PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

On the Segmental Excretory Organs of Certain Fresh-Water Ostracods

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Phil. Trans. R. Soc. Lond. B 1926 **214**, 1-27
doi: 10.1098/rstb.1926.0001

PHILOSOPHICAL TRANSACTIONS.

I. *On the Segmental Excretory Organs of Certain Fresh-Water Ostracods.*

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(Received February 11, 1925,—Read May 14, 1925.)

[Plates 1, 2.]

Introduction.

The origin of the duct of the segmental excretory organs of Crustacea is of critical importance in the consideration of the relationships of these organs to each other and to the similar organs in other Arthropoda. Up till now, it has been by no means certain what that origin is, or even whether the ducts have one common origin throughout the group. In a study of the development of Estheria it has been suggested by the author (1924, p. 422) that the divergences of opinion on this point may be due to the fact that segmental excretory organs are not completely homologous throughout the group. The end-sacs may be, and probably are, homologous, but not the ducts.

Burian and Muth (1921, p. 642) place these glands in two groups according to the constitution of their efferent ducts. In the first group the duct is built up of a large number of cells, and the lumen is intercellular; in the second it consists of a few cells only, and the lumen is intracellular. The efferent duct of the maxillary gland of Estheria is the first type and it is a true cœlomiduct, that is to say, it is completely mesodermal in origin.

To the second group belongs the antennal gland of Estheria. It was a comparison of the structure of the duct of this gland with that of the ectodermal labral glands of Estheria that led the author to suggest that in the second group the duct might be ectodermal in origin. It was thought probable that the antennal gland "represents a composite structure . . . of a cœlom sac in connection with an ectodermal gland."

In order more fully to investigate this hypothesis the forms selected were the fresh-water Ostracoda. In these, according to Bergold (1910, p. 18), the maxillary gland possesses an intracellular duct consisting of a few cells only, and, since the eggs hatch at the naupliar stage, it is possible to study the complete development of the gland in the larval stages without having to resort to embryonic stages, a point of considerable importance when dealing with any Crustacean form. The investigation led to the

 [Published August 5, 1925.

discovery of the real antennal gland and thence to the study of the "shell gland," which, until now, has always been taken as representing the antennal gland.*

What little is known of the segmental excretory organs of the Ostracoda is based chiefly on the observations of CLAUS, G. W. MÜLLER and BERGOLD. The earliest references to the "shell glands" of fresh-water Ostracods are given in BERGOLD'S paper (1910, p. 13). The first work of any importance is one by CLAUS in 1895. He figured and described in detail the "shell gland," and although he could not distinguish an efferent duct, deduced from its anatomy that it represented the antennal gland of other Crustacea. The maxillary gland he took to be a structure occurring at the base of the maxilla that has been more fully described by BERGOLD (1910, p. 22). It has, however, nothing to do with the true maxillary gland.

MÜLLER (1894, p. 173) observed that by giving carmine to *Cypris pubera* two organs were indicated as excretory—(1) in the labrum opening on the first joint of the antenna and (2) another in the base of the maxilla. The duct of the latter was not evident. He did not describe these organs further. Giving carmine to marine Ostracods yielded no results. Anatomical investigation of the latter forms was found to be very difficult owing to the difficulty of distinguishing the supposed organs from nervous ganglia, but in Paradoxostoma and in Bairdia he observed small epithelial sacs in the bases of the first limb posterior to the maxillule. These he considered segmental organs.

The most complete work on the subject is due to BERGOLD (1910). He worked out the anatomy of the organs in much more detail than had previously been done. He described for the first time the anatomy of the maxillary gland, as a typical Crustacean segmental excretory organ. The shell gland he described in detail and observed for the first time its exit. He considered that it represented a typical segmental organ and from the position of its exit concluded that it was the antennal gland. In addition to these, however, he observed a sac which, he states, was situated in the antennule. He could find no duct leading to the exterior, and so described the sac as the remains of an antennulary gland.

No observations whatever, as far as the author is aware, have been made on the development of any Ostracodan excretory organ.

The observations of BERGOLD will be dealt with in more detail later on. It will suffice here to point out in what directions they are, in the author's opinion, inaccurate.

First, the "shell gland" is clearly an ectodermal gland, in no way comparable with a typical segmental excretory organ of any crustacean. Secondly, the sac which he described as belonging to the antennule is actually situated in the basal joint of the antenna. It also possesses a complicated intracellular efferent duct. This organ and not the "shell gland" is the true antennal gland of the fresh-water Ostracods.

The author wishes to express his indebtedness to Prof. E. W. MACBRIDE, F.R.S., and to Dr. W. T. CALMAN, F.R.S., for much advice and criticism.

* The term "shell gland" is used here, as explained on p. 16, in its descriptive sense only. It does not imply any relationship to the shell glands of other Crustacea.

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Materials and Methods.

The forms used in the investigation were *Cypridopsis vidua* (O. F. Müller, 1785), *Cypris fuscata* (Jurine, 1820), and *Cyprinotus incongruens* (Ramdohr, 1808).

All larval and adult stages of *C. vidua* were gathered from a large, well-established tank in the laboratory. The youngest stages were easy to obtain, as they collected away from the light and at the surface of the water. The earliest larvæ of the other species which occurred in the same tank did not do this. The larval stages of the other two species used were obtained from cultures started from batches of eggs that had been laid by isolated adults.

The fixative used almost exclusively was Flemming-without-acetic-acid. It was used at ordinary temperature for the youngest stages and allowed to act for about two hours. The older specimens were placed in F.W.A. at the temperature of the embedding bath (59° C.) for about ten minutes only. For the adult anatomy alcoholic Bouin (Dubosq-Brasil) was sometimes used, and also the same fixative in which the acetic acid had been replaced by citric.

The method of embedding, except in the case of the first stage nauplii, was that described in the author's previous work on Estheria (1924, p. 396). In the case of the earliest nauplii, owing to their minute size (the first nauplius of *C. vidua* is about 100μ long), double embedding in celloidin and paraffin had to be resorted to. For this purpose the photoxylin method described by Murray (1924, p. 289) was employed. After the specimens had been brought up through the alcohols they were placed in clove oil, which dissolved the photoxylin and left the specimens in a syrup. Each specimen was then placed separately in clove oil-celloidin, orientated, and the usual procedure followed.

The sections were cut at 8μ. Thinner sections were not of much use. The organs to be studied were so small that it was preferable to have the organs occurring in a few sections and to study them with a binocular microscope rather than to have the organs cut up into many thin sections. The celloidin sections were cut at the same thickness and were flattened out in the presence of acetone vapour.

The stains used were iron hæmatoxylin and Mallory's triple stain.

The "Shell Gland."

The "Shell Gland" has been fully described by Bergold (1910, p. 13), and so here only the main points in its anatomy will be mentioned. It is its development which is of the greater interest for the purpose of this paper.

It consists, on either side, of three parts that may be termed reservoirs, viz.: an anterior, a middle and a posterior reservoir. The anterior and posterior are situated in the adult in the cavity of the shell fold and both open into the middle reservoir. The latter is situated in the body cavity immediately in front of the most anterior part of the liver. It is into this reservoir that an ectodermal intucking projects, which serves

as an exit for the whole gland. The complete gland is figured in its lateral aspect in text-fig. 1, *e*.

The middle reservoir is circular when viewed from the side. The cytoplasm constituting its walls is exceedingly vacuolated. In this apparently frothy mass there can be distinguished, in the best preparations, a single large flat nucleus resting against the main circular cavity of the reservoir. Bergold (1910, p. 14) describes four nuclei, but this is not the case in the forms studied. In living specimens, the cytoplasm is full of bright orange vacuoles. The latter are quite absent from the central cavity of the reservoir.

The anterior reservoir is the largest part of the gland. It extends from the middle portion into the shell fold in an antero-ventral direction. It consists of seven or eight cells each with a very large, approximately spherical nucleus. Bergold (1910, p. 13) describes it as syncytial, but in the forms studied the boundaries between the cells were quite obvious. The outermost parts of the cells are filled with small colourless vacuoles, grouped together in patches and of practically uniform size. The cavity of the reservoir is elongated with lateral bays and opens dorsally into the ventral part of the middle reservoir.

The posterior reservoir hangs down from the middle reservoir by an excessively thin-walled tube that connects its own cavity with the posterior part of the middle reservoir. Its length is slightly greater than that of the anterior reservoir, but the whole structure is narrow enough to hang into the shell-fold cavity without being laterally compressed as is the anterior reservoir. Its cytoplasm is sometimes filled with colourless vacuoles, irregularly scattered and of varying sizes. Its nuclei, which number about ten, are smaller than those of the anterior reservoir.

The exit of this complex gland is a cuticular tube formed by an ectodermal ingrowth from the inner face of the shell just lateral to the attachment of the second antenna to the body. It is flattened laterally and projects into the actual cavity of the middle reservoir.

The secretion of the anterior reservoir forms a coagulum after F.W.A. fixation, which is stained blue by Mallory. It can thus be distinguished streaming into the middle reservoir, which contains a secretion that is stained red by the same technique and that also sometimes exhibits a slight osmic reduction. The groups of vacuoles occurring in the outer parts of the anterior reservoir are apparently of a fatty nature, as they stain blue *intra vitam* with Nile Blue "hydrochloride," and sometimes appear as black dots after F.W.A. fixation.

The Development of the Shell Gland.

The developmental stages of the "shell gland" are figured in text-fig. 1. In the earliest larvæ of stage I,* only two parts of the gland are visibly represented and each is

* A concise account of the characteristics of the various larval stages of the Fresh-water Ostracoda is to be found in "Korschelt and Heider's Text-book of Invertebrate Embryology" (Translation), Vol. II, p. 205.

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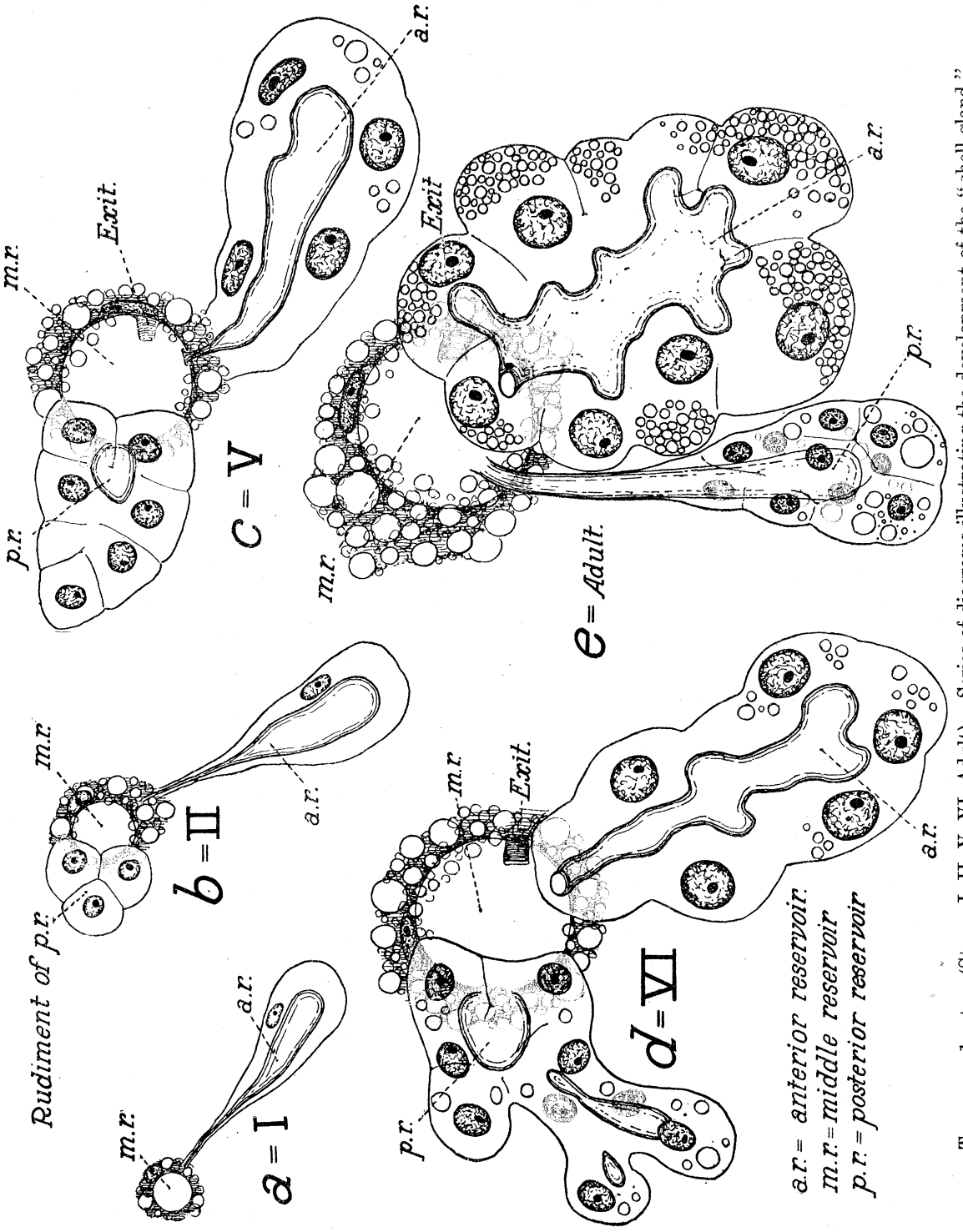


TEXT-FIG. 1 *a* to *e* (Stages I, II, V, VI, Adult).—Series of diagrams illustrating the development of the "shell gland."

constituted by a single cell (text-fig. 1, *a*). The anterior reservoir is an elongated flask-shaped cell protruding anteriorly into the cavity of the shell fold. The neck of this cell is very attenuated but can be traced backwards into a mass of vacuolated cytoplasm containing a single nucleus and surrounding a larger central vacuole that represents the middle reservoir. This vacuolated mass is situated in the body cavity just in front of the rudiments of the liver and rests against the ectoderm laterally. Both these cells, or more strictly the secretions in their reservoirs, show the adult staining reactions.

In stage II the structure of the gland is much easier to demonstrate as it is not so closely invested by the surrounding tissues (text-fig. 1, *b*). The anterior reservoir still projects forward as a single cell. Its lumen has enlarged and opens as before by a thin neck into the single cell representing the middle reservoir. The latter, however, is now connected with a group of three ectodermal cells which form the rudiment of the posterior reservoir. These are considerably larger than the neighbouring ectoderm cells, and are comparatively deep and thus project into the body cavity. They contain a few vacuoles but do not surround any central reservoir. Their cytoplasm stains comparatively deeply and so contrasts with that of the middle reservoir, that hardly stains at all.

In stage III the chief change is the increase in the number of nuclei in the anterior reservoir to about four. The method by which this increase takes place is not certain. From a statement by Bergold (1910, p. 14) referring to one of his figures (fig. 14) it is probable that he considered that the nuclei divided amitotically. This is probably the case in the forms investigated. Certainly no mitoses were found during the whole development of the gland.

The cells forming the rudiment of the posterior reservoir have also increased in number. They now surround a reservoir that is, however, completely separate from the middle reservoir. Their number is not definite as they merge into the surrounding ectoderm.

It is not until stage V is reached that any change other than growth takes place. In stage V there can be seen, just above the neck joining the anterior reservoir with the middle reservoir and close against the latter, an ectodermal ingrowth (text-fig. 1, *c*). This is the commencement of the exit tube. It arises from the inner face of the shell fold in the position described for the adult. In the next stage this ingrowth has passed into the middle reservoir and formed the distinct cuticular tube projecting into its cavity.

In stage VI, the cavity of the posterior reservoir, although distinct from that of the middle reservoir, opens into the latter by a broad mouth (text-fig. 1, *d*). The cells of the posterior reservoir form a well-defined group extending posteriorly above the junction of the stomach and liver. They have comparatively large nuclei and their cytoplasm stains fairly deeply. Anteriorly the cytoplasm in considerably vacuolated. This description applies to early specimens of stage VI. In later specimens this rudiment of the posterior reservoir shows a very marked change. Its cells commence to pass inwards partly through an overgrowth of the surrounding ectoderm cells, but in addition

some pass directly inwards, retaining connection with the main mass of cells by narrow necks of cytoplasm. At the same time subsidiary reservoirs appear in the cytoplasm, not in connection with the main reservoir but running towards it. As the cells become internal they pass ventrally as a group into the cavity of the shell fold. This change is the commencement of the formation of the elongated pear-shaped posterior reservoir. It is completed by stage VIII.

A change in the size of the nuclei takes place during this development. The nuclei of the cells of the posterior reservoir increase in size up to the time when they commence to pass inwards, but after this they become markedly smaller. The nuclei of the cells of the other two reservoirs increase in size progressively with development.

The Antennal Gland.

The details of the anatomy of the antennal glands can best be seen in stage III, at which stage the glands reach their fullest development. In earlier stages, owing to the surrounding tissues being closely packed around the glands, it is not possible clearly to define the details of the anatomy. The main features can, however, be made out, and it is certain that in all essential points the glands are complete in stage II. The anatomy could be seen most clearly in Cyprinotus, and in the following account the anatomy as seen in stage III of this form will be described.

The basal joint of the second antenna, from its point of attachment to the body just lateral to the most anterior part of the stomach, slopes ventrally and slightly forwards. About the middle of its length there can be seen a sac, slightly elongated and lying roughly horizontally with its long axis perpendicular to the body axis. This sac is the end sac of the antennal gland. It is very similar in histological detail to that of the maxillary gland as described by Bergold (1910, p. 20). Its cells bulge inwards into the cavity of the sac, and their cytoplasm is considerably vacuolated, the vacuoles sometimes being filled with granular deposits.

The sac is produced into three processes continuous with tendinous supporting strands (text-fig. 2). One short one runs laterally direct to the ectoderm. The other two are from the inner parts of the sac. One of these runs directly dorsally alongside the supra-œsophageal ganglionic masses, and in front of the stomach in order to become attached to the dorsal parts of the shell. The third divides into two soon after leaving the sac. The outer branch runs ventro-posteriorly to become attached to the tendinous plate which joins the transverse muscles of the mandibles. The inner continues as a muscle strand down the anterior wall of the œsophagus, where it becomes attached among the œsophageal musculature.

The duct system comprises two distinct parts, a complicated coil consisting of two cells and a single efferent duct cell. The coil cells are continuous with each other, and together form a U-shaped mass, the two arms of which lie alongside the lower anterior edge and the upper posterior edge of the sac respectively. The base of the U

is thus obliquely placed, and rests close against the lateral ectoderm. Inside this mass of cytoplasm there runs an intracellular duct, the configuration of which can best be studied by reference to text-fig. 2. It can be said to consist of an inner U continuous with an outer U. The commencement of the duct from the sac forms the anterior limb of the inner U. It runs first of all laterally, then dorso-posteriorly, and then medianly, the three parts thus forming the inner U. It then turns sharply back on itself and runs dorso-laterally to a point just in front of the anterior limit of the liver on the level of the reflexion of the ectoderm to form the inner face of the shell. This forms the posterior limb of the outer U. It then runs close inside the ectoderm antero-ventrally, and finally turns at a sharp angle, and runs medianly again parallel to the commencing limb of the duct. This completes the outer U. When this last limb has reached the median limit of the end sac, it turns back on itself at a very sharp angle and terminates by a very well-marked funnel opening into the efferent duct cell, the lips of the funnel projecting into the lumen of the latter. The anatomy of the duct can be seen clearly in figs. 14 and 15, Plate 2. These figures represent two consecutive sections that together contained the whole of the gland.

The actual commencement of the duct is not strictly at the surface of the coil cells, for the lumen continues for a short distance in a tangential direction through the cells of the end sac. This can be seen in figs. 13 and 15. The lumen exhibits a practically uniform diameter of about 1·3μ. At its opening into the efferent duct cell it enlarges, the mouth of the funnel measuring about 5μ across.*

The layer of cytoplasm immediately surrounding the lumen does not appear to be differentiated to form any definite walls, and certainly exhibits no radial striation. In the more dorsal parts of the loop there appears a layer of cytoplasm markedly homogeneous, that stains a uniform blue in hæmatoxylin preparations. It can be seen from fig. 14 that this layer does not surround the lumen completely, but occurs only on the outer side.

The two ovoid nuclei of the loop cells contain nucleoli and are very large, approximately 7μ in length. They are situated one on the dorsal and one on the ventral side of the end sac (fig. 15).

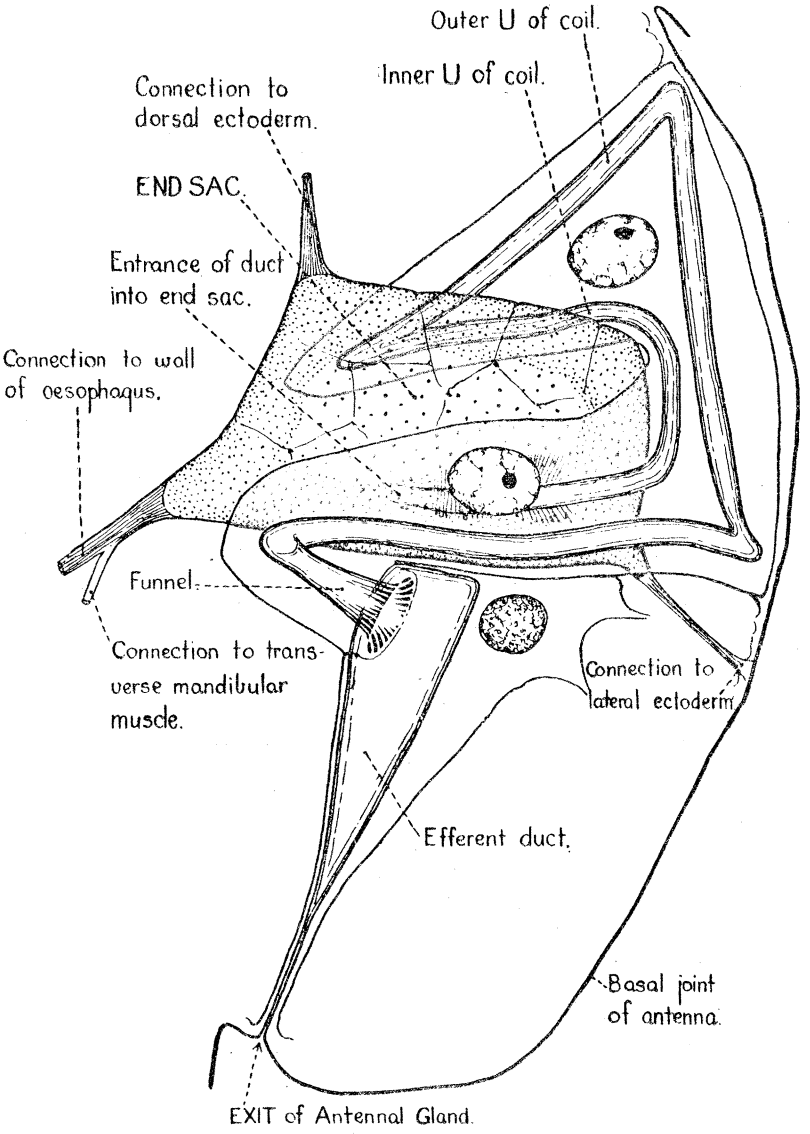
The efferent duct cell is a long cell running ventrally and tapering towards its opening at the inner edge of the attachment of the antenna (fig. 14). It is attached dorsally to the end sac as well as to the loop cells. Its nucleus (fig. 15) is situated very close to the funnel of the loop. This nucleus is smaller than those of the loop cells, and stains more intensely. Also it does not appear to contain a definite nucleolus.

The walls of the lumen show no differentiation from the surrounding cytoplasm. The lumen itself is comparatively wide at its dorsal end, as reference to fig. 14 will show, and it is into this wide region that the funnel of the loop cells opens. At this level it is about 4μ in diameter, while at its opening to the exterior it tapers to a minute tube

* The measurements were made from photomicrographs taken by Mr. M. T. Denne, V-P.R.M.S., to whom the author wishes to express his indebtedness.

estimated at about 0·3μ in diameter—that is, just within the limits of resolution of the ordinary system of lenses used.

The lumen does not occur centrally in the cell, but passes through its more median portions, there being only an exceedingly thin layer of cytoplasm along the inner side.



TEXT-FIG. 2.—Diagrammatic front view of Antennal Gland.

Owing to this fact and the fact that there are no differentiated walls to the lumen, in transverse sections of the larvæ, the efferent duct cell was not recognised as such for a considerable time. Its more lateral portion, together with the nucleus, appeared as a typical mesenchyme cell that occur throughout the body. The exceedingly thin median wall appeared as a minute connecting strand running from the loop cells to the ectoderm. This led to the anomalous conclusion that the duct of the loop cells opened by its funnel into the hæmocœle. It was, of course, chiefly on transverse

sections that the observations in this paper were made. Frontal sections of the larvæ, however, were then made, and these cut the efferent duct cell transversely. Here the cause of the difficulty became apparent, and the lumen could be traced as a continuous tube running from the funnel to the exterior.

Later stages of the larvæ show a progressive degeneration of the structure of the antennal glands. The first change noticeable occurs in stage V, where the funnel of the loop system becomes closed (fig. 12). At the same time the efferent duct cell shows signs of breaking down. Its lumen disappears and its nucleus becomes smaller and more darkly staining. In later stages the remains of this duct cell cannot with certainty be recognised. The cytoplasm of the loop cells, especially in the more ventral portions, gradually becomes filled with granules and larger ovoid bodies that stain grey with hæmatoxylin after F.W.A. fixation (fig. 13). These appear similar to those occurring in the terminal portion of the cytoplasm of the duct cells of the maxillary gland (fig. 9).

In stages V and VI the remainder of the funnel can still be seen as a blind ending to the loop duct (figs. 12 and 13). In the adult, however, the portion of the duct that originally led up to the funnel dwindles away into the cytoplasm, now full of the inclusions just mentioned.

In stage VI onwards, surrounding the actual commencement of the duct, that has already been described as passing through the cytoplasm of the end sac cells, there appears a fibrillar structure that apparently acts as a sphincter. It consists of three sets of fibrils. Two of these are placed on the side of the duct nearer the cavity of the end sac and together form a V-shaped gutter along which the lumen passes. The third is placed on the outer side of the lumen and nearer to the actual entrance of the duct into the cavity of the end sac, and this completes the triangle of fibrils. There always appear to be two nuclei close against the fibrils (fig. 13). This sphincter arrangement cannot be distinguished in the early stages when the loop duct is in continuity with the efferent duct cell. It is only after the latter has broken down that the sphincter can be demonstrated. In *Cypridopsis vidua* it was observed as early as stage VI, but in the other forms it was seen only in sexually-mature forms.

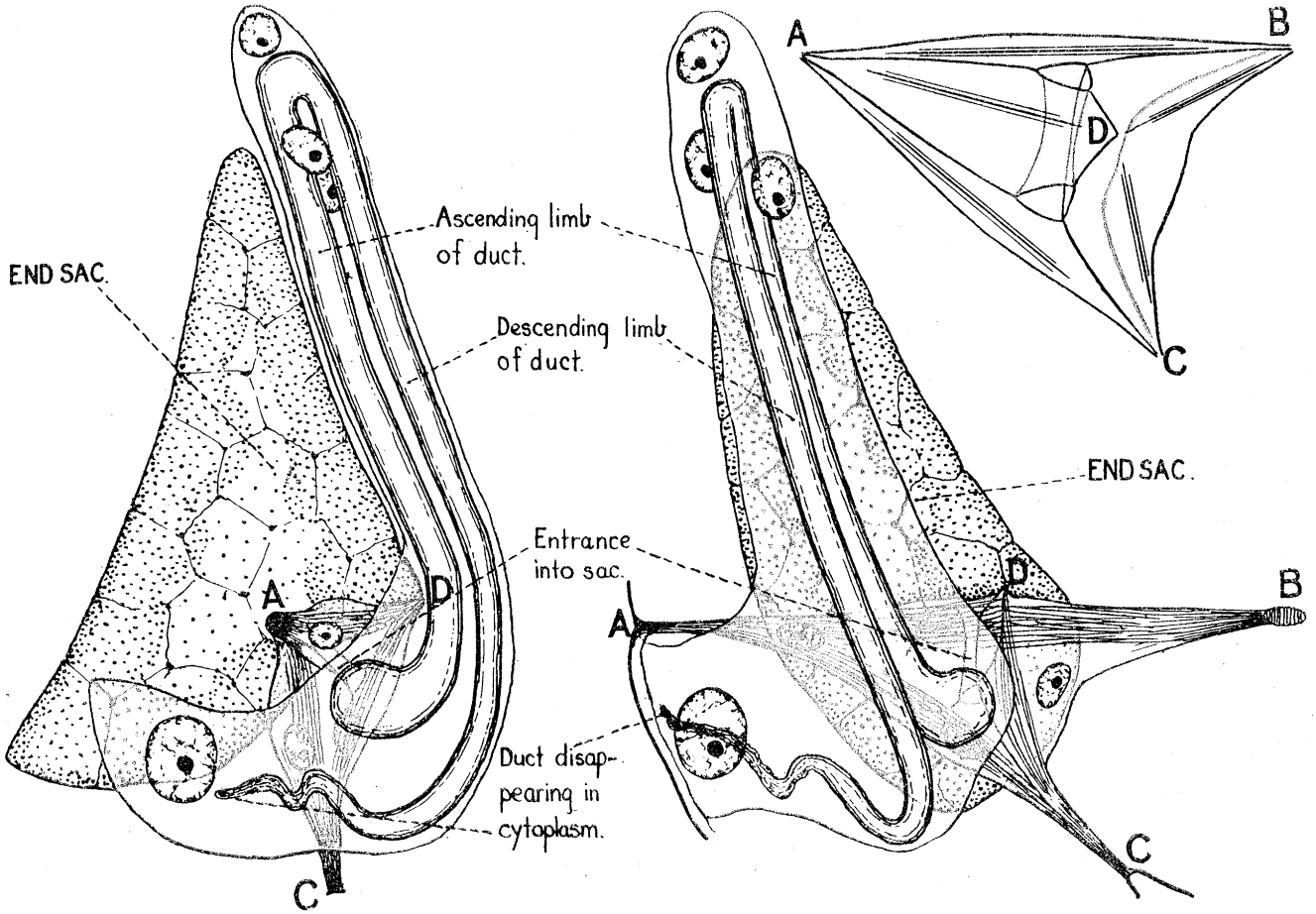
The Maxillary Gland.

The structures taken by Claus to represent the maxillary glands are a pair of glandular organs situated between the maxillulary and the maxillary segments immediately posterior to the hypostome. They have been described in detail by Bergold (1910, p. 22), and were called by him the " Maxillipedal Glands " (Maxillarfussdrüse).

The actual maxillary glands, as Bergold first pointed out, lie immediately behind and lateral to these structures. They extend posteriorly into the segment of the first trunk limb, and dorsally almost to the line of reflexion of the ectoderm, to form the shell fold. Each exhibits the typical structure of an end sac with an efferent duct leading towards the exterior. These ducts, however, do not open to the exterior, as maintained by Bergold (1910, p. 21). Also, their entrance from the end sac is

surounded by a complicated skeletal structure, probably acting as a valve. This is not mentioned in BERGOLD's description.

The end sacs show no marked peculiarities. Their walls consist of a single layer of cells projecting in an irregular way into the cavity of the sac. The protoplasm of the cells is considerably vacuolated, many of the vacuoles containing granular precipitates. The sacs are roughly pyramidal in shape, their apex pointing upwards. The most dorsal portions appear to be gripped in the general mesenchyme of the body cavity and to be fastened close against the lateral ectoderm. The base of the sac is fastened posteriorly by a connective strand to a tendinous plate lying between the first and second trunk limbs just above the ventral ectoderm, and to which also the muscles of these limbs are attached. These endoskeletal plates have been figured and described in an Ostracod by DADAY (1895).



TEXT-FIG. 3.—Two diagrammatic views of maxillary gland. That on the left shows the lateral aspect, that on right shows the same gland viewed from the front. At the top right-hand corner is a diagram representing the arrangement of fibrils in the valve system.

The anterior part of the base of the end sac is formed by the skeletal valve. This valve consists of three cells only that together form a structure tetrahedral in form. It can best be described by reference to text-fig. 3. The four corners of the tetrahedron

have been marked A, B, C and D The corners A and C are connected directly to the ectoderm, A to the lateral ectoderm just dorsal to the attachment of the maxilla to the body, and C to the ectoderm forming the most median part of this same attachment. The corner D is fastened to the anterior prolongation of the floor of the end sac. The corner B is attached to a complicated tendinous system. (This is figured diagrammatically in text-fig. 4, *d*, p. 14). It will be seen that this corner is held in position mainly by three connective strands. One of these runs backwards to the tendinous plate already mentioned as lying between the bases of the first and second trunk limbs. The other two pass forwards, one, the smaller, to attach to a similar plate lying between the maxillulary and the maxillary segments; the other attaches to the large tendinous plate forming the median part of the adductor muscle. On reaching this plate it turns sharply at right angles, and is continued as part of the adductor muscle to the lateral attachment to the shell.

The face ABD forms part of the floor of the end sac. From the middle of this face a tubular space, surrounded by the three cells, passes to the middle of the face ACD. The latter faces antero-laterally, and from it the efferent duct commences, the duct being continuous with the tubular space. In the cytoplasm of the three valve cells and surrounding this tube are two triangles of fibrils, probably contractile or elastic. The one forms the sides of the face ABD and so guards the entrance to the end sac while the other constitutes the sides of the face ACD, thus surrounding the entrance to the efferent duct. It will be seen that the fibrils running between the points A and B are common to both the triangles. The whole structure is represented in figs. 10 and 11. The cytoplasm of the three cells is markedly homogeneous and free from inclusions. In hæmatoxylin preparations it remains practically unstained.

The efferent duct consists of a syncytium of four cells. The continuous cytoplasm of this duct system extends from a point immediately dorsal to the apex of the end sac, runs ventrally in contact with the anterior edge of the sac, and then curves round posteriorly underneath the connection of the valve system to the lateral ectoderm. This last portion rests against the lateral ectoderm on a level with the base of the end sac. Three of the nuclei are situated near the apex of the system, while the fourth, which is much larger than the rest, is situated in the most ventro-posterior part of the system. The duct, from its origin at the valve runs forward for a short distance and then passes dorsally close against the end sac wall to the apex of the system, where it turns back on itself and passes ventrally to dwindle and disappear in the cytoplasm near the large nucleus at the posterior part of the duct system. There is thus an ascending and a descending limb of the duct. These two limbs are of equal and uniform calibre throughout their length. The commencement of the duct, however, is always swollen. This is usually not more marked than is indicated in text-fig. 3, but it may be very pronounced. Thus, in the fully adult Cyprinotus it is enlarged into a sac with much-folded walls.

The layer of cytoplasm immediately surrounding the lumen of the duct shows a

homogeneous structure. In hæmatoxylin preparations after F.W.A. fixation this layer attains a uniform blue contrasting with the surrounding cytoplasm that shows a more neutral tint. The radial striation described and figured by BERGOLD (1910, p. 21, and figs. 21–24) as a typical "Stäbchensaum," appeared in some preparations of fully adult specimens, but in these cases comparison with the other tissues indicated that such a structure is probably due to bad fixation. In no preparation was a layer of basal granules beneath the striated layer indicated.

The cytoplasm through which the ascending and descending limbs of the loop pass is slightly vacuolated. The terminal ventro-posterior cytoplasm in which the duct finally disappears is also vacuolated, but in the adult stages becomes crowded with granules and ovoid bodies similar to those already described as occurring in the cytoplasm of the antennal gland duct cells.

The whole gland, in common with all the other organs of the body is embedded in the general body mesenchyme. The latter does not, however, as maintained by BERGOLD (1910, p. 21), invest the gland completely like a sheath. No difficulty was found in distinguishing, in good preparations, the limits of the cytoplasm of the duct system. The difficulty experienced by BERGOLD was probably due to his using a sublimate fixative.

The Development of the Maxillary Gland.

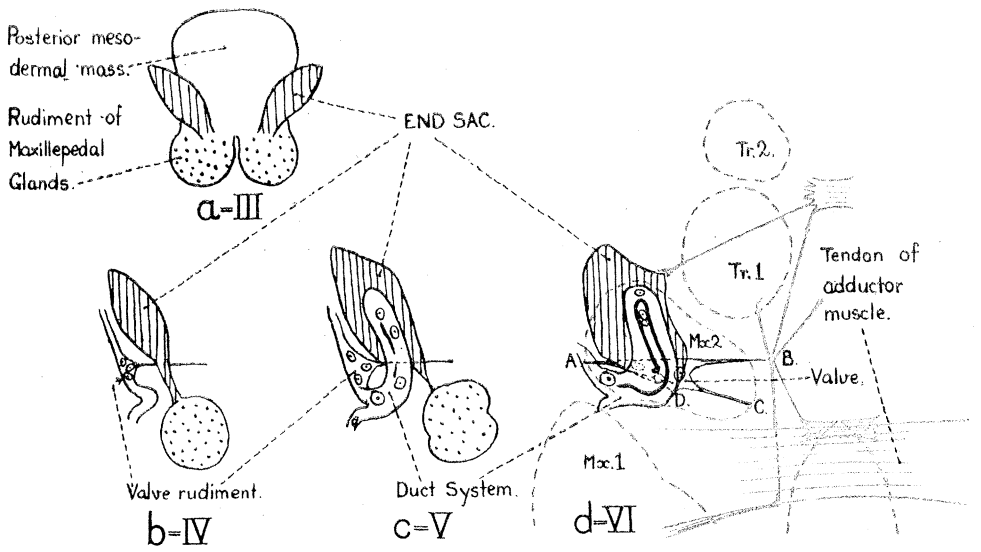
In stage I the post-mandibular mesoderm forms a continuous ventral mass of cells which extends somewhat dorsally at the sides of the gut.

In stage II, with the appearance of the caudal furca, the most posterior part of this mesodermal mass becomes differentiated into the furcal muscles. Anteriorly the rudiments of the maxillulæ are visible, but their musculature is not yet differentiated.

In stage III, the maxillulæ having appeared as free appendages, the most anterior part of the mesoderm has become differentiated as the maxillular musculature. In between the maxillular segment and the caudal furca, the mesoderm still forms, in the youngest specimens of this stage, an undifferentiated ventral mass. In older specimens, however, there is a marked differentiation in the more anterior portion (text-fig. 4, *a*). It appears divided into two lateral lobes overlying the most posterior part of the primordium of the nerve cord. These two lobes give rise to the glands called by BERGOLD the "Maxillipedal Glands." From these two lobes there extend posteriorly two groups of cells which constitute the primordia of the end sacs of the maxillary glands (fig. 1, Plate 1). They form elongated structures sloping upwards and outwards, in continuity anteriorly with the mesodermal mass, and dorsally connected to the lateral ectoderm by ectodermal connective strands. They appear to become separated from the more ventral mesoderm by being lifted up through the growth of the lateral ectoderm to which they are attached.

In stage IV, the stage in which the maxillæ first appear as free, movable appendages, the end sacs show the first indications of a cavity, fig. 2. Their connection with

the "maxillipedal glands" becomes drawn out into a single string of cells (text-fig. 4, *b*, and fig. 2, *c*), until in older specimens of this stage it forms merely a thin connecting strand.



TEXT-FIG. 4.—A series of four diagrams illustrating the development of the maxillary gland. The last diagram illustrates connections of maxillary gland with endoskeletal system.

A most important characteristic of this stage is the appearance of the primordia of the valves. These appear on each side as a group of three ectodermal cells (fig. 2, *p.v.s.*) placed immediately ventro-lateral to the most anterior portion of the end sac. They are arranged in a triangle the apex of which points inwards. The apex is connected by a very thin ectodermal strand with the median ventral ectoderm in the same transverse plane. The three nuclei are grouped close together, but at this early stage show no marked difference from the surrounding ectoderm nuclei. If anything, they are slightly larger.

Up to this stage the maxillary glands are represented only by the end sacs and the valves, the primordia of the duct systems not yet having appeared. These appear first in stage V.

In stage V there can be seen just anterior and slightly dorsal to the valve rudiment an intucking of ectoderm. From text-fig. 4, *d*, it will be seen that this occurs between the bases of the maxillulæ and the maxillæ. From it there extends an ingrowth of ectodermal cells that passes dorsally over the median connective of the valve rudiment, and then posteriorly to form a tongue of cells lying close against the dorsal surface of the end sac (text-fig. 4, *c*, and figs. 3 and 4, *d.c.*). In this tongue there can be seen three nuclei slightly elongated and larger than the ordinary ectodermal nuclei (fig. 4). These form the nuclei of the ascending and descending limbs of the duct. In the more lateral part close to the valve rudiment there can be seen a larger spherical nucleus (fig. 3, *t.n.d.s.*) that is the terminal nucleus of the duct system. In the specimen figured in figs. 3 and

4 there were indications in the cytoplasm of this ingrowth, of minute channels that undoubtedly represented the beginnings of the lumen of the duct system.

At this stage the valve rudiment has enlarged, the nuclei now being distinctly larger than the surrounding nuclei. Its median connection no longer runs direct to the median ectoderm but is raised up in connection with the complicated system already described in the account of the adult gland. The end sac has also enlarged. Its connection with the maxillipedal glands is sometimes broken, but sometimes, as in fig. 3, *c*, there is still a very thin connection passing over the valve rudiment and under the duct system rudiment. In all later stages it is broken completely.

In stage VI the adult facies of the gland is attained. The further development of the valve system is the most marked change that takes place during this instar. Instead of forming a triangle of cells with one side resting in the lateral ectoderm, it still forms a triangle but retains connection with the lateral ectoderm merely by one corner (fig. 8, *l.c.v.s.*), the other two corners extending inwards. One of these forms the median connection already described, while the other is connected to the inner ectoderm at the base of the maxilla. Here again this alteration appears to be brought about by a rapid growth of ectoderm in between two of the cells resulting in the pushing apart of those two cells, one cell thus passing from the outer side of the base of the limb to the inner side. This growth takes place during the VIth stage and not suddenly at an ecdysis. This can be seen from the series of figs. 5, 7 and 8, all of which are from stage VI larvæ. In fig. 5 the three nuclei still form the triangle characteristic of the earlier stages. Fig. 7 shows two of the nuclei moving apart, while in fig. 8 the connections of the valve are typical of the adult. Once the adult connections are made the development of the fibrils takes place. It is not, however, until the fully adult stage is reached that their arrangement can be made out with any certainty.

The change in the attachments of the valve that takes place during this instar appears at first sight very extraordinary, but it must be remembered that during this instar there must be, in the maxillæ, a complete reorganisation of the ectoderm underneath the cuticle, preparatory to the change that takes place at the succeeding ecdysis of the form of the maxilla from a simple pediform limb to a typical maxilliform appendage.

Pari passu with the alteration in the primordium of the valve, the terminal cell of the duct system grows backwards through the arch formed by the moving apart of the two valve cells (figs. 7 and 8). In so doing it grows over the inner surface of the more posterior ectoderm cells (fig. 8, *t.n.d.s.*), the nucleus passing at the same time to its adult position posterior to the valve system. In the earliest specimens of stage VI the lumen of the duct system can be seen as a minute channel in the form of an ascending and a descending loop (figs. 5 and 6). In the later specimens the ascending limb can be seen very clearly commencing from the centre of the three valve cells (fig. 8, *e.e.s.*), while the descending limb disappears near the terminal duct nucleus as in the adult (fig. 8, *t.d.*)

At this stage the portion of the duct system where the descending loop bends

posteriorly is still in the ectoderm. In later stages this portion becomes overgrown by the surrounding ectoderm so that in the adult the whole gland is internal.

Discussion.

It has already been pointed out in the introduction to this paper that it was CLAUS who first described the " shell gland " of the fresh-water Ostracods as corresponding to the antennal gland of other Crustacea. Apart from the question whether CLAUS was correct or not, his statements led to an unfortunate confusion of terms. The term " Shell Gland " was used originally to denote the segmental excretory organs of Daphnids because they happened to occur in the cavity of the shell fold. But in Daphnids these glands are the maxillary glands, and in using the same term in the case of the Ostracods, it naturally appeared to suggest that some homology existed between the Ostracod " shell gland " and that of the Daphnids. This, however, was not CLAUS' view, and it would have been better had he avoided the name " Shell Gland " and used the term Antennal Gland in the case of the Ostracods. Recent writers appear to be abandoning the term " Shell Gland " as applying to segmental excretory organs (*e.g.*, GIESBRECHT, 1913, p. 152), and so, in this paper, it has been used simply to imply a gland occurring in the shell fold.

CLAUS' view of the shell gland was based on his observation that it exhibited two main parts, one of which, the posterior reservoir, he identified as the end sac and the other as the efferent duct. Its opening to the exterior, however, he did not observe. BERGOLD'S conclusions were based on his own more complete description of the anatomy of the gland, and it has already been mentioned that the description given in the present paper agrees in all essentials with his.

Of the three main divisions of the gland described, BERGOLD also considered the posterior reservoir to represent the end sac. The anterior reservoir he took to represent the loop canal (Schleifenkanal) or ureter (Harnkanälchen), while the middle reservoir he considered as an enlargement of the latter. He termed it the hind-sac (hintern Sacke) (BERGOLD, 1910, p. 13).

The evidence upon which BERGOLD bases his conclusions is briefly as follows. The external opening of the gland is situated not far from the base of the second antenna. This is true, but the actual opening is formed by an ectodermal ingrowth from the inner surface of the shell, and it remains to be shown whether the shell or any part of it can be considered as belonging to the antennal segment.

The actual relationship of the shell to the segments of the Ostracod body is not at all clear. Some authors, *e.g.*, GIESBRECHT (1913, p. 227), maintain that the carapace of Crustacea has no value in phylogenetic comparisons. CALMAN, however (1919, p. 363), considers that it was present in the ancestral stock of the Crustacea, in which case the Ostracod shell must be considered as homologous with that of the Conchostraca. In the latter group there is evidence (CANNON, 1924, p. 408) that it is formed entirely as a paired outgrowth from the maxillulary segment. But, if this is so, it is difficult, assuming

the homology of the Ostracod and Conchostracan shell, to explain the fact that, while the newly hatched larva of the Ostracod shows only the three typical pairs of naupliar appendages and exhibits no trace of maxillulæ, it yet possesses a fully formed bivalve shell. However, the adductor muscle of this earliest stage is not the adult adductor muscle, but is a portion of the mandibular transverse muscle, separated laterally from the rest but attached to the same median tendinous plate. The adult adductor muscle appears first with the maxillulæ in the second larval stage. The small anterior mandibular adductor muscle persists throughout life separated from the larger maxillulary adductor muscle by the dorso-ventral muscles of the maxillulæ.

The fact that in the so-called nauplius the adductor muscle belongs to the mandibular segment does not necessarily indicate that the shell-fold must also be derived from that segment. It is equally probable that the precocious appearance of the shell is a larval adaptation, and that, in growing forward over the naupliar limbs, the shell-fold has utilised portions of the muscles, already differentiated in those segments, to serve as adductor muscles. It will be recalled that in Daphnids and Estheria the shell-folds grow backwards first of all, while in the Ostracod nauplius the greater part of the shell is in front of the mandibular segment, the adductor muscle being at its posterior end.

It is clear, then, that the segmental origin of the Crustacean carapace is still unsettled, and hence the fact that the opening of the "shell gland" occurs near the base of the antenna, but actually on the shell-fold, cannot be considered as evidence either in favour of or against the view that the "shell gland" is really the antennal gland.

Bergold's next point is that the ureter (the anterior reservoir) enlarges to form a "bladder" (the middle reservoir) before opening to the exterior, and this corrresponds closely with the ureter of the antennal gland of Mysis and Siriella, as described by Grobben (1880). In Mysis there is a long coiled tube which leads from the end sac to the exterior, and which, after many coils, enlarges to form a sac and then opens to the exterior. But even supposing that the posterior reservoir does represent a typical end sac, the anterior reservoir should not be termed a ureter, as it does not lead from the end sac to the exterior. It forms a *cul-de-sac* leading from the middle reservoir, as reference to text-fig. 1 (*e*), or Bergold's figure 14 (1910) will show. The latter, however, is not as critical as might be desired, but the statements in the text leave no doubt as to Bergold's conception of the anatomy of the gland. Thus he states (1910, p. 14): "Das vordere Ende des Schleifenkanals endet blind. Der hintere Teil mündet von auszen in den 'hintern Sack' ein, den man als Differenzierung der hintern Partie des Harnkanälchens anzusehen hat." It would have been more correct if the middle reservoir had been termed the ureter, as it is into this that the ectodermal exit tube projects, and thus it can, in a sense, be considered as a duct leading from the supposed end sac to the exterior. It has not the typical tubular form, but its bladder-like form might be compared with the "ureter" of certain Cirripedes, as described by Defner (1910).

Finally, Bergold states that the exit tube from the middle reservoir to the exterior

is lined by cuticle, and hence deduce that it must be formed by ectodermal cells. He indicates the similarity in this respect to the ducts of the antennal glands of Gammarus, Siriella and Mysis. However, as early as 1880, GROBBEN had described the antennal glands of Estheria, in which there is no cuticular lining, to the efferent duct.

It is thus clear that BERGOLD found little real evidence to urge in favour of considering the shell gland as a typical antennal gland. If now, the account of the development of the shell gland given in this paper is accepted as correct, it follows that the gland cannot in any way be considered as homologous with the segmental excretory organ of any other Crustacean. The one essential of any such organ is that its end sac must represent the remains of the cœlom and therefore must be mesodermal in origin. The origin of the efferent duct, as will be pointed out later, is different in different forms and hence has no critical value in this respect. Now that part of the gland considered by BERGOLD and others to represent the end sac is the one part of the gland of which it can be said definitely that it is ectodermal in origin, and therefore that it cannot constitute the end sac of a crustacean segmental excretory organ. What the origin of the other two parts of the gland may be cannot be said, but this is immaterial as it has never yet been suggested that either of these represents the end sac. Apart, however, from the development of the gland, the simple fact that in the base of the antenna there occurs an organ that, as will be shown later, agrees in all essentials with the antennal gland of Estheria, is alone sufficient to indicate conclusively that the shell gland cannot represent the antennal gland.

The actual function of the shell gland is not at all clear. A point in its development that suggests that its present function is secondary, is that it originates in connection with the outer ectodermal shell fold, but later develops a connection with the inner layer, and finally loses all connection with the outer. Thus it may represent an ectodermal gland that was present before the shell fold originated, and that, with the development of the latter, the inner end of the gland found itself in contact with the inner layer of ectoderm and acquired a new opening in this region.

BERGOLD'S description of what he terms the antennulary gland is not at all clear. His only description of its position in the body is in the sentence (1910, p. 12): "Dicht unterhalb der Leber liegt, horizontal ausgebreitet, die kleine sackförmige Drüse der 1. Antenne." From the first part of this statement it is difficult to imagine why he considered it as belonging to the antennulary segment at all. No part of the antennule lies underneath the liver. The antennules are attached to the body on either side of the median eye on a level that is dorsal to the upper surface of the gut. The liver, on either side, is produced from the antero-dorsal part of the stomach and slopes downwards and backwards in the cavity of the shell fold, so that no part of the liver can ever lie above any part of the antennule. On the other hand, the antennæ are attached to the body directly underneath and somewhat in front of the junction of the liver and the stomach. If then BERGOLD'S description of "dicht unterhalb der Leber" describes accurately the position of the sac that he discovered, then that is evidence

that the sac was actually the end sac of the real antennal gland. In addition to this, however, the figure that he gives of this sac agrees fairly well with those of the end sac of the antennal gland given in the present paper. Also he states that the hind part of the sac was drawn out into a point and attached to the endoskeleton (1910, p. 12). This is probably the tendinous strand that passes from the ventro-posterior part of the antennal gland end sac to attach to the median tendinous plate joining the transverse muscles of the mandibles. It can be taken then as practically certain that BERGOLD'S antennulary gland represents merely the end sac of the antennal gland. The reasons that he did not discover the duct system are probably, first, that he was working only on adult forms, and in these the duct system is always more or less degenerate, and secondly, that the methods of fixation used were not delicate enough to fix the duct cells.

The duct system of the antennal gland appears at first sight a very complicated structure, but comparison with the duct of the antennal gland of Estheria, Branchipus or Artemia shows that the duct system is built on the same fundamental plan in both cases. In these Branchiopods and in the Ostracods the duct is formed of three cells only. In both cases the two cells nearest the end sac contain a duct in the form of a loop that leads from the sac to the third cell which has the form of an elongated tube leading to the exterior. The loop is simple in the case of the Branchiopods, whereas in the case of the Ostracods it has the form of the double U that has been already described. If the simple Branchiopod loop is imagined as being pulled out and wrapped round three sides of the end sac then the condition found in the Ostracods is obtained. The peculiar angles in the Ostracod duct appear to be due to the duct cells being attached to the ectoderm, the rapid growth of the ectoderm pulling out the parts of the tube in between the points of attachment. In Estheria and in Chirocephalus no such attachment obtains. In part, no doubt, the shape of the loop is due to the end sac enlarging and pushing in between the two nuclei of the loop cells. In the Ostracods, in contrast to the Branchiopods there is not much room for the expansion of any one organ. In Estheria the end sac could enlarge considerably without pressing on the duct cells. In the Ostracods, on the other hand, if the end sac enlarges out of proportion to the surrounding organs it must press outwards in between the two loop cells.

The walls of the intracellular duct in the case of the Ostracods are remarkably similar to those of the homologous duct of Estheria. The cytoplasm of the loop cells, however, does not correspond in the two cases. In Estheria the more peripheral layer is full of darkly staining rods, radially placed, while a reservoir occupies the more central parts. No such arrangement can be seen in the Ostracods, the cytoplasm merely being considerably vacuolated.

The entrance of the duct into the end sac in both cases is surrounded by a triangular system of fibrils. In Estheria they appear very early, whereas in the Ostracods they can be seen only when the gland has commenced to degenerate.

The peculiar funnel opening of the duct of the loop cells into the efferent duct cell

has no counterpart in Estheria. In the latter the lumen of the duct of the antennal gland is of uniform calibre throughout and also the cytoplasm of all three cells of the duct system is continuous. However there appears no reason for considering this funnel to have any special morphological significance. Its presence is probably connected with the fact that the lumen of the efferent duct cell is considerably wider than that of the loop cells.

Apart then from these details of structure, the antennal gland of the Ostracods examined agrees very closely with the antennal gland of those Branchiopoda in which the anatomy has been described.

A comparison of the antennal gland with maxillary gland of the Ostracods described in this paper also shows a considerable degree of resemblance. The general construction is the same in the two glands and is, of course, typical. The end sacs are very similar in their histological details. The development of the maxillary end sac indicates clearly that it must represent a cœlomic sac. Its cavity appears as a split in a mass of mesoderm and never has any communication with the hæmocœlic body cavity.

It is in the ducts, however, that the most interesting similarity is to be found. The ducts are both intracellular and in both cases are constituted of a few cells only, three in the case of the antennal and four in the case of the maxillary gland. But whereas the former, at the maximum development of the gland opens to the exterior, the latter does not at any stage develop direct communication with the exterior. In all stages up to the adult it dwindles away in the cytoplasm of a cell in the ectoderm, while in adult stages this cell becomes overgrown by the surrounding ectoderm. This difference between the two ducts is probably not of much importance. There are several cases in which excretory ducts have been described as disappearing in an ectodermal cell without opening directly to the exterior. Thus in Dinophilus according to Shearer (1906, p. 525), the excretory canals end in vacuoles in the cytoplasm of ectodermal cells. In Sternaspis (Goodrich, 1897, p. 235) and in the genus Capitella (Eisig, 1887, p. 272) a similar condition obtains. In none of these cases, however, is the ectodermal cell in which the excretory duct terminates overgrown by the surrounding cells.

In the Ostracods it is quite probable that at the stage when the terminal duct cell becomes completely internal the maxillary gland has ceased to function as an excretory organ, excretion being carried out by the gut or some other organ. The histological details of the maxillary gland of the adult, compared with those of other organs, support this idea. The cells of the end sac are full of large vacuoles, while their nuclei are deeply staining degenerate masses. The outline of the end sac itself is difficult to distinguish. The cytoplasm of the duct cells also becomes choked up with deposits usually taken to represent excretory masses. According to Burian and Muth (1921, p. 678) no other excretory organs have as yet been demonstrated in Ostracods by the usual methods, but very little work has been done on the problem and most probably a less crude technique than that usually employed would indicate other organs of excretion than the segmental organs.

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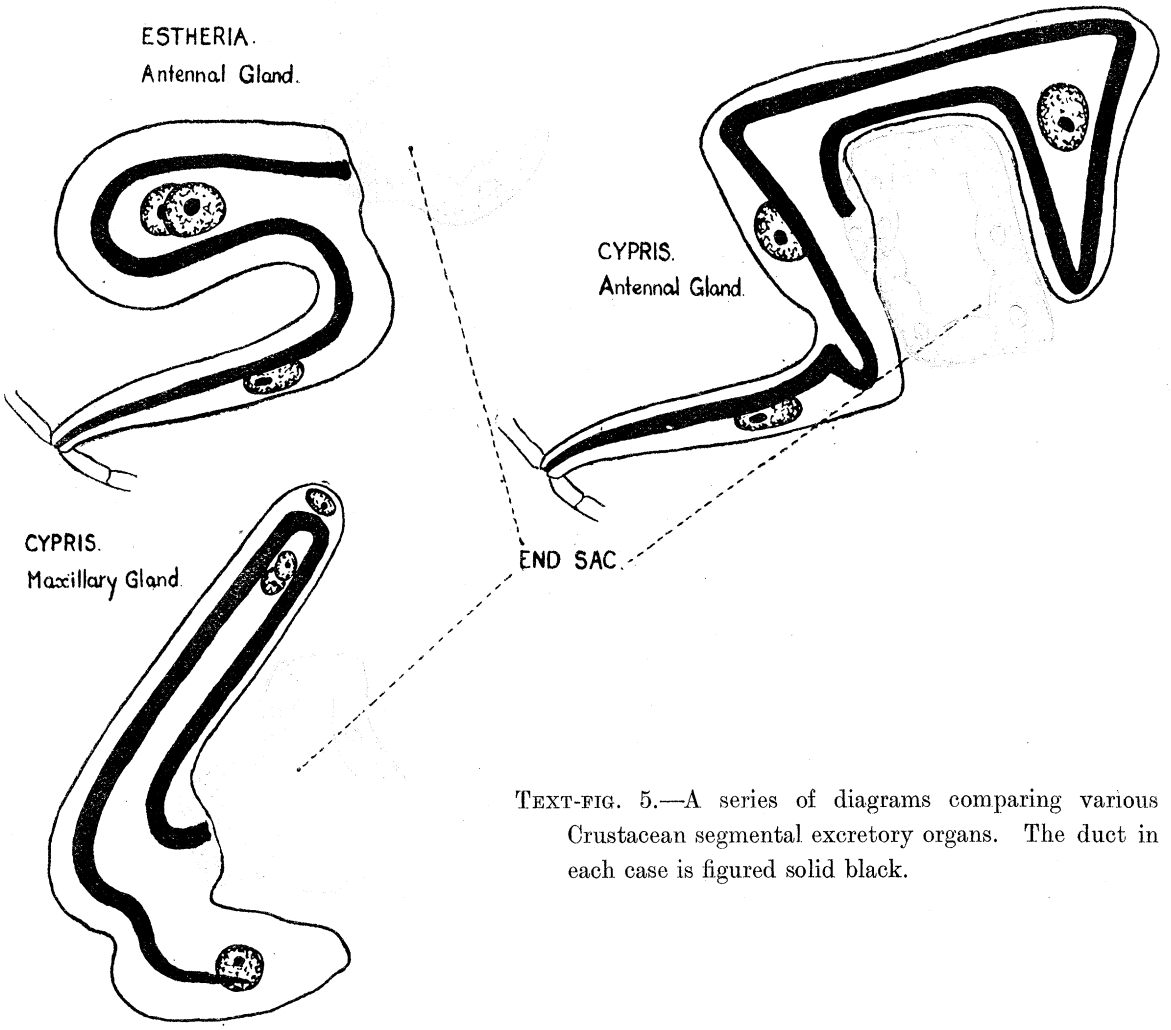
The commencement of the ducts from the end sac in both antennal and maxillary glands is surrounded by a structure containing a triangular network of fibrils, probably functioning as a valve. The two valves appear, however, very different. In the antennal gland the fibrils develop in cells that are apparently part of the end sac, and so mesodermal in origin, whereas the valve of the maxillary gland is entirely an ectodermal structure. This suggests that the two valves represent merely similar structures evolved in relation to similar physiological processes. The development of the antennal gland, however, has not been worked out, and it is possible that the cells containing the fibrils in this gland are similarly ectodermal cells, but have become incorporated in the end sac so as to be indistinguishable from the surrounding end sac cells. Leaving apart, however, the question of the origin of the valve, if the ducts of the two glands are compared it is obvious that they are really so similar that it is reasonable to deduce that they represent homologous structures. This similarity is emphasised in text-fig. 5.



TEXT-FIG. 5.—A series of diagrams comparing various Crustacean segmental excretory organs. The duct in each case is figured solid black.

In the case of the valve of the maxillary gland, its development and its close

connection with the complicated endoskeletal system suggest that it is a specialised portion of the latter. This view is supported by a close similarity between its development and that of the ingrowth of ectodermal cells to form plates serving as trochleas for the muscles of the second antenna as described by HUMPERDINCK (1922, p. 647) in Polyphemus.

The development of a muscular structure, such as the valve of the maxillary gland possibly is, from ectodermal cells, is not without parallel. STAFF (1910, p. 251) has shown in Criodrilus* that the circular muscle cells develop from ectodermal cells. It is equally probable, however, that the fibrils are elastic rather than muscular, as suggested by TER-POGHOSSIAN (1909, p. 14). In either case their connection forward by a thin tendinous strand to the adductor muscle is significant. It has been stated already that this connective, after running forwards to the tendinous plates of the adductor muscle turns sharply at right angles and is continuous with part of this muscle. Contraction then of the adductor muscle will presumably pull this connective strand forwards. From text-fig. 4 (*d*) it is clear that such a forward movement would exert a lateral tension on the valve. Probably by this mechanism, when the shell is closed the valve of the maxillary gland is also automatically closed, thus preventing the escape of the excretory fluids into the shell cavity.

Comparing now the maxillary gland of the Ostracod with that of Estheria, it is clear, if the development of the ducts is taken into account, that the two structures are quite different. They are only partly homologous. It was stated by the author (1924, p. 419), referring to the antennal and maxillary glands of Estheria, "There are certain points . . . that indicate that while the end sacs of both glands may be the remains of cœlomic cavities, the ducts may be of different origin, and hence not homologous." In the present paper it has been shown that this is the case if the maxillary glands of Estheria and the fresh-water Ostracods are considered. In Estheria the duct is a cœlomiduct outgrowth, whereas in the Ostracods it is an ectodermal ingrowth. Therefore, the glands must not be considered strictly as homologous. There appears no reason to assume that the Ostracods are unique in the development of their maxillary glands, and hence it is clear "why such diverse reports occur as to the germ-layer origin of the ducts of the Crustacean excretory glands."

What the ectodermal type of duct actually represents is not at all certain. The author suggested, from a comparison of adult structures alone, that this type of duct may represent an ectodermal gland with which a cœlomic sac has made connection (1924, p. 421). From the account of the development of the maxillary gland described in the present paper there appears no reason for withdrawing this suggestion.

An attractive alternative, however, advocated by many authors, is that the duct represents the Annelid nephridium. If, in making this comparison, only the more primitive nephridia of the Annelids are considered, then there is nothing in the

* Since writing the above the author has discovered that a whole series of muscles in *Chirocephalus diaphanus* are of ectodermal origin ('Nature,' vol. 115, p. 458).

development of the maxillary duct that is incompatible with this view. The duct, as in the case of the Annelid nephridium, is formed by a solid ingrowth of ectodermal cells, and in this an intra-cellular lumen develops later. A comparison of the figures of the developmental stages of the protonephridia of Polygordius (SHEARER, 1908, figs. 29 and 31, Pl. 27), or even of the larval nephridia of Dreissensia (MEISENHEIMER, 1900, figs. 87–91, Pl. 8) with the developmental stages of the maxillary gland duct of the Ostracod shows a very close similarity.

In addition to homologising the excretory gland ducts with the Annelid nephridium VEJDOVSKY (1901, p. 394) suggested, further, that the valve apparatus (Trichterventil) guarding the entrance of the duct to the end sac represented the Annelid nephrostome.

The Annelid nephrostome may be either simply a differentiation of the terminal cells of the nephridium and so purely ectodermal in origin, *e.g.*, as in Polygordius, or it may be a more complex structure into the constitution of which mesodermal elements enter, *e.g.*, as in Lumbricus. With regard to the latter, which was the type considered by VEJDOVSKY, there is no embryological evidence that the valve cells are ever mesodermal in origin. The evidence adduced by VEJDOVSKY was entirely from adult structures, and is not at all convincing. He did not, however, consider the question as settled. His main point in favour of the cœlomic origin of the valve apparatus was that it was continuous with and stained the same as the end sac wall. But, as was indicated earlier in this discussion in connection with the antennal gland valve, this is not proof that the valve cells are mesodermal in origin.

With regard to the purely ectodermal type of nephrostome, in those cases in which the development has been studied, *e.g.*, Polygordius, the nephrostome is formed from cells forming the tip of an ectodermal ingrowth, the remainder of the ingrowth forming the duct of the nephridium. In the maxillary gland of the Ostracod the valve cells and the duct cells are both ectodermal in origin, and hence together might be compared to the complete nephridium with its nephrostome. But the fact that the valve rudiment arises separately from the rudiment of the duct cells shows clearly that this cannot be the case. More definite evidence against this comparison is the fact that the valve arises before the duct. In stage IV the valve rudiment is distinct while there is still no sign of the rudiment of the duct cells. If then the valve did represent a nephrostome, the Ostracods would present the anomalous case of the nephrostome arising before the nephridial duct.

It has already been indicated that, in the opinion of the author, the valve system should be considered as a specialised part of the endoskeletal system. The fact just mentioned that the valve arises before the duct, fits in with such a view. Probably originally the valve cells only supported the base of the end sac just as do those cells that connect the end sac posteriorly to the endoskeletal plate or like those cells that support the antennal gland end sac. Later, while retaining their supporting function, they became incorporated into the excretory mechanism. Whether the junction of the duct system with the end sac was made through the three endoskeletal cells or whether

the latter only later surrounded the junction cannot be said with certainty. The developmental processes suggest that the former was the case, If the junction had been established before the valve control, it is clear from the method of development that the valve rudiment would have had to cut through the junction in order that the three valve cells might surround it. It appears more probable that the valve system was already established simply as a triangle of tendinous connectives, and that the duct system, whatever this may have constituted at this stage, made connection with the end sac through this triangle.

Summary.

(1) A typical arthropodan segmental excretory organ occurs in both the antennal and the maxillary segments of the fresh-water Ostracods.

(2) The antennal gland consists of an end sac with an intracellular duct leading to the exterior consisting of three cells only. The entrance of the duct into the end sac is guarded by a triangle of fibrils occurring in the substance of cells otherwise indistinguishable from the surrounding cells of the end sac. The gland attains its maximum development in the fourth larval stage, after which it loses connection with the exterior and degenerates.

(3) The maxillary gland consists of an end sac with an efferent intracellular duct consisting of four cells only. The four cells are completely covered by ectoderm and the duct dwindles away in the cytoplasm of the terminal cell. The entrance of the duct into the end sac is surrounded by a complicated valve. This consists of three cells in which occurs a double triangle of fibrils and which are connected to the ectoderm and endoskeletal system.

(4) The end sac develops from a mass of mesoderm that separates very early in the maxillary segment. A cavity develops in this mass which at no stage opens into the hæmocœle. The intracellular duct is formed from an ingrowth of ectodermal cells that finally become overgrown by the surrounding ectoderm. The valve is also ectodermal in origin and represents a development of the endoskeletal system.

(5) The " shell gland," which has previously been described as the antennal gland, is of unknown function and is in no way serially homologous with the true segmental excretory organs. It consists of three reservoirs, an anterior, a middle and a posterior reservoir. A cuticular invagination from the inner surface of the shell leads into the middle reservoir and serves as an exit for the secretion of the gland.

(6) The development of the " shell gland " has been partly described. The posterior reservoir which has previously been described as a typical end sac arises from a group of ectodermal cells in the outer layer of the shell fold.

LITERATURE LIST.

Bergold, A., 1910. "Beiträge zur Kenntnis des innern Baues der Süszwasser-Ostracoden." 'Zool. Jahrb., Abt. f. Anat.,' vol. 30, pp. 1-42.

Burian, R., and Muth, A., 1921. "Handbuch der vergleichenden Physiologie." Edited by H. Winterstein. 'Die Excretion—Crustacea,' Jena.

Calman, W. T., 1919. "Dr. Walcott's Researches on the Appendages of Trilobites." 'Geol. Mag.,' n.s., Decade VI, vol. 6.

Cannon, H. G., 1924. "On the Development of an Estherid Crustacean." 'Phil. Trans.,' B, vol. 212, pp. 395-430.

Claus, C., 1895. "Beiträge zur Kenntnis der Süszwasser-Ostracoden, II." 'Arb. a.d. Zool. Inst., Wien,' vol. 11, pp. 17-48.

Daday, E. v., 1895. "Die anatomischen Verhältnisse der *Cypris dispar*." 'Termesz. Fuz.,' Budapest, Beilage 18, pp. 1-133.

Defner, A., 1910. "Der Bau der Maxillardrüse bei Cirripeden." 'Arb. a.d. Zool. Inst., Wien,' vol. 18, pp. 183-206.

Eisig, H., 1887. "Fauna und Flora des Golfes von Neapel." 'Capitelliden.' Berlin.

Giesbrecht, W., 1913. "Handbuch der Morphologie." Edited by A. Lang. 'Crustacea,' Jena.

Goodrich, E. S., 1897. "Notes on the Anatomy of Sternaspis." 'Quart. Jour. Micr. Sci.,' vol. 40, pp. 233-245.

Grobben, C., 1880. "Die Antennendrüse der Crustaceen." 'Arb. a.d. Zool. Inst., Wien,' vol. 3, pp. 93-110.

Humperdinck, I., 1922. "Über Muskulatur und Endoskelett von *Polyphemus pediculus* de Geer." 'Zeit. f. Wissench. Zool.,' vol. 121, pp. 621-655.

Meisenheimer, J., 1901. "Entwicklungsgeschichte von *Dreissensia polymorpha*." 'Zeit. f. Wissench. Zool.,' vol. 69, pp. 1-139.

Müller, G. W., 1894. "Fauna und Flora des Golfes von Neapel." 'Ostracoden,' Berlin.

Murray, J. A., 1924. "The Open-tube-pyroxylin Method of manipulating Small Organisms." 'Journ. Roy. Micr. Soc.,' No. 268, pp. 289-291.

Shearer, C., 1906. "On the Structure of the Nephridia of Dinophilus." 'Quart. Jour. Micr. Sci.,' vol. 50, pp. 517-545.

Idem, 1908. "Studies on Larval Nephridia, II." 'Phil. Trans.,' B, vol. 199, pp. 199-230.

Staff, F., 1910. "Organogenetische Untersuchungen über *Criodrilus lacuum* Hoffmstr." 'Arb. a.d. Zool. Inst., Wien,' vol. 18, pp. 227-256.

Ter-Poghossian, F., 1909. "Beiträge zur Kenntnis der Exkretionsorgane der Isopoden." 'Hallesche Zeitschr. f. Naturwiss.,' vol. 81, pp. 1-50.

Vejdovsky, F., 1901. "Zur Morphologie der Antennen und Schalendrüse der Crustaceen." 'Zeit. f. Wissench. Zool.,' vol. 69, pp. 378-397.

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DESCRIPTION OF PLATES.

List of Abbreviations used.

a.l.d.s. = ascending loop of duct system.
a.m. = adductor muscle.
c. = connection of end sac with rudiment of maxillepedal gland.
c.e.n.m. = circum - œsophageal nerve mass.
d.c. = duct cells.
d.l.d.s. = descending loop of duct system.
d.n.d.s. = dorsal nucleus of duct system.
e.d. = efferent duct.
e.e.s. = duct entering end sac.
e.s. = end sac.
e.s.w. = wall of end sac.
ex.a.g. = exit of antennal gland.
f. = funnel.
f.d. = degenerate funnel.
i.l.c.d. = inner loop of coil duct.
l. = lumen of duct system.
l.c.v.s. = lateral connective of valve system.
l.n.c.c. = lower nucleus of coil cells.
m.c.v.s. = median connective of valve system.
n.e.d.c. = nucleus of efferent duct cell.
n.f. = neck of funnel
o.l.c.d. = outer loop of coil duct.
œs. = œsophagus.
p.e.s. = primordium of end sac.
p.mp.gl. = primordium of maxillepedal gland.
p.v.s. = primordium of valve system.
st. = stomach.
t.d. = duct dwindling away in cytoplasm of terminal duct cell.
t.n.d.s. = terminal nucleus of duct system.
u.n.c.c. = upper nucleus of coil cells.
v. = fibrils of valve system.
v.c.v.s. = ventral connective of valve system.
v.m.mx. = ventral mesoderm of maxillary segment.

All the figures are camera lucida drawings and the magnifications quoted are approximate.

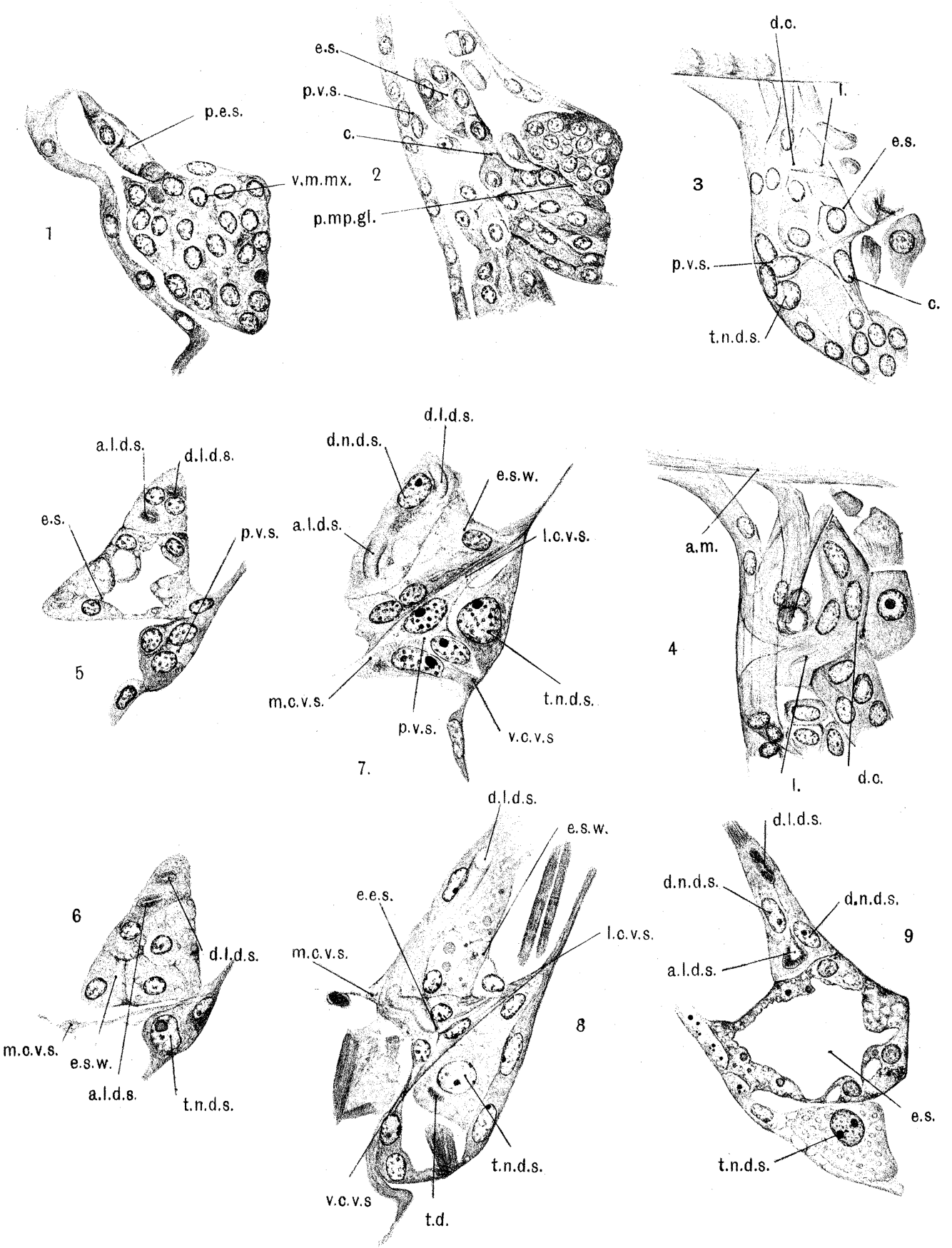
Plate 1.

Fig. 1.—A transverse section of a stage III larva of *Cyprinotus incongruens* passing through the earliest visible rudiment of the end sac of the maxillary gland. × 1680.

Figs. 2, 3, 5 and 7 form a series of transverse sections illustrating the development of the valve system of the maxillary gland.

Fig. 2.—A section of a stage IV larva of *Cypridopsis vidua* passing through the end sac and its connection to the rudiment of the maxillepedal glands. It shows the first sign of the three cells forming the rudiment of the valve system. × 1680.

Figs. 3 and 4 show two successive sections of a stage V larva of *Cypridopsis vidua*.



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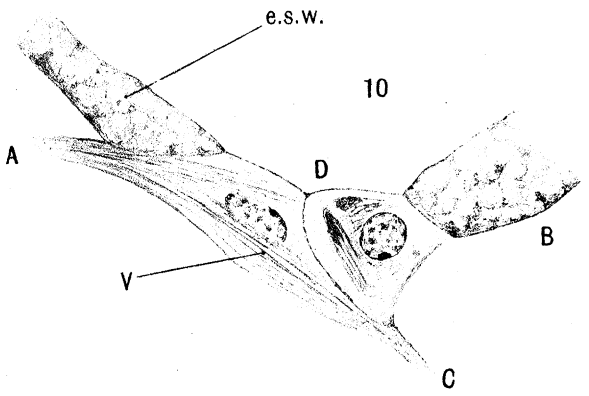

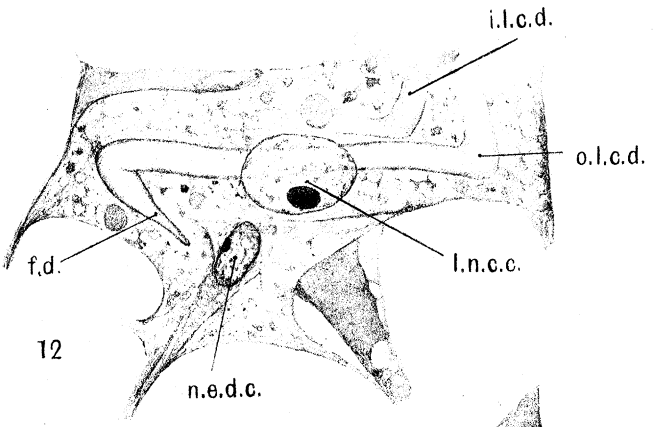

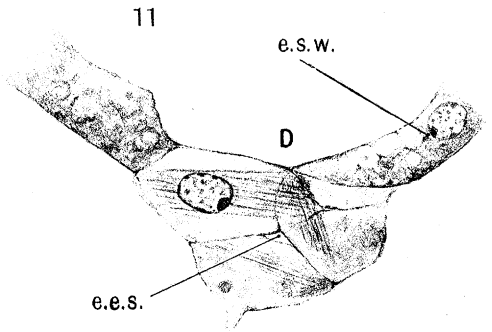

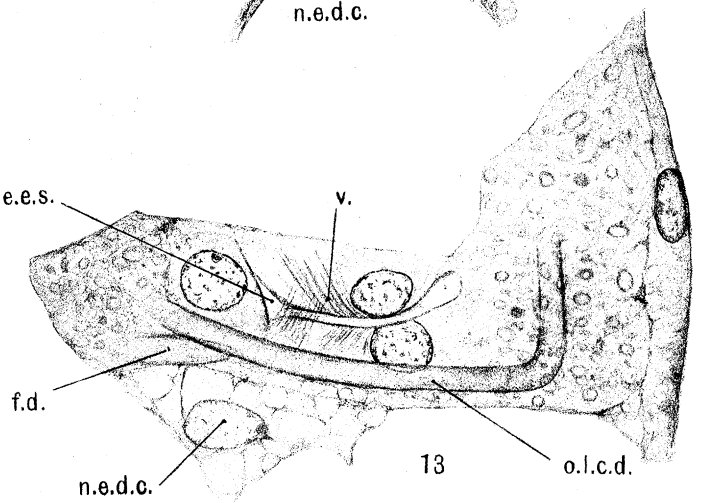

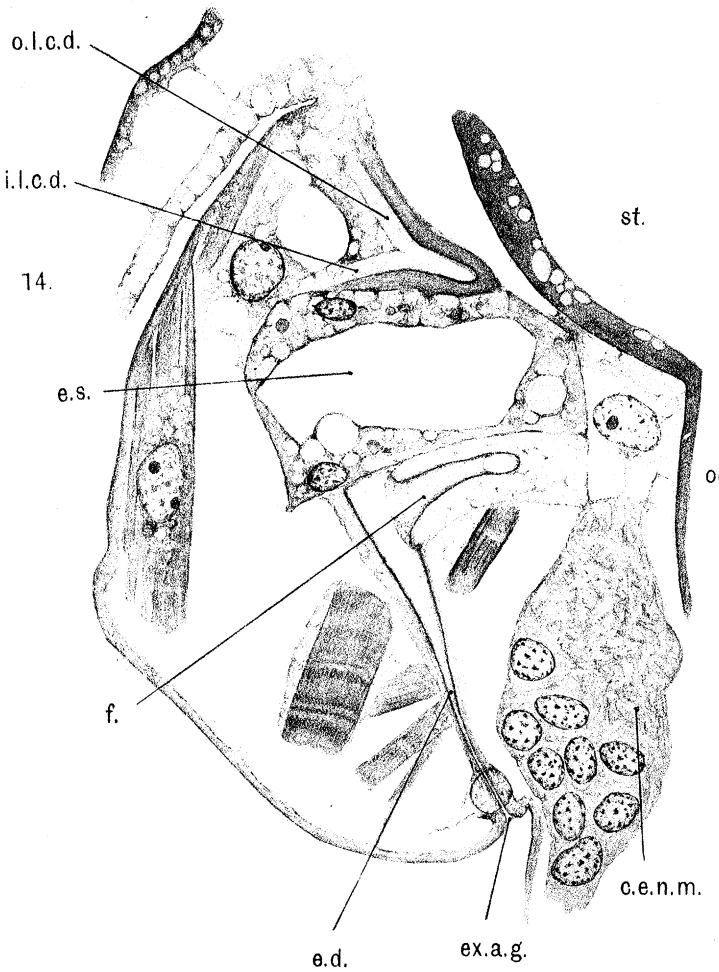

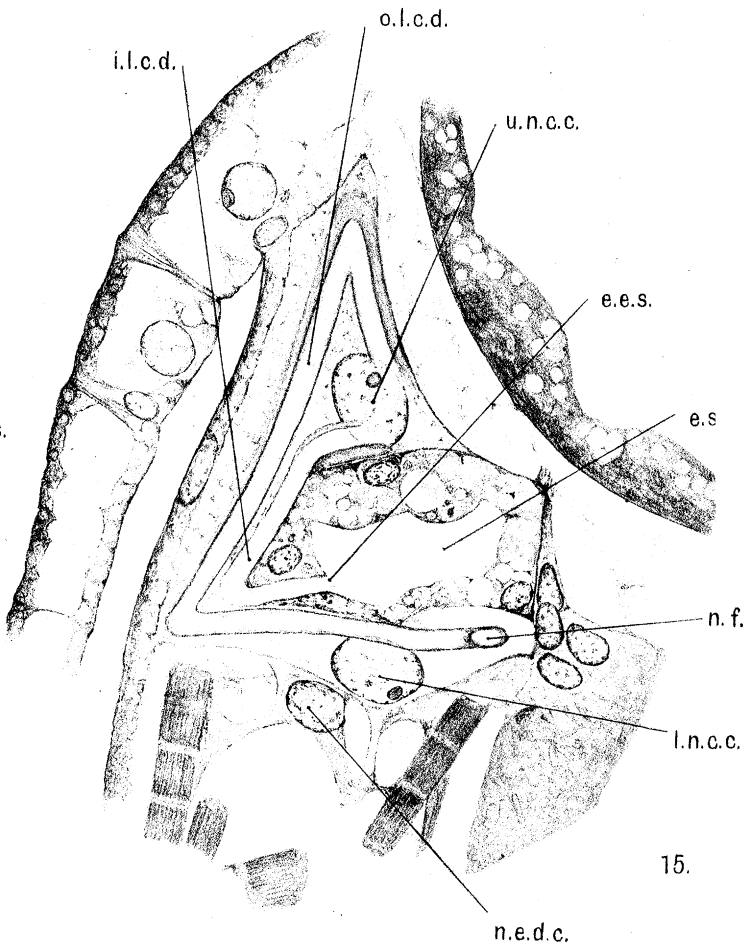

FIG. 3.—A section through the valve rudiment showing its median connective passing underneath the connection of the end sac to the maxillepedal glands. The dorsal part of the ingrowth forming the rudiment of the duct system can be seen on the dorsal side of the end sac. This section also contains the large terminal nucleus of the duct system. × 1280.

FIG. 4.—A section through the ingrowth forming the rudiment of the duct system × 1280.

FIGS. 5 and 6 are drawings of the anterior focus and the posterior focus of the same section of a stage VI larva on *Cypridopsis vidua*.

FIG. 5.—A section through the valve rudiment and end sac. × 1680.

FIG. 6.—A section through the anterior wall of the end sac and through the terminal nucleus of the duct system. × 1680.

FIG. 7.—A section through a stage VI larva of *Cypridopsis vidua* showing the later development of the valve system. The terminal cell of the valve system is seen growing back between the lateral and ventral connectives of the valve. × 1680.

FIG. 8.—A transverse section through a stage VI larva of *Cypris fuscata* showing the duct of the maxillary gland entering the end sac through the middle of the valve system. × 1680.

FIG. 9.—A transverse section through the maxillary gland of a nearly adult *Cypris fuscata*. It shows the terminal cell completely covered by ectoderm. × 840.

Plate 2.

FIGS. 10 and 11 represent the posterior focus and the anterior focus of the same transverse section of a mature *Cypris fuscata* showing the fibrillar structure in the valve. × 1680.

FIG. 12.—A transverse section through the basal joint of the antenna of a stage V larva of *Cyprinotus incongruens*, showing the degenerate funnel and efferent duct. × 1680.

FIG. 13.—A section of a stage VI larva of *Cypridopsis vidua* showing the fibrillar structure surrounding the entrance of the duct of the antennal gland into the end sac. × 1680.

FIGS. 14 and 15 represent two successive sections in a series of transverse sections of a stage III larva of *Cyprinotus incongruens*. In fig. 14 can be seen the funnel of the duct system and the efferent duct cell and part of the dorsal loop. The remainder of the latter and also the nucleus of the efferent duct cell are shown in fig. 15. × 1680.

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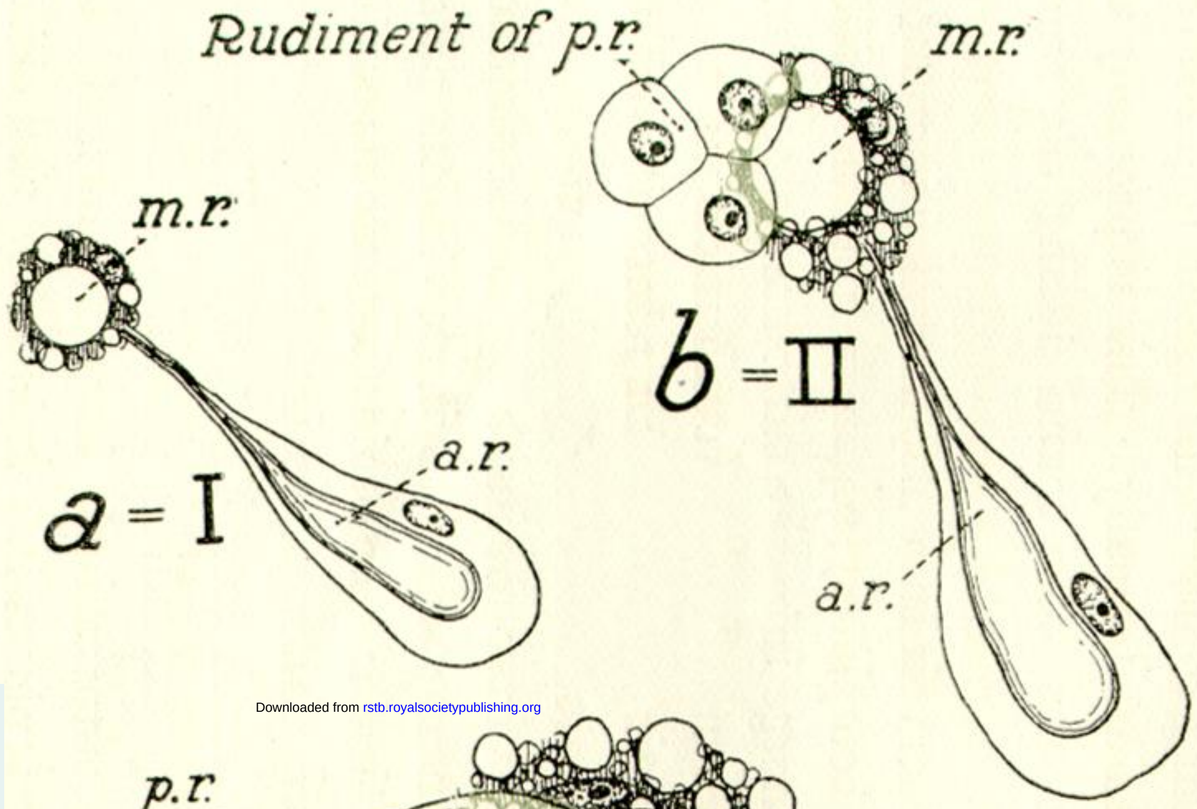
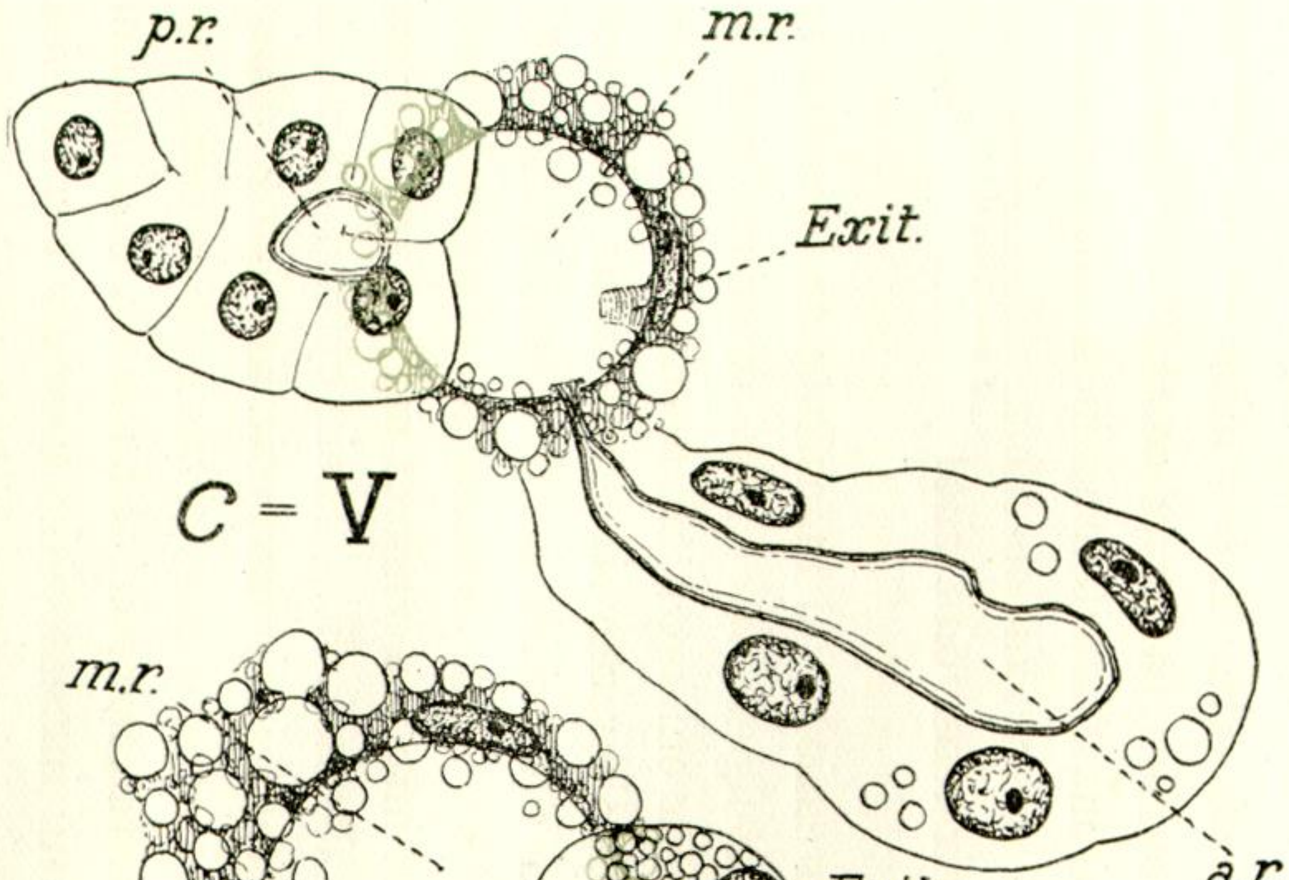
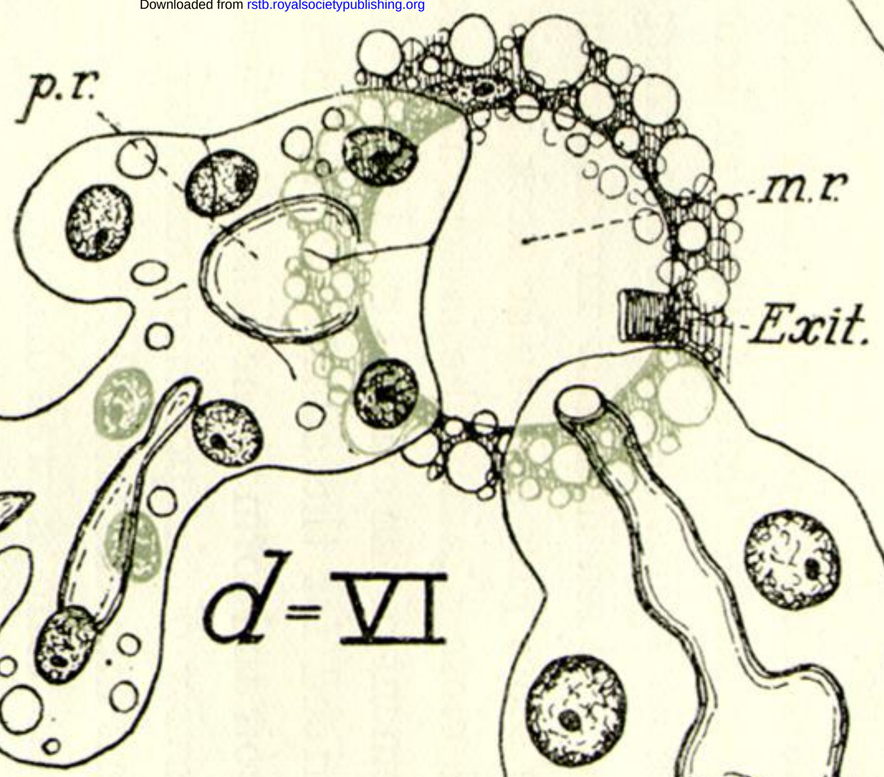
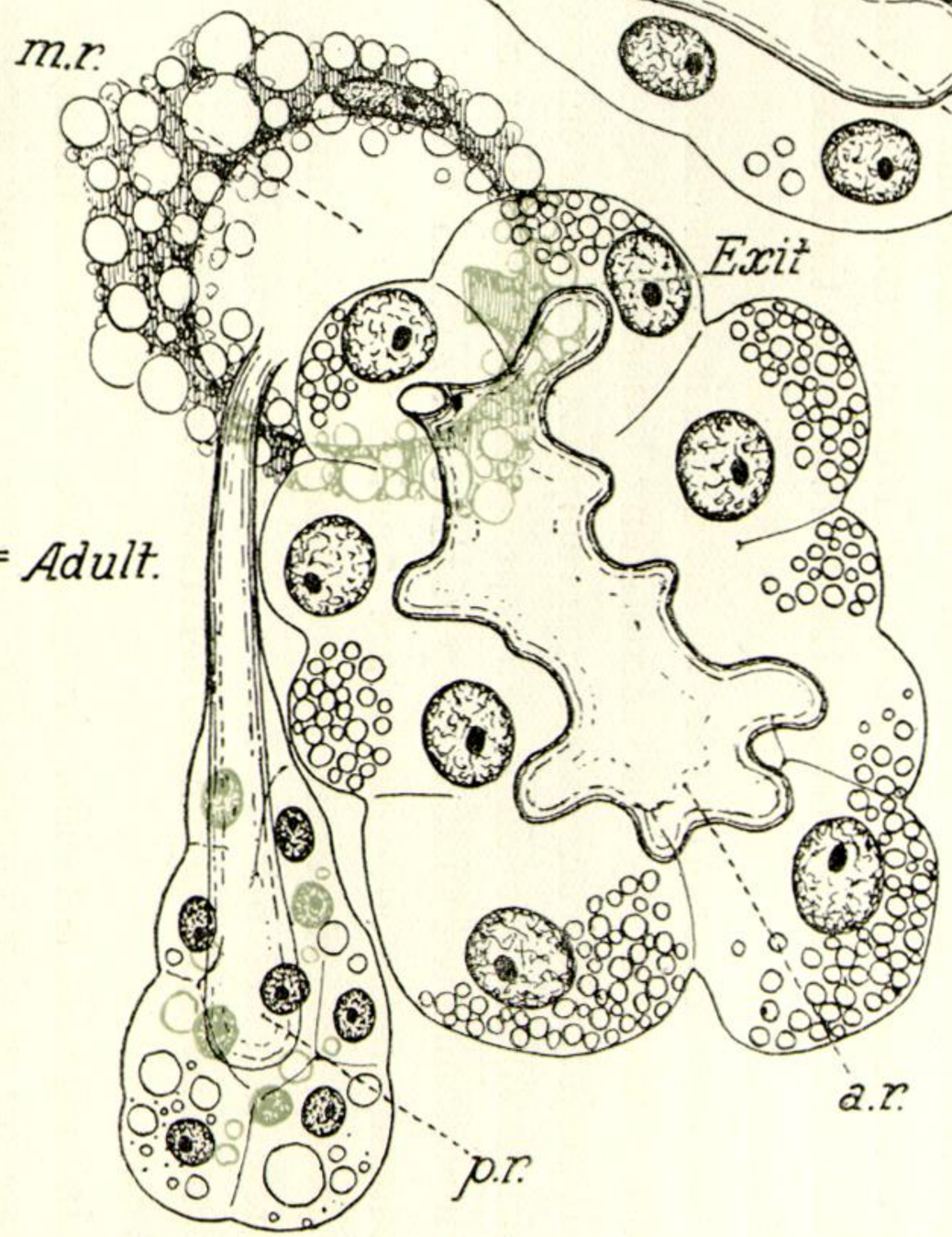




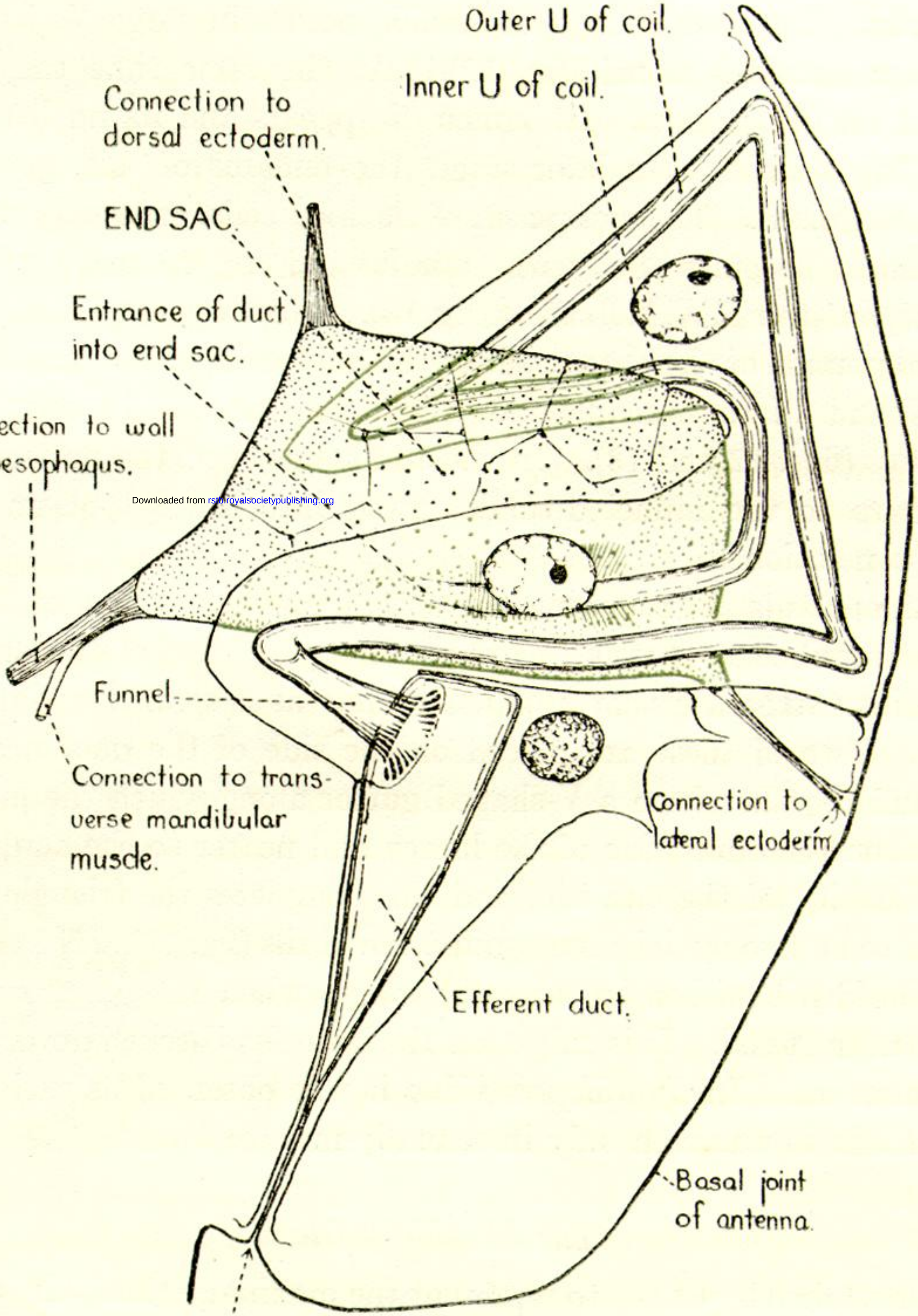




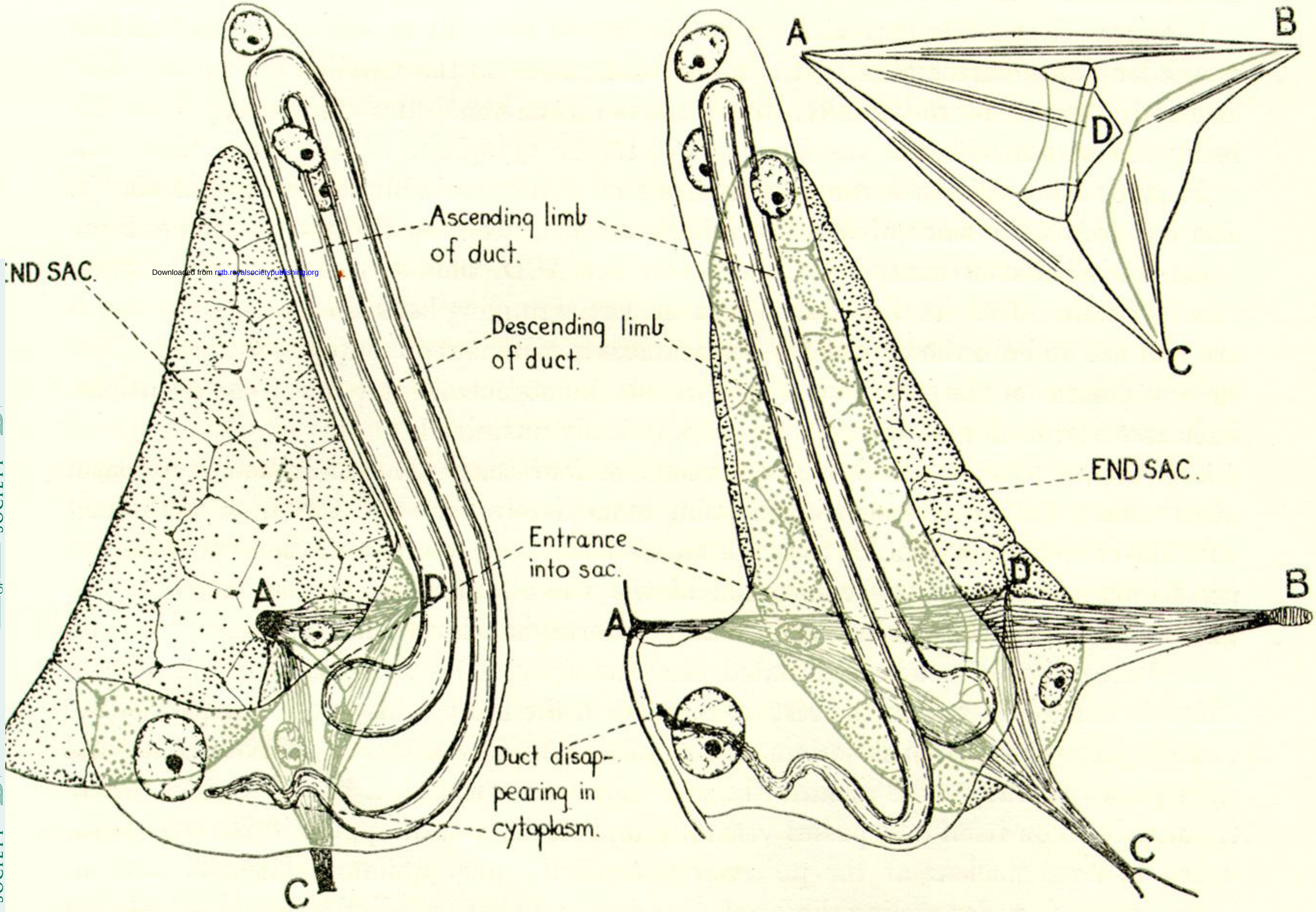
a.r. = anterior reservoir.
m.r. = middle reservoir
p.r. = posterior reservoir

a.r.

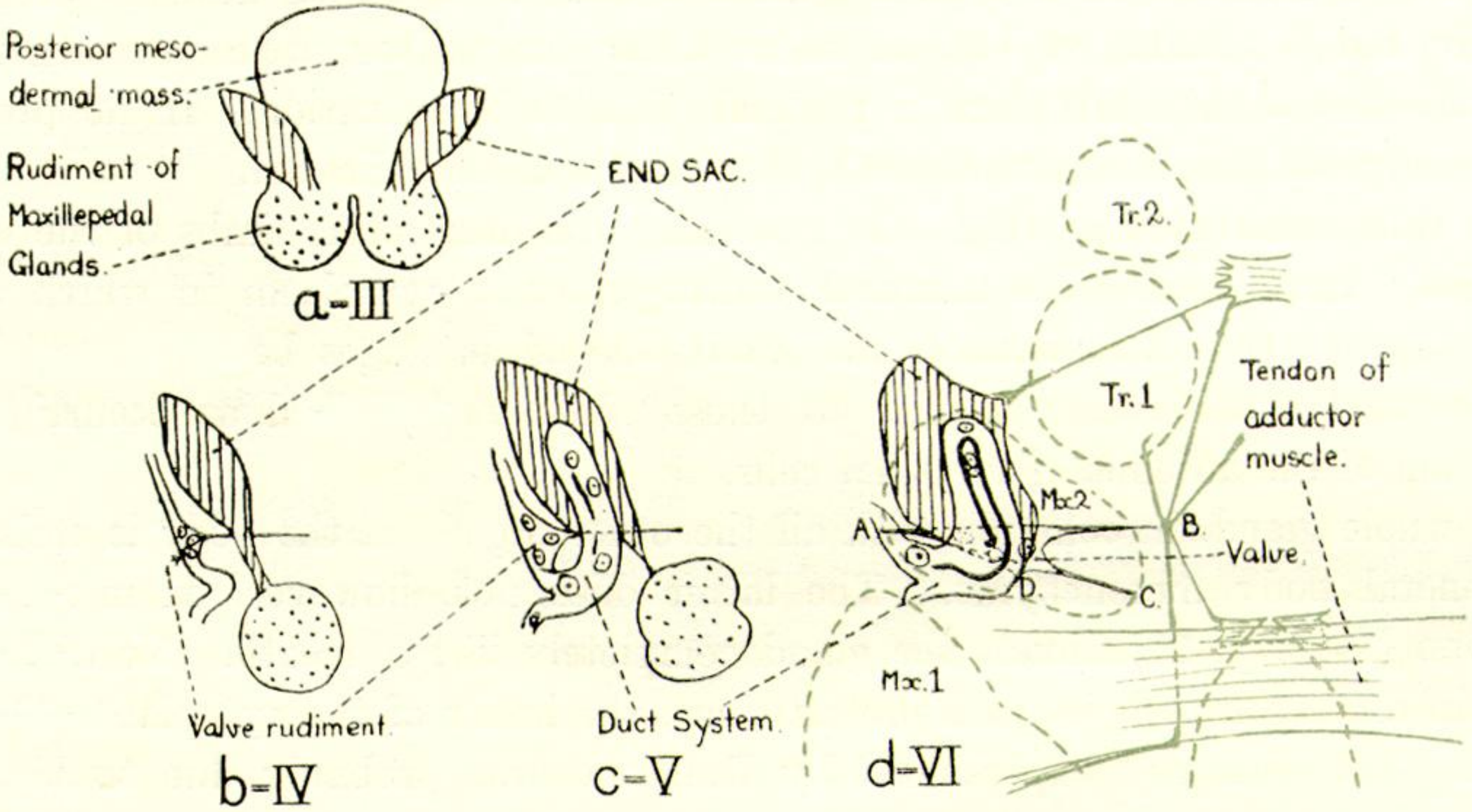
TEXT-FIG. 1 *a* to *e* (Stages I, II, V, VI, Adult).—Series of diagrams illustrating the development of the "shell gland."



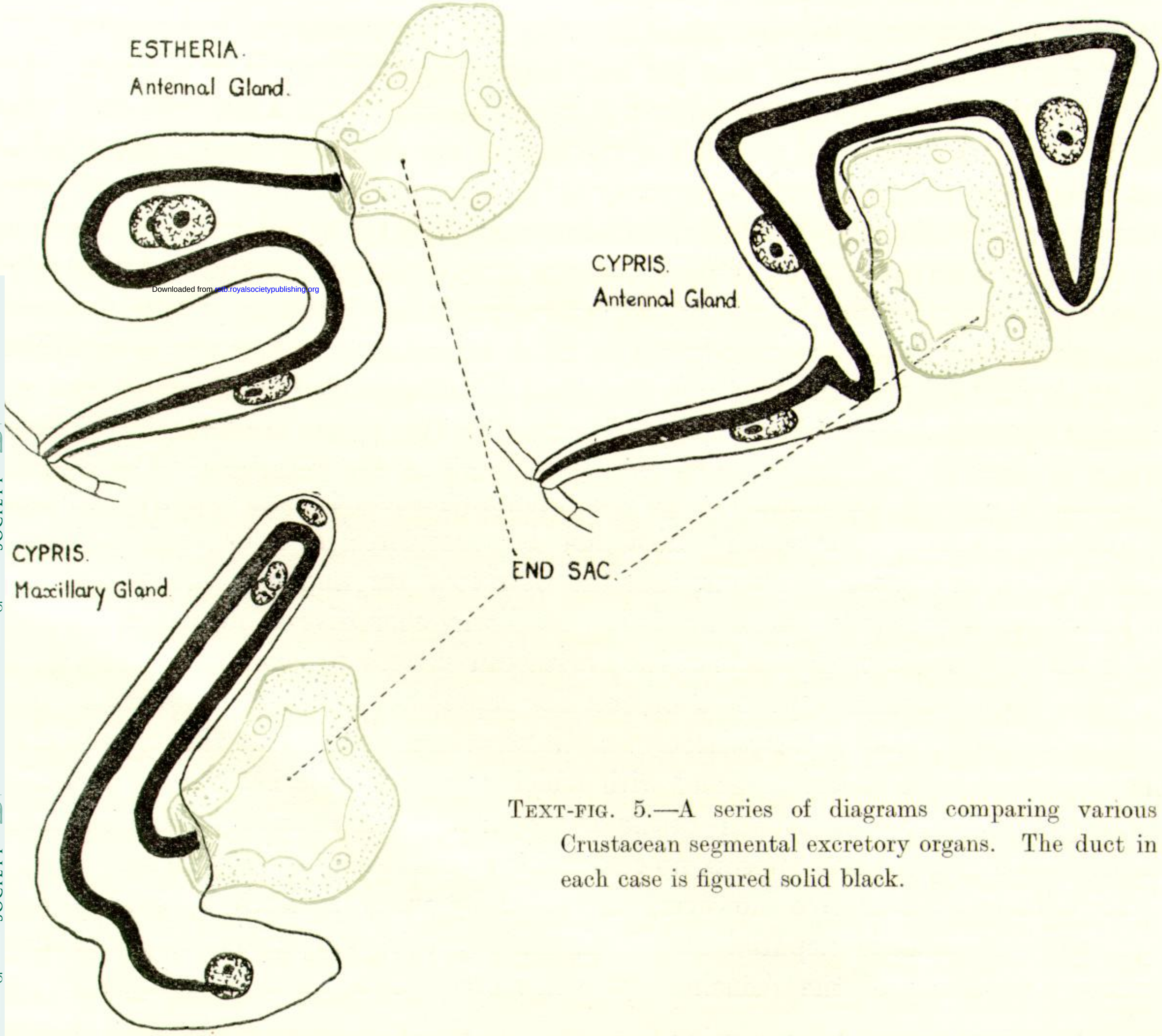
TEXT-FIG. 2.—Diagrammatic front view of Antennal Gland.



TEXT-FIG. 3.—Two diagrammatic views of maxillary gland. That on the left shows the lateral aspect, that on right shows the same gland viewed from the front. At the top right-hand corner is a diagram representing the arrangement of fibrils in the valve system.



EXT-FIG. 4.—A series of four diagrams illustrating the development of the maxillary gland. The last diagram illustrates connections of maxillary gland with endoskeletal system.



TEXT-FIG. 5.—A series of diagrams comparing various Crustacean segmental excretory organs. The duct in each case is figured solid black.

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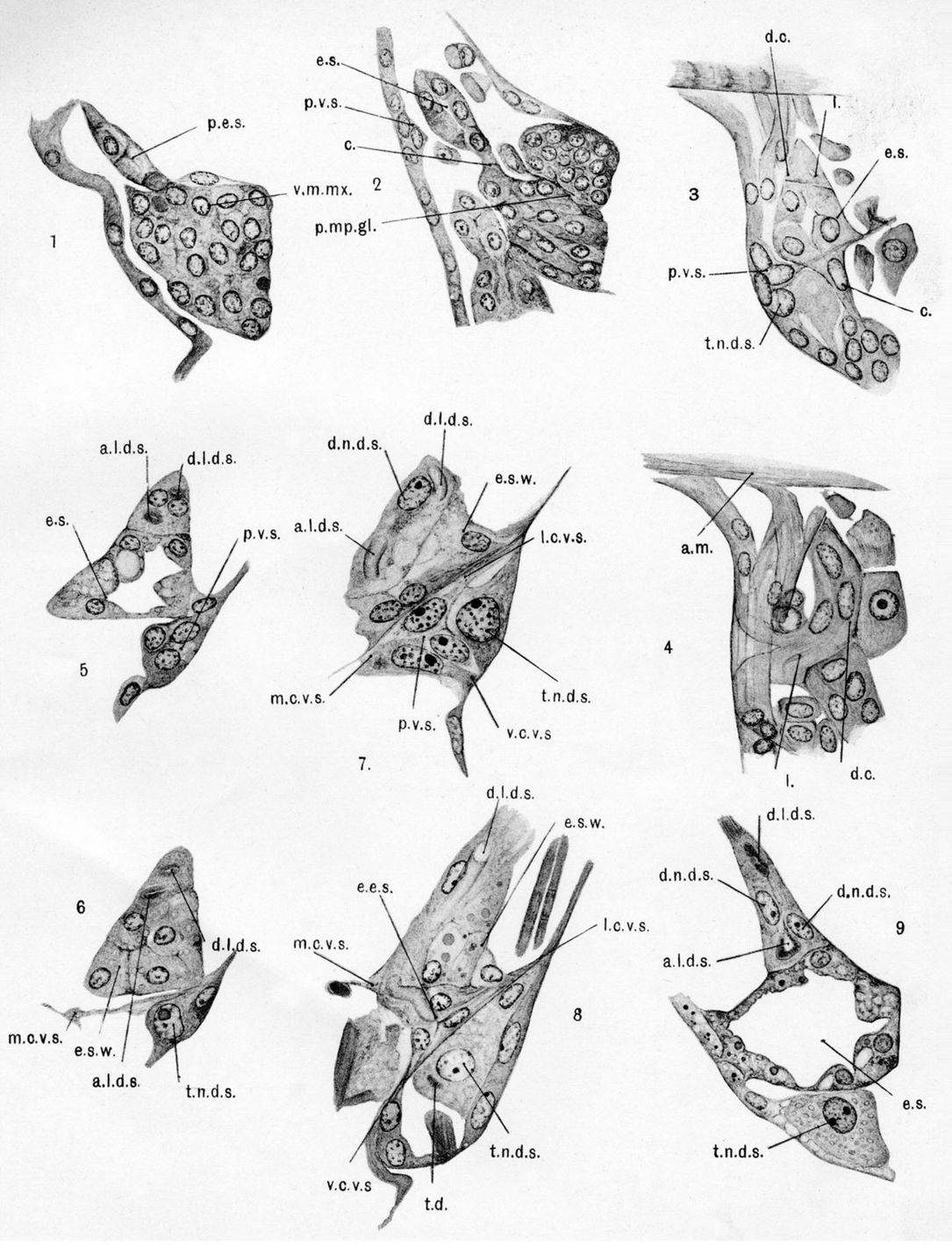

Plate 1.

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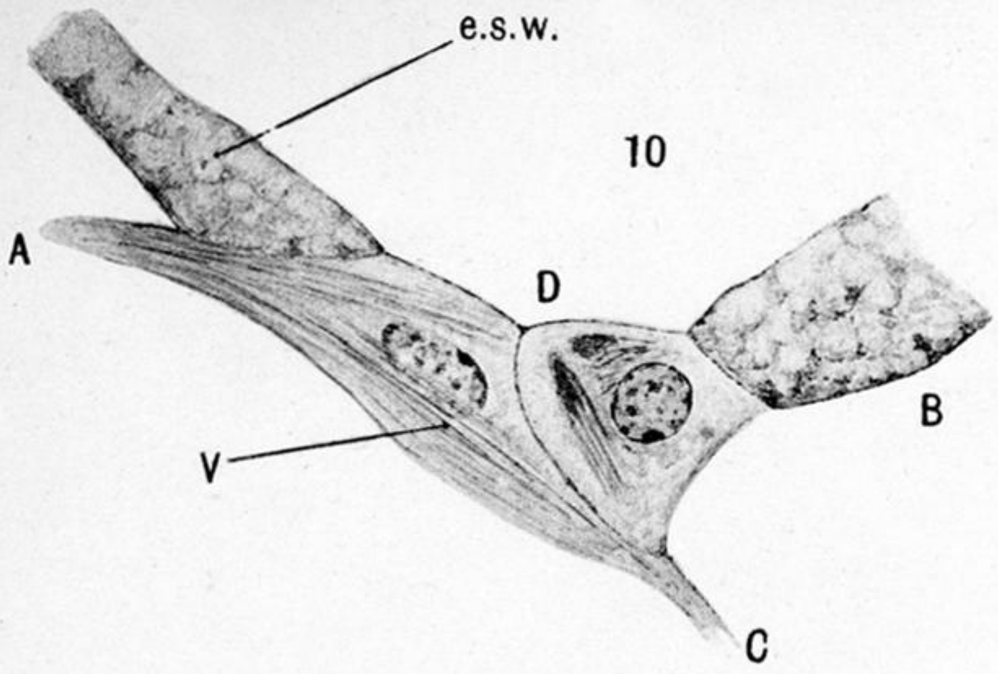
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



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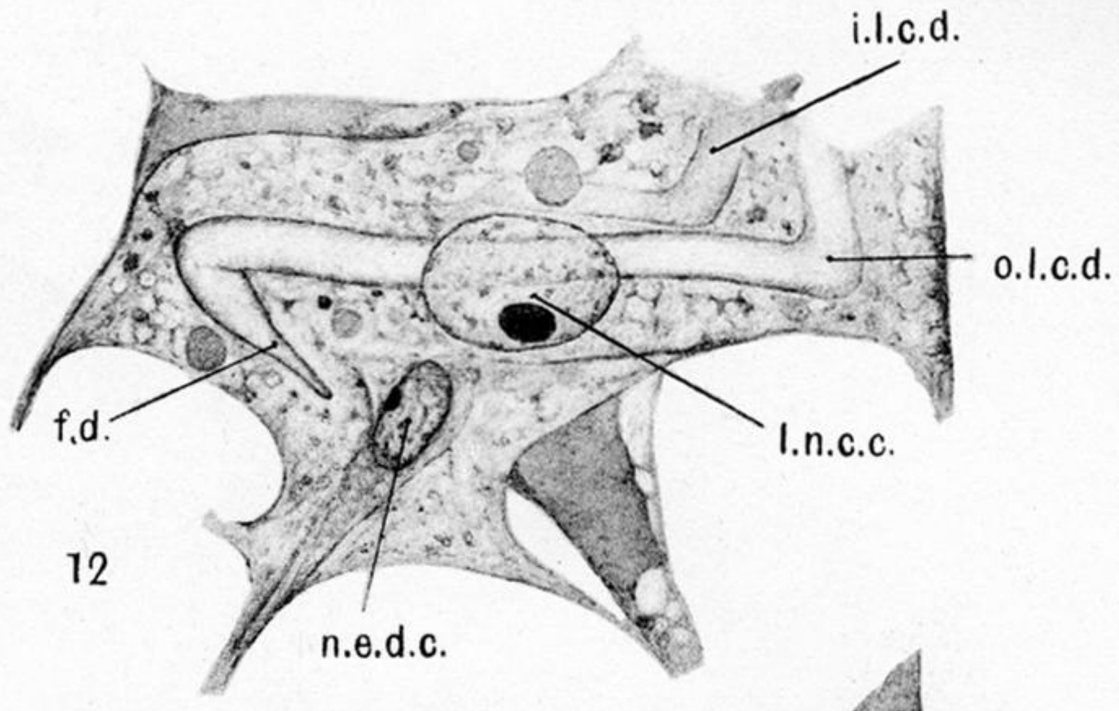

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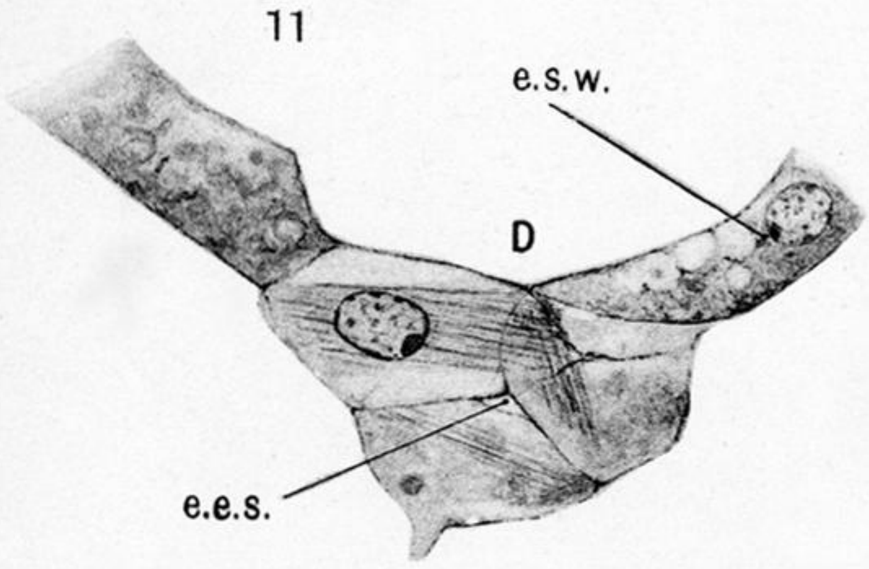

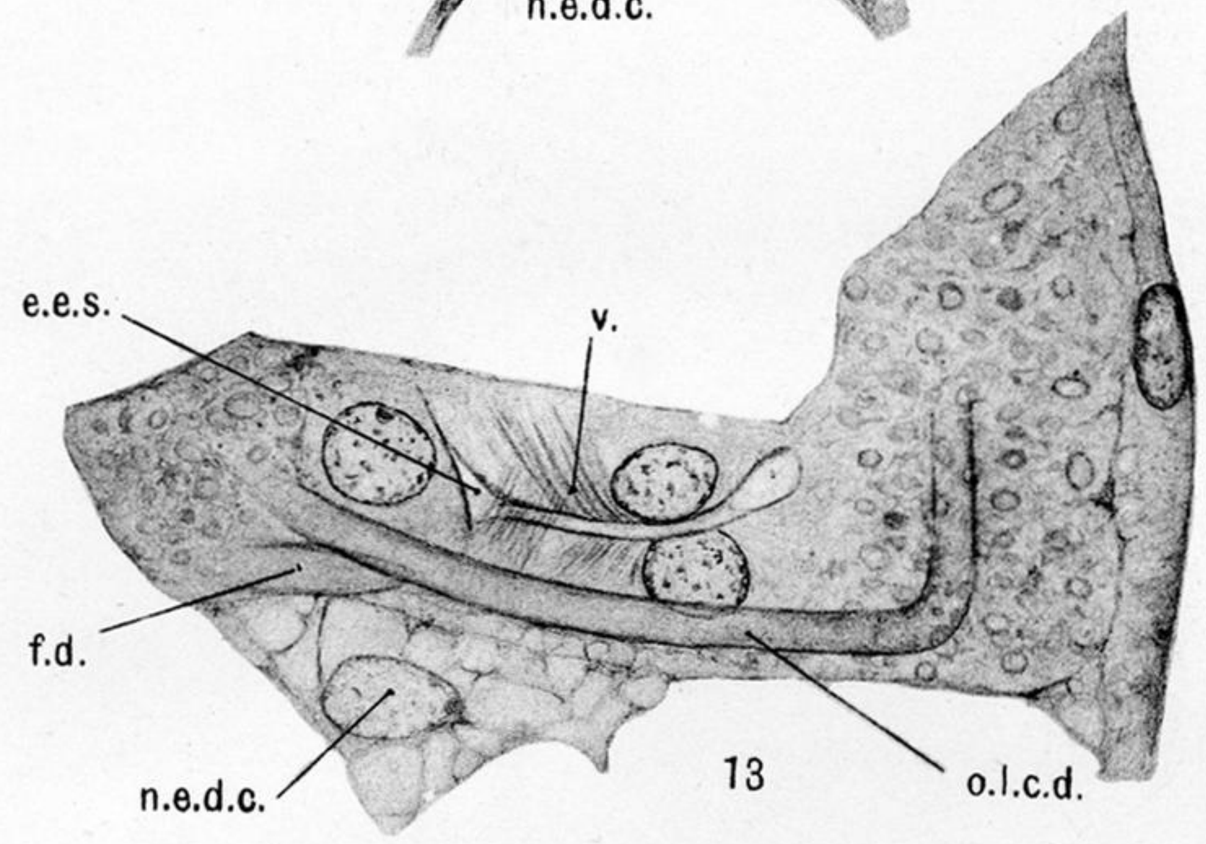

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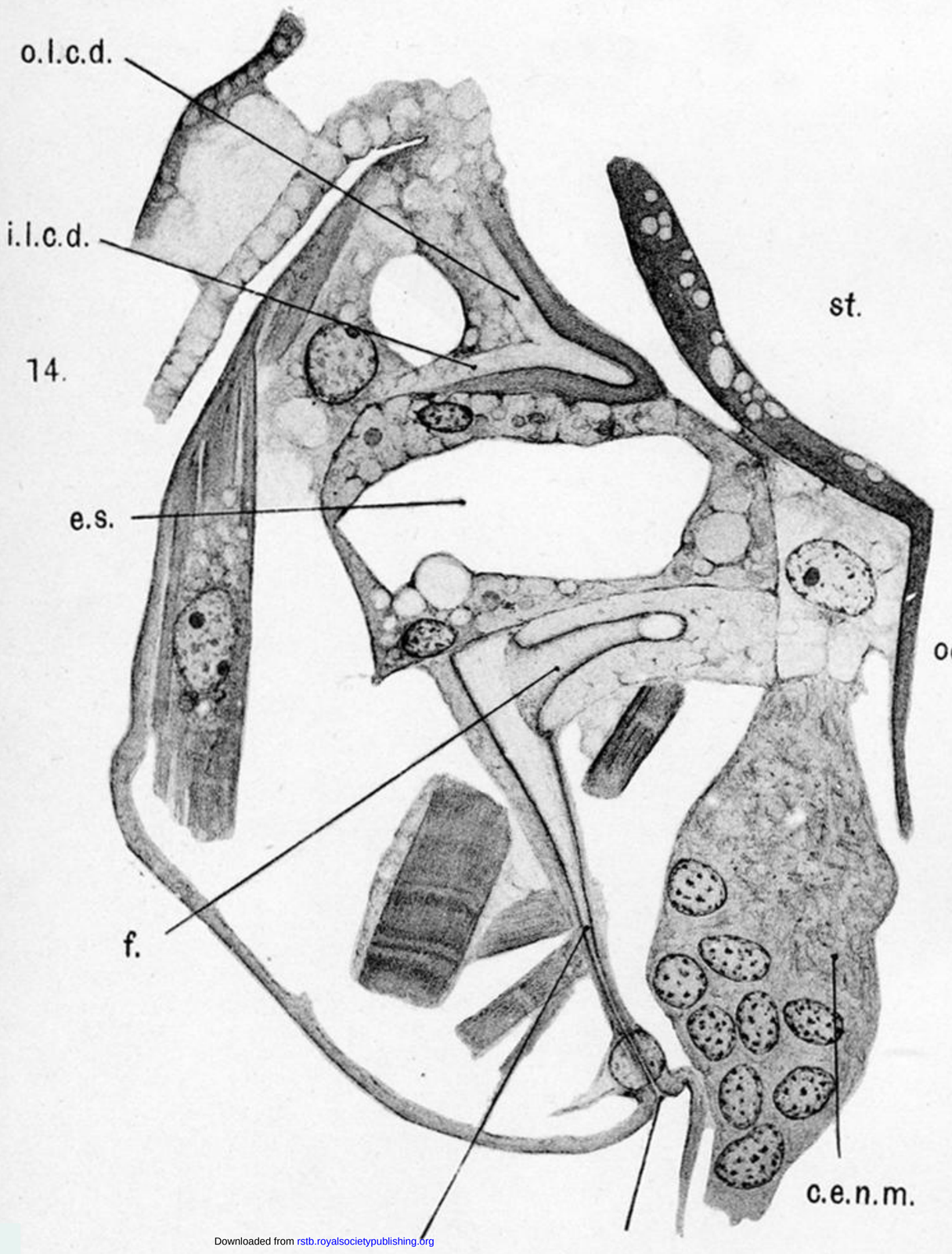

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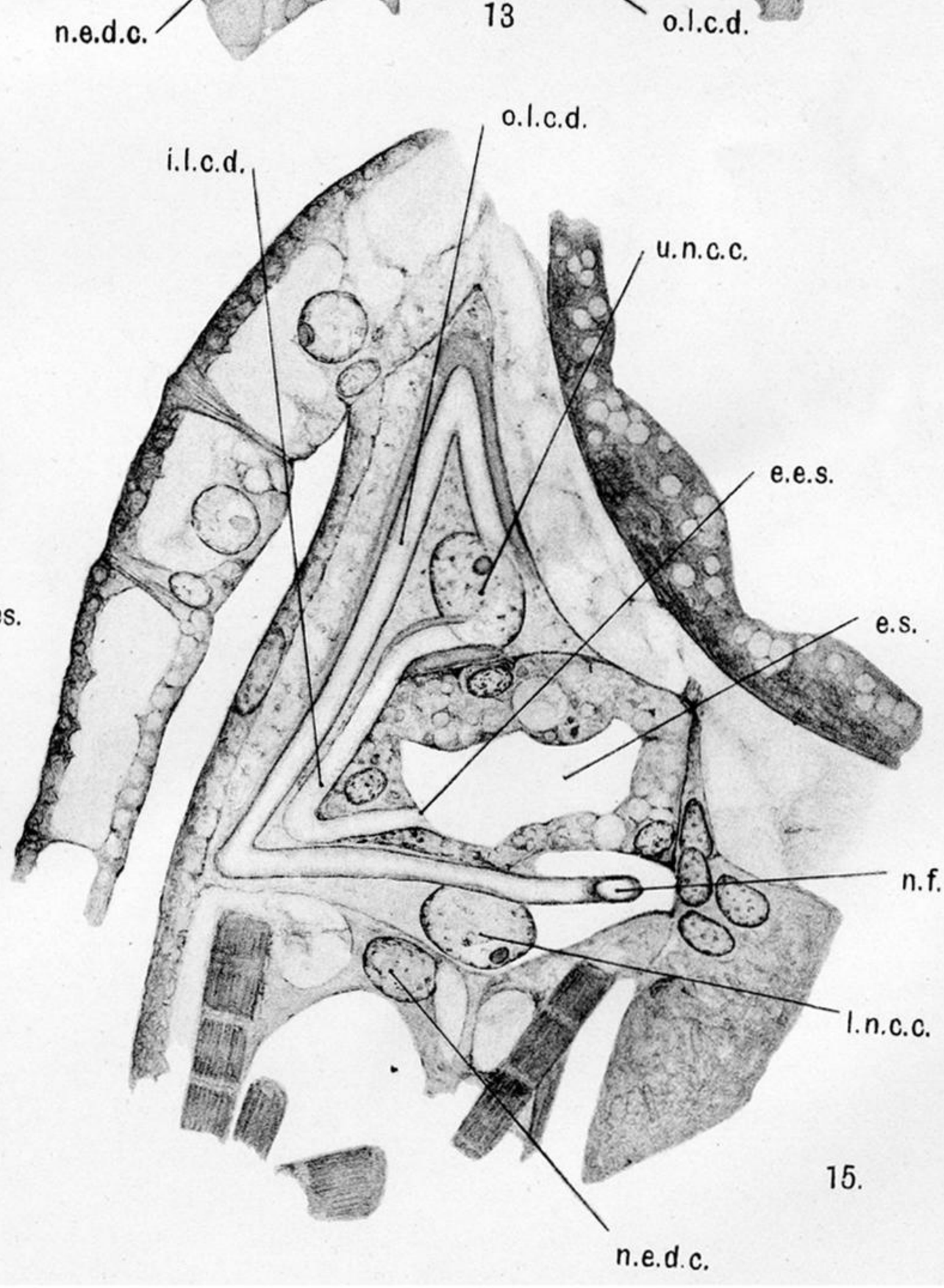

15.

Plate 2.

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