

Morphology, Behavior, and  
Histogenesis of the Pelagosphaera  
Larva of *Phascolosoma agassizii*  
(Sipuncula)

MARY E. RICE

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## ABSTRACT

Rice, Mary E. Morphology, Behavior, and Histogenesis of the Pelagosphera Larva of *Phascolosoma agassizii* (Sipuncula). *Smithsonian Contributions to Zoology*, number 132, 51 pages, 14 plates, 1973.—Morphology, behavior, and histology are described for the pelagosphera larva of *Phascolosoma agassizii* Keferstein. Observations are reported on histology, origin, and development of the following larval structures: body wall, coelom and coelomic elements, retractor muscles, digestive tract, buccal organ, lip gland, nervous system, nephridia, larval glands and terminal organ. Functional significance of certain structures, particularly organs associated with the mouth, is considered. Moreover, available information on developmental histology of other sipunculans is reviewed and intra- as well as inter-phyletic comparisons are discussed. An account is given of the general morphology of the larva and a few observations are noted on larval locomotion and feeding behavior in the laboratory.

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# Morphology, Behavior, and Histogenesis of the Pelagosphaera Larva of *Phascolosoma agassizii* (Sipuncula)

*Mary E. Rice*

## Introduction

As reported in an earlier paper (Rice, 1967), the development of *Phascolosoma agassizii* Keferstein is indirect with a pelagic, lecithotrophic trochophore followed by a prolonged pelagic, planktotrophic pelagosphaera stage.<sup>1</sup> The trochophore is characterized by an apical tuft, a prominent equatorial band of ciliated prototroch cells, mesodermal bands on either side of a closed gut and a stomodaeum closed to the exterior by an overlying egg envelope. Metamorphosis of the trochophore results in a second larval form, designated as a pelagosphaera, in which the metatrochal ciliary band has replaced the prototroch as the chief locomotory organ, the mouth and anus have opened to complete the gut, the coelom has expanded into a spacious cavity and a terminal attachment organ has formed. In this paper observations are reported on the morphology and behavior of the pelagosphaera larva of *Phascolosoma agassizii*, bred from known adults and reared in the laboratory. The histology and de-

velopment of the major organs and tissues of the larva are described, and intra- and interphyletic comparisons are discussed.

Sipunculan larvae were recognized as early as 1850 when Max Mueller, working at Trieste, described and illustrated a larva which he presumed to belong to the genus *Phascolosoma*. A year later, in 1851, A. Krohn criticized this work, asserting that the larva depicted was not that of *Phascolosoma*, but rather belonged to *Sipunculus nudus*. In 1883, Hatschek published a treatise on the development of *Sipunculus nudus*, describing in detail early development as well as the morphology and metamorphosis of the larval stages. His material was collected in the plankton at Messina, Sicily. Interest in larvae from oceanic plankton was elicited by Mingazinni's (1905) description of a form which he erroneously identified as an adult, creating a new genus and species, *Pelagosphaera aloysii*. The description was made from only one preserved and contracted specimen which had been collected in the Pacific between New Zealand and New Caledonia at a depth of approximately 500 meters by the planktonic expedition of the Italian Naval ship, *Liguria*. Working with material from the same expedition, but from waters off India and Ceylon, Senna, in 1906, described similar forms. He concluded that they were larval forms and thus corrected Mingazinni's interpretation. Spengel (1907), in an independent investigation, also exposed

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<sup>1</sup>A pelagosphaera has been defined as a sipunculan larva, resulting from the metamorphosis of a trochophore, which swims by means of a prominent ciliated metatroch and in which the prototroch either has been lost or has undergone a marked reduction (Rice, 1967:164).

Mingazinni's error. Comparing the larvae with those of *Sipunculus nudus* in the Bay of Naples, he suggested that the structures which Mingazinni claimed to be gonads might be instead glandular appendages of the esophagus. Thus Mingazinni's mistake was soon realized, but the name that he created, pelagosphera, remains well entrenched in the literature and is today used to designate the larval form of sipunculans which succeeds the stage of trochophore (Rice, 1967).

Other reports of planktonic larvae have been made by Heath (1910), Dawydoff (1930), Stephen (1941), Fisher (1947), Åkesson (1961a), Damas (1962), Jägersten (1963), Murina (1965), Scheltema and Hall (1965), and Hall and Scheltema (1966). Only two of these authors have attempted to assign adult specific affinities to the planktonic larvae. Fisher (1947) identified a larva collected off Cape Hatteras as *Sipunculus polymyotus* on the basis of the number of longitudinal muscle bands. Murina (1965) designated larvae from the Gulf of Aden as *Sipunculus aequabiliblis* and from the Northwest Pacific as *Sipunculus norvegicus*. Most of the earlier reports were based on preserved and contracted specimens and it is only in some of the more recent studies on living planktonic larvae (Jägersten, 1963; Murina, 1965; Hall and Scheltema, 1966) that larval morphology, particularly of the head region, has been correctly interpreted.

Four developmental patterns have been recognized within the phylum Sipuncula, only one of which includes the long-lived planktotrophic pelagosphera larvae of oceanic waters (Rice, 1967). The four categories of development are defined as follows: (1) direct development with no pelagic stages, (2) indirect development with one pelagic larval stage, the lecithotrophic trochophore, (3) indirect development with two pelagic larval stages, a lecithotrophic trochophore and a short-lived lecithotrophic pelagosphera, (4) indirect development with a lecithotrophic trochophore and a long-lived planktotrophic pelagosphera larva. Included in this last category are the development of *Phascolosoma agassizii*, *Sipunculus nudus*, and unidentified species which have oceanic planktonic larvae.

Previous studies of sipunculan development which include significant accounts of histogenesis have been reported for 6 species, representing all 4 developmental categories. The species are *Golfingia minuta*, category 1, with direct development (Åkes-

son, 1958), *Phascolopsis gouldi* and *Phascolion strombi* in category 2 (Gerould, 1907; Åkesson, 1958), and *Golfingia elongata* and *G. vulgaris* in category 3 (Åkesson, 1961b; Gerould, 1907). In category 4 histogenesis has been described for only one species, *Sipunculus nudus*; developmental features of this planktotrophic species have been found to differ markedly in several respects from the species in the first three categories with lecithotrophic development. The present account of histogenesis of the pelagosphera larva of *Phascolosoma agassizii* provides additional information on planktotrophic development in sipunculans and serves to broaden the basis for comparative evaluation of developmental features within the phylum and for interpretation of affinities with other groups.

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#### Materials and Methods

Adult specimens of *Phascolosoma agassizii* were collected from the San Juan Archipelago on the northwestern coast of the state of Washington and from Vancouver Island, British Columbia. They were found in intertidal habitats either under rocks or in a coarse gravel mixed with sand. Collections were made just previous to or during the breeding season which lasts from the middle of June to late August or early September (Rice, 1967). Animals were maintained at a temperature of 10° to 12° C in glass dishes in which the sea water was changed once or twice daily. When spawning occurred, the fertilized eggs were removed and placed in tall



covered glass petri dishes in which the embryos and larvae were reared. Dishes with developmental stages were maintained at 10° to 12° C on the sea water table or in a refrigerated incubator; water was changed daily for early embryonic stages, but less frequently for later larval stages. Food for the larvae was supplied by additions of unfiltered sea water, supplemented by mixed diatoms and dinoflagellates collected from tidal pools.

For histological studies, unless noted otherwise in the text, larvae were fixed in ice-cold 2 percent osmium tetroxide, buffered with 0.2 M S-collidine at a pH of 7.5 (Bennett and Luft, 1959) and embedded in Epon according to the method of Luft (1961). One or two micron sections were cut with glass knives on a Porter-Blum ultramicrotome, affixed to glass slides and stained with Richardson's stain (equal parts of 1 percent azure II and 1 percent methylene blue made up in 1 percent sodium borate) for examination with the light microscope (Richardson et al., 1960). Additional stains for specific structures are mentioned in the text. Before fixation the larvae were relaxed in a mixture of saturated aqueous chloretone and sea water (1:2) (Clement and Cather, 1957).

### Review of Early Development

The egg of *Phascolosoma agassizii*, ovoid in shape, but somewhat flattened sagittally, is relatively low in yolk content and undergoes spiral, unequal cleavage to give rise to a freely swimming blastula with modified blastocoel (Rice, 1967). Gastrulation occurs chiefly by epiboly, although invagination plays a minor role, forming a narrow archenteron (Plate 1a,b). Mesodermal bands, derived from two large mesodermal teloblasts, are situated on either side of the endoderm cells which are distinguished by their small green cytoplasmic granules. At the site of the closed blastopore, the stomodaeum is formed by an invagination of rapidly dividing ectodermal stomatoblasts. From the rosette cells in the center of the apical plate, the cilia of the apical tuft extend through the pores of the overlying egg envelope and the embryo, at its equator, is encircled by two equatorial rows of ciliated prototroch cells (Plate 1a-d). A remnant of the blastocoel persists on the inner side of the prototroch cells as the "prototrochal cavity" into which

yolk granules from the prototroch are discharged during early development (Plates 1c,d, 2a-d).

The trochophore stage extends from 2½ to 9 or 10 days of age (Plates 1c,d, 2a,b). In the early trochophore the essential size and shape of the egg envelope are retained, but at approximately 5 days of age gradual changes begin to take place. This period of gradual change in the trochophore, extending over 4 or 5 days and leading up to the more rapid changes of trochophoral metamorphosis, is referred to here as premetamorphosis (Plates 2a-d, 3a). During premetamorphosis alterations occur in size and shape of the trochophore, a narrow coelomic cavity is formed by the splitting of mesodermal bands, and the differentiation of larval organs such as gut and retractor muscles is initiated. Metamorphosis of the trochophore into the pelagosphera larva occurs at the age of 9 or 10 days and lasts for a period of 12 hours or less at 10° C (8 days at 12° C). It is marked by a rapid increase in length (30 percent), expansion of the coelom, opening of mouth and anus, reduction of the prototroch, formation of the metatroch, and emergence of the terminal organ (Plate 3a-c).

In the following report on morphology, behavior, and histogenesis of the pelagosphera larva of *Phascolosoma agassizii*, descriptions, unless otherwise indicated, will refer to the larva as it appears at 20 days of age. Larvae have been maintained in the laboratory up to an age of 7 months, with relatively few observed changes other than an increase in size, a change in relative proportions, and a modification of cuticular patterns. Duration of larval life under natural conditions is unknown. Transformation to the juvenile worm has not been observed and all efforts to collect larvae from the plankton for comparative studies have been unsuccessful.

### Morphology and Behavior of the Larva

The body of the pelagosphera larva of *Phascolosoma agassizii* is marked by four distinct regions: head, thorax, elongated trunk, and terminal organ (Plates 3c, 4a-f, 5). The average length of the larval body at 20 days of age is between 400 and 500 microns; by stretching or contracting, a given larva may increase or decrease this length as much as 50 percent. The average maximum width is approximately 100 microns.

The head of the larva is covered by cilia on the ventral surface and divided into two ventral lobes by a median ciliated groove which leads into the mouth. The groove begins as a wide channel at the tip of the head and becomes narrower as it approaches the mouth. Below the mouth is a ciliated lobe (Plates 4*c,d*, 5, 8*b*) which will be referred to as the "lip" in conformity with Jägersten's (1963) terminology for pelagosphaera larvae. The lip usually projects out at right angles from the ventral surface of the head, but when the head is withdrawn into the trunk, the lip is pulled in parallel to the head. When the larva is feeding on the substratum this lip may be pushed back so that it is flattened out against the substratum in the same plane as the ventral surface of the head. The dorsal surface of the head lacks ciliation with the exception of the prototrochal cilia which are now reduced in size and number. Located in a dorsolateral position about half of the distance down the length of the head are a pair of eyespots, hemispherical in shape and of a deep orange pigmentation (Plates 4*b*, 11*b,d*). The entire head may be withdrawn into the trunk when the larva is inactive or when it is startled by some stimulus such as a movement of the surrounding medium.

Not only may the head be withdrawn into the trunk, but also the region immediately posterior to the head, known as the "thorax," may be withdrawn when introversion is complete. In the terminology of Jägersten (1963) the head plus the thorax make up the introvert. Hence the thorax may be defined as that part of the larval body adjoining the head which is retractable into the posterior portion of the larva. It includes the metatroch and is characterized by a thinner cuticle than that of the trunk.

The trunk includes that portion of the larval body posterior to the thorax with the exception of the terminal organ which is considered to be a distinct region of the body. When extended the trunk is wedge-shaped, being broadest anteriorly and becoming increasingly narrow toward the posterior end. It is at the same time curved so that the dorsal surface is slightly convex and the ventral surface concave (Plates 3*c*, 4*a,c,f*, 5). The anus is clearly visible on the dorsal surface of the trunk, usually about halfway along its length, but its relative position varies according to the degree of body extension (Plates 4*a,c*, 5). Other openings, not so

easily observed, occur in the proximity of the anal opening. Slightly anterior to the anus in ventrolateral positions are the two nephridiopores. Dorsolaterally, on either side of the anus, are the openings to the pair of large sacciform glands (Plates 4*b*, 14*a-c*). Three pairs of dorsolateral papillae, each papilla bearing a single bristle, appear between 10 and 20 days. The most anterior pair is in the midtrunk region at the level of the stomach and the other two pairs are located in the posterior third of the trunk (Plate 14*d*). When the larva has reached the age of 28 days the cuticle of the trunk is covered over its entire surface with numerous small papillae (Plates 4*c,e*, 5, 7*b-d*, 10*d*, 13*d*). These cuticular papillae must not be confused with the epidermal papillae described above. The cuticular papillae are responsible, in Jägersten's (1963) terms, for the "rough" appearance of the body surface. On the basis of the appearance of body surface, Jägersten divided the pelagosphaera which he examined into two species: "rough" and "smooth."

From the posterior end of the trunk there extends a narrow appendage, usually 50 microns in length, but extensible to a length of 170 microns. This appendage is known as the terminal organ (Plates 3*c*, 4*a,c,e,f*, 14*e,f*). Such an organ has been described by Hatschek (1883) in the larva of *Sipunculus nudus* and has been noted by Jägersten (1963) in some unidentified pelagosphaera. The terminal organ can be retracted into the posterior end of the trunk and this is the usual position when the larva is swimming or when it is quiescent. The organ manifests adhesive properties and its primary function seems to be the attachment of the larva to the substratum.

The larva of *Phascolosoma agassizii* begins to feed as soon as it completes metamorphosis. At this stage it is chiefly a bottom feeder, although it is also able to feed on small planktonic organisms. Most commonly the larva attaches by the terminal organ to some debris on the bottom of the container and from this point of attachment it may stretch out great distances, either parallel to the bottom or upward at any angle. One frequent pattern of feeding is for the larva to stretch out along the bottom and then, with mouth applied to the substratum, to pull the head backward toward the point of attachment; the body is arched as the head progresses toward the terminal organ and the

mouth takes in diatoms and various sorts of debris as it moves along the bottom. The larva may also assume an arched position as it sweeps around the attached terminal organ in a complete circle. The ciliated ventral surface of the head is flattened upon the bottom, the lip bent backward, while the larva grazes over the substratum and clears the debris from the area surrounding it. From time to time the larva may release itself from its posterior attachment and swim with the ventral surface of the head applied to the substratum and the terminal organ pointing upward. In this manner it can cover more territory than would be possible in a fixed position, or this may be only a means of finding a new location for settling. The larva may also move about by swimming along the bottom in an arched position with the terminal organ dragging along behind, unattached; or it may crawl along in the manner of an inch worm. At other times it may swim freely through the water, propelled by the activity of the metatrochal cilia, or it may remain quiescent on the bottom, unattached with the introvert retracted. The larva appears to feed for the most part when it is attached with the introvert extended. Food intake is apparently accomplished by ciliary activity on the ventral surface of the head and by the occasional eversion of the buccal organ. Possible functions of the buccal organ will be discussed in more detail in the treatment of the histogenesis of this organ (pp. 11-13). Occasionally the larva bends both the head and the posterior end in a ventral direction so that the lip and terminal organ touch, or else the terminal organ is pushed past the lip to one side of the head. Whatever may be accomplished by this activity is not obvious; in fact, the activity may be abnormal since it is most likely to occur when there is no substratum on which the larva may settle. It is possible also that a secretion is transferred from the posterior sacciform glands or from the terminal organ to the lip, or from the lip to the terminal organ. Jägersten (1963) reported that the terminal organ was pushed into the mouth, but in *Phascolosoma agassizii* this was not observed.

The metatrochal cilia function mainly in locomotion. When the larva is attached, these cilia aid in the movement of the anterior end around the point of attachment, but they do not beat continuously. When the introvert is retracted the metatrochal cilia usually cease to beat.

### Larval Histogenesis

The description of histogenesis in each organ or structure in the larva of *Phascolosoma agassizii* will be preceded by an account of its histology and followed by a comparative review of the information available on the histology and development of the structure in other sipunculans. The larval structures to be described are the following: body wall, coelom and coelomic elements, retractor muscles, digestive tract, buccal organ, lip gland, nervous system, nephridia, larval glands, and terminal organ. In addition to the descriptive considerations, functional significance of many of the organs will be discussed.

#### BODY WALL

*Cuticle.*—The cuticle of the larva is distinctive in each region of the body; hence the four regions will be discussed separately.

In the head there are two distinct types of cuticle: the dorsal cuticle differs from the ventral and has a separate origin from it (Plates 3*b,c*, 11*d*). The dorsal cuticle of the head is very thin and no structure can be resolved within it. It is derived from the inner layer of the vitelline envelope, the outer layers being sloughed off soon after metamorphosis. The dorsal cuticle stains very lightly with Richardson's stain as does the ventral cuticle and both react positively to the periodic acid-Schiff stain. The ventral cuticle, penetrated by numerous cilia, is slightly thicker than the dorsal and is divided into two layers by a darkly staining central striation. It originates from the lining of the stomodaeum and bears no affinity to the original vitelline envelope.

The cuticle of the thorax is structurally the same as that of the trunk, but it is much thinner (Plate 5). There is no sharp demarcation between the two, but rather a gradual transition. This cuticle, composed of two layers, is derived from the vitelline envelope (Plate 13*b*). The inner layer, wider and more lightly staining, represents the combined inner and middle layers of the vitelline envelope and the outer layer of the cuticle is derived from the outer layer of the envelope. The inner layer gives a faint positive reaction to the periodic acid-Schiff stain and the outer layer is characterized by metachromasia.

Between 3 and 4 weeks of age small papillae form

in the cuticle of the trunk (Plates 4*c,e*, 5, 7*b-d*, 13*d*, 14*e*). These are filled at their base by the substance of the inner layer, but within the apex, staining bright blue with Richardson's stain, a new substance makes its appearance. The papillae are covered externally by the outer cuticular layer.

The cuticle of the terminal organ is identical to that of the trunk and continuous with it. It differs only in that it is much thinner.

The fate of the egg envelope and the formation of the cuticle are not uniform in all species of sipunculans. Gerould (1907) observed the shedding of the egg envelope in *Phascolopsis gouldi* and *Golfingia vulgaris* at the time of metamorphosis. The envelope ruptured at the postoral cirrlet of cilia and the entire posterior portion was immediately lost. Anteriorly the envelope remained attached longer but was eventually sloughed off by the repeated introversions of the head. A fine granular cuticle beneath the egg envelope became the larval cuticle after the shedding of the envelope. Selenka (1875) described a transformation of the egg envelope into the larval cuticle in *Golfingia elongata*. In the light of his own observations Gerould doubted the validity of Selenka's description, but Akesson in 1961(b) confirmed Selenka's original report. Akesson (1958) also had reported that the egg envelope in *Phascolion strombi* and *Golfingia minuta* was distended to form the larval cuticle. Beneath the larval cuticle he observed a second narrow cuticle, presumably the definitive cuticle of the juvenile form. The egg envelope, although distended, did not become elastic and when the larva contracted the envelope bulged out from the body. Bulges in the larval cuticle of *Phascolosoma agassizii* have been seen only in abnormal larvae or in larvae that have been subjected to relaxants and fixatives. The egg envelope in *P. agassizii* is transformed into an elastic larval cuticle and no second cuticle has been observed.

In *Sipunculus nudus* the egg envelope is cast off at the initial metamorphosis along with the remnants of the prototroch cells (Hatschek, 1883). In this species the relationship of the egg envelope to the embryo is quite different from that in other sipunculans (Gerould, 1903). The trochoblasts spread around the inside of the egg envelope forming a complete envelope or "serosa" surrounding the developing embryo. The cells degenerate and

are shed with the egg envelope at the beginning of the pelagic larval stage.

*Epidermis.*—In the larva the epidermal cells are marked by their vacuolar cytoplasm and large nuclei with homogeneous, darkly staining nucleoplasm and prominent nucleoli (Plates 3*c*, 5, 7*c*). Very fine cytoplasmic extensions of the epidermal cells penetrate into the cuticle.

The epidermal cells of the trunk are derived largely from the descendants of the second cell. In the early stages these cells are filled with yolk, but by the end of the trochophore stage much of the yolk has disappeared. After the formation of the coelom, large intercellular spaces appear in the epidermis between the points of the epidermal attachment to the peritoneum (Plate 3*a*). As the cells expand into these spaces, their cytoplasm becomes intensively vacuolated (Plate 3*b*). At the metamorphosis of the trochophore to the pelagosphaera the epidermis is stretched out into a much thinner layer (Plate 3*c*).

The epidermal glands will be considered in a later section on larval glands (pp. 18–19).

*Muscles of the Body Wall.*—The muscles of the body wall are small and inconspicuous and are not easily identified in any stage that has been studied (Plate 7*b,c*). In the larva the longitudinal muscles appear in sagittal section as long, very fine, darkly staining fibers on the outer side of the peritoneum and the circular muscles are represented by small darkly staining dots, apparently attached to the outer side of the longitudinal muscles. The adult arrangement of longitudinal muscles into bundles of fibers is not discernible in the larva.

Immediately posterior to the metatroch there is a broad circular sphincter which increases in breadth with age (Plate 7*b*). In a larva of 2 months this sphincter may reach from the metatroch to the level of the stomach. At fixation it often contracts, pushing the stomach into an abnormal anterior position. The normal function of the sphincter is to close the anterior opening of the body after the head has been retracted. In sagittal section the sphincter is seen as a number of relatively large spherical fibers well separated from each other and located to the inside of the epidermal cells.

Nothing can be said concerning the origin of the muscles of the body wall. In prelarval development small nuclei, resembling those of muscle, are seen scattered among the epidermal cells, but it has

not been possible to demonstrate whether these give rise to the circular and longitudinal muscles.

There is little agreement among other authors as to the derivation of the muscles of the body wall. Gerould (1907) reported the origin of the circular muscles in *Golfingia vulgaris* and *Phascolopsis gouldi* to be ectomesodermal, but he does not mention the source of the longitudinal muscles. Hatschek (1883), on the other hand, believed that all of the body wall musculature in *Sipunculus nudus* was derived from endomesoderm. In his study of *Golfingia minuta* and *Phascolion strombi* Åkesson (1958) found that the circular muscles were formed from ectomesoderm and the longitudinal muscles from endomesoderm.

*Peritoneum.*—The peritoneum lines the coelom of the larva, covering the inner side of the body wall and continuing around the esophagus and rectum to surround the gut. The cytoplasm of each peritoneal cell extends out from the nucleus as a thin membrane which joins that of neighboring cells to form the peritoneal lining. The nuclei of the peritoneum are distinguished from those of the epidermis by their pronounced chromatin granules and their lightly staining nucleoplasm.

The peritoneum is derived from the lateral bands of mesoderm which in turn arise from the early mesodermal teloblasts (Plate 1*b*). The lateral bands spread around the embryo and split into inner and outer layers, splanchnic and somatic, respectively, to form the coelom (Plates 2*b-d*, 7*a*). The splanchnic mesoderm surrounds the gut and the somatic forms the inner lining of the body wall. At first the nuclei of the peritoneum are very close together (Plate 3*a*), but with the elongation of the body they are pulled apart and the cytoplasm is stretched out into a thin mesothelium (Plate 3*c*).

The ciliated peritoneal cells of the adult have not been identified in the larva.

#### COELOM AND COELOMIC ELEMENTS

The expansive coelom of the larva extends without interruption from the most anterior part of the head into the terminal organ and it is open to the exterior only by way of the nephridial organs. The coelom is formed between 6 and 7 days by a splitting of the splanchnic and somatic layers of mesoderm.

Within the coelom there are freely suspended

coelomic cells which are moved about by the nearly continuous contraction and expansion of the larval body. There are two types of coelomocytes (Plates 7*a-d*, 14*c*). One is spindle-shaped with occasional variations in form that suggest amoeboid activity. The cytoplasm of this cell type stains darkly and in older larval stages it is packed with basophilic granules. The second cell type is round and flat and the cytoplasm in younger larvae stains lightly with relatively few inclusions. At two months the cytoplasm of the second cell type is characterized by clusters of granules and vacuoles and the formerly central nucleus is in an eccentric position (Plate 7*c*).

In the premetamorphic larva, cells resembling the second cell type appear to be budding off from the splanchnic peritoneum into the newly formed coelom (Plate 7*a*). These coelomocytes increase in number with age, but there is no evidence for their later derivation or for the derivation of the spindle-shaped cells. The round, flat cells of the larva are similar to and may be identical with the red cells of the adult, whereas the spindle cells may represent the small amoebocyte of the adult. There are 3 types of adult coelomocytes, however, that have not been recognized in the larva. They are the large amoebocytes with large granules, the vesicles, and the urns.

The coelomocytes described by Gerould (1907, pl. 9: fig. 80*b, c*) in *Golfingia vulgaris* and *Phascolopsis gouldi* are very similar to the round flat cells of *Phascolosoma agassizii*. Although he presented no evidence for their origin, Gerould suggested that they were probably derived from the peritoneum in the posterior coelom. In addition to these coelomocytes, Gerould also found within the coelom 19 cells with large nuclei and scant cytoplasm, which he believed to be the remains of the discarded prototroch cells.

#### RETRACTOR MUSCLES

Spanning the coelom lengthwise are four retractor muscles, two dorsal and two ventral, which function to withdraw the head into the trunk. In the early larva up until the age of one month each muscle is composed of eight fibers and each fiber is a single cell with only one nucleus. A cross-section of the muscle reveals a circular arrangement of fibers joined together by a thin peritoneal mesen-

tery enclosing a central cavity (Plates 12a, 13b).

The fibers of each of the dorsal retractors begin in a dorsolateral position near the base of the brain, attached in a straight row to the peritoneal lining (Plate 13a). The most median fibers arise more anteriorly than the outermost which arise slightly posterior to the level of the mouth. At the level of the ventral lip the fibers and their surrounding peritoneum pinch off from the peritoneal lining forming a circle with a central space (Plate 13b). The muscle then extends posteriorly through the coelom to the first posterior bristle where it again attaches to the peritoneal lining of the dorsal body wall, fanning out into a row of fibers. Just below the bristle the two outermost fibers insert into the body wall (Plate 14d). Two more fibers are inserted at the level of the posterior glands and the last four fibers in the region of the anus.

The fibers of the ventral retractors arise from the body wall on either side of the mouth near the emergence of the circumesophageal connectives from the brain (Plate 13a). The four most anterior fibers lie between the epidermis and the connectives and it is only posterior to the connectives that the fibers come into contact with the peritoneum. The four anterior fibers are joined by four additional fibers in the region of the buccal organ and the eight fibers plus the interconnecting peritoneum separate from the peritoneal lining, forming a circular muscle similar to that of the dorsal retractor (Plate 13b). The ventral retractors pass posteriorly between the buccal organ and the lip glands to the level of the first bristle where each of the ventral retractors gives off two fibers, one close to the ventral nerve cord and another more laterally. The circle of fibers then flattens out and becomes continuous with the ventral peritoneal lining. At the level of the nephridia two more fibers are inserted (Plate 13c) and the four remaining fibers in each of the ventral retractors insert far posteriorly at the level of the second bristle, thus exceeding by far the dorsal retractors in length.

The nucleus of each muscle fiber is located anteriorly along the length of the fiber and all of the nuclei of a muscle are relatively close together (Plate 6d). In both dorsal and ventral retractors the nuclei are found at the level of the lower esophagus or upper stomach, although the ventral nuclei may extend slightly farther posteriorly.

The retractor muscles of *Phascolosoma agassizii* appear to be derived from ectoderm, but it has not been possible in this study to identify with certainty the rudiments in very early stages. At the time of coelom formation groups of cells with nuclei which resemble those of the later muscles are found bulging into the coelom in the position of the future ventral retractors (Plate 6a, b). These cells are located at the anterior end of the newly forming posttrochal coelom and appear to be connected by cytoplasmic extensions to the ectodermal cells of the stomodaeum and the ventrolateral apical plate, just above the prototroch. In the position of the future dorsal retractors the early coelom is narrow and a cluster of cells corresponding to that in the ventral region has not been identified; however, a few cells of similar cytology have been recognized just anterior to the coelom. Within a period of 24 hours after the ventral cell clusters first are seen, both ventral and dorsal retractors make their appearance as long fibers with central nuclei which extend from the lateral ectoderm of the apical plate through the coelom to the posterior somatic mesoderm (Plate 6c). The ventral retractors with their very thick fibers are much larger when first formed than the dorsal retractors with their relatively thin and tenuous fibers. Both ventral and dorsal retractors are intimately associated with peritoneal cells, the nuclei of which, although usually smaller and rounder, are not always easily distinguished from those of the muscle.

The above observations are interpreted to signify that the retractor muscles originate from four areas of ectomesoderm located in dorsolateral and ventrolateral positions in the apical plate. These rudiments grow downward, retaining their cytoplasmic connections in the head, and push into the coelom taking with them an investing layer of peritoneum. Long cytoplasmic processes or fibers are extended through the coelom and attach posteriorly to the outer layer of coelomic mesoderm.

If the observations of this study are complete and if the interpretations are correct, then the formation of retractor muscles in *Phascolosoma agassizii* differs from that observed in studies of other sipunculans. Gerould (1907) in his study of *Golfingia vulgaris* and *Phascolopsis gouldi* stated that the muscles were apparent in the early trochophore prior to the division of the coelomomesoblast, extending from the sides of the apical plate, from

which they were derived, posteriorly to the region of the postoral circler. He suggested that the muscles passed through the loose layer of mesoderm at the time of metamorphosis.

Hatschek (1883) proposed that in *Sipunculus nudus* the retractors were derived from somatic mesoderm. He was unable, however, to trace their early development and recognized them only after they were already formed within the coelom.

Confirming Gerould's observations, Akesson (1958, 1961a) found in three species of sipunculans, *Phascolion strombi*, *Golfingia minuta*, and *Golfingia elongata*, that the retractor muscles were derived from ectomesoderm and that they were formed outside of the mesoderm in very early stages before the formation of the coelom. In *Phascolion strombi* and *Golfingia minuta* the development of the muscles was reported in detail and it was observed that each retractor had two rudiments, an anterior and a posterior, which grew toward each other and fused to form the completed muscle. This manner of development could not occur in *Phascolosoma agassizii* where each fiber of the larval muscle is a single cell which extends the entire length of the muscle.

The reported incongruities demand further studies on *Phascolosoma agassizii* and a reinvestigation of *Sipunculus nudus* before conclusions can be drawn as to the mode of development of the retractor muscles of sipunculans and the specific variations.

The larval retractor muscles are retained as the retractor muscles of the adult sipunculan. In *Phascolosoma agassizii* it has been demonstrated that the muscle fibers increase in number with the age of the larva. In the larva of 28 days each muscle is composed of only eight fibers arranged as a tube with a central cavity, whereas at 2 months the number of fibers has greatly increased, completely filling the central cavity (Plate 13d).

#### DIGESTIVE TRACT

The gut of the larva is regionalized into four distinct morphological and cytological portions: the long, broad esophagus with posterior sphincter, the expanded and bulbous stomach, the thin, looped intestine, and the short rectum.

*Esophagus.*—The epithelial lining of the esophagus is composed of cuboidal cells with dense, long

cilia which form a central swirl in the lumen (Plates 7b, 13d). The epithelium is lined with an inner cuticle which is continuous with that of the ventral head and stains brightly with periodic acid-Schiff. The nuclei are relatively large and the cytoplasm appears to be homogeneous with small lipid droplets scattered through it. On the outer side of the epithelium is a thin layer of muscle fibers covered by peritoneum. The muscle layer increases in thickness at the junction with the stomach, forming a tight sphincter (Plate 7b). When the head is retracted into the body the esophagus is pushed into a lateral position.

The esophageal lining is formed from the stomodaeal invagination and is therefore ectodermal in derivation. The invagination begins at approximately 60 hours and by the end of the trochophore stage it has elongated in an anterodorsal direction and the cells have developed cilia (Plate 1d). As the stomodaeal invagination sinks inward the anterior end of the stomach moves into a more dorsal position. At the beginning of the premetamorphosis stage (6½ days) the invagination opens into the cavity of the stomach and, as elongation proceeds, the stomach is moved into a position directly posterior to the esophagus (Plate 2b).

*Stomach.*—The stomach is very large and bulbous in shape and its maximum width is nearly equal to that of the coelom (Plates 3b, 5). Its diameter may exceed that of the mid-esophagus two or three times and that of the esophageal sphincter over four times. The lining of the stomach is constructed of a single layer of cuboidal epithelium enclosing an expansive central cavity and, in contrast to the esophagus and intestine, there is no obvious intervening muscle between the epithelium and the surrounding peritoneum (Plates 7b-d, 10c, 12b). The apical border of the epithelial cells is striated as well as weakly ciliated, the cilia being much less concentrated and shorter than those of the esophagus (Plates 7b, 12b, c). Within the cytoplasm of the epithelial cells there are numerous lipid droplets of varying sizes, all larger than those of the esophagus. In larvae older than one month, the lipid droplets coalesce to form one huge sphere of lipid in each cell (Plate 7c, d). The remainder of the cytoplasm is highly vacuolated, giving the appearance of a reticulum. The epithelial nuclei have a large eccentric nucleolus and the nucleoplasm, with prominent chromatin granules, stains

more lightly than that of the esophageal cells. Although the cuboidal epithelial cell is by far the most common, there is a second cell type that is rather sparsely scattered throughout the epithelium and is most readily observed in the older stages. This is a smaller cell, pyramidal in shape and with homogeneously staining basophilic cytoplasm.

The larval stomach is derived from endoderm and is the first portion of the gut to be differentiated. It can be distinguished at two and one-half days when characteristically small, green cytoplasmic granules appear in the endoderm and a cavity, the future lumen of the stomach, appears in the midst of the endoderm cells. The cavity elongates as the cells of the posterior portion of the stomach undergo division. In the premetamorphosis stage the cells of the posterior stomach give rise to the rudiment of the intestine which makes its appearance as a small round knob at the end of the stomach (Plate 2*b*).

In the early trochophore the green granules increase slightly in size and, along with the relatively densely concentrated yolk granules, mark the cells of the stomach as distinct from all others (Plate 7*a*). In the late trochophore some of the yolk granules coalesce and, when the coelom is formed, these large clusters of yolk are cast off into it. During premetamorphosis the remainder of the yolk disappears, its fate undetermined. The green granules now vary in size; some are quite large and are recognizable as lipid droplets. After metamorphosis the small granules disappear and the lipid droplets increase in size and decrease in number until there is in each cell only one very large sphere (Plate 7*c, d*). From this evidence it is assumed that the green granules described in the early stages were in actuality small lipid inclusions. Whether these lipid inclusions are responsible for the green coloration in the living larva or whether there is some other constituent that has not been defined cytologically is not known. The two are, however, linked together by circumstantial evidence in that they both appear at approximately the same stage of development. The esophagus, although not colored in the living larva, does contain some lipid, but it is not present in the same concentration as in the stomach and intestine, both of which are marked by green coloration.

At the time of metamorphosis the cavity of the stomach increases in size and the bulbous shape is

assumed. In older larvae (2 months or more) the stomach enlarges and elongates to form a long cylinder which may extend more than half the length of the body. The fate of the larval stomach is unknown. Although it occupies a place of great prominence in the larva, it has no morphological counterpart in the adult. Since only those sipunculan larvae with a prolonged pelagic life, such as *Sipunculus nudus* (Hatschek, 1883) and other unidentified pelagosphera (Åkesson, 1961*a*; Damas, 1962), possess a well-defined stomach, it is likely that the stomach is of functional significance to a pelagic mode of existence.

*Intestine.*—The stomach narrows abruptly into the thin intestine which in the larva up to age of 60 days is looped but not coiled. There is a descending portion which extends anteriorly toward the dorsal anus. As it leaves the stomach the intestinal lining is composed of a relatively thick, ciliated cuboidal epithelium with a densely basophilic cytoplasm. Within a short distance the wall becomes exceedingly thin, the cells are flattened and the cytoplasm vacuolated (Plates 12*d, 13c*). The intestinal epithelium is ciliated and there are small lipid granules scattered throughout its length. The wall of the intestine is completed by a thin muscle layer and a peritoneal covering.

The intestine originates from endoderm which grows out as an extension from the posterior end of the stomach in the late trochophore stage (Plate 2*b*). Growing dorsally it attaches to the dorso-posterior ectoderm in the position where the future anus will form (Plate 10*b*). At metamorphosis there is an elongation of the body posterior to this point of attachment, extending the coelom far below the anal region and as the intestine continues to grow it loops downward into the posterior coelom. From the time of its formation there are lipid droplets dispersed throughout the cytoplasm of the intestine, but they never become as large or as concentrated as those of the stomach.

*Rectum.*—The larval rectum is a short tube surrounded by a thin layer of muscle and an outer covering of peritoneum (Plates 5, 10*c*). The epithelium of the rectum is very much thicker than that of the preceding intestine and the cells are heavily ciliated. The cytoplasm is basophilic with no visible inclusions other than yolk granules in the early stages, and the inner border of the cells stains strongly with periodic acid-Schiff, as does



that of the esophageal cells. The rectum continues into the anus and at the anal opening the larval cuticle and its associated epidermis turn inward to meet the rectal epithelium (Plates 5, 10*b*, *c*). Surrounding the anus there is a thick sphincter muscle.

The rectum is formed during premetamorphosis from a dorsoposterior ectodermal proliferation. The rudiment of the rectum is composed of only a few cells which are soon joined by the growing intestine (Plate 10*b*). A ciliated cavity forms among the ectodermal cells and before metamorphosis this opens into the intestinal lumen. A slight depression appears in the cuticle opposite the rectal rudiment and at this point the cuticle ruptures during metamorphosis, forming the anus and thus opening the rectum to the exterior.

In all species of sipunculans that have been studied the esophagus is formed from a stomodaeal invagination, the rectum from a cluster of ectodermal cells referred to as the proctodaeum, and the remainder of the gut from the entomeres of the blastoporal region. In the different species of sipunculans there is a great difference in the time at which a lumen appears within the gut and the time at which the gut is completed. *Golfingia minuta*, with a large egg rich in yolk, remains lecithotrophic for 2 months and *Phascolion strombi* for one month (Akesson, 1958). In *Golfingia vulgaris* and *Phascolopsis gouldi* a lumen appears within the archenteron at 5 or 6 days, but they do not begin to feed until the second or third week of age (Gerould, 1907). The gut of *Sipunculus nudus* is functional at 3 days (Hatschek, 1883) and that of *Phascolosoma agassizii* at 9 or 10 days.

#### MOUTH AND ASSOCIATED STRUCTURES

The term mouth will be used in a broad sense to include the anterior ciliated channel of the ventral head, the ventral, posterior lip, and the buccal groove through which the buccal organ may be protruded.

The ciliated channel divides the ventral surface of the head into two lobes and continues into the esophagus, forming the dorsal and lateral boundaries of the esophageal opening. The ventral boundary of the opening is formed by the union of two nonciliated folds. The channel of the ventral surface of the head is derived from the roof of the stomodaeum at metamorphosis.

Posteriorly the mouth is expanded into the ventral lip (Plates 5, 8*b*), a lobe which usually extends out perpendicularly from the head, but which may be withdrawn along with the head into the trunk or may, when the larva is feeding, be flattened out against the substratum in a 180° angle from the head. The lip is partially bifurcated by a deep ciliated groove which begins near the distal end of the lip and extends to a ciliated pore near the proximal end of the lip. The lip glands, to be described later, open into this pore. Distally the ciliation of the lip is limited to the central groove and a few lateral cilia, whereas that portion of the lip proximal to the pore is evenly ciliated. When the mouth opens at metamorphosis, the floor of the stomodaeum is extended outward to form the surface of the lip (Plates 3*b,c*, 5, 9*d*). The cuticle surrounding the lower portion of the mouth is stretched out with the stomodaeal extension to form the sides of the ventral lip.

*Buccal Organ.*—At the base of the lip is a long, transverse slit, the width of the mouth, which extends downward as a nonciliated infolding for some distance along the ventral side of the esophagus (Plates 8*a*, *b*, 11*c*). The slit, which will be termed the buccal groove is expanded in the center of its posterior extremity and ventrally it may show one or more folds. The entire invagination is lined by a very thick cuticle which is stained by periodic acid-Schiff. On the dorsal side, the cells of the epithelium are very thin and lie in close association with the esophagus along much of its length. On the ventral side of the infolding and extending around under the posterior end is the buccal organ; when the infolding is everted the buccal organ protrudes to the exterior.

The buccal organ hangs down into the coelom of the larva as a large muscular sac within which there are two main cavities: posterior-dorsal and anterior-ventral; the latter may be divided again, making a small third cavity. The organ is circumscribed by a sheet of muscle which attaches ventrally to the proximal portion of the lip and, looping down around the posterior extremity of the buccal groove, attaches dorsally to the muscle layer of the esophagus (Plates 3*c*, 5, 8*a*, *b*, 11*b*, *c*). The epithelium of the organ lines the ventral side of the buccal groove and, with its overlying cuticle, is the first portion to reach the exterior when the organ is protruded. The epithelial cells are very elongate

with thin necks leading to rounded tips which bulge out into the cavity of the organ. The partition that divides the cavity into chambers is formed by an extension of epithelial cells across the cavity, connecting to the outer muscle wall. The cytoplasm of these cells stains lightly and appears to be fibrous (Plate 8*b*).

The formation of the buccal organ is not entirely understood. The outer muscular layer is formed from cells which grow downward from the sides of the anterior esophagus and posterior stomodaeum and join beneath in a solid mass (Plate 9*c,d*). The cells are first recognized at the beginning of the pre-metamorphosis stage and by the end of metamorphosis they have spread out as a single layer to form a muscular sac which hangs down from the esophagus. It has not been possible to discern whether the cells originate from the ectoderm of the apical plate above the esophagus or whether they are derived from the mesoderm along the sides of the esophagus. The buccal groove has not been observed until the day preceding metamorphosis. At this time it extends downward as an invagination from the floor of the stomodaeum, but in contrast to the stomodaeal lining, it has a heavy cuticle and lacks ciliation (Plate 9*d*).

The buccal organ was first described in 1883 by Hatschek in the larva of *Sipunculus nudus*. Hatschek referred to this organ as the "Schlundkopf," a word which can be translated as the upper pharynx, esophagus, or buccal mass. The structure was described as a fold in the esophageal wall which extended posteriorly as a groove, then curved upward with the two layers of the fold tightly apposed to surround a round vesicle (Hatschek, 1883, pl. 5: fig. 57*a*). The lumen of the vesicle was traversed by spindle-shaped cells. The vesicle was reported to arise from mesoderm and the fold from an ectodermal evagination of the esophagus. In *Phascolosoma agassizii* the fold does not continue around the organ, but instead the organ is enclosed by a muscular sac.

In a study of pelagosphera collected by the *Dana* Expedition, Damas (1962:13-14) again described this organ and called it the "machoire." Damas' representation of the organ agrees in its essential aspects with the findings in *P. agassizii*. He described a transverse groove in the posterior mouth with a thick cuticle, covered ventrally by a unique epithelium of tall, cylindrical cells. Beneath the

epithelium was an oblong transverse mass of turgid tissue composed of muscle fibers and within this mass there were numerous cavities. The histological details of the turgid tissue as presented by Damas may not be entirely reliable since his specimens were fixed aboard ship in diluted formalin 30 years prior to his investigation.

Jägersten (1963) observed the organ in living pelagosphera and referred to it as the "pharyngeal bulb." Although he noted that it was in a position at the base of the lip, he gave no information on the structure of the organ.

The term buccal organ has been used in the present study because it does not imply a molluscan homology as does "buccal mass" (Hatschek, 1883), nor does it attribute a speculative function as is implicit in the term "machoire" (Damas, 1962). Jägersten's term, "pharyngeal bulb," is not appropriate, since there is no differentiated pharynx in the larva and, even if there were, this term would connote a muscular expansion in the pharyngeal tube, rather than a saccular evagination.

Investigators have pondered the possible functions of the buccal organ, but thus far no one has provided a conclusive demonstration of its significance in larval life. Hatschek's (1883:39) only comment on the function was "Die Function dieses sogenannten Schlundkopfes ist mir ziemlich räthselhaft geblieben." Although he did not observe the eversion of the organ, Hatschek noted that pressure on an overlying coverslip resulted in the extrusion of both the esophagus and the "Schlundkopf." Damas (1962) did not examine living larvae, but he proposed that the organ might act as a rocker, pushing food accumulated in front of it back into the esophagus, Jägersten (1963) observed in the living pelagosphera a "kneading" action of the buccal organ and he supposed that such an action might serve to break up large pieces of food into smaller pieces; however, he did not give evidence to support this supposition.

In the present study a cinematographic record of a larva of *Phascolosoma agassizii* was made as it moved along a filamentous diatom, continually everting the buccal organ. The diatom was of too great a length to be ingested intact by the larva. The mouth of the larva was applied to the diatom, the body arched, and the terminal organ attached to the substratum. At each eversion of the buccal organ the head of the larva moved forward. After

a series of eversions the diatom strand was bent inward, appearing to be directed into the esophagus. In a final eversion the strand was pushed out of the mouth and the larva abandoned the diatom. From these observations it would appear that the buccal organ is not only able to push food into the esophagus as proposed by Damas (1962), but also it can reject objects, pushing them away from the esophagus. The latter action might be of value if large and extraneous objects adhere to the presumably sticky secretion of the lip gland. The extrusion of the buccal organ, moreover, blocks the esophageal opening and, if living organisms were being ingested, this motion might prevent their escape. Another function, possibly performed by the continual eversion of the buccal organ against a substratum, could be the loosening of small diatoms and debris which would then be swept into the digestive tract by the directed ciliary movement. Although a function in the feeding process seems to be the primary significance of the buccal organ, it may also play some role in locomotion. The fact that the head moves forward when the buccal organ is applied to the substratum suggests this possibility. On the other hand, the head can also move forward by means of ciliary activity alone. A possible sensory function cannot be eliminated even though the heavy cuticle of the organ makes such a function seem improbable. An innervation has not been demonstrated, but the connectives from the brain are very closely associated with the organ at either end of the buccal groove (Plate 12a).

*Lip Gland.*—In the larva of *Phascolosoma agaszizii* the lip gland, composed of four pendulous lobes, hangs down from the inner surface of the lip into the anterior coelom (Plates 3b, c, 5, 8a, b). The two median lobes of the gland are approximately 50 microns in length and are slightly longer than the two lateral lobes. Each lobe, suspended from the lip by a long neck, ends in a bulbous expansion. At their point of attachment to the lip, the necks of the lobes surround the lip pore (Plate 8b). Because of this arrangement it is inferred that the glands discharge a secretion into the pore.

In larvae up to an age of 20 days each lobe of the lip gland is composed of a single large cell, but in later larvae the number of cells increases so that a single lobe may consist of several cells. Each cell is characterized by a large nucleus with an ex-

ceptionally large nucleolus and a nucleoplasm that stains deep blue with Richardson's stain. The cytoplasm is highly vacuolated and penetrated by a fine reticular network (Plate 8b). Large patches of darkly staining material are scattered through the cell, sometimes concentrated at the periphery or around the nucleus, and from these patches thick strands of similar material radiate into the surrounding cytoplasm. In larvae that have been embedded in methacrylate the gland gives a negative reaction to periodic acid-Schiff and alcian blue, but with safranin O small areas within the gland stain metachromatically.

The lip gland is derived from ectoderm. At the beginning of the premetamorphosis stage, four large cells are distinguishable among the cells of the ventral wall of the stomodaeum by their large size, basophilic cytoplasm, and large nuclei each with a single prominent nucleolus (Plate 9a). As the cells increase in size the cytoplasm becomes progressively vacuolated; by the time of metamorphosis the characteristic cytology of the larval gland has been assumed (Plates 3b, c, 9b). At their first appearance the cells are widely scattered and at this time the lip pore cannot be identified. The process by which contact is established between the gland cells and the lip pore is not understood.

The lip glands were first described by Max Mueller (1850) in a larva which he presumed to belong to the genus *Phascolosoma*. The glands were designated as the unpaired organ and described as a bilobed body opening into a ciliated excretory duct beneath the introvert. The significance of the organ was not clear to Mueller, but he suggested that it was either a secretory gland or a gonad. Later, in 1883, Hatschek depicted the gland as the "Anhangsdruse" in the larva of *Sipunculus nudus* and reported it to be an esophageal invagination, the cells at the blind end of which grew toward the coelom to form the glandular masses. The gland was found to be composed of two glandular appendages, each with tall prismatic cells that ended at the lumen of a ciliated excretory duct. The duct opened near the posterior wall of the mouth. Mingazinni (1905) figured the glands in a pelagosphaera larva that had been collected at a depth of 500 meters in the South Pacific between New Caledonia and New Zealand. Unaware of Hatschek's work, he believed the glands to be gonads and therefore considered the animal to be an adult sipunculan, creating a new genus

and species, *Pelagosphaera aloysii*. Senna (1906) in a study of other planktonic sipunculan larvae, recognized the glands to be the esophageal evaginations described by Hatschek. In pelagosphaera larvae from Monterey Bay, California, Heath (1910) found similar structures and identified them as larval glands, rather than gonads. In his examination of 1,900 pelagosphaera from the *Dana* Expedition, Damas (1962) reported a typical bilobed gland suspended in the coelomic cavity from the end of a ciliated canal. The canal opened to the exterior on the ventral surface of the anterior introvert. The cells of the gland were arranged in the shape of a fan around a central cavity, a continuation of the ciliated canal. Åkesson's (1961a) description of the glandular organ of four pelagosphaera collected by the *Galathea* Expedition agrees essentially with that of Damas. The term lip gland was proposed by Jägersten (1963) who was the first to observe that the duct of the gland opened onto a ventral lobe of the mouth which he designated as the lip. Jägersten's observations have been confirmed here in the larva of *Phascolosoma agassizii*; thus his term, lip gland, has been adopted in this report.

The structure of the lip gland of *P. agassizii* differs from that described in the literature in that it is divided into four appendages, all of which converge at a pore on the base of the lip. There is thus no long "excretory duct" as found in other pelagosphaera and there is no central cavity within the gland. Rather than numerous cells around a central cavity, in the early larva of *P. agassizii* there are only four cells in the gland, each lobe consisting of a single cell suspended from the region of the pore.

There is very little information available on the nature of the glandular secretion. Åkesson (1961a) reported a basophilic staining with the Azan stain and Damas (1962) demonstrated a weak reaction with periodic acid-Schiff. In the gland of *Phascolosoma agassizii* there was no reaction with periodic acid-Schiff, but scattered patches of cytoplasm manifested an intense basophilia with Richardson's stain. Areas of the gland were stained a bright orange with safranin O, a reaction characteristic of mucus (Lillie, 1954:285). The results are confusing, however, in that there was no manifestation of metachromasia with Richardson's stain and there was no reaction to alcian blue. Further work is necessary before the nature of the secretory product can

be defined. If it should be mucus, then such a secretion might function in a ciliary-mucus pattern of feeding or in adhering the ventral surface of the head to the substratum as the larva moves along in search of food.

Both the buccal organ and the lip gland are larval organs with no known fate in the adult. Åkesson (1961a) hypothesized that the lip gland is homologous to the ventral sensory organ of the adult in the genera *Phascolosoma*, *Siphonosoma*, and *Aspidosiphon*. The ventral sensory organ has been described as a tubular organ which opens on the ventral midline beneath the collar and terminates at the anterior end of the ventral nerve cord. In some species the termination is expanded into a cavity with a central statocyst (Åkesson, 1958). The ventral sensory organ in the adult of *Phascolosoma agassizii* has been found in the present study to be a simple, narrow, nonciliated tube ending blindly at the anterior end of the ventral nerve cord at the point of junction of the circumesophageal connectives. In the larva the position of the lip pore is similar: the connectives from the brain approximate each other on either side of the lip pore and continue along the sides of the lip groove. The transformation of the larva to the adult form has not been observed in this study; hence it has not been demonstrated whether the lip pore and lip groove of the larva are in any way related to the sensory organ of the adult.

#### NERVOUS SYSTEM

The central nervous system of the larva of *Phascolosoma agassizii* consists of a cerebral ganglion, circumesophageal connectives, and an unsegmented ventral nerve cord.

The cerebral ganglion or brain is located dorsally in the head in close association with the overlying epidermis (Plate 11a, b). Localized in the most dorsal portion of the brain are small nuclei with dense chromatin granules and a lightly staining nucleoplasm. Ventral to the nuclei is a mass of cytoplasmic processes, the neuropile of the brain (Plate 11b). Two pairs of nerves originating from the neuropile have been identified: one pair extends to the dorsoanterior surface of the head and another pair to the esophagus. Embedded laterally near the surface of the head in the posterior portion of the brain is a pair of eyes, each composed of

a hemispherical pigment cup which opens in an anterolateral direction (Plate 11*b, d*). The optic nerve has not been identified.

The circumesophageal connectives leave the brain near the level of the eyes and extend ventrally to either side of the ciliated channel of the mouth (Plates 12*a, 13a*). They pass posteriorly along the channel, then laterally around the buccal groove and, finally, ventrally on either side of the lip pore and lip groove. Beyond this point they become very thin and their union has not been observed in younger larvae. The connectives are characterized by a few fibers and many small nuclei, similar to those of the brain. Usually intimately associated with the epidermis or esophagus, the circumesophageal connectives are not well-defined structures.

The circumesophageal connectives are joined at the distal end of the lip by the ventral nerve cord (Plates 3*b, c, 5, 8b*). Up until the age of one month the nerve cord is double throughout most of its length. In the region of the thorax it is very slender, composed of two longitudinal rows of nuclei joined by a transverse, slightly thickened mesentery which probably represents the neuropile. The two rows of nuclei are attached by mesenteries to the peritoneum of the body wall and the lip glands. The nerve cord is thickened in the trunk region where it lies within the epidermal layer of the body wall as two longitudinal cords (Plates 12*b-d, 13c*). The neuropile of each cord is dorsal or dorsomedial to the nuclei or in some regions the fibers of the neuropile may be scattered among the nuclei. The separation between the two neuropiles is not always distinct in the most anterior regions, but posteriorly the neuropiles are clearly separate.

At the age of two months in most larvae the nerve cord has united. In the thorax it is bilobed, the two lobes joined at their bases with nuclei in a lateral position on the outer side of each lobe (Plate 13*d*). In the trunk the two longitudinal cords have fused to form a single cord, suspended by a thickened peritoneum. The nuclei of the cord are ventral and the neuropile dorsal as in the adult. Posteriorly the nerve cord becomes extremely thin and it has not been possible to trace it to the posterior end of the trunk.

The central nervous system is ectodermal in origin. The rudiments of the brain are formed from the lateral cells of the apical plate by an inward proliferation in the early trochophore and

by the end of the trochophore stage the brain rudiments are well developed (Plate 11*a*). The cells of the brain surround the rosette cells and extend downward to the prototroch, forming the sides of the apical cavity. Beneath the cavity the lateral brain rudiments are united by a central commissure, the future neuropile. The nuclei of the brain rudiments are small and ellipsoidal, measuring approximately 4×5 microns, with prominent chromatin granules and very little surrounding cytoplasm. When the mouth opens at metamorphosis the ventral portion of the brain is pushed into a more dorsal position, the apical cavity is obliterated, the rosette cells degenerate, and the two lateral lobes of the brain are united.

The pigment granules of the eye appear late in the trochophore stage in a dorsolateral cell on either side of the apical plate. The two cells are located directly above the prototroch at the external surface beneath the egg membrane. During the premetamorphosis stage the granules increase in number and assume the form of a cup with the opening directed posterolaterally. At metamorphosis the opening of the cup is turned in an anterolateral direction, retaining its position near the surface.

The circumesophageal connectives appear to originate from the brain, growing down on either side of the esophagus. By the time of metamorphosis the connectives are complete.

The nerve cord is proliferated inward from the ventral ectoderm posterior to the stomodaeum. At the end of the trochophore stage a few scattered cells, very similar in cytology to those of the brain, make their appearance in this position. In the premetamorphosis stage at the time of post-trochal elongation the ventral nerve cord is clearly visible as two loosely arranged rows of small nuclei near the ventral surface of the developing embryo. The two rows are separated posteriorly by epidermal cells, but in the region beneath the stomodaeum the cells are more scattered and there is no distinct separation. The neuropile has not been identified in prelarval stages.

In all other studies on sipunculans the ventral nerve cord has been reported to arise from a median unpaired thickening of the ectoderm of the trunk, beginning in the anterior trunk region and progressing posteriorly (Hatschek, 1883; Gerould, 1907; Åkesson, 1958, 1961*a*). In *Phascolosoma agas-*

*sizii* the development of the ventral nerve cord begins as a ventral ectodermal proliferation of scattered cells in the anterior trunk region, but it differs from other sipunculans in that it progresses posteriorly as two separate longitudinal cords. By the time the larva has reached the age of 2 months, the two cords have united.

Gerould (1907) reported a transitory metamerism of the nerve cord and the mesoblasts into two to four segments. Later in letters to W. K. Fisher and G. Pickford, he repudiated this report, explaining the apparent metamerism to be the result of larval contraction (Hyman, 1959:657). Neither Hatschek nor Åkesson found any evidence for segmentation in the ventral nerve cord, nor was any such evidence found in *Phascolosoma agassizii*.

Both Gerould (1907) and Hatschek (1883) asserted that the circumesophageal connectives were formed by a bifurcation and forward growth of the anterior end of the ventral nerve cord. Åkesson (1958) was unable to determine whether the connectives originate from the brain or the ventral nerve cord. Because in *Phascolosoma agassizii* the connectives appear before the nerve cord is well developed, it is suggested that they arise from the brain.

Eye spots similar to those of *Phascolosoma agassizii* have been described in the embryos of *Golfingia vulgaris*, *Phascolopsis gouldi* (Gerould, 1907), and *Golfingia elongata* (Selenka, 1875). The eye spots as figured by Gerould are of the inverse type, unlike the everse adult eye which is embedded within the brain at the inner end of an ocular tube. In the larva of *Phascolosoma agassizii* it has not been possible to demonstrate whether the larval eyes are inverse or whether they are rudiments of the adult eyes. Åkesson (1958) observed rudimentary ocular tubes in the larvae of *Phascolion strombi* and *Golfingia minuta*, but he did not find pigmented eye spots in these species. Two pairs of eye spots, one large and one small, have been reported in the larva of *Sipunculus nudus* (Hatschek, 1883) and in unidentified pelagosphaera (Senna, 1906; Jägersten, 1963). In *Sipunculus nudus* one pair of eye spots appeared in the early gastrula and a second in the older larva. Åkesson (1958) hypothesized that the first pair might be the inverse type and the second the everse eye characteristic of the adult.

#### NEPHRIDIA

In the larva of *Phascolosoma agassizii* there is a single pair of nephridia, each member of the pair with a U-shaped ciliated canal opening at one end to the exterior by way of an epidermal pore and at the other end to the coelom through a ciliated funnel (Plate 10*d*). The external pores are situated in a ventrolateral position on either side of the ventral nerve cord, slightly anterior to the level of the anus. From their point of attachment in the region of the pore the nephridia are suspended freely within the coelom, usually extending posteriorly, although they may be pushed anteriorly by the movement of the gut or the introversion of the head (Plate 4*b*). At the age of one month the nephridia measure approximately 40 microns in length.

The arm of the nephridium that is associated with the funnel is narrower in diameter than the arm that terminates at the external pore, the difference being more marked in the region of the external openings (Plate 7*d*). The nephridium is four cells in width, with two cells surrounding the funnel canal and two cells around the pore canal. The number of cells along the length of the nephridium increases with its growth. The cells of the pore arm are characterized by green granules, often enclosed within large vacuoles. The cytoplasm of both the pore and funnel arms is densely concentrated with mitochondria and the nuclei are large with finely dispersed chromatin and prominent nucleoli (Plate 7*d*). The nuclei of the cells of the funnel are small and resemble those of the peritoneum that surrounds the remainder of the nephridium.

The rudiments of the nephridia have been identified in the premetamorphosis stage at the time of coelom formation (Plates 2*d*, 10*a*). A nephridial rudiment consists of two enlarged cells located ventrally within the mesoderm bands in the middle of the post-trochal region of the developing embryo. The distinctive nuclei are large and lobate with a lightly staining nucleoplasm and one or two prominent nucleoli. In the premetamorphosis stage the somatic and splanchnic bands of mesoderm split anteriorly and posteriorly to the nephridial rudiment to form the coelom, leaving the nephridial cells as a bridge connecting endoderm and ectoderm (Plates 2*d*, 3*b*, 10*a*, *b*). At

the time of metamorphosis the nephridial cells are separated from the gut, but retain their connection with the body wall as they are overgrown by cells of the somatic peritoneum (Plate 10c). It appears probable that the more interior nephridial cell gives rise to the funnel arm of the nephridium and the more exterior cell to the pore arm. A proliferation of small cells within the epidermis joins the outer nephridial cell to form the external pore, and cells resembling those of the peritoneum surround the nephridium to form the funnel. The pores and the funnels were not observed until several days after metamorphosis.

There is no protonephridium apparent at any time during the development of *Phascolosoma agassizii* and the larval nephridium, as described above, undoubtedly becomes the definitive nephridium of the adult. The exact origin of the larval nephridium has not been conclusively determined. The position of the nephridial cells in the mesoderm bands and the relationship of the developing nephridium to the coelom suggest that the nephridium of *Phascolosoma agassizii* is mesodermal in origin, growing out from the coelom toward the periphery. The source of the nephridial cells, however, has not been traced and the possibility of their migration inward from the ectoderm cannot be excluded. Moreover, the exact derivation of the cells of the coelomic funnel is not known, and it is possible that the funnel is formed from coelomic epithelium as a separate structure and at a later stage than the tubular portion of the nephridium.

In the literature on sipunculan development there are discrepancies concerning the mode of formation of the nephridium. Hatschek (1883) reported a purely mesodermal origin of the nephridium of *Sipunculus nudus*. He was able to recognize two nephroblasts within the mesoderm at a very early stage by their distinctive yellow pigmentation. The division of each yellow cell was found to give rise to a U-shaped chain of cells with a central lumen opening at one end through the epidermis and at the other into the coelom. The anlage of the coelomic funnel he reported to be composed of cells of the peritoneal lining. The reported origin of the nephridia of *Sipunculus nudus* from cells in the mesoderm bands is thus similar to that observed for *Phascolosoma agassizii*. In the species studied by Akesson (1958) and Gerould (1907), the nephridium was not observed

at its inception because of large amounts of coelomic yolk. Both authors assumed, nevertheless, that the nephridium proper was formed as an ectodermal invagination and that the funnel arose from peritoneum. Akesson's assumption of an ectodermal participation in nephridial formation was based on the morphology of the larval nephridium in *Golfingia minuta*. The central cavity of the nephridium in this larva is lined with a cuticle that is continuous with that covering the body wall. Akesson supposes, therefore, that the cells surrounding the cavity are ectodermal and that the more peripheral cells and the cells of the funnel are derived from mesoderm.

The observations of Akesson and Gerould, although incomplete, suggest that the nephridia of the species they studied are composite structures, conforming to the concept of mixonephridia by Goodrich (1945). Observations on *Phascolosoma agassizii* and *Sipunculus nudus*, on the other hand, do not rule out the possibility that the nephridial structure may be a coelomoduct, derived entirely from entomesoderm. In view of the information available to him from the studies of Hatschek (1883) and Gerould (1907), Goodrich (1945) assumed the sipunculan nephridium to be a mixonephridium. Further evidence on the exact source of the "nephroblasts," the derivation of the coelomic funnels, and the comparative cytology of the cellular components is now necessary to clarify the nature of the nephridia of the Sipuncula.

#### LARVAL GLANDS

The glands in the larva of *Phascolosoma agassizii* are designated as the lip glands, the posterior sacciform glands, the epidermal glands, and the glands of the head. The lip glands have been discussed previously as an associated structure of the mouth.

*Posterior Sacciform Glands.*—The posterior sacciform glands are thus termed because of their shape and their position in the posterior half of the larva. There are two glands, one on either side of the anus in a dorsolateral position (Plate 4b). The glands are cellular sacs, composed of a single layer of cells surrounding a central cavity that opens to the exterior through a wide aperture in the body wall (Plate 14b, c). In the larva of one month of

age each gland projects into the coelom to a length of approximately 30 microns.

The cells of the gland are of two different types: one presumed to be secretory and the other supportive. The nucleus of the secretory type is round, approximately 4.5 microns in diameter, with a homogeneous, darkly staining nucleoplasm and a very large, eccentric nucleus (Plate 14*b*). There is only one such cell in each sacciform gland and it is located at the rounded extremity of the gland. The cytoplasm of this cell is thicker than that of the other cells of the gland and it is intensely basophilic. The nucleus of the second cell type, the supportive cell, is smaller, measuring 2×3 microns, with fine chromatin granules. Many cells of this type form the wall of the gland surrounding the central cavity. The cytoplasm of the cells, also basophilic, becomes increasingly thin as the central cavity enlarges and the gland increases in size. The central cavity is filled with tightly packed globules, the secretory product of the gland. These globules are stained a light blue with Richardson's stain, but they are not stained by alcian blue or periodic acid-Schiff. Hence, the chemical nature of the secretion has not been determined and the possible function of the sacciform glands in larval life remains enigmatic.

The posterior sacciform glands develop from ectoderm late in the premetamorphosis stage. An ectodermal cell on either side of the rectal rudiment increases in size; the cytoplasm of the cell becomes basophilic and the nucleus moves into a position toward the coelom (Plate 14*a*). In that part of the cytoplasm nearest the body wall, small globules, similar to those later found in the central cavity of the gland, make their appearance. This cell is identical to the cell at the extremity of the mature gland, and it is presumed to be the secretory element of the gland. Smaller ectodermal cells are proliferated around the base of the larger cell, and as the gland grows these cells are extended to form the walls of the gland, surrounding the central cavity. The aperture through the body wall is completed a few days after metamorphosis.

Glands such as the posterior sacciform glands in the larva of *Phascolosoma agassizii* have not been described in any other species of sipunculans.

*Epidermal Glands.*—There are three pairs of epidermal glands in the larva at 20 days of age (Plate 14*d*). Located in dorsolateral positions on

the trunk, one pair is near the middle of the body and two additional pairs are relatively close together in a more posterior region. In living larvae each gland appears as a small epidermal papilla from which a very fine bristle, 5 to 10 microns in length, protrudes to the exterior. In sectioned material the glands are found to be pyriform in shape, the more pointed end leading to a narrow canal which passes through the larval cuticle. The rounded base of the gland, covered by peritoneum, projects for a distance of approximately 10 microns into the coelom. The gland is comprised of a cluster of small epidermal cells surrounding a diminutive cavity which opens into the cuticular canal. Within the cavity is a homogeneous secretory material, staining light blue with Richardson's stain, and from the base of the cavity the bristle extends outward through the canal. In contrast to the other epidermal cells, the cytoplasm of the gland cells is scant and the nuclei are characterized by fine chromatin granules and an absence of nucleoli.

The epidermal glands are derived from ectodermal proliferations, first evident late in the premetamorphosis stage. The most anterior pair of glands, the earliest to appear, is fully developed with extended bristles at the end of metamorphosis. By 17 days bristles are also apparent on the two posterior pairs of glands. All of the bristles have vanished in larvae older than one month and many additional epidermal glands have formed without bristles (Plate 13*d*).

Epidermal glands similar to those in *Phascolosoma agassizii* have been described in all studies of sipunculan larvae; however, associated bristles have been reported only in *Golfingia elongata* (Selenka, 1875; Åkesson, 1961*b*) and *Phascolion strombi* (Åkesson, 1958). In *Phascolion strombi*, studied by Åkesson (1958) at Kristineberg, the bristles were not arranged in pairs, but rather in six circlets, each circlet with four bristles. *Golfingia elongata*, also from Kristineberg, showed the same arrangement (Åkesson, 1961*b*), but larvae of the same species at Roscoff entirely lacked bristles (Gerould, 1907; Åkesson, 1961*b*) and those at Nice possessed only three pairs (Selenka, 1875). In an effort to explain this variation among different populations of the same species, Åkesson (1961*b*) pointed out that *Golfingia elongata* at Kristineberg lived at depths of 30 meters, whereas those at Roscoff in-



habited the intertidal zone. Such a correlation between depth of habitat and presence of bristles was not found in the present study in which larvae from an intertidal population of *Phascolosoma agassizii* were observed to possess three pairs of bristles.

Akesson (1958) speculated on the functional significance of the glands and bristles, suggesting that the secretion of the gland in *Phascolion strombi* might aid in dissolving the holes which the larvae inhabited in gastropod or scaphopod shells, or that it might have a protective effect against enemies. Although he was unable to demonstrate a sensory element in the gland, Akesson proposed that the bristles might be tactile organs. In the study of *Phascolosoma agassizii* neither sensory cells nor sensory nerves could be demonstrated in the glands; however, larval nerves are very difficult to trace and sensory cells might not have been recognized, therefore it cannot be concluded that a sensory element does not exist. Epidermal organs in the adult of this species have a sensory function as well as secretory, and it is not unlikely that the organs of the larva might also have a dual function.

*Glands of the Head.*—There are many different glands in the head of the larva of *Phascolosoma agassizii*. Some of the glands secrete a material that stains with periodic acid-Schiff, in others neurosecretory granules can be demonstrated, and in still others the secretion has not been characterized.

Opening on the ventrolateral surface of the head are two pairs of long, thin glands, approximately 7 microns in width, packed with small globules that stain very intensely with periodic acid-Schiff (Plate 11d [g<sup>1</sup>, g<sup>2</sup>]). One pair opens directly above the lateral extremities of the buccal groove, passing posteriorly along either side of the esophagus for a distance of 40 microns. A second pair, somewhat shorter, opens more anteriorly and extends into the head coelom. Also staining with periodic acid-Schiff is a third pair of smaller, club-shaped glands, approximately 15 microns in length, that opens farther anteriorly on the ventral surface of the head (Plate 11d [g<sup>3</sup>]). These glands are filled with loosely packed globules, smaller than those of the long, thin glands. The secretion of these three pairs of glands is discharged onto the ventral surface of the head and may aid in the trapping of

food particles which would then be swept by ciliary action into the esophagus.

Neurosecretory granules have been demonstrated in cells in the brain and in cells along the sides of the esophagus. After fixation in osmium tetroxide and embedding in methacrylate the larvae were sectioned at 1 micron and stained in paraldehyde fuchsin by the method of Clark (1955). Prominent neurosecretory granules were stained a very deep red. In larvae embedded in Epon and stained with Richardson's stain, very large cells in the brain and along the esophagus were assumed, because of their position and cytology, to be the neurosecretory cells of the methacrylate-embedded material. The cytoplasm of these large cells was intensely basophilic with vacuolar inclusions and the nucleus contained a prominent nucleolus (Plates 8b, 11d [g<sup>2</sup>]).

There are other glands with an unidentified secretion that are apparently unicellular and open to the exterior. The secretion, foamy in appearance, does not stain with Richardson's stain, periodic acid-Schiff, or paraldehyde fuchsin. One pair opens on the ventral surface of the head between the apertures of the long, thin glands described above, and the others open on the anterior head along the ventral boundary of the brain (Plate 11c, d [g<sup>4</sup>]).

The origin and fate of the glands of the larval head in *Phascolosoma agassizii* are not known. In the larvae of *Phascolion strombi*, *Golfingia minuta*, and *Golfingia elongata*, Akesson (1958, 1961b) described a larval cerebral organ, derived from the rosette cells, with a secretion characterized in the first two species by basophilic, argentaffine staining properties. The more proximal cells of the organ are eventually incorporated into the brain, whereas the remainder form the cerebral organ of the adult. The cells that are incorporated into the brain are not secretorily active in the adults of these species; however, Akesson (1961b) points out that cells in the same position in the brain of the adult *Sipunculus nudus* are neurosecretory and he assumes, because of their identical position, that they are homologous to the incorporated cells of other species. From these observations and assumptions, Akesson concludes that primitive epidermal secretory cells transform into neurosecretory cells within the brain. Since Akesson did not examine the larva of *Sipunculus nudus* and Hatschek (1883), in his

study of the larva of this species, did not describe a larval cerebral organ, further observations on the larva are necessary before Akesson's conclusion can be confirmed. In the larva of *Phascolosoma agassizii* no distinct cerebral organ was found, but neurosecretory cells, of uncertain origin, were already present within the brain of the larva. Whether other glandular cells later transform into additional neurosecretory cells has not been determined in the present study.

#### TERMINAL ORGAN

The terminal organ is a retractable appendage located at the posterior end of the larva (Plates 3c, 4a,c,e,f, 14e,f). Usually a bulb-shaped organ, 25 microns at its widest dimension and 50 microns in length, the terminal organ may be elongated to a length of 170 microns. The function of the organ is the attachment of the larva to the substratum. When the larva is swimming or lying unattached on the substratum the organ may be retracted within the body.

Withdrawal of the terminal organ into the posterior portion of the trunk is accomplished by the contraction of two retractor muscles. These muscles originate within the terminal organ and insert dorsally on either side of the dorsal midline at the level of the middle pair of epidermal glands (Plate 14f). Each retractor muscle is composed of six fibers and differs from the retractor muscles of the head, each of which has eight fibers.

The terminal organ is covered by a thin cuticle, a continuation of the cuticle of the trunk. The cuticle at the posterior tip of the organ is interrupted by an aperture into which several gland cells open (Plate 14e,f). Gland cells, vacuolar in appearance, are not stained by Richardson's stain, periodic acid-Schiff, or alcian blue. Even though the secretion has not been characterized by its staining properties, it is assumed that it is adhesive in nature because of the attachment of the larva to the substratum by means of the terminal organ.

The terminal organ and its retractor muscles form from an ectodermal proliferation, first observed during the premetamorphosis stage in a ventral position near the posterior tip of the body. An invagination appears in the egg membrane opposite the ectodermal proliferation and at the time of metamorphosis the ectodermal thickening

is pushed out through the invagination, taking with it the surrounding cuticle, to form the terminal organ (Plate 14e). The cuticle at the posterior tip of the organ is ruptured, thus creating the aperture through which the secretion is discharged. At the end of metamorphosis the larvae settle on the substratum, an indication that the gland cells have become active in secretion.

A terminal organ has been reported in other benthopelagic or pelagic sipunculan larvae. In the larva of *Sipunculus nudus* Hatschek (1883) described an ectodermal thickening to which bristles were attached. The thickening was connected internally to the ventral nerve cord by thin fibers and he supposed it to be a tactile organ. Usually retracted, the organ was only rarely observed to be extended. Akesson (1961a) described a similar organ in unidentified, preserved pelagosphaera larvae and noted the similarity to the terminal organ of adult species of *Sipunculus*. In the larvae of an intertidal population of *Golfingia elongata*, Akesson (1961b) found a terminal glandular organ, but in a deep-water population of the same species at Kristineberg he did not find such an organ. Larvae in the former population were observed to adhere to the substratum by means of the organ, but no mention was made of retractility. In more than half of the species of pelagosphaera that Jägersten (1963) examined, he observed a retractile "tail," considered to be identical with the terminal organ described by Akesson (1961a). Jägersten reported an odd behavior in which the "tail" was placed into the mouth possibly, he speculated, to discharge a secretion. In *Phascolosoma agassizii* a similar behavior was observed whereby the body was bent so that the terminal organ touched the lip or else was pushed past the head (p. 5). This action was most frequently noted when there was no substratum on which the larva could settle, and what role, if any, it served in normal behavior was not determined. The primary function of the terminal organ in this species is attachment of the larva to the substratum. It would be of interest to ascertain whether the pelagosphaera studied by Jägersten ever use the terminal organ under natural conditions to attach to a substratum, or to some debris floating among the plankton. If not, then the behavior that Jägersten reports might be explained as an adaptation to planktonic life and the

terminal organ in such forms may serve an entirely different function from that of attachment.

### Comparisons with Other Phyla

The phylum Sipuncula with spiral cleavage and a trochophore larva is, by definition, a member of the Protostomia. Close affinities with both annelids and mollusks are apparent from developmental studies and much of this evidence has been reviewed in detail by Hatschek (1883), Gerould (1907), Åkesson (1958), and Clark (1969).

Comparisons of cuticle formation reveal that in many polychaetous annelids, as in sipunculans, the egg envelope may undergo a transformation into the larval cuticle. Such a transformation is found in the sipunculan species *Golfingia elongata*, *Phascolion strombi*, *Golfingia minuta* (Åkesson, 1958; 1961b), *Phascolosoma agassizii*, *Golfingia pugettensis*, and *Themiste pyroides* (Rice, 1967) and in numerous polychaetes, some of which are *Clymenella torquata*, *Arenicola cristata* (Wilson, 1892), *Pomatoceros triqueter* (Segrove, 1941), *Nereis diversicolor* (Dales, 1950), *Diopatra cuprea* and *Autolytus fasciatus* (Allen, 1959; 1964).

A larval structure, the metatrochal band of cilia, common to all pelagosphera larvae of sipunculans, is homologous in sipunculans and polychaetes, as indicated by the cell lineage studies of Gerould (1907). In contrast to that of most polychaetes, however, the metatroch of the pelagosphera is the primary locomotory organ and at the time of its formation, or soon thereafter, the prototroch is lost or diminished. In *Sipunculus nudus* the prototroch is unique in that it completely envelops the embryo. This structure has been compared by Gerould (1907) to a similarly modified prototroch that constitutes the velum of some aplacophorans and primitive lamellibranchs.

A protonephridium, found in the trochophores of the many polychaetes and some mollusks, is lacking in sipunculans. The larval nephridium of the sipunculan, a U-shaped tubular structure opening at one end to the exterior and at the other to the coelom, becomes the nephridium of the adult with no essential change. Its precise derivation has yet to be determined, but studies of *Phascolopsis gouldi*, *Golfingia vulgaris* (Gerould, 1907) and *Golfingia minuta* (Åkesson, 1958) suggest that it is a composite structure, formed from ectoderm

and coelomic epithelium. In *Sipunculus nudus* (Hatschek, 1883), however, and in *Phascolosoma agassizii* a purely mesodermal origin is suggested, but not proved. Goodrich (1945) considered the sipunculan nephridium to be a mixonephridium, a structure characteristic of most polychaetes but not of mollusks.

The nervous system develops similarly in sipunculans and annelids and is comprised in both of a cerebral ganglion, circumesophageal connectives, and a ventral nerve cord. The nerve cord develops as a ventral ectodermal thickening which is double in annelids, but in most sipunculans is median and single. An exception is the species *Phascolosoma agassizii* in which the nerve cord is paired at its inception, although later it is united into a single cord. Gerould (1907) considered the unpaired ventral nerve cord to be a primitive feature, relating the sipunculans to primitive annelids such as *Polygordius*. Åkesson (1958) took an opposite view, pointing out that among the turbellarians, annelids, and mollusks the orthogonal nervous system of turbellarians should be considered the primitive one and that the nerve cord of sipunculans therefore was not a primitive but rather a derived feature. The rudimentary, paired nerve cord in *Phascolosoma agassizii* is interpreted in this study as a reminiscence of a double nerve cord that probably appeared in the development of the ancestors of extant sipunculans, relating the sipunculans more closely to the annelidan stem.

The anus of the sipunculan larva does not arise at the posterior region of the blastopore as in most polychaetes, but rather it arises at the site of a dorsal ectodermal proliferation, the rectal rudiment. In a few polychaetes, such as *Hydroides* (Shearer, 1911), the anus develops similarly.

A striking and important difference from the annelids is the complete lack of segmentation of the nerve cord and the mesoderm. Although Gerould in 1907 described a temporary division of the nerve cord and mesoderm of *Phascolopsis gouldi* into two to four segments he later retracted this report (Hyman, 1959:657). No other investigator has noted any indication of mesodermal or nervous segmentation in sipunculan development. The arrangement of epidermal bristles into several circlets in the larvae of *Golfingia elongata* and *Phascolion strombi* is regarded by Åkesson (1958, 1961a) as evidence of a transitory metamerism. The papillae from which

the bristles arise, however, are ectodermal and there is no involvement of the mesoderm. The bristles, a secretion of the papillae, are readily dissolved by fixatives and are in no way homologous to the chaetae of polychaetes.

Jägersten (1963) noted the likeness of the lip of the pelagosphera to the foot of a gastropod larva. Previously Gerould (1907) had suggested a possible homology of the lip gland (glandular organ) and the buccal organ ("Schlundkopf") to two similarly placed invaginations in the veligers of chitons: the pedal gland and radular sac. These organs, however, have not been observed in the lecithotrophic pelagosphera which are assumed to represent a more primitive development (Rice, 1967); hence, it seems probable that they are adaptations of the planktotrophic larva for feeding activity.

### Summary

The pelagosphera larva of *Phascolosoma agassizii*, as observed from living specimens reared in the laboratory, is characterized by a ventrally ciliated and bilobed head, a thorax with prominent metatroch which, along with the head can be withdrawn into the body, an elongated, wedge-shaped trunk which in older larvae is marked by characteristically papillated cuticle, and a posterior terminal organ. The larva attaches to the substratum by the terminal organ and in this position it feeds on bottom-dwelling diatoms and debris. It is able to release itself from the substratum and swim freely through the water by means of the metatrochal circlet of cilia. Larvae were not observed to transform into the adult form.

In a histological study of the structure, origin, and development of larval organs, the following observations were made: The stomodaeum is formed at the site of the closed blastopore and the proctodaeum arises later as a new formation in a dorsal position. The coelom results from the splitting of the mesoderm layers and the retractor muscles, originating from ectomesoderm, make their appearance soon after the formation of the coelom. At the time of metamorphosis of the trochophore into the pelagosphera larva the egg envelope is transformed into the elastic, extensible cuticle of the larva. The nephridium arises from two conspicuous cells within the mesoderm bands. The ventral nerve cord, derived from trunk ectoderm, is double

at its inception, but by the age of 2 months the two longitudinal cords have united. There are three pairs of epidermal glands with bristles and, in addition, there is one pair of posterior sacciform glands opening to the exterior on either side of the anus. Two organs of the mouth, the buccal organ and the lip gland, previously described only in the larva of *Sipunculus nudus* and in unidentified pelagosphera larvae, are present in the pelagosphera larva of *Phascolosoma agassizii*.

Finally, the histogenesis of selected structures in sipunculans shows many similarities to the polychaetous annelids and, to a lesser extent, to the mollusks.

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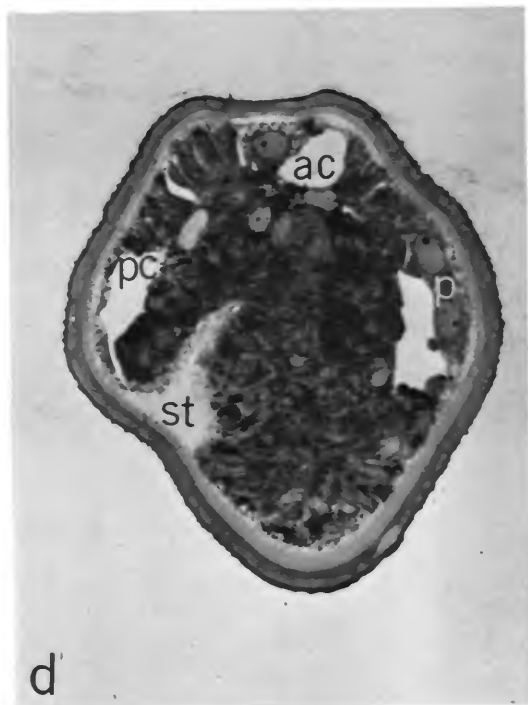
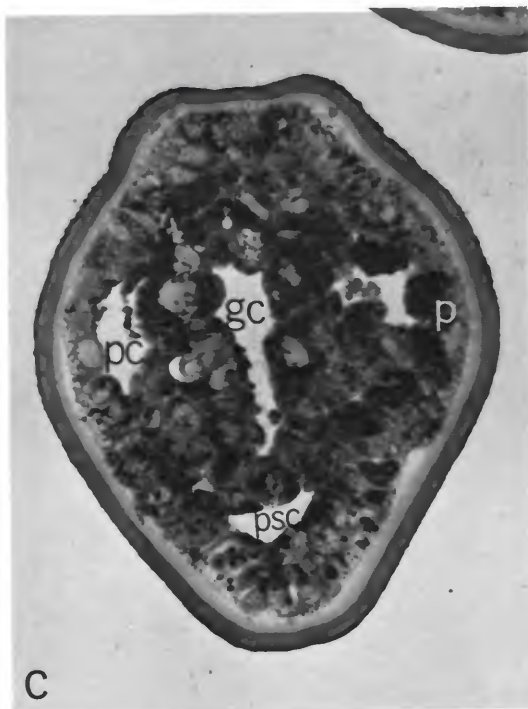
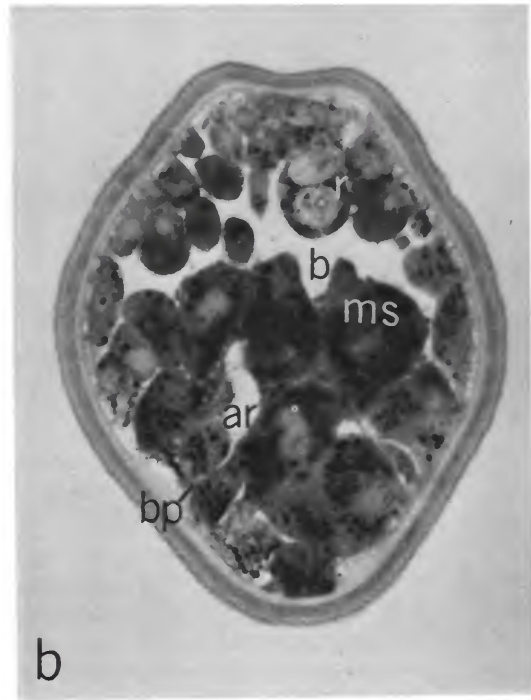
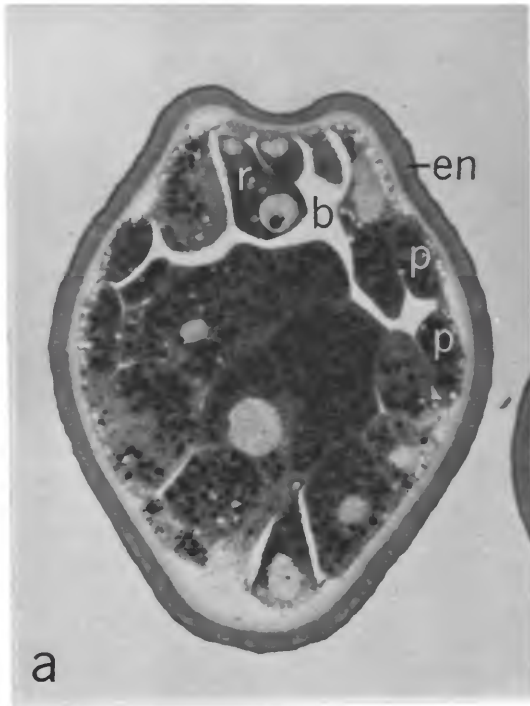
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**PLATE 1**

Early developmental stages of *Phascolosoma agassizii*: blastula, gastrula, trochophore. Epon, one micron, Richardson's stain.

- a. Sagittal section of a 24-hour embryo showing rosette cells (r) with apical cilia, modified blastocoel (b), prototroch cells (p) and three-layered egg envelope (en) perforated by fine pore canals.
- b. Sagittal section of a 48-hour embryo showing rosette cells (r), blastocoel (b), blastopore (bp), mesentoblast (ms), and archenteron (ar).
- c. Sagittal section of trochophore showing gut cavity (gc), prototrochal cavity (pc), prototroch cells (p) and posterior cavity (psc). Note yolk granules in the prototrochal cavity. 4 days (10°C).
- d. Oblique sagittal section of trochophore. 5½ days (10°C). Note apical cavity (ac), prototrochal cavity (pc) and stomodaeum (st). The prototroch cells (p) have lost most of their yolk granules at this stage. Prototrochal cilia are clearly shown in this photograph.

[ac=apical cavity, ar=archenteron, b=blastocoel, bp=blastopore, en=egg envelope, gc=gut cavity, ms=mesentoblasts, p=prototroch cell, pc=prototrochal cavity, psc=posterior cavity, r=rosette cell, st=stomodaeum.]



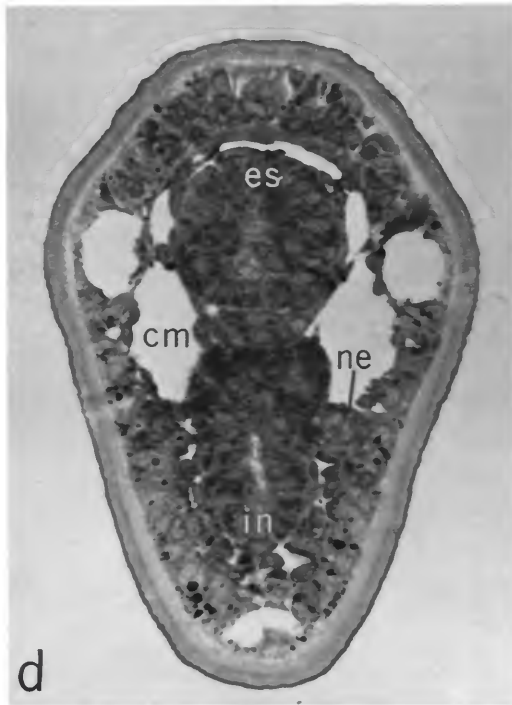
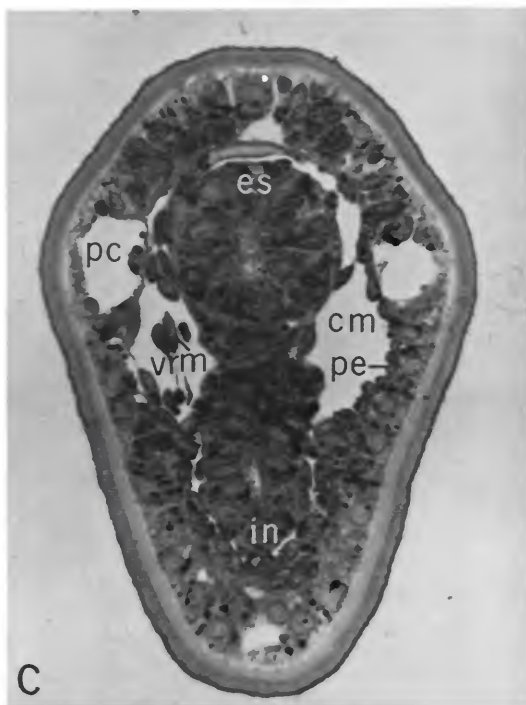
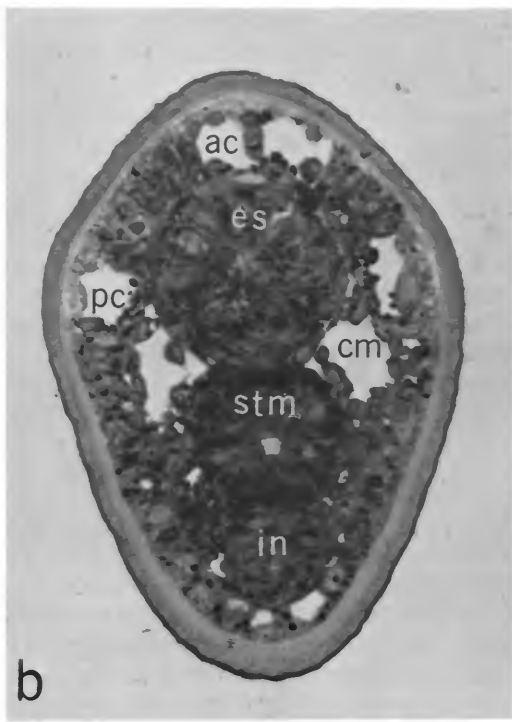
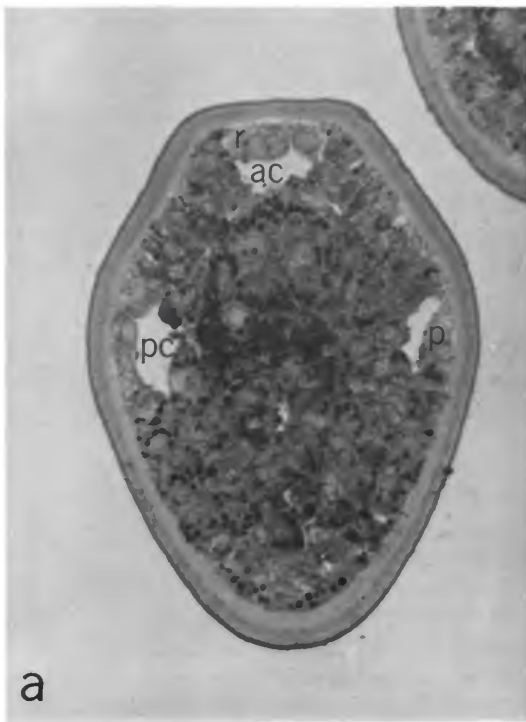
**PLATE 2**

Late trochophore and premetamorphosis stages of *Phascolosoma agassizii*. Epon, one micron, Richardson's stain.

- a. Frontal section of late trochophore showing rosette cells (r) with apical cilia, apical cavity (ac), prototrochal cavity (pc), and prototroch cells (p). The prototroch cells and rosette cells have lost their yolk granules. 6 days (10°C).
- b. Frontal section of premetamorphosis stage. 7 days (10°C). Note apical cavity (ac), prototrochal cavity (pc), coelom (cm), and the peritoneal cells lining the coelom. Three regions of the gut can be identified: esophagus (es), stomach (stm) and intestine (in).
- c. Frontal section of premetamorphosis stage showing cells of the ventral retractor muscles (vrn) traversing the coelom (cm). 7½ days (10°C).
- d. Frontal section of premetamorphosis stage through nephridial rudiment (ne). Posterior to the nephridial rudiment the coelom (cm) is apparent as a narrow slit between splanchnic and somatic mesodermal layers. 7½ days (10°C).

[ac=apical cavity, cm=coelom, es=esophagus, in=intestine, ne=nephridial rudiment, p=prototroch cells, pc=prototrochal cavity, pe=peritoneum, r=rosette cells, stm=stomach, vrn=ventral retractor muscles.]



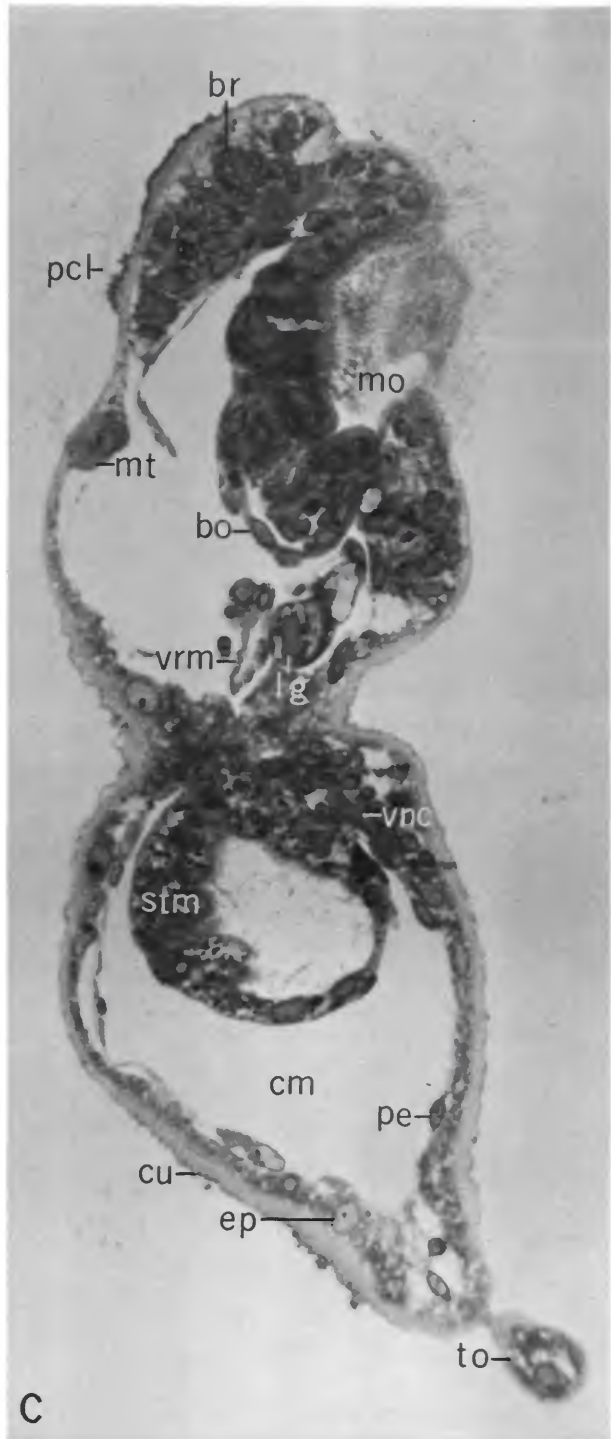
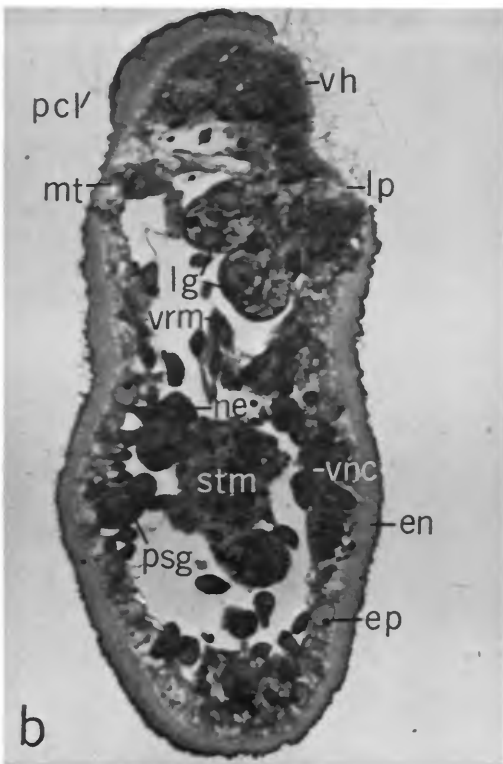
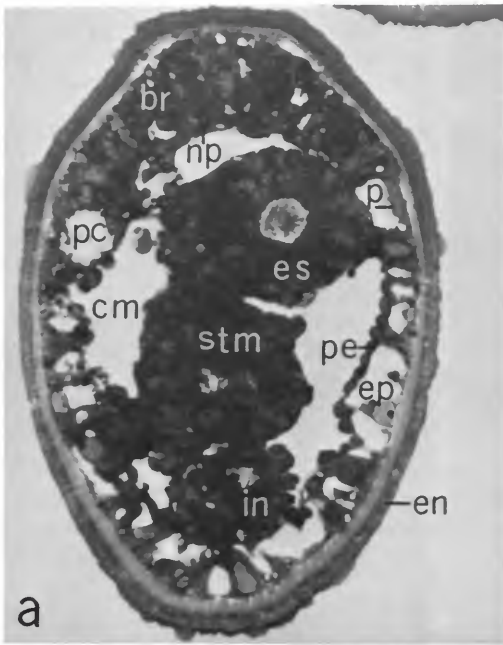


## PLATE 3

Metamorphosis from late trochophore stage to pelagosphera larva of *Phascolosoma agassizii*. Epon. One micron. Richardson's stain.

- a. Sagittal section of premetamorphosis stage. 6 days (12°C). Note the intercellular spaces in the epidermis. The egg envelope (en) posterior to the prototroch (p) has begun to lose its porosity and lamellation.
- b. Parasagittal section of the beginning metamorphosis stage. 8 days (12°C). The egg envelope (en) has ruptured in the region of the mouth and the stomodaeal lining has everted to form the ciliated ventral surface of the head (vh).
- c. Sagittal section of recently metamorphosed larva. 9 days (12°C). The coelom (cm) has expanded, the metatroch (mt) has become the functional locomotory organ, the terminal attachment organ (to) has emerged, the gut (evident here only in part) has been completed, and the egg envelope (en) has undergone a transformation into the larval cuticle (cu).

[bo=buccal organ, br=brain, cm=coelom, cu=cuticle, en=egg envelope, ep=epidermis, es=esophagus, in=intestine, lg=lip gland, lp=lip pore, mo=mouth, mt=metatroch, ne=nephridium, np=neuropile, p=prototroch, pc=prototrochal cavity, pcl=prototrochal cilia, pe=peritoneum, psg=posterior saciform gland, stm=stomach, to=terminal organ, vh=ventral ciliated head, vnc=ventral nerve cord, vrm=ventral retractor muscle.]

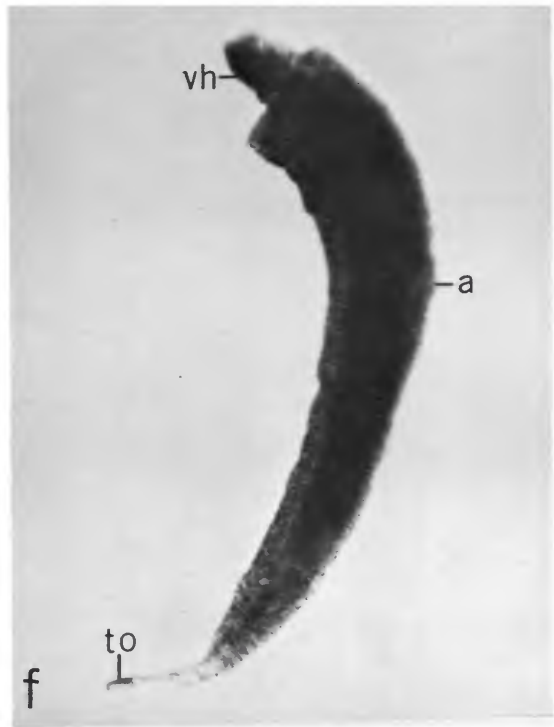
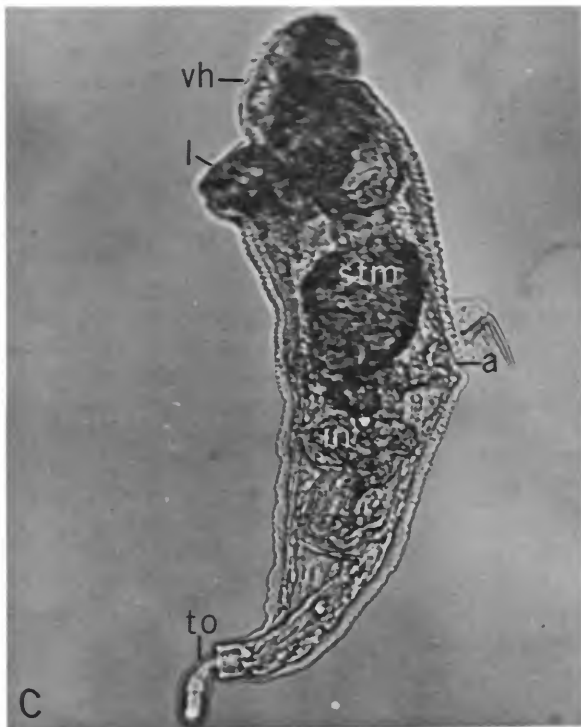
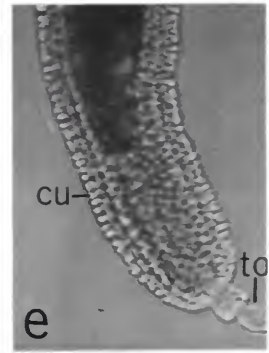
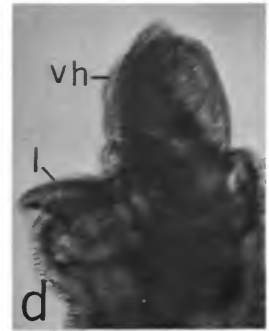
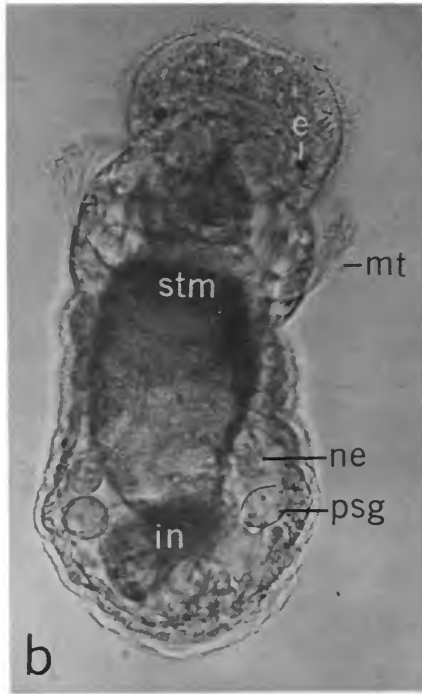
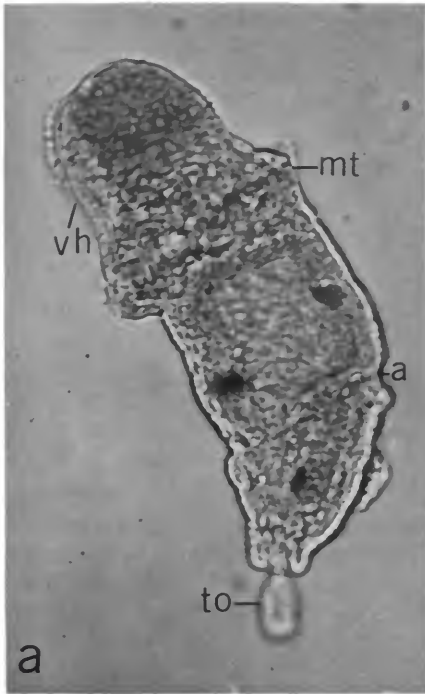


**PLATE 4**

Photographs of pelagosphaera larvae of *Phascolosoma agassizii*: living larvae and whole mounts.

- a. Eleven days. Whole mount, fixed in 10 percent buffered formalin, mounted in euparal. No stain. Lateral view.
- b. Four weeks. Fixed in osmium vapor (2 minutes) after relaxation in a 1:2 solution of saturated chlorethane in sea water (3 minutes). Dorsal view.
- c. Four weeks. Living larvae. Lateral view. Metatroch is retracted.
- d. Ten weeks. Living larva. Lateral view of head. Metatroch is retracted.
- e. Ten weeks. Living larva. Lateral view of posterior terminal organ (to). Note papillated cuticle (cu).
- f. Ten weeks. Living larva. Lateral view of entire larva with retracted lip and metatroch. Note change from 4-week stage (4c) in relative proportions of body.

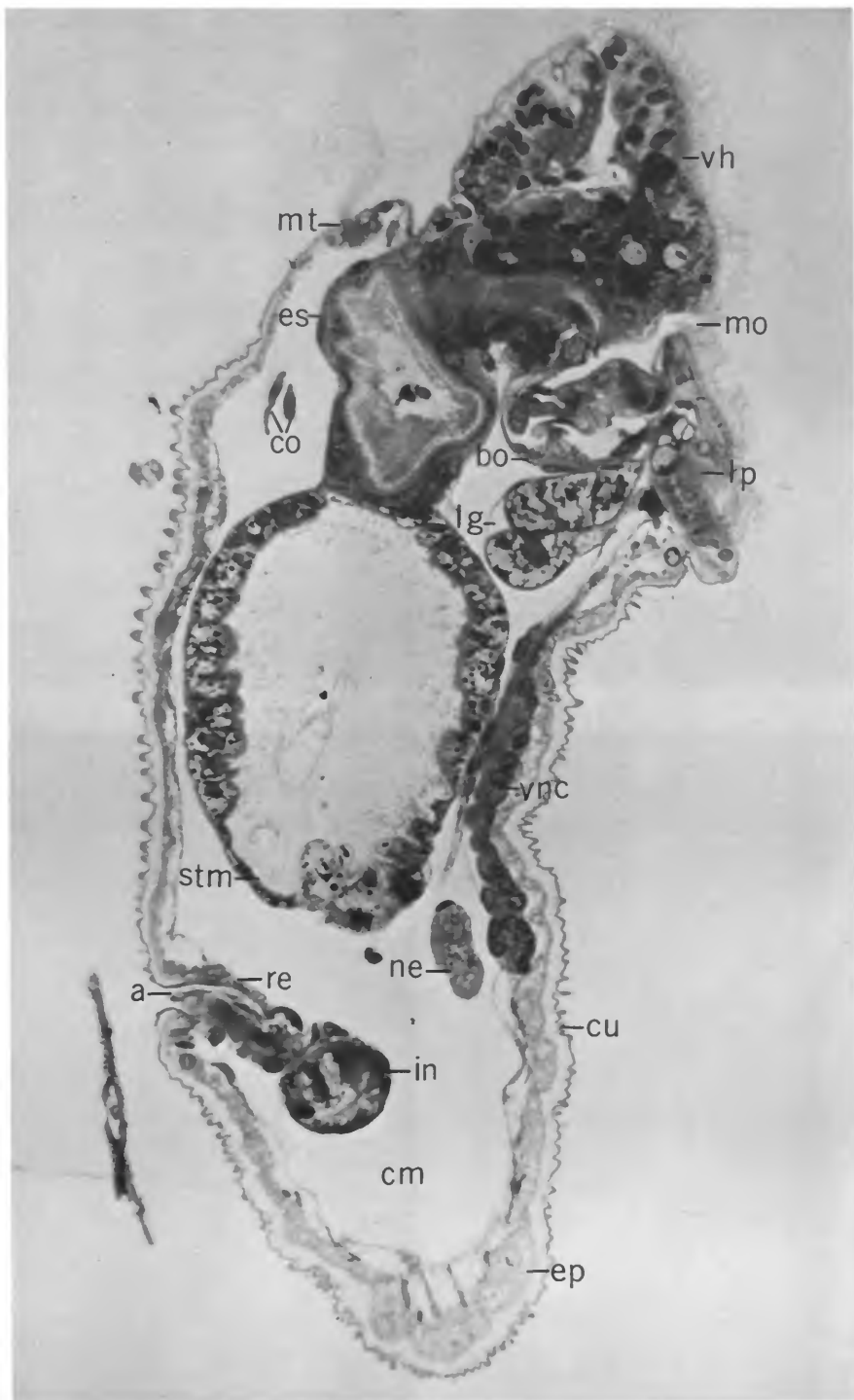
[a=anus, cu=cuticle, e=eye, in=intestine, l=lip, mt=metatroch, ne=nephridium, psg=posterior sacciform gland, stm=stomach, to=terminal organ, vh=ventral ciliated head.]



**PLATE 5**

Sagittal section of larva of *Phascolosoma agassizii*. 28 days. The terminal organ, present at this stage, is not shown in this section. Epon. One micron. Richardson's stain.

[a=anus, bo=buccal organ, co=coelomocytes, cm=coelom, cu=cuticle, es=esophagus, ep=epidermis, in=intestine, lg=lip gland, lp=lip pore, mo=mouth, mt=metatroch, ne=nephridium, re=rectum, stm=stomach, vh=ventral ciliated head, vnc=ventral nerve cord.]



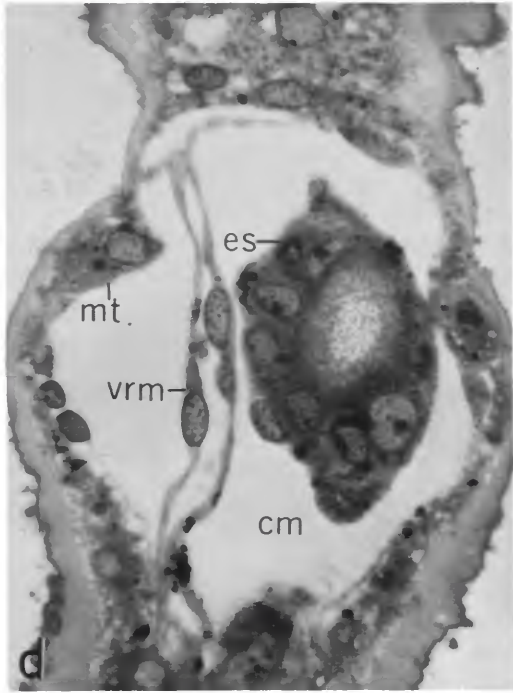
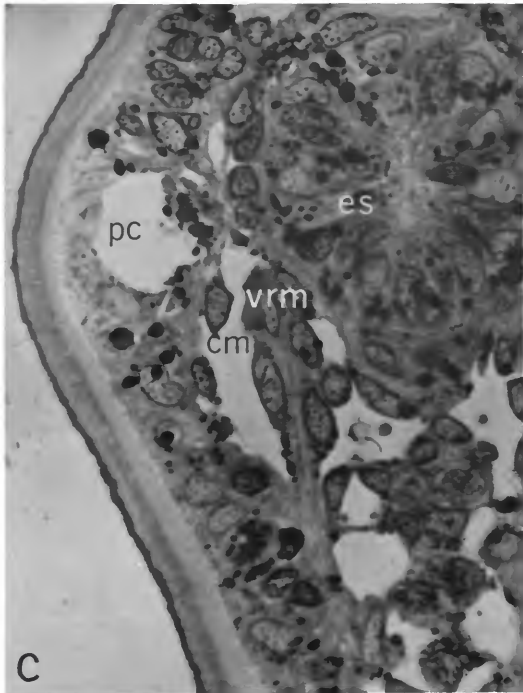
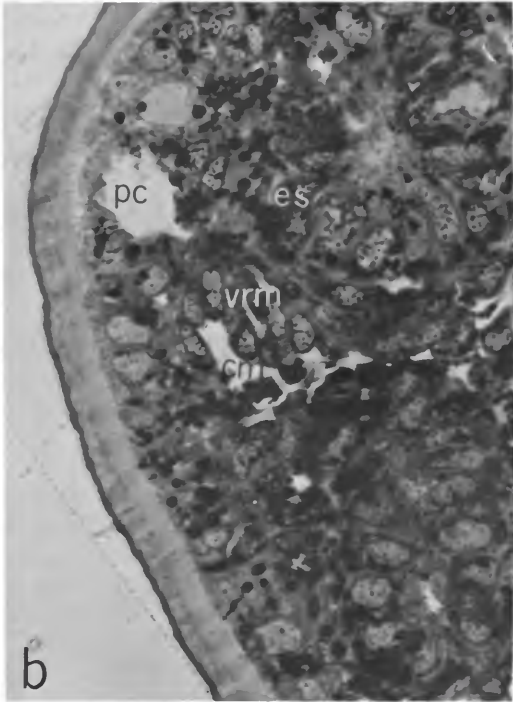
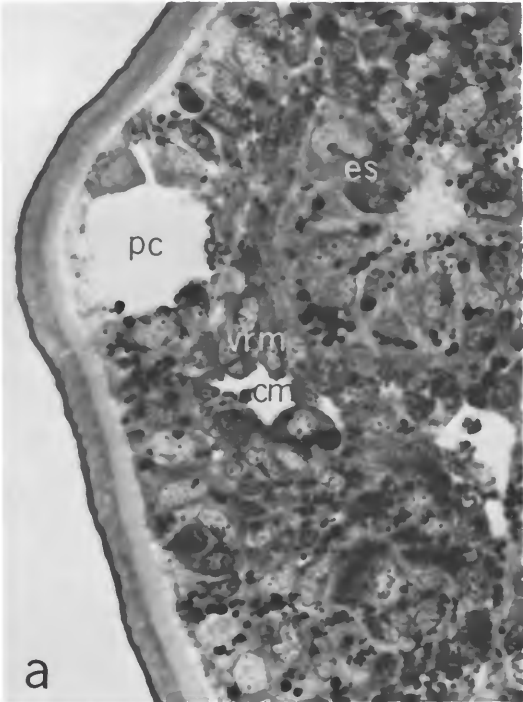
**PLATE 6**

Sections showing formation of ventral retractor muscles and coelom in *Phascolosoma agassizii*. Epon. One micron. Richardson's stain.

- a. Frontal section of an early premetamorphosis stage showing the cells of the ventral retractor muscle (vrm) growing into the newly formed coelom (cm). 6½ days (10°C).
- b. Frontal section of premetamorphosis stage, slightly later than above. 7 days (10°C). Cells of the ventral retractor muscle (vrm) have invaded the coelom (cm).
- c. Frontal section of premetamorphosis stage showing completely formed ventral retractor muscle (vrm) traversing the coelom (cm). 7½ days (10°C).
- d. Oblique frontal section of metamorphosed larva. 11 days (10°C). The coelom (cm) has expanded and the cells of the ventral retractor muscles (vrm) have elongated.

[cm=coelom, es=esophagus, mt=metatroch, pc=prototrochal cavity, vrm=ventral retractor muscle.]



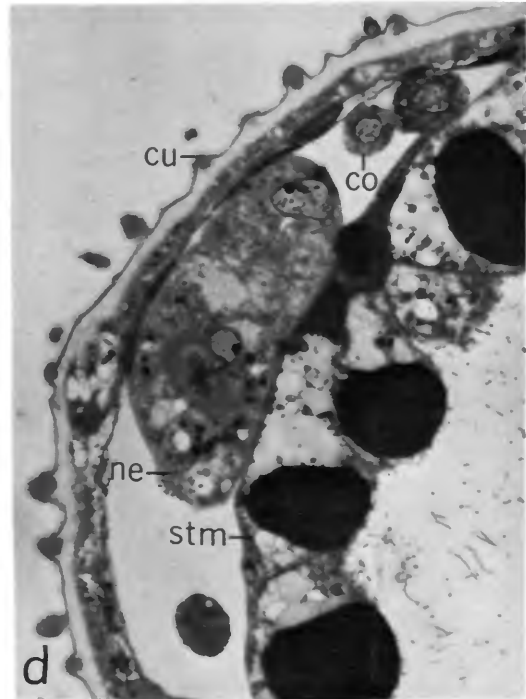
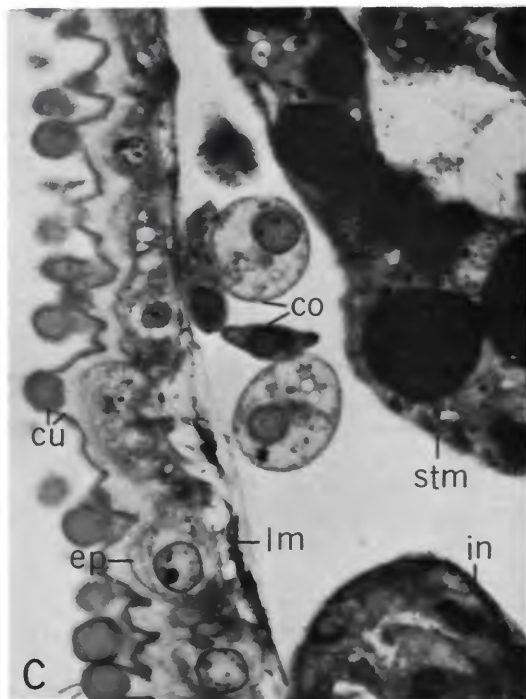
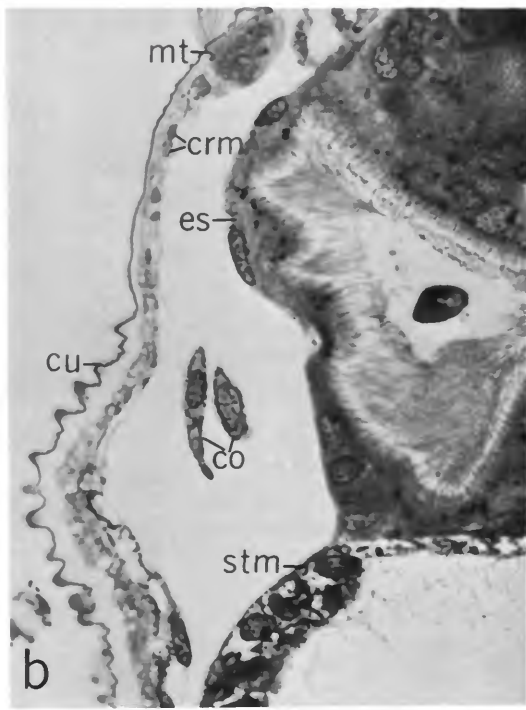
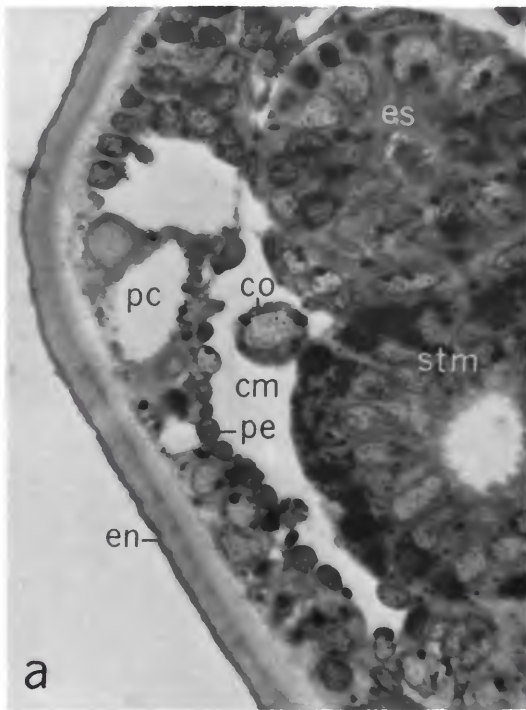


**PLATE 7**

Sections showing gut, coelomocytes and cuticle in premetamorphosis and larval stages of *Phascolosoma agassizii*. Epon. One micron. Richardson's stain.

- a. Frontal section of premetamorphosis stage. 7½ days (10°C). Note coelomocyte (co) which appears to be attached to the splanchnic peritoneum. The stomach (stm), in contrast to the esophagus, is marked by a dense concentration of darkly staining granules of varying sizes.
- b. Sagittal section of 28-day larva showing spindle-shaped coelomocytes (co), heavily ciliated esophagus (es), weakly ciliated stomach (stm), and circular muscles of the postmetatrochal sphincter (crm).
- c. Sagittal section of 43-day larva. Both round and spindle-shaped coelomocytes (co) are evident as well as the longitudinal musculature of the body wall and the papillated cuticle.
- d. Cross-section of 57-day larva through the stomach (stm) and nephridium (ne). Each cell of the wall of the stomach contains one large darkly staining granule or globule. In the nephridium the ciliated canals of the two arms are apparent: the upper is associated with the coelomic funnel and the lower terminates at the external pore.

[cm=coelom, co=coelomocyte, crm=circular muscle, cu=cuticle, en=egg envelope, ep=epidermis, es=esophagus, in=intestine, lm=longitudinal muscle, mt=metatroch, ne=nephridium, pc=prototrochal cavity, pe=peritoneum, stm=stomach.]

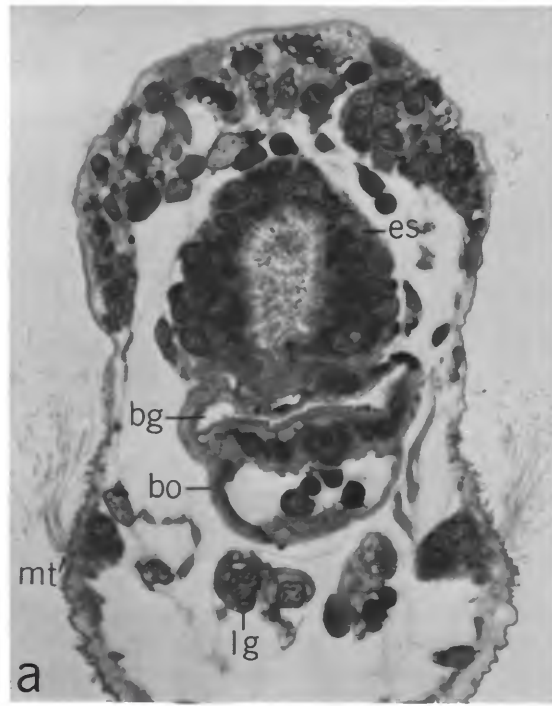


**PLATE 8**

Sections of larval organs of the mouth. Larvae of *Phascolosoma agassizii*. Epon. One micron. Richardson's stain.

- a. Frontal section of 60-day larva showing buccal organ (bo) with buccal groove (bg) and lip glands (lg).
- b. Sagittal section through lip and lip pore (lp) and associated organs of the lip of a 28-day larva. The arrow points to a neurosecretory cell overlying the esophagus.

[bg=buccal groove, bo=buccal organ, es=esophagus, lg=lip gland, lp=lip pore, mt=metatroch, vnc=ventral nerve cord.]

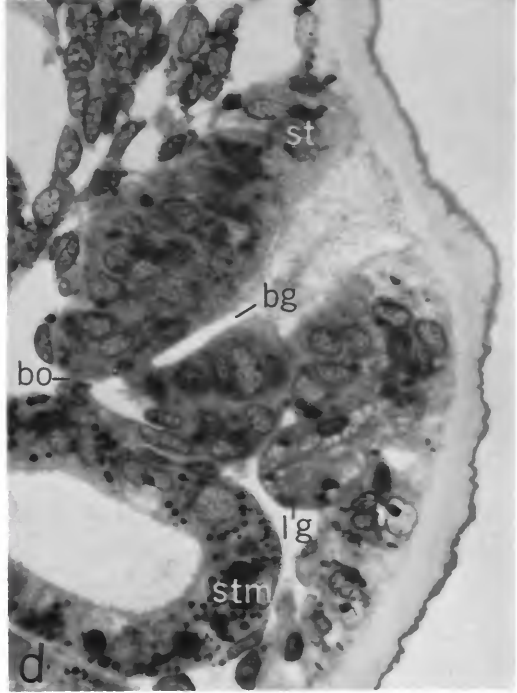
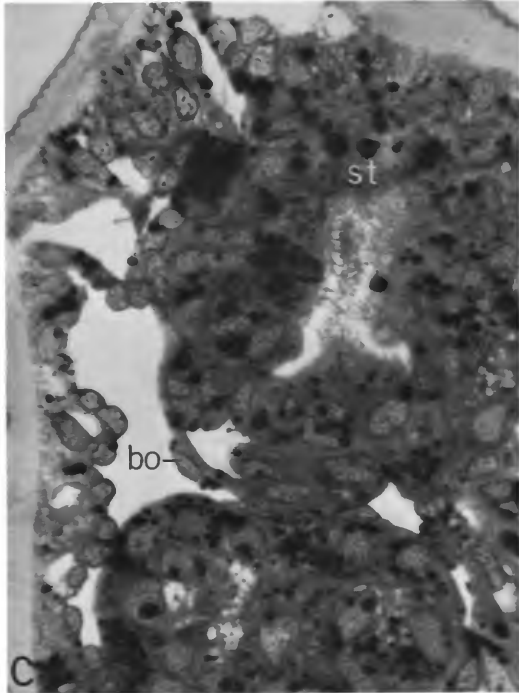
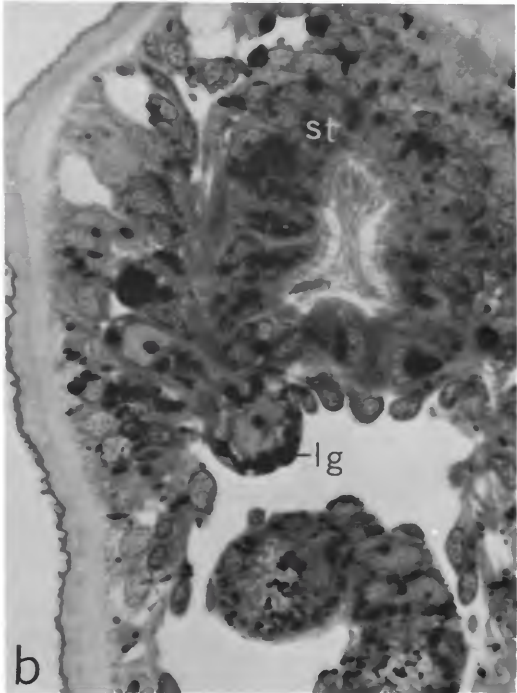
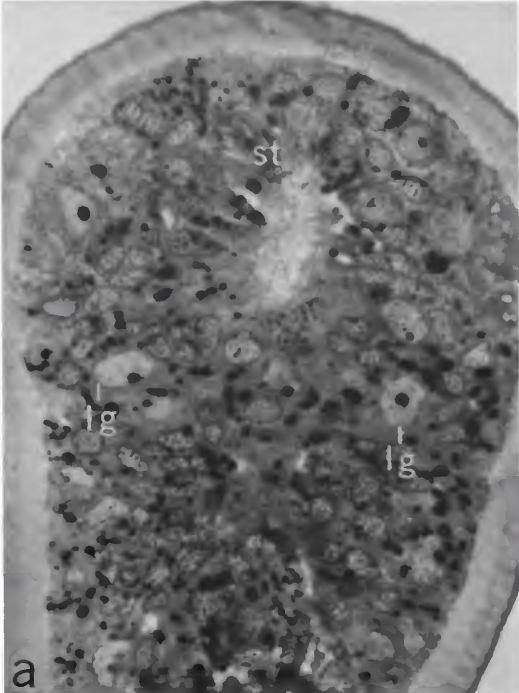


**PLATE 9**

Sections showing formation of organs of the mouth. *Phascolosoma agassizii*. Epon. One micron. Richardson's stain.

- a. Frontal section of premetamorphosis stage. 7 days (10°C). Note the two large cells of the future lip gland (lg) and the stomodaeum (st).
- b. Frontal section of premetamorphosis stage showing lip gland (lg) with cytoplasmic vacuoles and inclusions and stomodaeum (st). 9½ days (10°C).
- c. Frontal section of premetamorphosis stage showing buccal organ (bo) and stomodaeum (st). 9 days (10°C).
- d. Sagittal section of premetamorphosis stage. 9½ days (10°C). Note stomodaeum (st), buccal groove (bg), buccal organ (bo), lip gland (lg), and stomach (stm).

[bg=buccal gland, bo=buccal organ, lg=lip gland, st=stomodaeum, stm=stomach.]



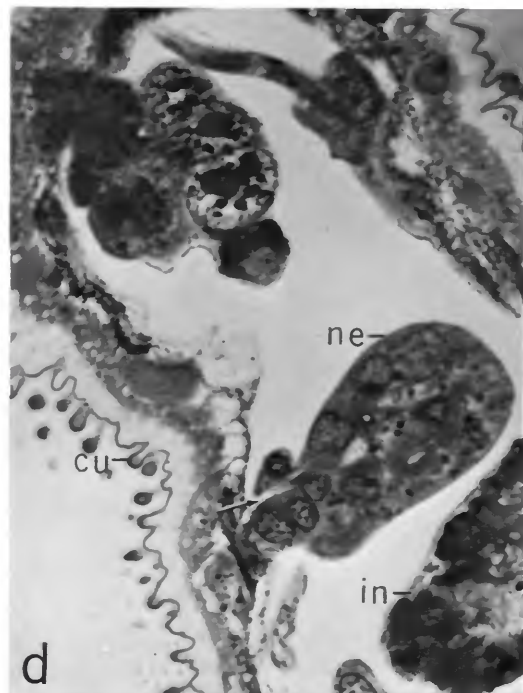
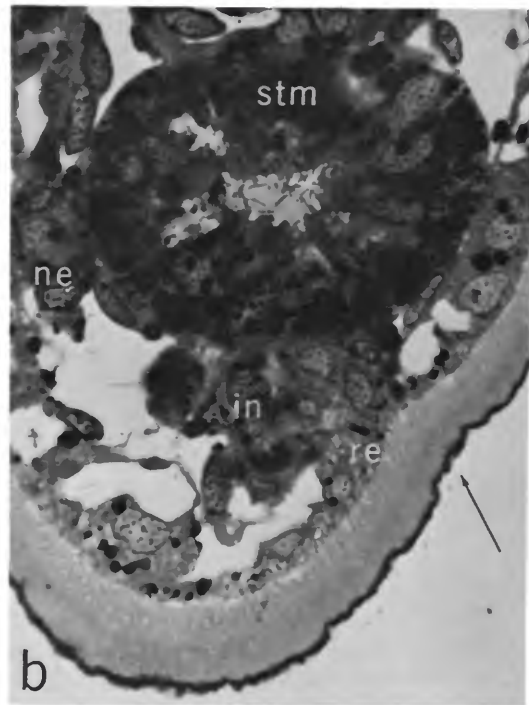
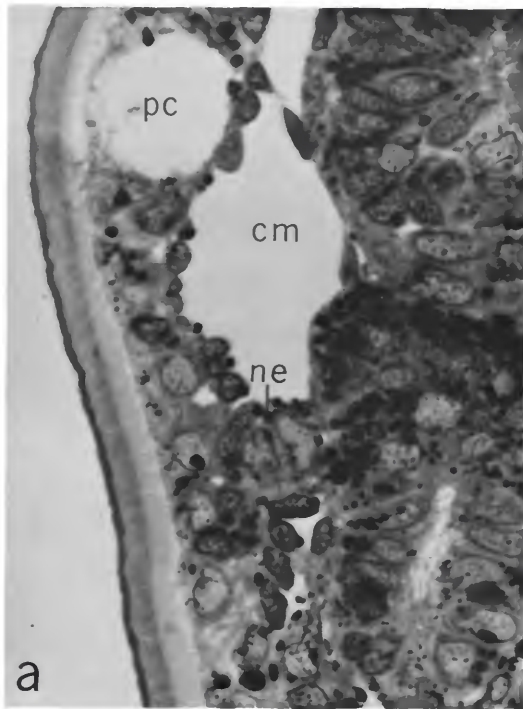
**PLATE 10**

Sections showing formation and structure of nephridium and anus of *Phascolosoma agassizii*. Epon. One micron. Richardson's stain.

- a. Frontal section of premetamorphosis stage. 7½ days (10°C). The nephridial rudiment (ne), consisting of two enlarged cells, is located within the mesodermal band which has split anterior and posterior to the developing nephridium to form the coelom (cm).
- b. Sagittal section of premetamorphosis stage showing rectal rudiment (re), intestine (in), stomach (stm) and nephridial rudiment (ne). Note a slight invagination of the egg envelope indicated by the arrow opposite the rectal rudiment; the envelope later will rupture at the site of the invagination to form the anus. 8½ days (10°C).
- c. Sagittal section of recently metamorphosed larva. The anal opening (a) has been formed, completing the intestine (in), and the nephridial rudiment (ne) has enlarged. 10 days (10°C).
- d. Sagittal section of 28-day larva showing the well-developed nephridium (ne). The arrow points to the ciliated funnel opening into the coelom.

[a=anus, cm=coelom, cu=cuticle, in=intestine, ne=nephridium, pc=prototrochal cavity, re=rectum, stm=stomach.]



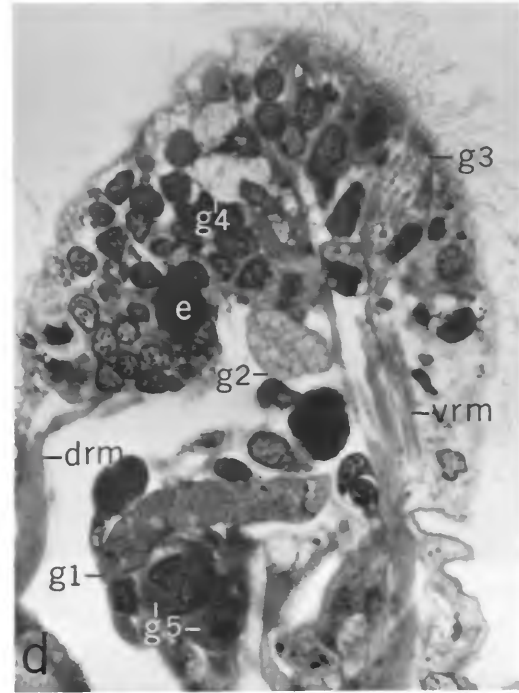
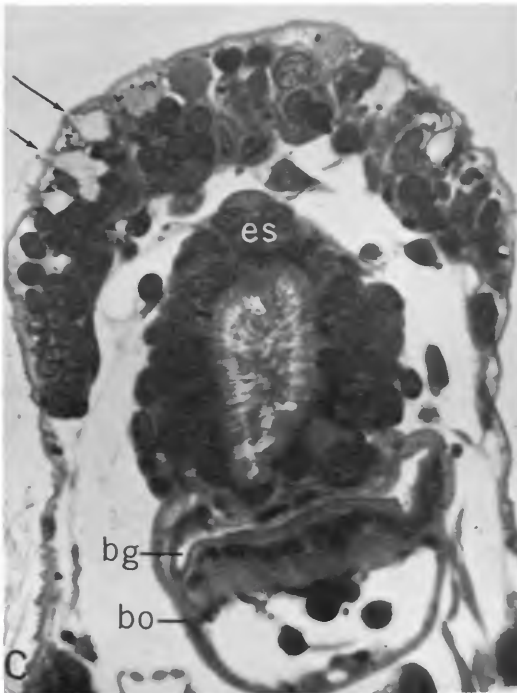
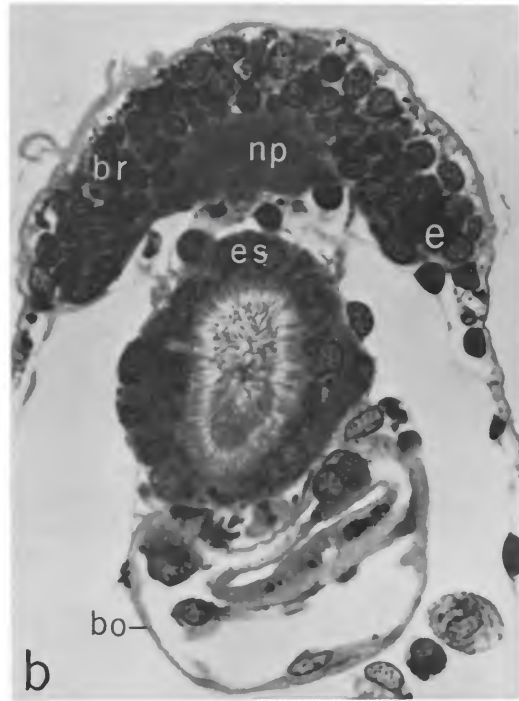
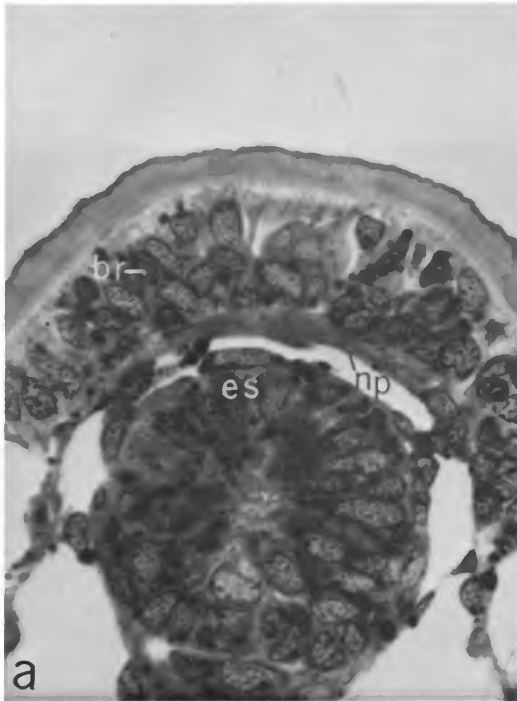


## PLATE 11

Sections of premetamorphosis stage and pelagosphera larvae of *Phascolosoma agassizii* showing development of the brain and the structure of the buccal organ and glands of the head. Epon. One micron. Richardson's stain.

- a. Frontal section of premetamorphosis stage through the region of the developing brain (br) and neuropile (np). 7½ days (10°C).
- b. Frontal section of 60-day larva showing brain (br), neuropile (np), eye (e), esophagus (es), and buccal organ (bo).
- c. Frontal section of 60-day larva. Head glands, designated by the arrows, open to the exterior. They are the same as that labeled g4 in the sagittal section pictured in 11d.
- d. Sagittal section of 28-day larva. Five different glands of the head are apparent in this section: g1, g2, and g3 are PAS-positive; g4 are the "foamy" glands with unidentified secretion; g5 have been characterized as neurosecretory cells.

[bg=buccal groove, bo=buccal organ, br=brain, drm=dorsal retractor muscle, e=eye, es=esophagus, g1-5=glands of the head, np=neuropile, vrm=ventral retractor muscle.]

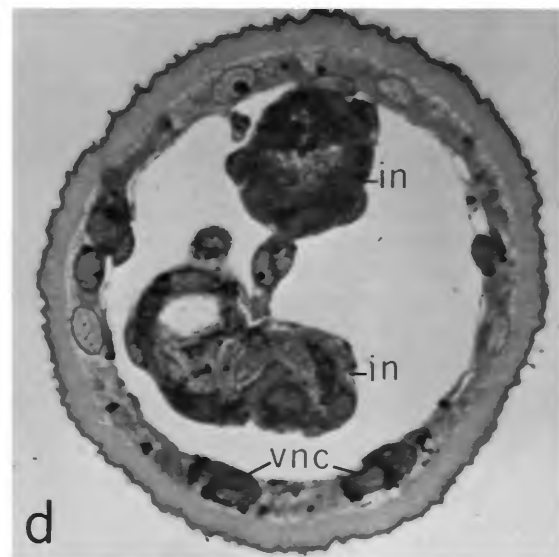
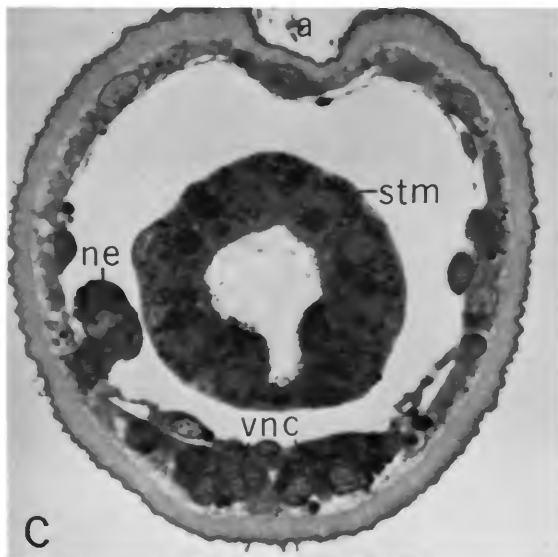
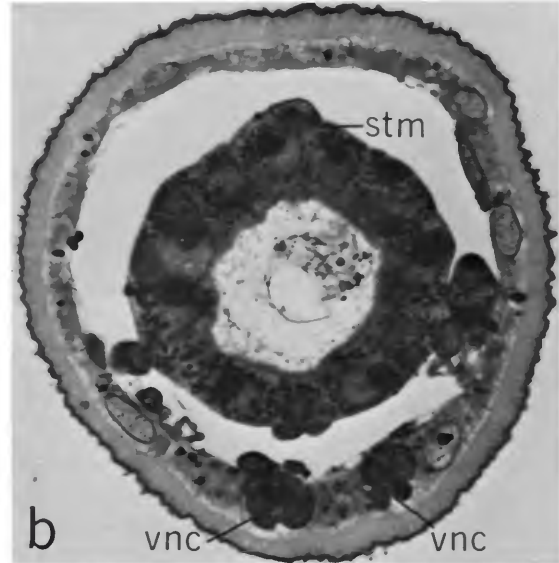
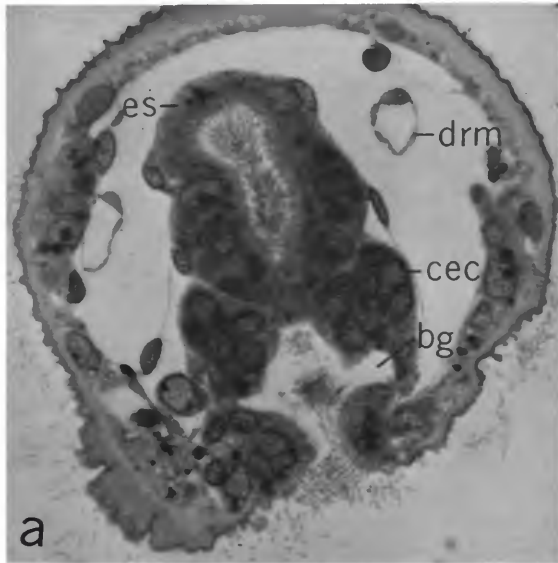


**PLATE 12**

Cross-sections of a recently metamorphosed pelagosphera larva of *Phascolosoma agassizii*. 10 days (10°C). Epon. One micron. Richardsons' stain.

- a. Cross-section through region of the lip.
- b. Cross-section through region of the mid-stomach. Double nature of the ventral nerve cord (vnc) is clearly apparent.
- c. Cross-section through anus and nephridium.
- d. Cross-section of posterior body through intestine.

[a=anus, bg=buccal groove, cec=circumesophageal connective, drm=dorsal retractor muscle, es=esophagus, in=intestine, ne=nephridium, stm=stomach, vnc=ventral nerve cord.]

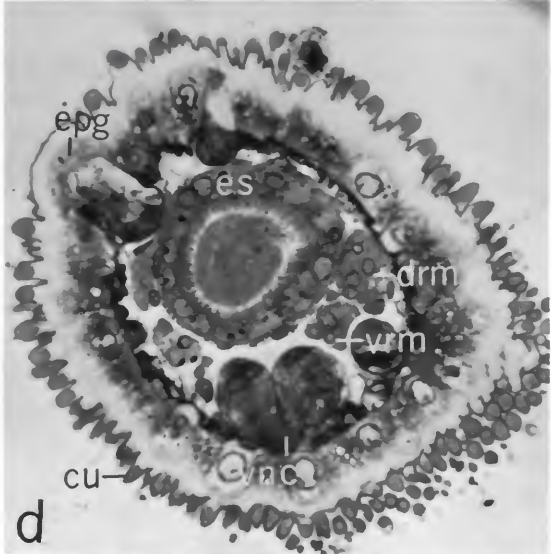
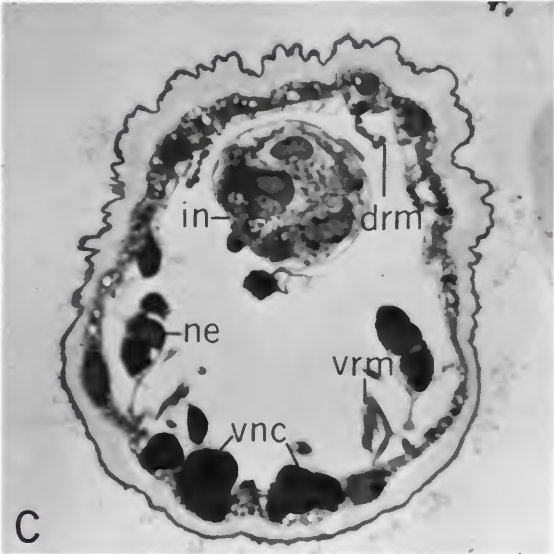
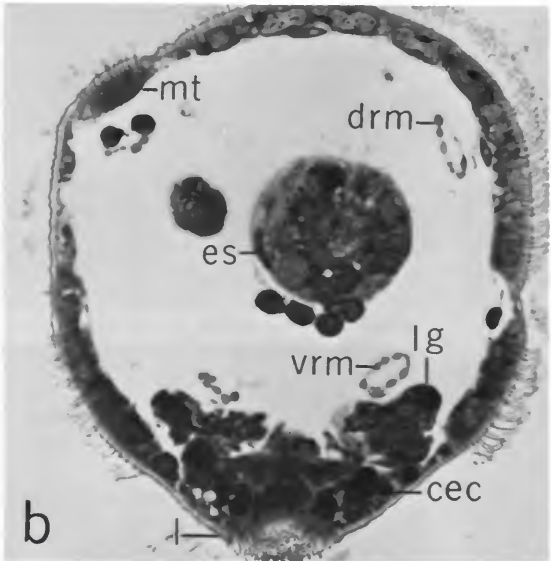
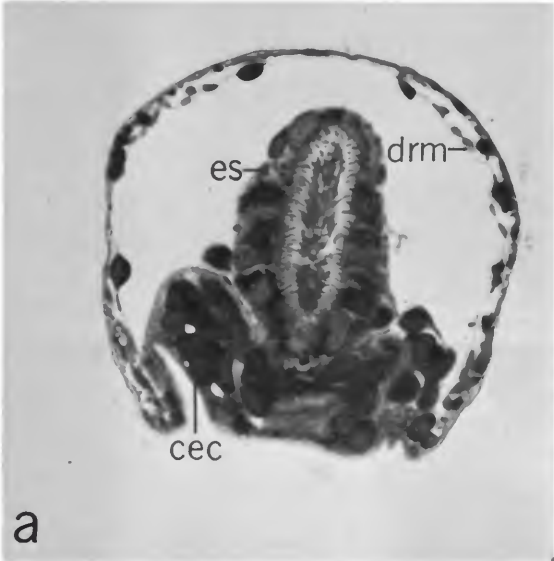


**PLATE 13**

Cross-sections of pelagosphera larvae of *Phascolosoma agassizii*. Epon. One micron. Richardson's stain.

- a. Cross-section of a 22-day larva through the region of the head. Note that in this region the dorsal retractor muscles (drm) are attached to the peritoneum of the body wall.
- b. Cross-section of a 22-day larva through the metatroch (mt) and bilobed lip (l). Dorsal and ventral retractor muscles (drm, vrm) are seen suspended in the coelom as circles of fibers.
- c. Cross-section of a 22-day larva through the region of the nephridia (ne). Some of the fibers of the ventral retractor muscles (vrm) insert into the body wall in this section.
- d. Cross-section of a 57-day larva through the region of the contracted postmetatrochal sphincter. At this age the epidermal glands (epg) are well developed, the retractor muscles (drm, vrm) have increased in thickness and number of fibers and the nerve cord is bilobed.

[cec=circumesophageal connectives, cu=cuticle, drm=dorsal retractor muscle, epg=epidermal gland, es=esophagus, in=intestine, l=lip, lg=lip gland, mt=metatroch, ne=nephridium, vnc=ventral nerve cord, vrm=ventral retractor muscle.]



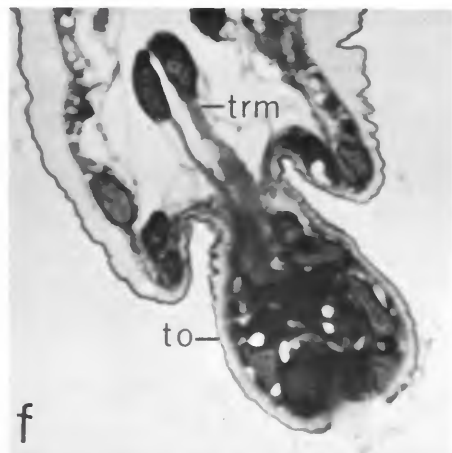
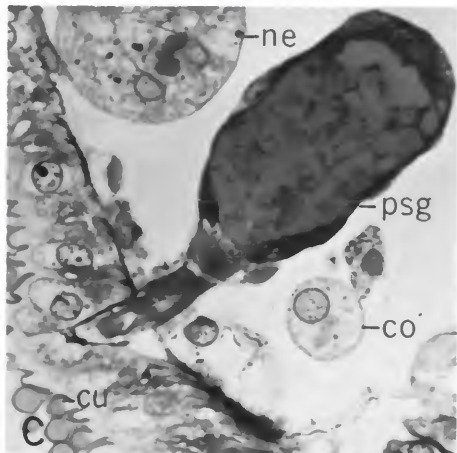
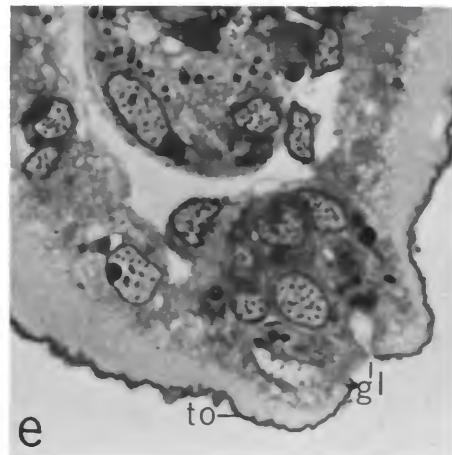
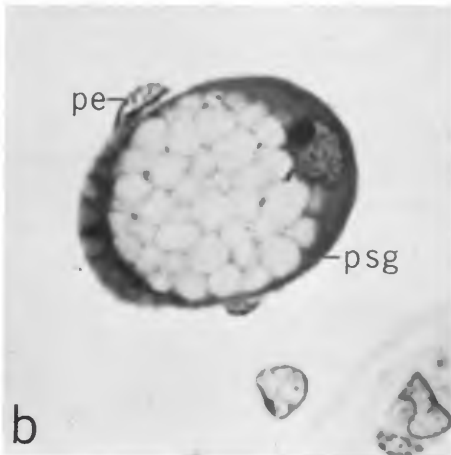
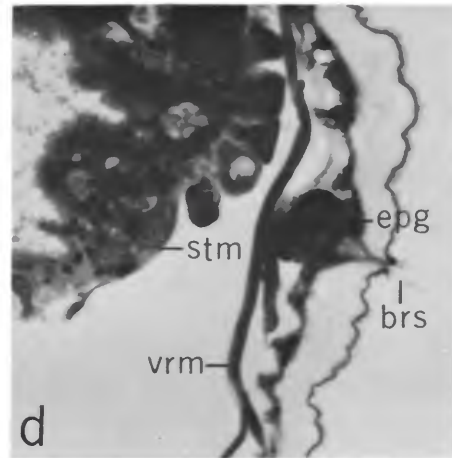
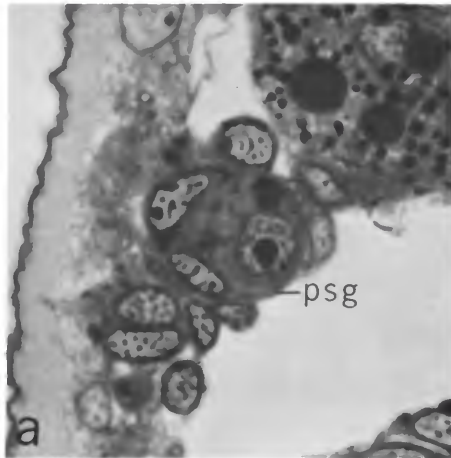
## PLATE 14

Sections of premetamorphosis and larval stages of *Phascolosoma agassizii*. Stages in the formation of the posterior sacciform glands and the terminal organ are illustrated. Epon. One micron. Richardson's stain.

- a. Sagittal section of beginning metamorphosis stage showing posterior sacciform gland (psg). The nucleus of the gland cell is distinguished by the large eccentric nucleolus.
- b. Oblique cross-section of the distal end of the posterior sacciform gland (psg) of a 28-day larva through the secretory cell with its characteristic nucleus. The large, unstained granules of the central cavity of the gland are of unidentified composition.
- c. Sagittal section of posterior sacciform gland (psg) in a 43-day larva. Filled with amorphous secretory material, the central cavity of the gland is surrounded laterally by supportive cells and distally by the secretory cell. The cavity is continuous with the exterior by way of the proximal canal which penetrates the body wall.
- d. Sagittal section of 22-day larva showing anterior epidermal gland (epg) with bristle (brs).
- e. Sagittal section of beginning metamorphosis stage showing the terminal organ (to) in an early stage of formation. Note the gland cells (gl) which open to the exterior through the aperture at the distal end of the terminal organ.
- f. Sagittal section of the terminal organ (to) of a 22-day larva. This section shows the aperture at the end of the terminal organ and the retractor muscles of the terminal organ (trm).

[brs=bristle, co=coelomocyte, cu=cuticle, epg=epidermal gland, gl=gland of terminal organ, ne=nephridium, pe=peritoneum, psg=posterior sacciform gland, stm=stomach, to=terminal organ, trm=retractor muscle of terminal organ, vrm=ventral retractor muscle.]







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