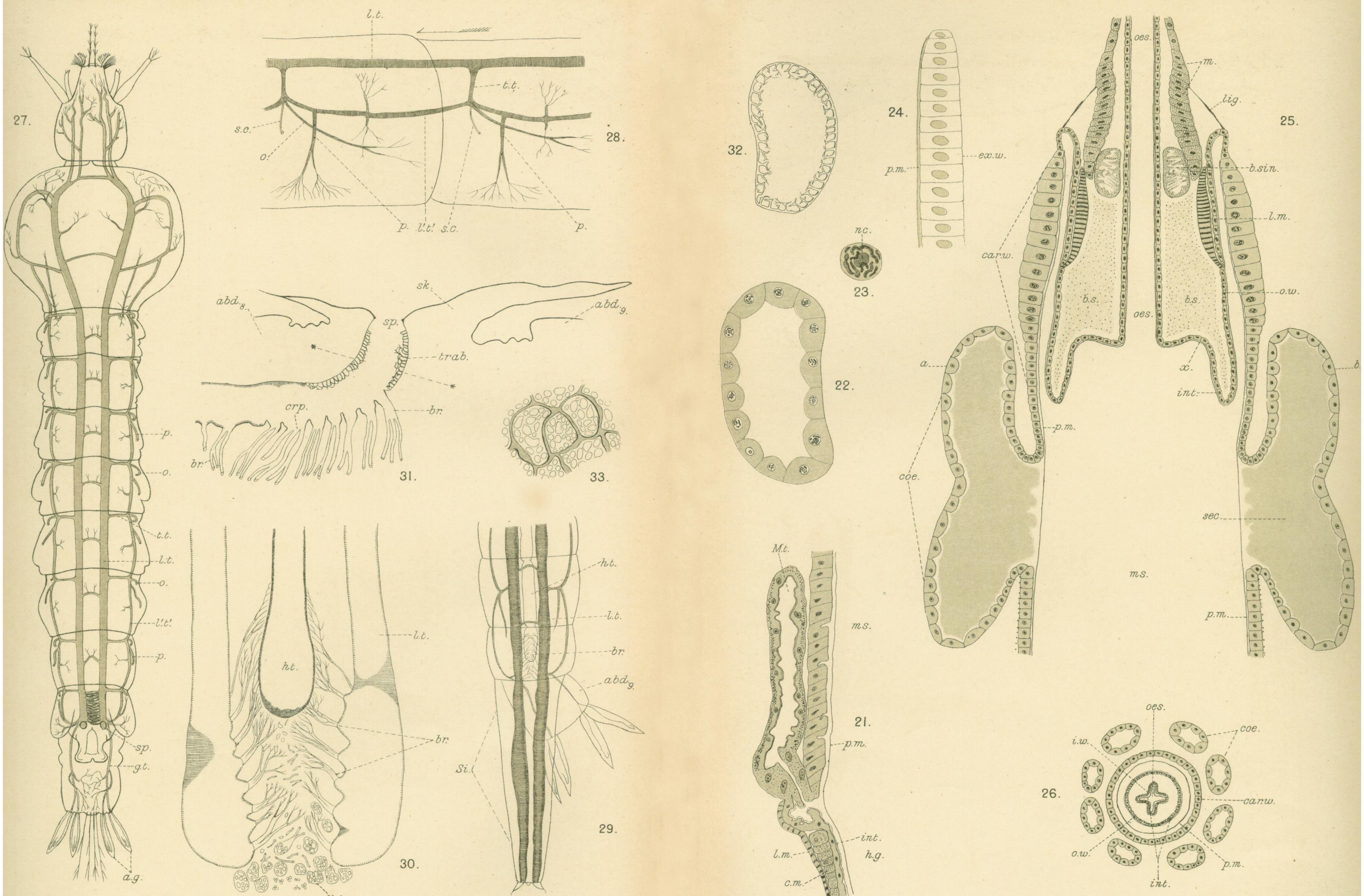
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