# EMBRYONIC DETERMINATION IN THE ANNELID, SABELLARIA VULGARIS

## I. The Differentiation of Ectoderm and Endoderm When Separated through Induced Exogastrulation

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### Introduction

The method of transplantation has brought to light the abilities of certain embryonic parts to induce, or organize, the development of other embryonic structures in adjacent tissues. The use of this technique has, however, only recently been extended to the so-called "mosaic" eggs. Tung (1934) reported experiments involving the fusion of eggs at the two-cell stage, fusion of micromeres and macromeres, and rotation of micromeres in the eggs of *Ascidiella*. He found no deviation from the normal in the development of the cells giving rise to notochord and muscle. However, there was evidence that the sense organ and the ectodermal adhesive organ were induced. Two years later, there appeared abstracts by Hörstadius (1936) and by the writer (1936) describing the development of transplanted blastomeres of *Cerebratulus* and *Sabellaria* respectively. Hörstadius (full report, 1937) found no evidence of induction in fusions of various quartets of the 16-cell stage. The essentially similar results of the writer will be described in greater detail in the second paper of the present series.

It is the purpose of this first paper to describe the results of an experiment which, though similar to isolation experiments, possesses certain of the added advantages of the transplantation method. The experiment consists of the production of larvæ in which the endoderm cells grow out, during gastrulation, from their normal position underlying the ectoderm. This affords a way of testing the independent differentiation of the two layers and, by inference, the effect of one layer upon the other during normal development.

### Material and Methods

The egg used is that of the marine annelid, *Sabellaria vulgaris*. That this is an egg of the classical mosaic type is shown by the completely partial development of isolated blastomeres (Hatt, 1932). The procedure for obtaining fertilized eggs of this species has been described elsewhere (Novikoff, 1937). The larvæ of this species are closely similar to those described by Wilson (1929) for the two European species, *alveolata* and *spinulosa*.

Exogastrulae can be produced in considerable numbers by a treatment which removes the vitelline membranes of fertilized ova. A variation of the method employed by Hatt (1931) has proved to be quite effective. At any time before they have begun to change their form in preparation for cleavage, the eggs are washed once with an isotonic NaCl solution brought to pH 9.6 by the addition of $Na_2CO_3$ (.98 gram in 1 liter of solution) and are then placed into 5 cc. of the same solution. The eggs adhere to each other in this medium, forming large aggregates, but as the egg membranes disappear, the aggregates break up. When this has occurred, 5 cc. of acidified NaCl solution (0.45 cc. 1.0 N HCl in 100 cc. 0.53 N NaCl) are added, bringing the medium to pH 8.2. When the eggs have settled to the bottom of the dish, they are washed once in sea water and transferred to a Syracuse dish containing fresh sea water. Among the swimming larvæ which develop, the exogastrulæ can readily be distinguished from the normal larvæ in the dish.

Representative stages in the development of exogastrulæ and control larvæ are described in the text. All text figures are camera lucida drawings of living larvæ; the description accompanying each figure refers not to that specific larva but is a general description of larvæ at that stage in development. The time intervals given are approximate, for temperatures ranging from 19° to 24° C.

## Description of Results

It is possible to follow the details of ectodermal and endodermal differentiation in living larvæ. In the normal course of development the ectoderm gives rise to the following structures: a prototroch of long active cilia, at first encircling the animal but later incomplete on the dorsal surface; chromatophores, which are yellow when they form but later change to green; an apical tuft which disappears when replaced by short non-motile cilia; paired chætæ-sacs from which extend long serrated bristles; a single, short, non-motile posterior cilium; several long, non-motile dorsal cilia in the region of the prototrochal gap; a single orange-red eye spot on the left side, towards the dorsal surface; and rapidly moving cilia ("neurotroch") in a ventral depression leading to the mouth cavity of the fully formed trochophore. The endoderm differentiates into a tripartite gut consisting of œsophagus, stomach, and intestine. The œsophageal cells are lined internally with many long actively-moving cilia. The stomach and intestine are also ciliated but the motion of their cilia differs from that of the œsophagus; they move more slowly and remain more rigid during the effective stroke.

In order to compare the development of the eggs from which the membranes have been removed with that of the normal eggs, a series of stages in the development of both are described. Those stages have been chosen which show definite advances in structural differentiation.

The early cleavages of the membraneless eggs are similar in all respects to those of normal eggs. Although the details of the later cleavages have not been described for this species, any marked difference in this process between the two types of eggs would have been noted. But no such variations were apparent. The first noticeable departure from normal development appears at the time when the larvæ show the first signs of movement. This is usually between six and seven hours after fertilization and probably corresponds to the time when gastrulation is normally occurring.

*Comparison of Complete Exogastrulæ with Normal Larvæ*

*Six to Seven-hour Larva.*—Normal (Fig. 1*A*). There is no regularity in the shape of the larvae at this time; it may vary from a spherical to a roughly conical form. The vitelline membrane, retaining the many wrinkles characteristic of the earlier stages in development, is still far removed from the body of the larva. Through the membrane, there project a group of long cilia at the future anterior end of the larva, and many short, rapidly moving cilia which form a narrow girdle completely encircling the larva.[1] These cilia constitute the apical tuft and the prototroch respectively. The larva is rather opaque and it is with difficulty that the large internal cells can be distinguished. Directed towards the inner cells and situated slightly posterior to the prototroch is the blastoporal indentation.

Exogastrula (Fig. 1*B*). These larvæ are more uniformly regular than the normal larvæ of the same age. Not obscured by the vitelline membrane as they are in the normal larvæ, the cilia of the apical tuft and prototroch are more easily distinguished. The distance between the apical end and the prototroch (i.e., the pretrochal region) is the same as it is in the normal larva, but the post-trochal region is considerably lengthened by a posterior outgrowth of cells. No blastopore is present.

*Thirteen-hour Larva.*—Normal (Fig. 1*C*). The larva has now rounded out and there are only slight irregularities in the surface.

[1] The cilia in the early larvæ may be brought out more clearly with darkfield illumination.

It has elongated somewhat in the antero-posterior direction and is just beginning to assume the typical trochophore appearance. The apical tuft is directed forward as the larva swims, rotating on its longitudinal axis. The prototroch, which formed a complete ring about the animal at the preceding stage, is interrupted by a short region with no cilia; this gap marks the dorsal surface of the trochophore. At the posterior, broader end there projects a fine non-motile cilium. The surface of the larva now has a general gold-yellow coloration when viewed by transmitted light. Through the outer ectoderm cells the "anlage" of the gut is discernible. From a crescent-shaped cavity at the apical end, a narrow canal leads through the gut cells to open on the surface at the posterior end. The blastopore, which has become progressively smaller up to the eleventh hour of development, has completely disappeared.

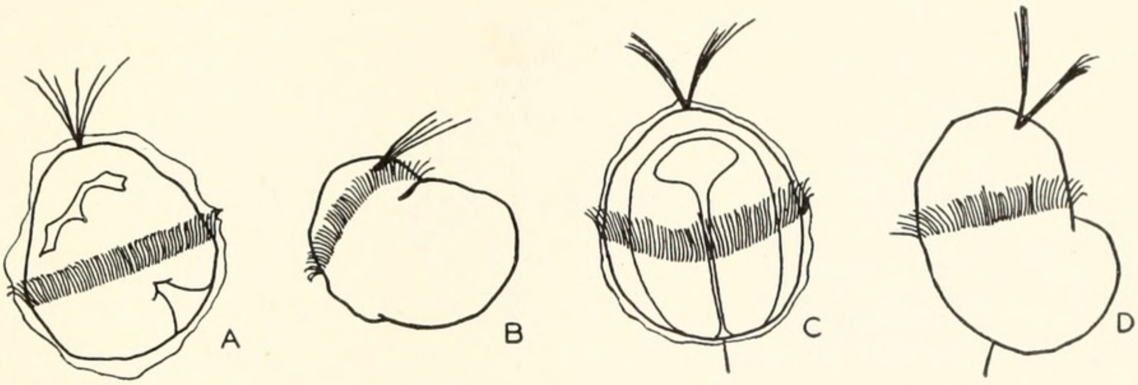


FIG. 1. *A*. Normal larva. Seven hours after fertilization. Shows apical tuft and prototroch. Posterior to the prototroch is the blastoporal indentation. *B*. Exogastrula. Seven hours. Viewed from posterior end. Shows apical tuft, prototroch, and elongate post-trochal region. *C*. Normal larva. Thirteen hours. Shows apical tuft, prototroch, posterior cilium, and differentiating gut. *D*. Exogastrula. Thirteen hours. Shows apical tuft, prototroch, posterior cilium, and elongate post-trochal region.

Exogastrula (Fig. 1*D*). This larva is considerably more elongate, antero-posteriorly, than the normal larva of the same age. The structure of the apical tuft, prototroch, and posterior cilium is normal, and the apical tuft is in the usual location. On the other hand, the relative positions of both the prototroch and the posterior cilium are strikingly different. The former is situated more anteriorly in the larva; the latter is to one side of the larva and projects laterally instead of posteriorly. The general yellow coloration of the embryo does not extend very far beyond the equator; the cells at the posterior end are colorless. The crescent-shaped cavity and the longitudinal canal present in the endoderm cells of the normal larva at this stage are not present.

*Eighteen-hour Larva.*—Normal (Figs. 2*A* and *B*). Since the preceding stage, the larva has become more spherical and more regular in outline. The membrane, almost free of irregularities, is more closely applied to the larval surface. The cilia of the apical tuft are now considerably longer and they move slowly in two distinct groups. The prototrochal gap has grown in size; the posterior cilium is still present, and the yellow pigment has become localized into a number of larger areas on the surface of the body. The endoderm cells within are now assuming the form of the gut; one can recognize the three main divisions—the ventral œsophagus runs anteriorly into the wider stomach, which in turn is continuous with the intestine. The only lumen visible in the gut is the narrow canal described in the earlier larva; it runs through the stomach and intestine and opens as the anus. Slightly anterior to the prototroch and on either side of the

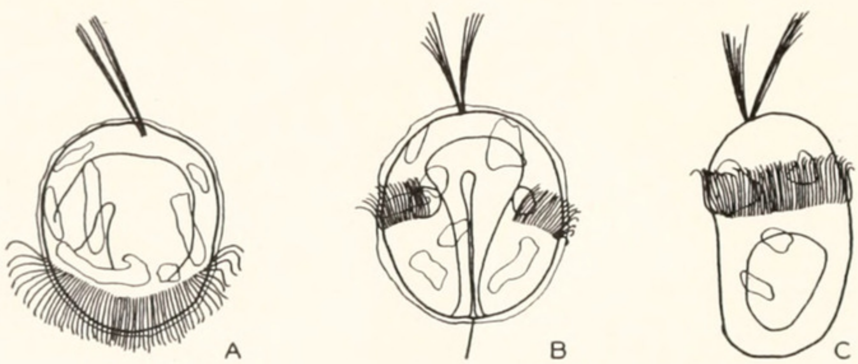


FIG. 2. Larvæ, eighteen hours after fertilization. *A*. Normal larva. Side view. Shows apical tuft, prototroch, gut, and pigmented areas. *B*. Normal larva. Dorsal view. Shows prototrochal gap, stomach and intestine portion of gut, and posterior cilium. *C*. Exogastrula. Post-trochal region is elongated and hollow.

stomach there has developed a spherical structure, the chæta-sac, from which the long bristles later grow.

Exogastrula (Fig. 2*C*). Changes similar to those described for the normal larva have occurred in the apical tuft, prototroch, and pigmentation of the exogastrula. The apical tuft is composed of two groups of long cilia. The prototrochal gap extends over the dorsal surface. The yellow pigment has become more localized, but since the pigment was originally restricted to the more anterior part of the larva, the posterior portion is completely colorless. The difference between this larva and the normal one of the same age is, however, much more pronounced than at the preceding stage. In contrast to the fairly spherical form of the normal, it is narrow and long. It possesses no internal gut and in the posterior half there appears a large cavity. The posterior cilium is again laterally displaced.

*Twenty-four-hour Larva.*—Normal (Figs. 3*A* and *B*). Important changes have occurred between this and the preceding stage of development. In the apical region there have appeared, alongside the apical tuft, a number of short, non-motile cilia. On the dorsal surface of the larva, in the region of the enlarged prototrochal gap, there are two or three long non-motile cilia. The posterior cilium is still short and non-motile. The large pigmented areas have been broken up into well-defined yellow chromatophores. The gut has acquired a continuous lumen, lined with rapidly moving cilia. The cilia of the œsophagus differ from those of both the stomach and intestine in that they are slightly longer, are more numerous, per unit surface area, and move more rapidly. In their movement, they are bent more than are the stomach-intestinal cilia. The œsophagus opens to the exterior through

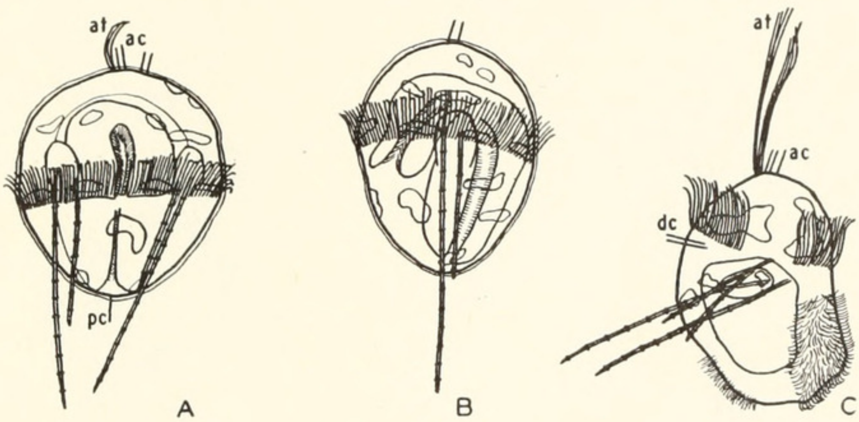


Fig. 3. Larvæ, twenty-four hours after fertilization. Abbreviations: *at*, apical tuft; *ac*, apical cilia; *pc*, posterior cilium; *dc*, dorsal cilia. *A*. Normal larva. Ventral view. Mouth, œsophagus, and intestine visible. The pigmented areas are broken up into well-defined chromatophores. Two pairs of bristles are present. *B*. Same larva as *A*. Side view. *C*. Exogastrula. Shows elongate, hollow, post-trochal region. On the surface, toward the posterior end, short cilia are visible.

the mouth, the intestine through the anus. From each of the chætæ-sacs there now extend two bristles. The position and structure of these bristles are very characteristic. Arranged at intervals along their length are serrated "collars." As the larva swims about, the bristles, projecting posteriorly, are seen being rotated about the long axis of the animal. That muscles have developed can be deduced from the observed contraction of the larvæ and the movement of the chætæ-sacs and bristles.

Exogastrula (Fig. 3*C*). At this stage, the exogastrulæ can easily be distinguished, even with low magnification, from the normal animals. The most striking feature is the behaviour of the bristles when the animal is in motion. The larva rotates as usual as it moves in a forward direction. But the bristles project laterally instead of posteriorly, and they are accordingly turned over and over in a wide arc, rather than rotated on the longitudinal axis of the larva. Viewing the animal from above, the bristles therefore appear and disappear from sight. No gut is present, and the cavity of the preceding stage has increased noticeably in size. On the surface of the posterior portion of the larva there are now rapidly beating cilia, of the same size as the cilia in the gut of the normal larva. They are usually found in two distinct extensive areas, with the larger of the two areas covered with cilia characteristic of the normal œsophagus. It is possible to distinguish the single asymmetrically-placed posterior cilium. The larvæ show the following changes over the preceding stage, which are similar to the changes in normal development: (1) the appearance of short, non-motile cilia at the apical end; (2) the formation of smaller, yellow chromatophores, although in the exogastrulæ they are

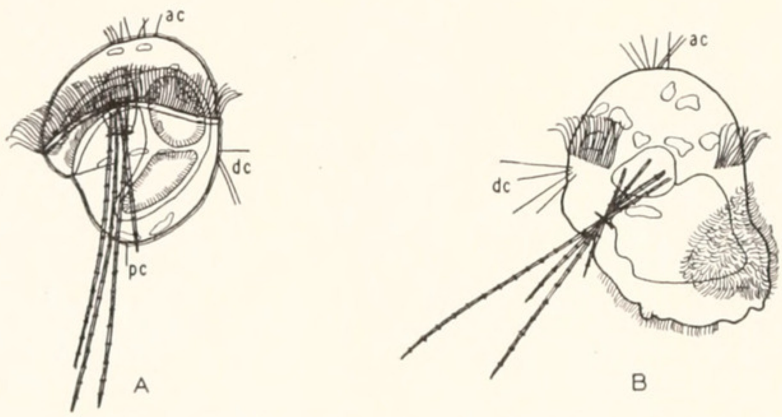


FIG. 4. Larvæ, twenty-nine hours after fertilization. Abbreviations as in Fig. 3. *A*. Normal larva. Side view. Mouth, stomach, and intestine are well-formed. The bristles on the left side only have been drawn. *B*. Exogastrula (slightly flattened). Dorsal view. Only four of the eight bristles have been drawn.

limited to little more than the anterior half of the larva; (3) the development of dorsal cilia near the gap in the prototroch; (4) the appearance of contractions of the larva, albeit these contractions are considerably weaker than those of the normal larva; (5) the formation of two pairs of bristles of the same notched structure as described for the normal.

*Twenty-nine-hour Larva*.—Normal (Fig. 4*A*). The larva is beginning to assume the appearance of the fully-developed trochophore. The membrane is closely applied to the surface of the larva. The apical tuft has disappeared, and only the non-motile cilia remain at the anterior end. The prototroch is raised slightly, forming the so-called "hood fold" (Wilson, 1929). The single posterior cilium persists. The small yellow chromatophores are taking on a green tinge. The stiff dorsal cilia are longer and more numerous than in the previous stage. The œsophagus, lined with many active cilia, leads into the stomach. The stomach is partially separated from the intestine by an incomplete shelf; the intestine is lined with cilia of the type found in the stomach. The number of bristles varies from three to four pairs. On stimulation (i.e., when the slide or the dish is tapped gently), the larva rapidly contracts and the bristles are spread into a fan-like arrangement. After several seconds in this position, the animal returns to its usual form.

Exogastrula (Fig. 4*B*). The general appearance of the larva is similar to that of the preceding stage. The three or four pairs of bristles project laterally and, as the animal moves, are rotated in the manner described above. On stimulation, they are spread out as in

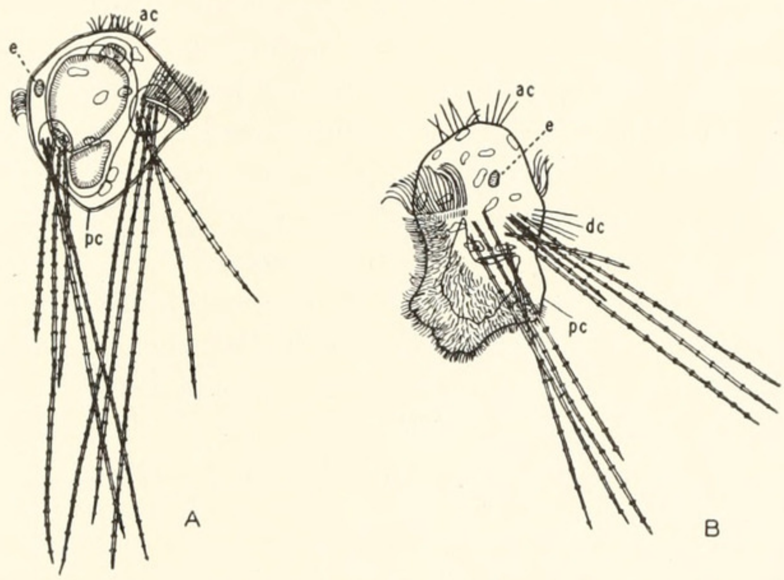


FIG. 5. Larvæ, forty-eight hours after fertilization. Abbreviations as in Fig. 3. *A*. Normal larva. Dorsal view. To the left of the stomach is the eye spot (*e*). *B*. Exogastrula. Dorsal view. A large part of the post-trochal region is covered with short cilia.

the normal larva, although to a lesser extent. There is no internal gut, and a large cavity is present inside the larva. The chætæ-sacs project into the cavity. As in the normal larva of this age, only the apical cilia persist at the anterior end. The dorsal cilia have elongated and increased in number. The chromatophores, limited to about the anterior half of the larva, are becoming greener in color. The cilia on the exterior of the post-trochal region are found in two regions. Of these only the ventral region has the cilia of the œsophageal type.

*Forty-eight-hour Larva.*—Normal (Fig. 5*A*). This is a well-developed trochophore. The post-trochal region has lengthened somewhat. The apical cilia are numerous and the number of dorsal cilia has also increased. There is a single posterior cilium. A second, posterior row of short cilia has appeared on the hood fold, just posterior to the long prototrochal cilia. On either side of the mouth there has developed a fold covered externally with cilia, the lip fold. The chromatophores have a more pronounced green coloration. On the left side of the larva, near the stomach, there has appeared a single eye spot, composed of closely packed orange-red granules. The gut is clearly differentiated into œsophagus, stomach, and intestine. The stomach has increased in size and has become more spherical. Running posteriorly from the mouth, in a groove along the ventral surface, are the long cilia which constitute the neurotroch. A larva at this time ordinarily has four pairs of bristles, which project posteriorly.

Exogastrula (Fig. 5*B*). Changes similar to those occurring in the normal larvæ have taken place between this and the preceding stage. The apical cilia have become more numerous. The prototroch is better developed and a row of short cilia has been added posterior to it. The dorsal cilia have increased in number and size, and the posterior cilium is single and dorsal. On the dorsal surface, anterior to the prototroch, may be seen the eye spot. Four pair of bristles project laterally as do the bristles of the earlier larvæ. There is no internal gut. The cilia characteristic of the œsophagus and the intestine, together with the cilia of the neurotroch and the lip fold, cover the post-trochal region almost completely. Special mention must be made of the eye spot, since it was not seen in all the larvæ examined. It could not be found in thirteen of the ninety-three larvæ studied.

*Partial Exogastrulæ*

There are always found in the culture dishes containing the membraneless larvæ a few larvæ which are intermediate in structure between those described as complete exogastrulæ and the normal. These larvæ are illuminating for an understanding of the structure of the more extreme type. Figure 6 shows two views of a well-developed trochophore of this type. Not all the structures are visible in the drawings, but the larva possesses every structure found in the normal animals, although these structures have in some cases differentiated in abnormal positions. The most outstanding feature of these larvæ is the gut structure. The stomach and intestine are clearly normal in form, position, and type of ciliation. The intestine opens posteriorly, as usual, through the anus. At the anterior end of the stomach there is a circular opening lined with cilia which move in a manner characteristic of the cilia of the œsophageal-stomach opening in the normal larva. But this opening leads here not to the œsophagus, but to the outer surface of the larva. In fact, there is no internal œsophagus at all. On the ventral surface of the larva is a long, conical outgrowth, completely covered with long actively-moving cilia which are characteristic of the œsophagus.

## Discussion of Results

There are two important points of difference between larvæ which develop from eggs without membranes and normal larvæ. The first is the complete absence of an internal gut and the second the striking deviation from the normal form of the larva. Instead of possessing the fairly spherical shape, they have elongate bodies in which the posterior structures, the posterior cilium and the paired bristles, are dorsally displaced. The distortion of the posterior part of the larva may be accounted for if we assume that the endoderm cells, which differentiate normally inside the ectoderm to form the gut, grow out so that they come to lie on the surface of the larva.

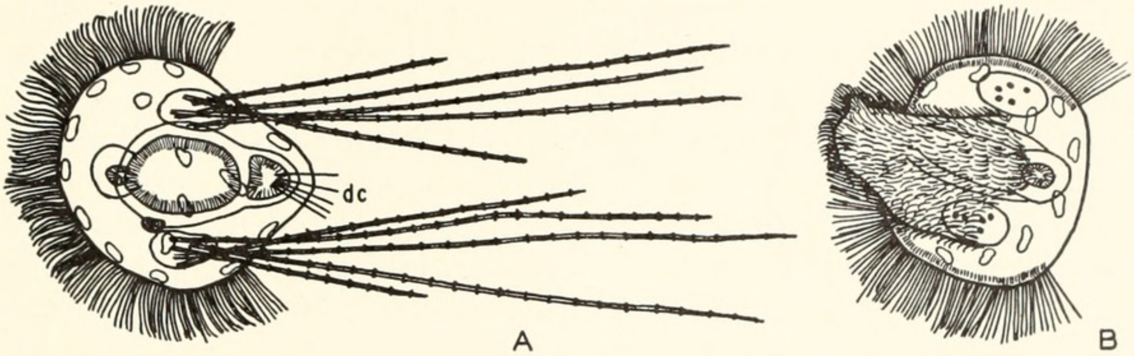


Fig. 6. Partial exogastrulæ. Seventy-two hours after fertilization. *A*. Dorsal view. The opening to the stomach, the stomach, and the intestine are visible. *B*. Ventral view. The bristles have been omitted in the drawing; only their points of origin in the chætæ-sacs are indicated. The opening to the stomach may be seen at the base of the long conical projection covered with short cilia.

That this posterior outgrowth of the endoderm cells has indeed occurred is shown by the following considerations: 1. The gut is completely lacking in these larvæ, and within the post-trochal region of the larva there is a large cavity. 2. The cells situated at the posterior end of these larvæ are not pigmented and never develop chromatophores. In the normal larva the chromatophores are found in the ectoderm; only the endoderm is colorless. 3. In the normal larva, motile cilia do not appear on the external surface of the post-trochal region until approximately thirty-five hours after fertilization. When they do develop, they are restricted to a narrow region, the neurotroch. But in the membraneless larva cilia begin to appear on the surface of the post-trochal region before the end of the first day of development. By twenty-four hours after fertilization they have covered two wide areas. That these cilia are not those of the neurotroch is clear from the fact that they cover two extensive regions and that they develop twelve hours earlier than do the neurotrochal cilia. Their appearance coincides precisely with the development, normally, of cilia on the inner surface of the endoderm cells. It is also possible to detect differences in the cilia which correspond to the differences between the œsophageal cilia and the cilia of the stomach and intestine.

We may therefore conclude that in these eggs the endoderm has been turned inside out. Whatever the mechanism involved, the end result of this process is comparable to the exogastrulæ that have been described in the sea-urchin (Herbst, 1893; Driesch, 1893) and in the amphibia (Holtfreter, 1933*a*, *b*).

In the partial exogastrulæ, the stomach and intestine develop normally, and only the œsophagus is exogastrulated. And, as we would expect on the basis of our assumption, the general form of the larva is more like the normal than is that of the complete exogastrula.

The most significant feature of exogastrula development in *Sabellaria* is the complete self-differentiation of both endoderm and ectoderm. In the amphibian egg, Holtfreter found that normal differentiation of the ectoderm is dependent upon contact with the mesendoderm. When the latter, instead of coming to lie beneath the ectoderm as it does normally, evaginates, it leaves the ectoderm a wrinkled, hollow sac in which no trace of differentiation into nervous tissue appears. On the other hand, the mesendoderm, although turned inside-out, undergoes self-differentiation; it produces gut, thyroid, pancreas, liver, notochord, musculature, kidney, and gonad. Thus, in the normal course of development, the endoderm differentiates independently of the ectoderm, but the ectoderm must be in contact with the endoderm, or gut roof, to differentiate normally. It is this gut roof which acts in the capacity of an organizer—which induces the formation of a nervous system in the ectoderm.

In *Sabellaria*, on the other hand, there is no deviation from normal differentiation of either the ectoderm or endoderm in the exogastrulæ. The endoderm cells retain their morphological polarity, so that they develop cilia on what is in these larvæ their external surface. And although the tripartite nature of the gut is lost, the type of cilia developed by the exogastrulated œsophagus cells differs from that of the stomach and intestine in the same way as do the normal œsophageal cilia from the normal stomach-intestinal cilia. The ectoderm, too, differentiates normally. The pigment develops and concentrates into yellow areas, which later break up into smaller chromatophores and become distinctly green in color. The apical cilia form at the anterior end and the apical tuft disappears. The serrated bristles develop normally, although the cells from which they arise have been displaced to one side (dorsally). On the dorsal side there develops the orange-red eye spot. Muscles, as evidenced by contractions of the larva, are also differentiated.

Thus, we find no evidence of any inducing effect of the endoderm upon the ectoderm, or vice versa, in the development of *Sabellaria*. There may possibly be some question concerning the self-differentiation of the eye spot in the exogastrula since in 13 out of 93 cases no eye spot was observed. In more than one hundred normal control larvæ studied, none was found without the eye spot. However, when individual untreated eggs are isolated in small drops of sea water beneath cover-slips it is found that they develop into larvæ in which only the eye spot fails to develop. In these isolates, it is clear that the absence of the eye spot is not due to the morphological absence of an inducing region below the ectoderm. The failure of the ectoderm to form the red pigment may perhaps be due to some general factor such as increased pressure or lower oxygen tension. The same is probably true of those exogastrulæ in which no eye spot is seen.

Thus, the absence of the eye spot in some of the exogastrulæ does not constitute a serious objection to the conclusion that there is no inducing ability of either the ectoderm or endoderm, as tested by their effects upon one another during development. It is possible that in the exogastrulæ the ectoderm has not been completely isolated from the endoderm. The cells which will give rise to the two layers are in contact with each other at one point. Through the study of exogastrulæ alone one can not exclude the possibility of diffusion of materials from one part of one layer across cell boundaries to any part of the other layer; nor can it be denied that the cells may exercise inducing effects upon each other during cleavage. These criticisms merely point to the limitations of this mode of approach. To overcome them, another method may be used—that of transplantation of blastomeres. The results of such experiments in the egg of *Sabellaria vulgaris* form the subject-matter of the second paper in this series.

## Summary

1. A simple method for the production of complete and partial exogastrulæ in *Sabellaria vulgaris* is described.

2. In the complete exogastrulæ, no internal gut is formed. In the partial exogastrulæ there is no internal œsophagus, but the stomach and intestine develop normally.

3. Both the ectoderm and endoderm show complete self-differentiation in the exogastrulæ, indicating that one layer exercises no inducing

effect upon the other between six and forty-eight hours of development, from gastrulation to the well-developed trochophore.

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