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The Development of Patella vulgata

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III—The Development of *Patella Vulgata*

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I—Introduction

The problems of Gastropodan structure and phylogeny can only be solved with the help of an adequate knowledge of the embryology of the more primitive members of this group. It is unfortunate that the only Gastropod whose development has been followed in detail from the egg to the adult is *Paludina*, the relatively specialized form which was the subject of Erlanger's classic investigations (1891). Among the more primitive forms, *Acmea* and *Haliotis* have been examined, though only superficially, by Boutan (1899). Another type almost as primitive is represented by *Patella*, in which the originally paired kidneys are both retained. The development of *Patella* has an added interest inasmuch as the egg possesses comparatively little yolk and the larva is free-swimming from the beginning. Under these conditions it is reasonable to expect a much greater retention of primitive characters than otherwise. Our information regarding the development of *Patella* is unfortunately confined to an account of the cell lineage of *P. coerulea* by E. B. Wilson (1904), and a

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still earlier paper by PATTEN (1885), in which is described the development of the same species previous to larval torsion. PATTEN does not give the history of development after torsion, and his work has not been repeated since publication some fifty years ago. Nevertheless, it is upon his work that we have to rely for our knowledge of the development of the primitive Gastropods. For this reason it was considered of the utmost importance that a fuller investigation should be carried out, covering the whole period of larval development, and that the more important points of PATTEN's paper should be reinvestigated.

The investigations were carried out in the Huxley laboratory of the Imperial College of Science and Technology, under the direction of Professor E. W. MACBRIDE, F.R.S., who originally suggested the problem, and to whom I am greatly indebted for his kind encouragement. I wish also to acknowledge the kindness of Dr. E. J. ALLEN, F.R.S., in giving me facilities for collecting material at the laboratory of the Marine Biological Association, Plymouth.

II—BREEDING HABITS AND REARING OF LARVAE

A paper dealing with the artificial fertilization of *P. coerulea*, the species common at Naples, was published by PATTEN in 1868 as the first recorded case of successful artificial fertilization among the Gastropods. The difficulties in rearing the larvae thus obtained were very great, and PATTEN mentions that on one occasion only did he succeed in obtaining a post-torsional veliger, corresponding to the four-day *P. vulgata*. During the present investigation the larvae of *P. vulgata* have been reared in a few cases as far as metamorphosis, and metamorphosis itself has been observed in a two to three weeks old larva.

For purposes of artificial fertilization, the adults were collected from Drake's Island, Plymouth, in which locality only the one species is found. During the winter 1932–33, the breeding season was found to extend from the middle of October to the end of the following March. There seems to be an annual variation in this, however, since the gonads of limpets examined at the end of March, 1933, were all spent, whereas some of the limpets collected early in April, 1932, possessed ripe gonads.

While collecting the adults, an effort was made to ascertain whether the gonads showed cyclic activity, which in many of the littoral invertebrates is related to lunar or tidal phenomena. At intervals of not more than two days samples were taken from the same locality at or near low water, fifty being examined on each occasion. The sampling was continued over a period embracing two consecutive sets of spring tides. Although the number taken was far too small to justify a statistical analysis, it nevertheless established that at all stages of the lunar and tidal cycles the majority of animals possess full and active gonads. Though the actual ratio of spent to full gonads may vary somewhat, it is always very small except at the end of the breeding season.

The occurrence of hermaphrodites, mentioned by GEMMIL (1896), AINSWORTH DAVIS and FLEURE (1903), and ORTON (1929), was confirmed by the discovery of an individual whose gonad contained both eggs and sperm. Only the larger limpets were used and the majority (about 2 : 1) were found to be female, while only one hermaphrodite was taken in the total of over a thousand limpets examined during the two seasons 1932–33–34. These observations give some support to ORTON's conclusion that *Patella* is a protandrous hermaphrodite.

No difficulty was experienced in bringing about artificial fertilization, so long as care was taken to sterilize vessels and instruments in the usual way, and to use only ripe gonads. The unripe gonads of both sexes and the spent gonads are of a brick-red colour, and the ovaries ripen to a dark olive-green tint. The ripe testis is easily distinguished by its pale cream colour. Whereas eggs from partially ripe ovaries were capable of being fertilized, it was always necessary to examine sperm suspensions under the microscope before a testis containing a sufficient number of active sperms could be selected.

Normally the sea-water used was " outside water," *i.e.*, water collected outside the Plymouth breakwater, filtered by means of a charcoal press. The limpet was removed from the shell by cutting through the horseshoe-shaped shell muscle with a scalpel. The entire ovary was then removed from beneath the thin mantle membrane and immersed in a finger-bowl of sea-water for a few seconds without stirring so that the ripe ova could separate of their own accord. The remainder of the ovary was then lifted out. The sperm suspensions were made in a similar manner. Neither ova nor spermatozoa were ever treated with alkaline sea-water as this was considered likely to produce abnormal larvæ. Ten drops of sperm suspension were added to each finger-bowl of ova, and two hours later the excess of sperm decanted off and fresh sea-water added. Four hours after fertilization, the first divisions of the egg could be observed, and, when twenty-four hours old, the larvae were swimming freely at the surface. These were then removed, so as to separate them from the unfertilized ova and the abnormalities.

During the third day food was provided by the addition of small quantities of the diatom *Nitzschia* from a culture kindly supplied by Dr. ALLEN. At this stage, before torsion had taken place, some of the cultures were treated for a short time by immersion in hypertonic sea-water, in the hope of producing abnormally symmetrical larvae. The only effect was a delay in the beginning of torsion, with no internal changes.

At the ninth day the larvae began to crawl at intervals on the glass sides of the containing vessel, by means of the ciliated foot. Later on the velum degenerated and finally disappeared during the third week, when the larvae were actively crawling. An examination of the tow-nettings both from Plymouth Sound and from the sea outside yielded a fair number of larvae which corresponded in development with the artificially reared nine-day and older larvae. The oldest of these planktonic larvae seemed to be identical with the fifteen-day larvae of the artificial culture. This is therefore the most probable estimate of the age at which the larva adopts a more

sedentary life and metamorphosis begins to take place. Though the planktonic larvae have not hitherto been described, they are present in the catches of tow-nets, fine and medium, throughout the breeding season and are readily to be identified.

By the time the velum had degenerated and finally disappeared, the adult shell was forming as a fringe or peristome to the larval shell. Two of the artificially reared larvae were able to reach this stage of development, but soon showed signs of degeneration. Some of the planktonic larvae were kept in plunger-jars and sometimes they grew to the post-larval stage. In March, 1933, the rocks of Drake's Island were examined for post-larvae, and a number of them were found varying in size from about 0·2 mm upwards. The smallest of these were only found after a prolonged search, and the metamorphosing animal, which is less than 0·2 mm long, was not taken.

The eggs of *Patina pellucida*, a closely allied form, were also fertilized and developed into minute larvae similar in all essential respects to those of *Patella*. This resemblance made an external examination necessary in order to be able to distinguish between the larvae in the tow-nettings. Their description is to be found elsewhere.

Investigations were carried out with a view to establishing the factors most important in the rearing of these larvae. The first cause of mortality to become evident was the presence of ciliate protozoa, which were also present in the ovaries of some of the adults and were not, therefore, easily to be avoided.

Physical factors did not appear to be the cause of the great difficulty experienced in rearing the larvae. Plunger-jars placed in various intensities of light, such as an underground cellar and in direct daylight (winter-time), showed the same numbers of survivals. Larvae reared under various conditions of constant temperature showed no appreciable difference in mortality. The temperatures were all within three degrees of the mean sea temperature. On the other hand, when the culture was inadvertently subjected to a sudden change in temperature the larvae would sometimes become abnormal or would all die. It is interesting to note that whenever the mortality became high, a test showed that the p_H of the sea-water remained the same as that for fresh sea-water, and was never too alkaline or vitiated by possessing too high a CO_2 content.

The greatest relative mortality occurred just after larval torsion, when the larvae appear to start feeding. Since the physical and chemical factors of the environment seemed to be satisfactorily provided for, attention was turned to the food supply. *Nitzschia* did not seem to be a suitable food organism, and many larvae were reared equally well without adding this diatom to the water. Occasionally the addition of *Nitzschia* was actually harmful, as too large a quantity would cause the larvae to become enmeshed in tangled masses of the diatom. Taking these facts into consideration, it would seem that the factor limiting the rearing of these larvae is that of food. The use of sea-water which had not been filtered was tried in an attempt to provide the natural food, but the appearance of small crustacea and other harmful intruders invariably followed. Another method adopted was that of placing the larvae in a breffet jar covered with bolting silk (fine silk net) and placing the jar in

the circulating sea-water of the laboratory. The most successful attempts, resulting in the partial metamorphosis of several animals, were carried out in this way.

Further attempts to find a suitable food, by adding encrusting growths of algae from the sides of old plunger-jars, gave negative results.

The larvae ready to metamorphose were sometimes supplied with rock chippings as a substratum, but this was found to be unnecessary, since the larvae could always be made to crawl on the glass sides of the containing vessel.

III—General Technique

The larvæ of *Patella* when contracted into the shell are difficult to examine either by means of sections or by means of whole mounts. It was, therefore, necessary to kill them in an extended state. The most satisfactory method of doing this was found to be the immersion of the larvae in a 5% solution of magnesium chloride for ten minutes prior to fixation.

For histological purposes very good results were obtained by using Bouin's fixative, in which the objects were immersed for 12 hours before washing and finally storing them in 70% alcohol. In order to examine whole mounts some of the larvae were treated by the glycerine-acetic acid method, using light green as a light stain. These preparations, however, were of a temporary nature only. Others were prepared by fixation in 40% formalin, which forms a colourless compound with the protoplasm. After an hour in this fixative they were washed in 90% alcohol and preserved in the same fluid. For these, staining was carried out with Mayer's haem-alum or Delafield's haematoxylin. Owing to the difficulty experienced in killing the animal in an extended state, whole mounts were only used to supplement the examination of the living animal. Post-larval stages were fixed in Bouin's solution and after washing and dehydrating were cleared in clove oil and mounted in balsam without staining.

Since so few larvæ were available it was necessary to adopt a more than usually efficient method of section cutting. In order satisfactorily to orientate these small asymmetrical objects a modification of the clove oil-celloidin method was employed similar to that adopted by D. P. Wilson (1934) for the orientation of small polychaet larvae. It was found, however, that this method did not satisfactorily overcome the difficulty of flattening the sections whereby valuable series are liable to be spoilt, and further modifications had to be introduced before the difficulty was surmounted.

IV—Larval Development

Segmentation and cell lineage

The course of segmentation in *Patella vulgata* closely follows that of *P. coerulea* as described by E. B. Wilson (1904), and a detailed account need not therefore be given.

The egg segments with great regularity, the first two divisions forming four cells which then divide by a process of spiral cleavage similar to that found in the primitive Annelida and in the Platyhelminthes. These cells, the macromeres, bud off towards the apical plate successive quartettes of smaller cells which are the micromeres.

The first quartette gives rise to cells forming the apical plate (1·111, using the nomenclature adopted by WOLTERECK, 1903). In *P. coerulea* WILSON was able to distinguish radiating from the apical plate eight groups of cells arranged in the form of two alternating crosses, the Annelidan Cross (1·11), and the Molluscan Cross (1·12). These are not so readily distinguished in *P. vulgata*, but still can be made out, *An.X*, *Mol. X*, fig. 1.

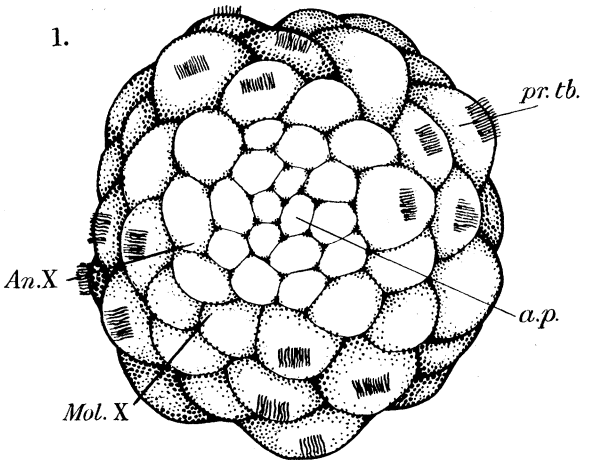


FIG. 1—View of 13-hour larva from apical pole, showing primary trochoblasts. *An. X*, cells forming the Annelidan Cross; *a.p.*, apical plate; *Mol. X*, cells forming the Molluscan Cross; *pr.tb.*, primary trochoblasts

In each quadrant of a ten-hour larva, cilia are acquired by a lozenge-shaped group of four cells, situated near the position of the tip of the Annelidan Cross, between the arms of the Molluscan Cross. The cilia borne by each of these cells, which are the primary trochoblasts *pr.tb.*, fig. 1, are not diffusely scattered but arranged in a single row in each cell parallel to the equatorial plane. They thus bear a resemblance to the ciliated bands of a Ctenophore, a resemblance that led WILSON to use the term "ctenophore stage." Between the groups of primary trochoblasts appear the secondary trochoblasts. They are formed from first and second quartette cells at the tips of the Molluscan Cross, which acquire the same Ctenophore-like arrangement of cilia, but which are smaller in size than the primary trochoblasts. There are only three of these secondary trochoblasts in each quadrant in *P. vulgata*.

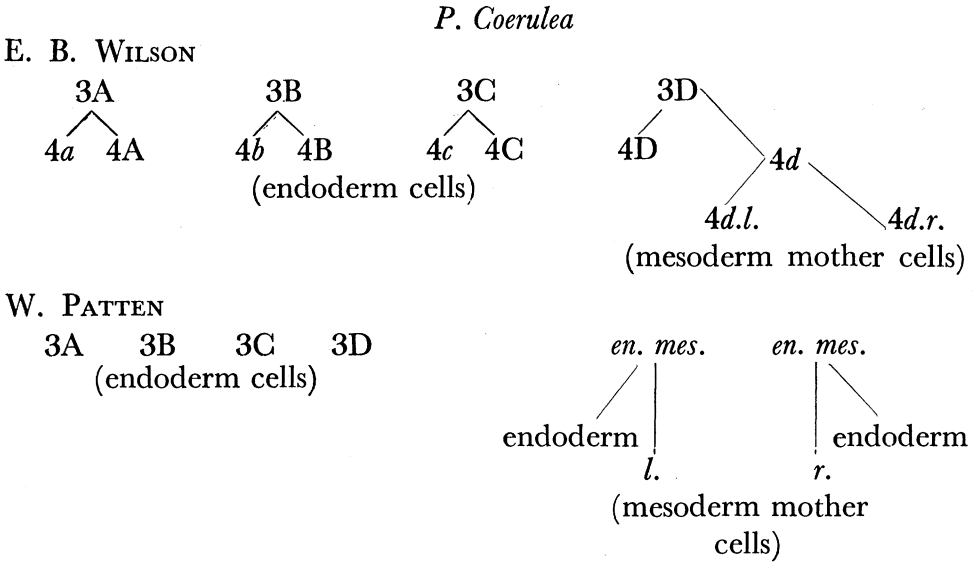
The remainder of the second quartette forms ectoderm, including that from which the stomodaeum later arises. All the adult and larval ectoderm is derived from the first three quartettes.

Gastrulation and formation of the germ layers

So far my observations agree with the more detailed account of E. B. WILSON, but with regard to gastrulation and formation of the germ layers there is a divergence between us. According to E. B. WILSON, who worked on optical sections of whole mounts, endoderm is formed by the macromeres 4A, 4B, 4C, and 4D, together with the micromeres 4*a*, 4*b*, and 4*c*. He states that 4*d* gives rise during the process of gastrulation to two daughter cells which are the mother cells of the mesoderm. PATTEN states that :

> "Two large cells soon appear, one at each side of the four endoderm cells, destroying the previous radial symmetry, and transforming the embryo into a bilateral organism . . . During their present condition they may be called the endo-mesoderm cells (*en. mes.*), since the subsequent division of each of these cells gives rise to two cells, one of which becomes the primitive mesoderm cell and the other, after remaining in the mouth of the blastopore for some time, finally becomes pushed inwards by the narrowing of the blastopore, and forms one of the endoderm cells lining the cavity of the mesenteron."

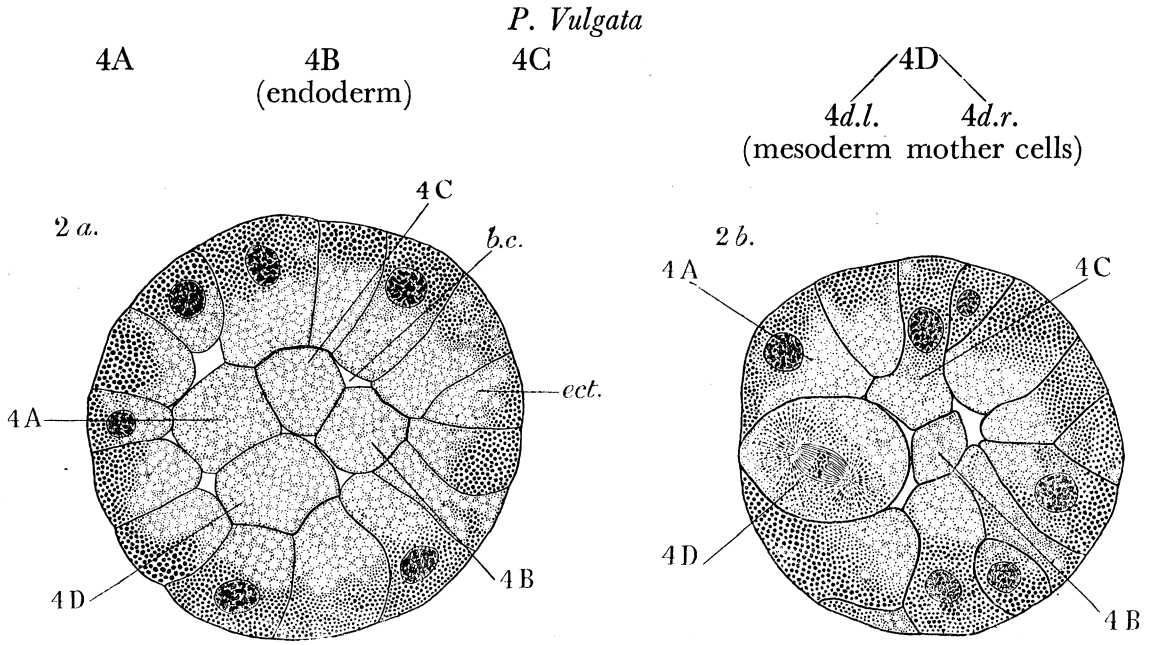
These views are summarized in the following diagram.



PATTEN does not say how his "endo-mesoderm" cells arise and WILSON has assumed that they are the same as his mesoderm mother cells. WILSON is more clear about the formation of the latter, but as this happens inside the larva, the observations of whole mounts upon which he based his statements cannot give such reliable information as observations on serial sections.

The present investigations show very clearly that in *P. vulgata* the whole of the

macromere in quadrant D gives rise to mesoderm and that endoderm is formed from the macromeres of the quadrants A, B, C alone.



FIGS. 2*a*, 2*b*—Transverse sections of 13-hour larva, showing division of cell 4D. *b.c.*, blastocoele ; *ect.*, ectoderm

Figs. 2*a*, 2*b* show transverse sections through a 13-hour old larva. In the first may be seen the four macromeres as they appear inside the blastocoele, into which

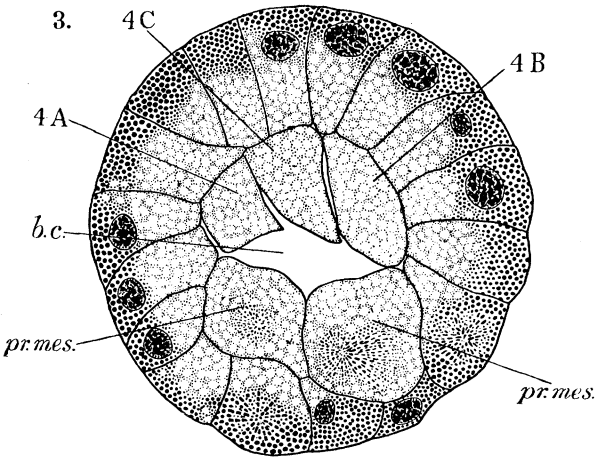
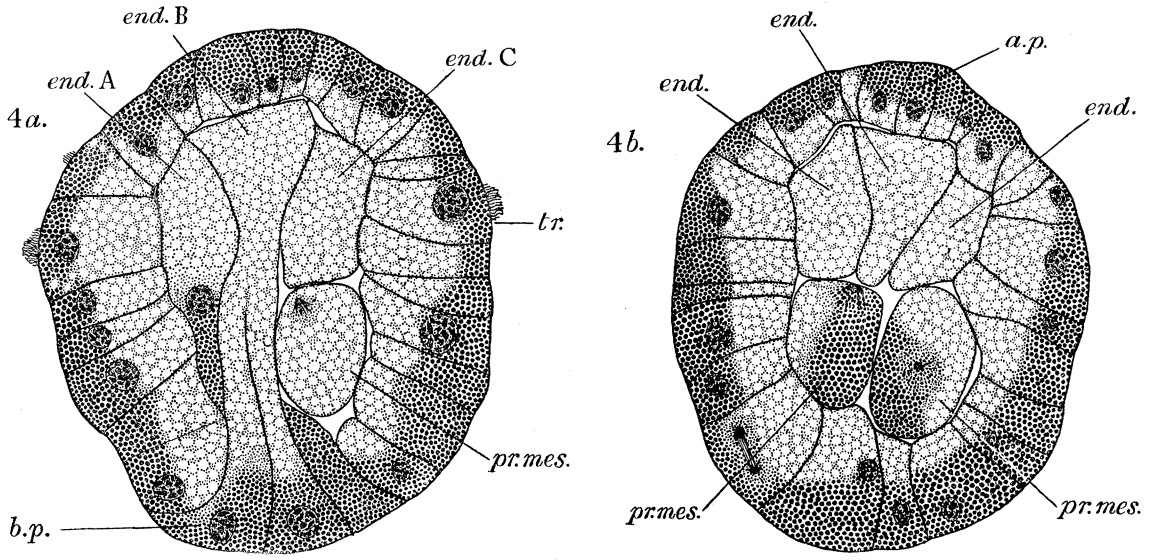


FIG. 3—Transverse section of 14-hour larva, appearance of mesoderm mother cells. *b.c.*, blastocoele ; *pr.mes.*, primitive mesoderm cells

they grow during the process of gastrulation. The same macromeres in fig. 2*b* are cut nearer the surface, and it will be seen that 4D has begun the transverse division whereby it is entirely transformed into the paired mesoderm mother cells. In a

larva 14 hours old this division has taken place and a transverse section, fig. 3, shows the products of division together with the three endoderm cells, 4A, 4B, and 4C. The mother cells of the mesoderm, 4*d.l.* and 4*d.r.* are sub-ovoid in shape so that they may be recognized at all stages until just before larval torsion takes place, figs. 4*a*, 4*b*, 5, 6. The three endoderm cells may also be recognized at a later stage, when they have begun to divide longitudinally, figs. 4*a*, 4*b*.



FIGS. 4*a*, 4*b*—Frontal section of 16-hour larva, longitudinal division of endoderm cells. *a.p.*, apical plate; *b.p.*, position of blastopore; *end.*, endoderm; *pr.mes.*, primitive mesoderm cells; *tr.*, prototroch

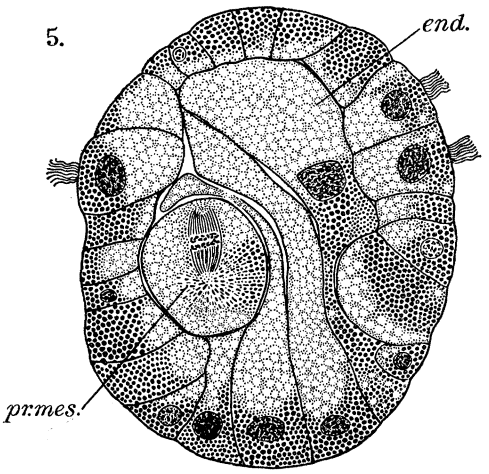


FIG. 5—Parasagittal section of 16-hour larva seen from right side. *end.*, endoderm; *pr.mes.*, primitive mesoderm cell

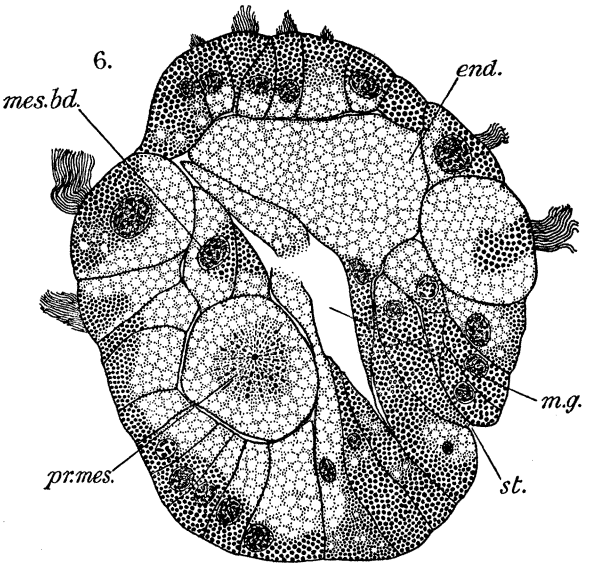


FIG. 6—Parasagittal section of 21-hour larva seen from right side. *end.*, endoderm; *mes.bd.*, mesodermal band; *m.g.* incipient cavity of the mid-gut; *st.*, stomodaeal groove

The discrepancy between the statements of PATTEN and WILSON and the above account is the more readily accounted for when it is realized that the division of 4D to form the mesoderm mother cells takes place in less than two hours, and that the further division of each of these cells to form the mesodermal bands of the larva might easily be mistaken, in its early stages, for a division whereby the mesoderm rudiments are cut off from endoderm cells. So far as PATTEN is concerned it seems clear that he has missed the stage described here, whereas WILSON, working on whole mounts and optical sections, has made a mistake in the identity of cells lying inside the larva, a mistake difficult to avoid unless observation is also made on serial sections.

Gastrulation begins before formation of the mesoderm mother cells, by the inward growth of the macromeres 4A, 4B, 4C, and 4D. When the cells 4*d.l.* and 4*d.r.* have been formed, the inner ends of the three endoderm cells expand, the nuclei remaining in the drawn-out part of the cells closing the blastopore. At 16 hours, longitudinal division of the endoderm cells takes place, the plane of division being at right angles to the surface, figs. 4*a*, *b*. After this the nuclei travel inwards and further division takes place transversely, so that a group of large yolky cells is formed within the blastocoele. A slight separation of these cells from each other causes a small space to appear between them, and this later forms the cavities of the mid-gut *m.g.*, fig. 6, and the liver.

Trochophore larva

The larva is at first spherical, but after gastrulation has taken place it becomes elongated in a polar direction. The prototroch, consisting of primary and secondary trochoblasts, at first forms two irregular rows of large ciliated cells, which alternate with each other. Later on the arrangement becomes more regular and takes the form of an anterior girdle of larger cells behind which can be distinguished a row of smaller cells, less powerfully ciliated. The cilia of the large cells spread until they form a continuous circle around the larva, while in front of them very much smaller cilia appear covering the entire embryo cap. These fine cilia remain until just before the mantle and shell are formed. The cells immediately in front of the prototroch form a definite row called by PATTEN the " anterior support cells " of the velum, *v.s.a.*, fig. 7. They do not, however, acquire cilia like the prototroch, as PATTEN suggests, and they can only be distinguished from the other cells of the embryonal cap by their larger size and their regular arrangement. A row of similar cells behind the prototroch is termed by PATTEN " posterior support cells " *v.s.p.*, fig. 7.

At this stage the apical plate is furnished with long fine cilia with no apparent motor function, *a.p.*, fig. 7. Whether they are sensory in function or whether they help to preserve equilibrium in the actively moving larva is not clear. In a larva of 25 hours old, a cell grows out on each side of the apical plate, carrying fine stiff hairs, and having an optically refractive character. There can be little doubt that they carry out some sensory function in the larva, but they do not persist for more than a day after larval torsion begins to take place, *ref.b.*, fig. 7*b*.

After formation of the blastopore and the beginning of division in the endoderm cells, two cells dorsal to the blastopore acquire stiff coarse hairs, forming the anal tuft or telotroch, *tel.*, fig. 7*c*. It is near this region that the anus finally opens after torsion. A flattening and division of the cells between the anal tuft and the prototroch causes the blastopore to travel forwards along the ventral surface, and later the process is continued by the division of cells between the anal tuft and the blastopore. The latter sinks into a pit, wide behind but narrow in front, where it deepens as the ectoderm grows in to form the stomodaeum. As the blastopore travels forwards, the shallow posterior part of the groove in which it is situated does

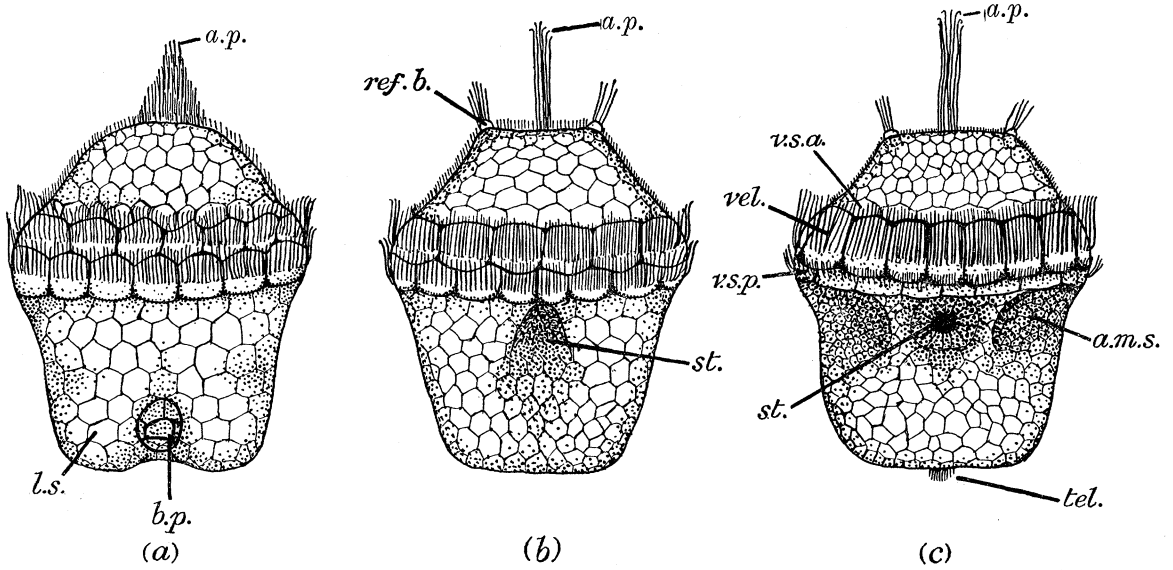


FIG. 7*a*. FIG. 7*b*. FIG. 7*c*.

Stages in development of trochophore larva. Ventral view. *a.m.s.*, anterior mantle sacs; *b.p.*, blastopore; *l.s.*, lateral swellings (so-called "foot rudiments"); *ref.b.*, refractive body; *st.*, stomodaeum; *tel.*, telotroch; *vel.*, velum; *v.s.a.*, *v.s.p.*, anterior and posterior velar support cells

not shallow out and disappear immediately, fig. 7. For this reason there is an apparent paired outgrowth of the postero-ventral part of the larva, *l.s.*, fig. 7. This is described by PATTEN as the paired foot rudiment, since in this position the foot appears. There does not seem to be any foundation for this assumption in *P. vulgata*, since when the foot is formed it is a median outgrowth and there is no trace of a median groove such as PATTEN described "persisting for a considerable period." The pair of elevations figured here are no longer in existence when the foot grows out, as a median protuberance. A section through this region shows that the growth of the mesoderm bands by division of the mesoderm mother cells is restricted posteriorly because of the development of the shell gland on the dorsal surface, and they are thus a contributory factor in the formation of the so-called pedal rudiments. The dorsal invagination of columnar cells which is the shell gland is shown in fig. 8.

The mesoderm mother cells divide so as to form a pair of mesodermal bands extending longitudinally at the sides of the archenteron, figs. 9, 10*a* and the anterior portions of these bands give off small spindle-shaped cells which constitute a kind of

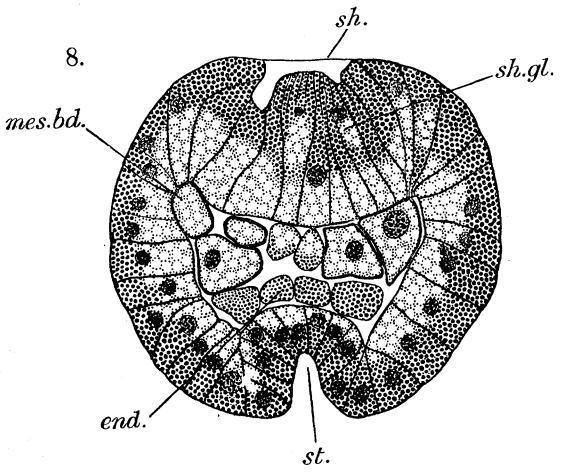


FIG. 8—Transverse section of 28-hour larva. Development of shell gland, stomodaeum, and apparent "pedal lobes." *end.*, endoderm cells; *mes.bd.*, mesodermal band; *sh.*, first trace of larval shell; *sh.gl.*, shell gland; *st.*, stomodaeum

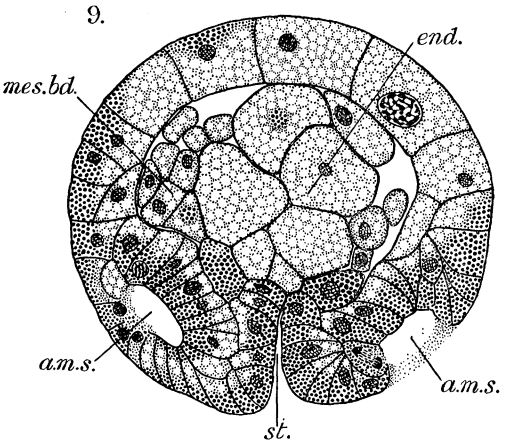


FIG. 9—Transverse section of 32-hour larva. Development of so-called otocysts. *a.m.s.*, anterior mantle-sac; *mes.bd.*, mesodermal band

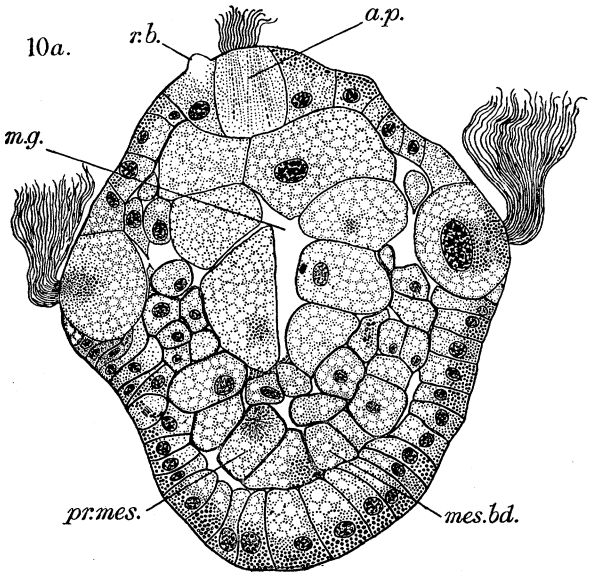


FIG. 10*a*—Frontal section of 32-hour larva showing the mesoderm bands. *a.p.*, apical plate; *mes. bd.*, mesodermal band; *m.g.*, mid-gut; *pr. mes.*, primitive mesoderm cells; *r.b.*, refractive body

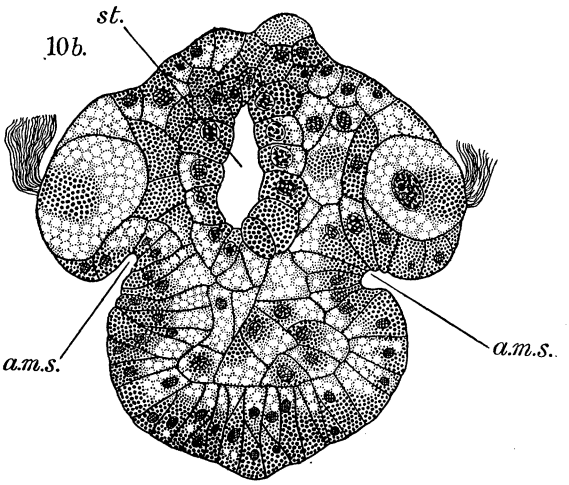


FIG. 10*b*—Frontal section of the same larva showing the stomodaeum and the rudiments of the mantle cavity; *a.m.s.*, anterior mantle-sac; *st.*, stomodaeum

mesenchyme from which the larval muscles are formed. No mesenchyme is formed from the ectoderm of the trochophore.

At the sides of the stomodaeum and immediately behind the prototroch a pair of sacs is formed by invaginations of the ectoderm. PATTEN suggested that these might give rise to the otocysts, but the present investigations show that this is not so. They appear before the development of the mantle cavity, which is formed a few hours later by the eversion of the shell gland and the downgrowth of its thickened edge. A close study of these pits, shown in fig. 7, and in section in figs. 9 and 10*b*, suggests that they are a precocious formation of the mantle cavity, especially as they retain their connection with the mantle cavity and never show any signs of being constricted from it, even temporarily.

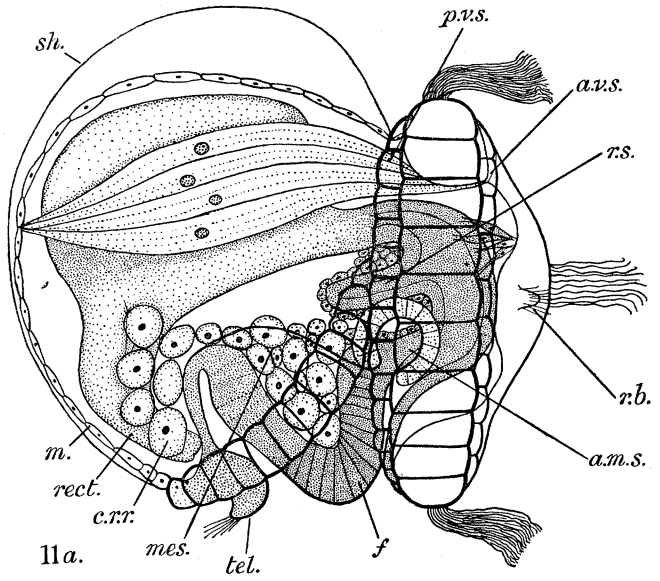


FIG. 11*a*

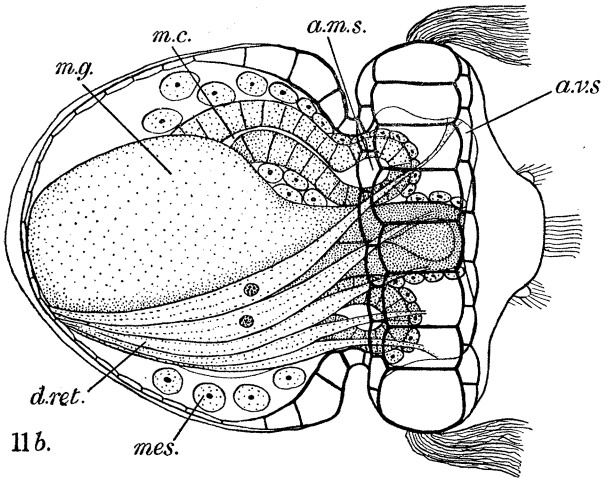


FIG. 11*b*

Reconstruction of 48-hour larva (*a*) from right side, (*b*) from above. *a.m.s.*, anterior mantle-sac; *a.v.s.*, anterior velum support cells; *c.r.r.*, right coelomic rudiment; *d.ret.*, dorsal shell retractor muscle; *f.*, foot; *m.*, mantle; *m.c.*, mantle cavity; *m.g.*, mid-gut; *mes.*, mesoderm; *p.v.s.*, posterior velum support cells; *rect.*, rectum; *r.s.*, radula sac

Pre-torsional veliger larva

With the evagination of the shell-gland and growth of its thickened edge to form the mantle and mantle cavity, the trochophore becomes a veliger larva. A reconstruction of a 48-hour veliger is shown in figs. 11 and 12. It will be seen that the foot has grown out to form a well-marked ventral organ, and that the shell is dorsal and cap-shaped. Further growth of the shell takes place at the mantle edge, particularly at the postero-ventral border, giving the characteristic nautiloid type of shell.

Other changes that take place in the formation of the veliger larva are the loss of cilia by the posterior row of cells of the velum or prototroch, and the loss of the small cilia which clothed the pre-velar area. The velum now consists of a single row of large cells bearing powerful cilia, supported by a row of smaller cells in front

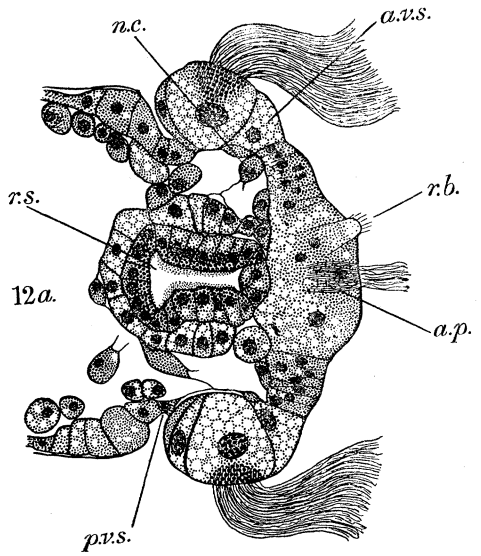


FIG. 12a

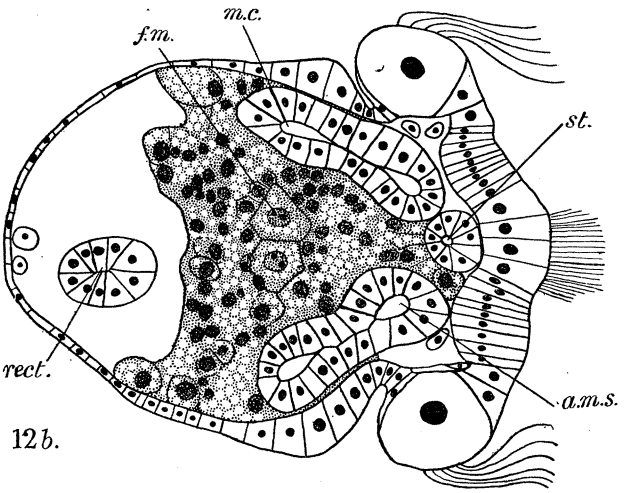


FIG. 12b

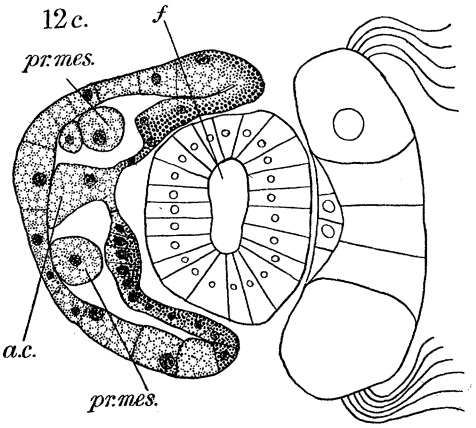


FIG. 12c

Frontal sections of 48-hour larva, showing anal cell, mesoderm in foot and primitive nerve cells. *a.c.*, anal cell ; *a.m.s.*, anterior mantle-sac ; *a.p.*, apical plate ; *a.v.s.*, anterior support cells ; *f.*, foot ; *f.m.*, large cells of unknown fate ; *m.c.*, mantle cavity ; *p.v.s.*, posterior velum support cells ; *rect.*, rectum ; *r.b.*, refractive body ; *r.s.*, radula sac ; *st.*, stomodaeum

and another behind, *a.v.s.*, *p.v.s.*, figs. 11*a*, 11*b*, and 12*a*. The telotroch is borne by a prominent pair of cells at the edge of the mantle cavity, *tel.*, fig. 11, and consists of stiff, coarse hairs. This probably functions as a rudder-like organ in the actively swimming veliger.

From the ventral wall of the stomodaeum, a pouch grows out which is destined to form the radula sac, *r.s.*, figs. 11, 13. This was suggested by PATTEN, who was unable, however, to follow its further development.

The growth of the mantle has caused the body cavity to increase in size, and there is now a space between the well-defined gut and the body wall. The alimentary canal consists of the stomodaeum and radula sac forming the ectodermal fore-gut, a large sac-like endodermal mid-gut or larval stomach formed of large cells containing many yolk vacuoles, and a rectal rudiment endodermal in origin and not yet in communication with the exterior. It terminates in the outer wall of the mantle cavity, in the mid-posterior region, as a well-defined anal cell, *a.c.*, fig. 12*c*, formed from an ectodermal cell near the telotroch.

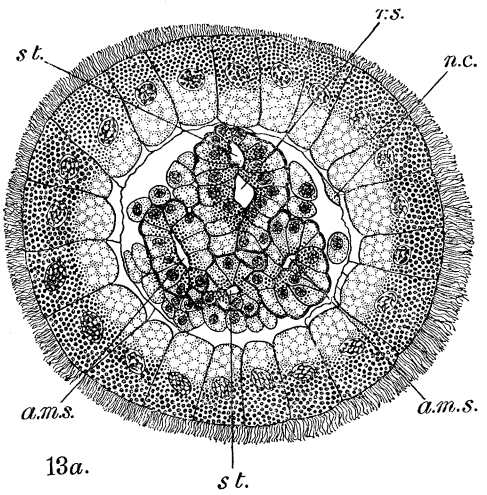


FIG. 13*a*

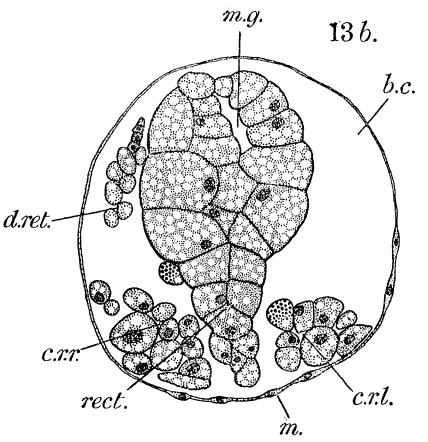


FIG. 13*b*

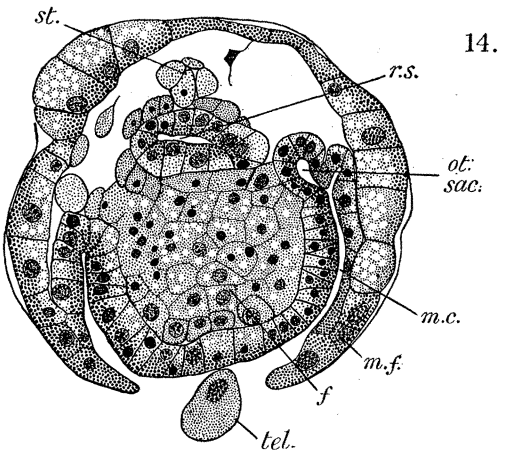
Transverse sections of 48-hour larva, (*a*) seen from in front, (*b*) to show retractor muscle and paired coelomic rudiments. *a.m.s.*, anterior mantle-sac ; *b.c.*, blastocoele ; *c.r.l.*, *c.r.r.*, left and right coelomic rudiments ; *d.ret.*, dorsal retractor muscle ; *m.g.*, mid-gut ; *m.*, mantle ; *n.c.*, primitive nerve cell ; *rect.*, rectum ; *st.*, stomodaeum

On each side of the anal cell may be seen the original pair of mesoderm mother cells, forming part of a pair of mesodermal blocks of tissue lying at the sides of the rectum, fig. 13*b*. These blocks are the coelomic wall rudiments. The anterior parts of the mesodermal bands have given rise to the musculature of the larva. This consists of small muscle cells joining the stomodaeum to the pre-velar region, and some very large spindle-shaped cells extending from the region of the velum to the posterior part of the mantle and shell. This muscle is asymmetrical, having a median point of insertion at the anterior end, but running backwards along the right side of the mid-gut or larval stomach. The posterior point of attachment is on the right side, *d.ret.*, figs. 11, 13*b*. There is no trace in development of another member of a pair, and it seems most likely that the muscle was originally, in a more primitive ancestor,

a median one which has become diverted to one side by the presence of a large yolky endoderm mass in the larva. The larva has become asymmetrical because of this, even before the beginning of torsion.

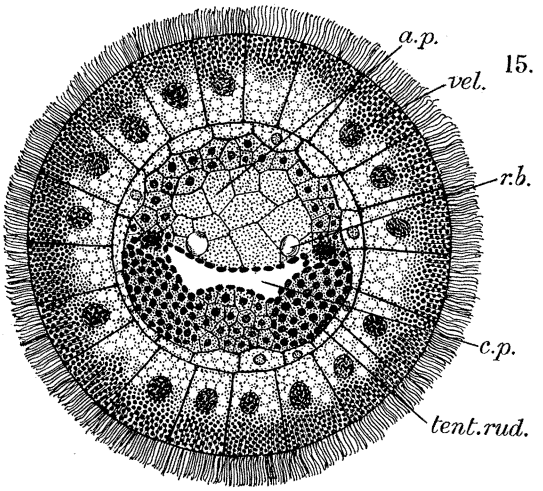
The remainder of the mesoderm has formed an investing layer of cells covering the stomodaeum, radula sac, and the anterior mantle diverticula, together with a mass of undifferentiated cells in the foot. Among the latter are two very prominent cells, larger than their fellows, and arranged as a pair close to the middle line. Before torsion has taken place they disappear and their ultimate fate is unknown, *f.m.*, fig. 12*b*.

In the space bounded by the velum and the embryo cap are some cells that appear to be in the nature of a primitive nervous system. They consist of a circle of fine fibres and cells, *n.c.*, fig. 12*a*, passing close to the inner surface of the velar



14.

Fig. 14—Transverse section of 53-hour larva, showing origin of otocysts. *f.*, foot ; *m.c.*, mantle cavity ; *m.f.*, mantle fold ; *ot. sac.*, auditory sac ; *st.*, stomodaeum ; *tel.*, telotroch



15.

Fig. 15—Diagram of pre-velar area of veliger. *a.p.*, apical plate ; *c.p.*, cerebral pit ; *r.b.*, refractive body ; *tent. rud.*, rudiment of tentacle ; *vel.*, velum

cells, to which pass finer branches. The cells of this system are also connected with the drawn-out ends of the anterior and posterior velar support cells, fig. 13*a*, and with larger cells situated in the space between the body wall and the radula sac and stomodaeum. These cells are usually rounded in shape with a pair of fibrous processes at the same end of the cell.

A similar arrangement is described by Patten as a primitive musculature, but the rounded cells with processes at one end only could hardly function as muscles. Furthermore, the velum has never shown any signs of being contractile in the living larva. The probability is that the whole system is a primitive nervous net, since the rudiments of the permanent nervous system do not develop until after torsion has started.

The otocysts are formed from paired invaginations which develop by the sides of the foot, at the posterior angles of the mantle cavity, in the 53-hours-old larva, fig. 14. Shortly after this, at the end of the third day, torsion begins to take place.

The larva during torsion

Torsion takes place between the third and fifth days after fertilization. Before describing the process in detail it will be convenient to consider the other changes that take place in the larva during this period.

The foot, at first a simple wedge-shaped protuberance, grows rapidly both by the division of the ectoderm and by the increase in number of the enclosed mesoderm cells. The anterior face of the foot increases in area until it forms an inturned ventral surface, the future creeping sole of the foot, which acquires cilia, but which does not subserve a creeping function until after torsion. On the anterior face of the foot there appears a paired ectodermal thickening, which is the rudiment of the pedal ganglia, *p.g.*, fig. 17*a*.

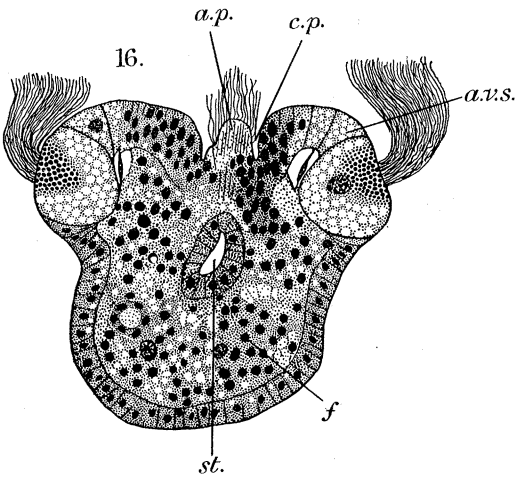


FIG. 16—Frontal section of 70-hour larva. Formation of cerebral ganglion. *a.p.*, apical plate *a.v.s.*, anterior support cells of the velum; *c.p.*, cerebral pit; *st.*, stomodaeum

The cerebral ganglia are initiated by the appearance of a pair of pits connected by a shallow trough, and situated in the prevelar area immediately ventral to the apical plate, figs. 16, 17*b*, 25. At the bases of the pits the ectodermal cells migrate inwards, forming a pair of densely nucleated masses from which the cerebral ganglia develop. The cerebral commissures are developed shortly afterwards and in a similar way from the cells at the base of the trough. The development of the ganglia and commissure is completed by the end of torsion.

The pedal ganglia and commissure originate from the paired rudiments by a process of ectoderm proliferation similar to that described for the cerebral ganglia, but at a slightly later stage. No pits are formed and the process is complete by the end of torsion, figs. 20, 21.

The pleural ganglia only begin to develop towards the end of torsion and there is no sign of the visceral ganglia until after this process is completed.

Above the pedal ganglia, the otocyst invaginations become detached from the foot ectoderm and form small spherical sacs at the sides of the foot. At this stage no otoliths are present, *ot.*, figs. 20 and 21.

Little change takes place in the alimentary canal, but the radula sac elongates and becomes folded in the form of a U, so that it takes up a great deal of space in the body

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cavity. From the sides of the rectum, the coelomic wall rudiments grow downwards and fuse with each other beneath it, forming a bilobed mass of cells.

The mesoderm cells of the foot grow out at each of the posterior angles as a pair of bulb-like projections into the body cavity. A lumen appears in each of these projections and the cells lining it lose their yolk vacuoles and acquire deeply staining protoplasmic contents, *ped.gl.*, figs. 17*a*, 20. The lumen branches among the foot mesoderm, the branches terminating near the ectoderm of the ventral surface. The origin of these glands appears to be mesodermal, and no ectodermal invaginations have been observed in connection with them. Their future development shows that they are mucous glands.

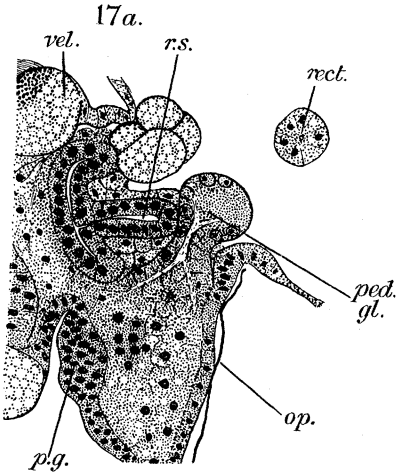

FIG. 17*a*

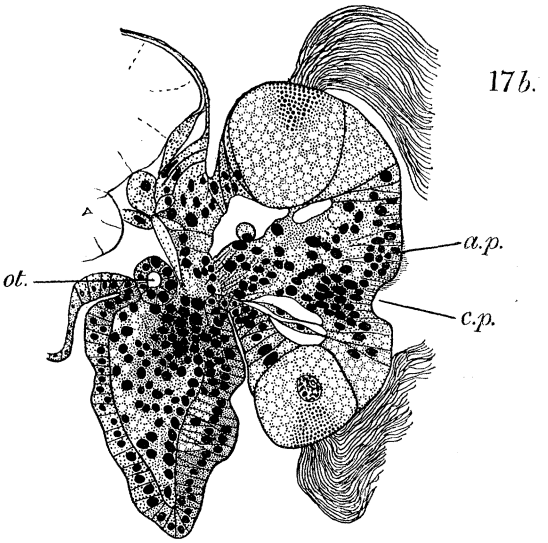

FIG. 17*b*

Parasagittal sections of 70-hour larva. (*a*) Origin of pedal gland. (*b*) Front right side. Otocyst formed. *a.p.*, apical plate ; *c.p.*, cerebral pit ; *op.*, operculum ; *ot.*, otocyst ; *ped. gl.*, pedal gland ; *p.g.*, pedal ganglion ; *rect.*, rectum ; *r.s.*, radula sac ; *vel.*, velum

Besides these paired glands, there also appears a median structure in the foot, *m.gl.*, fig. 23*a*. This organ, whose origin and fate have not been determined, consists of a short rod of cells extending into a small median cavity in the foot mesoderm near the posterior surface. It is possible that this may be a glandular structure associated with the secretion of material for the operculum. The latter is secreted by the posterior surface of the foot during torsion, and before it can be of any service to the larva.

The torsion process

The first part of torsion takes place slowly, beginning at between 60 and 70 hours after fertilization. A half-way stage is reached at between 100 and 110 hours, and the final stages are passed through quickly during the next few hours.

At the beginning of the process the mantle encloses a deep cavity at the back of the foot, fig. 11*a*. This cavity is shallower at the sides of the foot, but deepens to form

the anterior mantle sacs at each side of the mouth. The mantle fold does not at this stage of development extend dorsally.

When torsion takes place the mantle and shell as a whole rotate through nearly 180 degrees relative to the head and foot, carrying the posterior part of the alimentary canal with them. During the first period, the pallial complex, consisting of the posterior part of the mantle cavity together with the anal cell, rectum and coelomic wall rudiments, moves along the right side of the larva until it reaches a point half-way towards its final position, which is antero-dorsal, fig. 20. In the pre-torsional stages, the retractor muscle holds the shell and mantle cavity over the foot, fig. 18 (*a*). At the half-way stage, however, growth of the foot has taken place, causing the postero-ventral rim of the shell and mantle to be pushed backwards and upwards

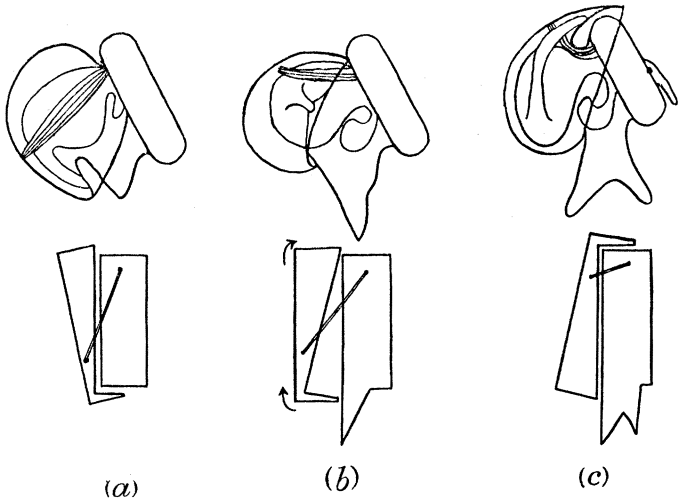

FIG. 18—The larva during torsion, (*a*) before, (*b*) during, (*c*) after torsion

and increasing the tension in the retractor muscle, fig. 18 (*b*). At the same time it stretches the mantle and causes the mantle cavity to become shallow.

As the pallial complex passes the half-way position, it slips forward towards the velum, no longer being held back by the foot. The movement takes place quickly, resulting in the reappearance of a deep mantle cavity, into which the velum may now be retracted, fig. 18(*c*).

During the whole of the torsion process differential growth occurs in the region where the rotation takes place, being greater to the left of the mantle cavity. It does not necessarily follow, however, that this is the cause of the torsion process. The sequence of events detailed above shows that the action of the retractor muscle plays a major part in bringing it about, even if it is not the main cause. This action, tending to draw the larva in towards the shell apex, is prevented by the growth of the foot, adjacent to the mantle cavity, fig. 18 (*b*). This produces an unstable condition, since a rotation of the visceral hump will enable the dorsal part of the larva to be withdrawn into the mantle cavity, fig. 18 (*c*).

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The result is that a rotation takes place, similar to that produced in an eccentric disc by the pressure of a rod acting on its surface, fig. 19. The movement of the rod towards the disc—and at right angles to it—corresponds to the movement of the velum towards the shell under the influence of the retractor muscle. The resulting rotation of the disc is equivalent to the rotation during torsion. The direction of the torsional rotation is determined by the position of the asymmetrical retractor muscle (on the right side of the larva).

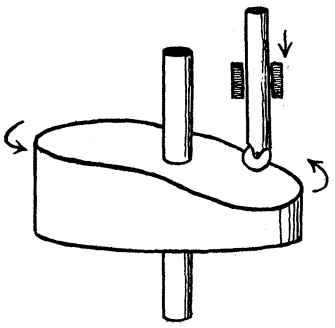

FIG. 19—Diagram of a mechanical model to replace torsion

Post-torsional veliger larva

Several important changes have taken place in the larva by the end of torsion. The shell has grown slightly by additions to its antero-dorsal (pre-torsional postero-ventral) edge, but does not so far show any difference in shape.

The position of the retractor has been altered and its point of attachment to the shell, previously situated on the right hand posteriorly, has now been rotated to a position on the left dorsal surface, nearer to the velum. The anterior attachment is still in the velar area near the stomodaeum. There appears after torsion, however,

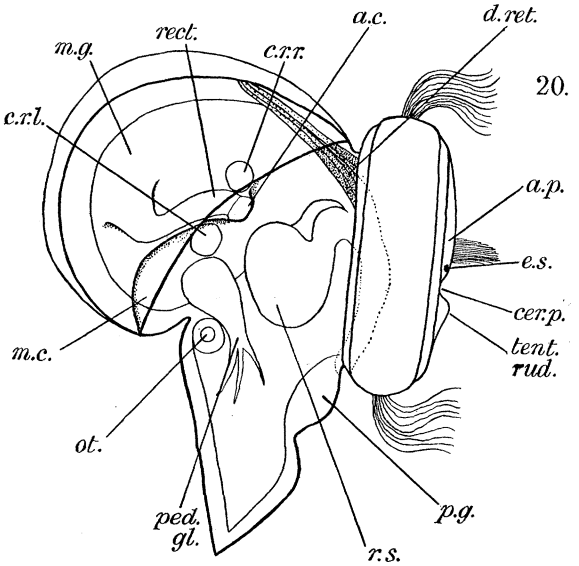

FIG. 20—Diagram of a larva with torsion half completed. *a.c.*, anal cell; *a.p.*, apical plate; *cer.p.*, cerebral pit; *c.r.l.*, *c.r.r.*, left and right coelomic rudiments; *e.s.*, eye; *m.c.*, mantle cavity; *m.g.*, mid-gut; *ot.*, otocyst; *ped.gl.*, pedal gland; *p.g.*, pedal ganglion; *rect.*, rectum; *r.s.*, radula sac; *tent.rud.*, rudiment of tentacle

a new muscle, extending from the region ventral to the dorsal retractor to a position on the shell on the right ventral surface, *ret.v.*, figs. 22*a*, 22*b*. The fibres or cells composing these muscles lose the yolk vacuoles and the cell contents stain uniformly.

The pedal ganglia and the otocysts are fully developed by the end of torsion, and at about 124 hours there appears a single otolith in each of the sacs.

The pleural ganglia originate from ectoderm cells of the body wall at the sides of the larva, just above the foot, and are quite close to the cerebral and pedal ganglia,

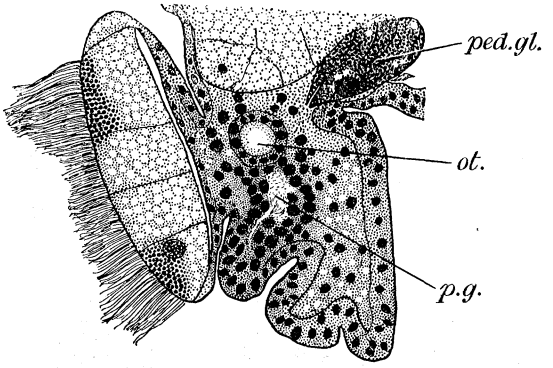

FIG. 21—Parasagittal section of 100-hour larva. Pedal ganglion. *ot.*, otocyst ; *ped.gl.*, pedal gland; *p.g.*, pedal ganglion

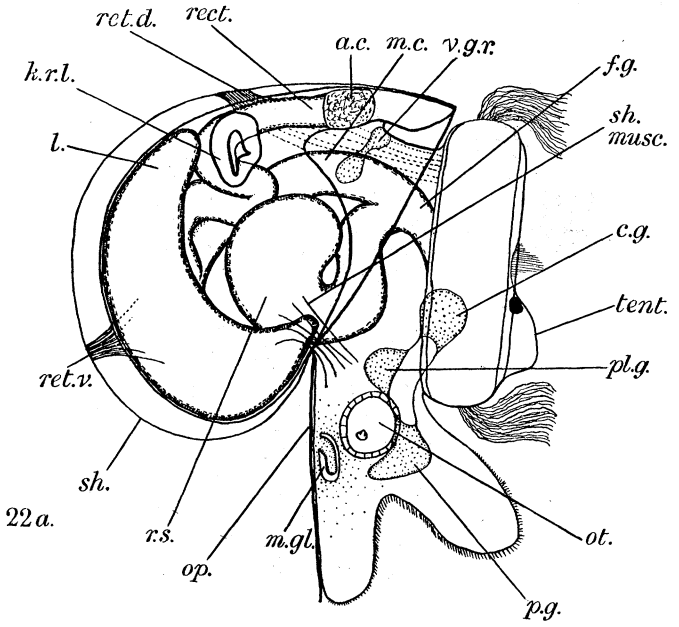

FIG. 22*a*

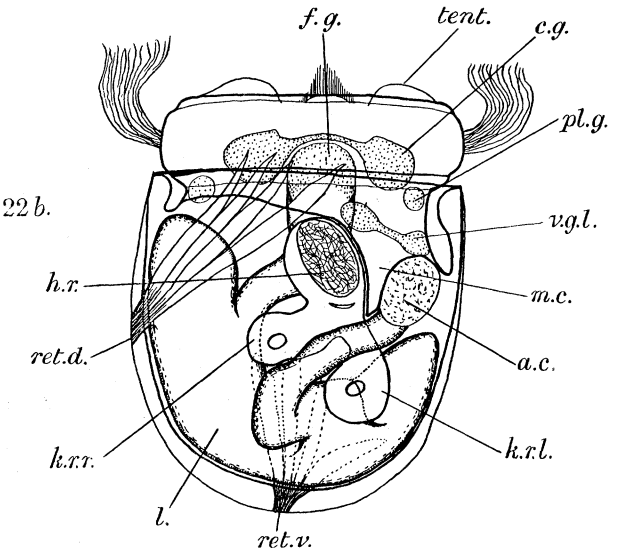

FIG. 22*b*

Diagram of post-torsional larva (*a*) from right side, (*b*) from above. *a.c.*, anal cell ; *c.g.*, cerebral ganglion ; *f.g.*, fore-gut ; *h.r.*, heart rudiment ; *k.r.l.*, *k.r.r.*, left and right kidney rudiments ; *l.*, liver ; *m.gl.*, median gland of foot ; *m.c.*, mantle cavity ; *op.*, operculum ; *ot.*, otocyst ; *p.g.*, pedal ganglion ; *pl.g.*, pleural ganglion ; *ret.d.*, dorsal retractor muscle ; *ret.v.*, ventral retractor muscle ; *r.s.*, radula sac ; *sh. musc.*, shell muscle ; *v.g.l.*, *v.g.r.*, left and right visceral ganglia

pl.g., figs. 22*a*, 22*b*, 23*b*, 23*c*. Soon after their formation, they become united with the last named by nerve fibres growing out from them. The visceral ganglia *v.g.l.*, *v.g.r.*, figs. 22*a* and 22*b*, develop in the floor of the mantle cavity after torsion has taken

place. They are also developed from the ectodermal body wall, and are situated a little to the right-hand side of the larva. The buccal ganglia do not develop until later, while the labial ganglia are not visible until the larva has metamorphosed.

It is of particular importance to note that, though the pedal and pleural ganglia

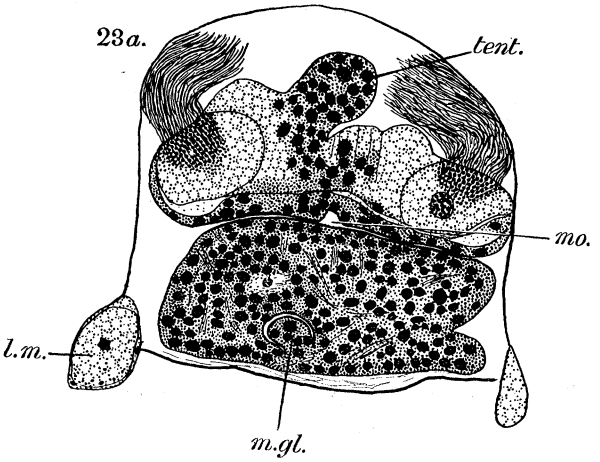

Fig. 23*a*

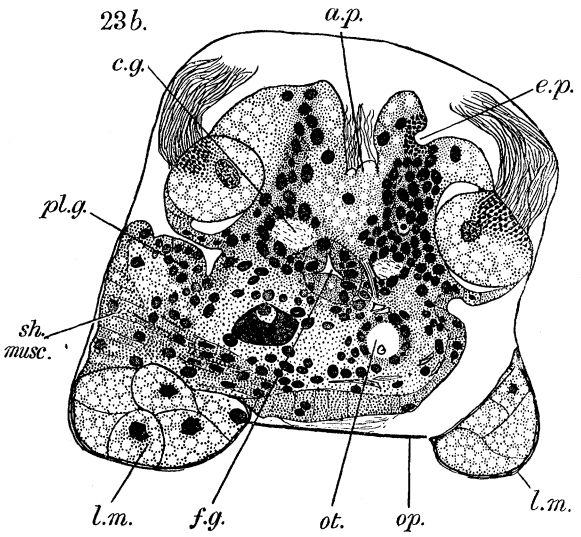

Fig. 23*b*

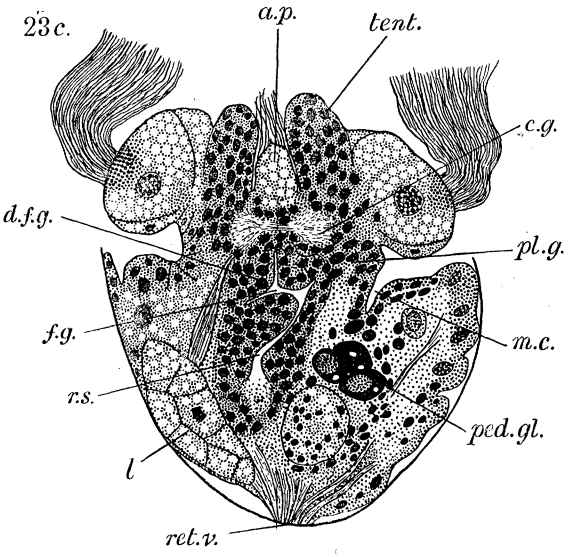

Fig. 23*c*

Frontal sections of 124-hour larva showing (*a*) tentacle, and unpaired gland of foot, (*b*) origin of eye, extension of liver, (*c*) cerebral and pleural ganglia. Abbreviations as in previous figures. In addition: *d.f.g.*, dorsal fold of buccal cavity; *e.p.*, eye-pit: *l.m.*, lobe of liver extending into mantle fold; *mo.*, mouth

begin to develop before the torsion process is complete, it is not until afterwards that the visceral and pleural ganglia are formed, so that the twist in the visceral loop is not produced during the torsional rotation. It may be that although the visceral ganglia are not formed at that time, yet the ectoderm to which they owe their origin

has already become differentiated physiologically. It is also interesting to note that the transverse commissures have their origin in the ectoderm of the body wall, whereas the lateral connectives arise by outgrowth from the already formed ganglia.

The eyes are represented during the torsional stages by small patches of pigment in the velar area near to the refractive bodies, which disappear before torsion is complete. At the end of torsion the pigment patches become larger and better defined and they eventually sink into a pair of pits, fig. 23*b*. The adult eye is virtually the same as the larval eye in structure and only differs in size. The pit is at first shallow and open, but as the larva grows into the adult the pit becomes partially closed.

At the sides of the velar area and just below the eye pits the tentacles arise as rod-like outgrowths of the ectoderm, figs. 23*a*, 23*c*. The topographical relations of the organs of the velar area are shown in a diagram of the prevelar area given in fig. 15.

The alimentary canal is twisted so that the intestinal portion passes up the right side of the larva and the anal cell is situated inside the mantle cavity dorsally and

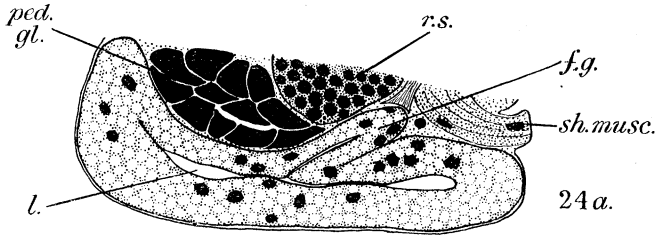

FIG. 24*a*

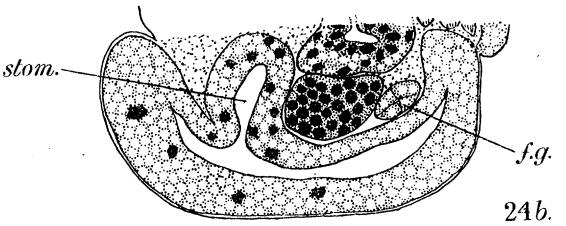

FIG. 24*b*

Frontal sections of 124-hour larva, from below, showing origins of the liver and stomach. Abbreviations as in previous figures. In addition : *f.g.*, fore-gut (gullet): *sh. musc.*, shell muscle (adult retractor muscle)

slightly to the right. The mid-gut or larval stomach is situated posteriorly and the stomodaeal portion of the canal grows in length so as to extend from the mouth towards the dorsal surface and then bend down to the point where it is attached to the mid-gut, figs. 22*a*, 22*b*. The part of the stomodaeum anterior to the radula sac is later to form the buccal cavity of the adult, and in the six-day larva the dorsal folds of the buccal cavity are already formed, *d.f.g.*, fig. 23*c*.

From the mid-gut the adult stomach and gullet are developed by constriction, figs. 24*a*, *b*. The constriction at the base of the gullet is shown in fig. 24*a*, while in fig. 24*b* can be seen the base of the intestine, which is constricted from the mid-gut as a large sac, later to form the adult stomach. The remainder of the mid-gut forms the adult liver, the portions on each side of the median constrictions constituting a pair of lobes. The extremities of these lobes extend into the lateral portions of the mantle folds and are a marked feature of the living larva, fig. 23*c*.

The radula sac develops rapidly and soon forms an S-shaped coil. This, together with the pedal glands and the large liver lobes, takes up a great deal of space in the

body cavity, so that the remainder of the organs are very much compressed. This may account for the manner in which the liver lobes have grown into the mantle fold.

The most important changes that take place in the larva during the few hours following torsion, however, are those concerning the development of the kidney and pericardial rudiments from the coelomic wall rudiments. The latter originate as paired mesodermal blocks of tissue, one at each side of the rectum, and become joined during torsion by the development of a median mass of cells extending below the rectum. These cells are formed from the original paired masses by outgrowth and fusion. At the end of torsion, a small cavity appears in each of the original

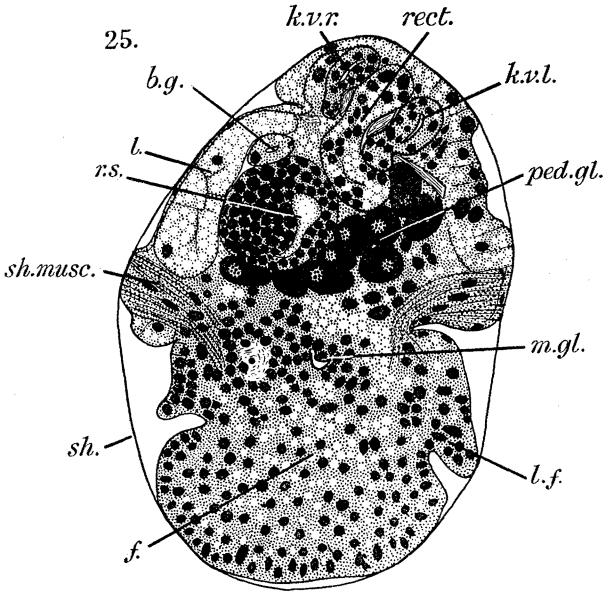

FIG. 25—Transverse section of 124-hour larva, from behind showing the origin of kidneys and pericardium. Abbreviations as in previous figures. In addition : *l.f.*, lateral fold of the foot; *sh.musc.*, shell muscle (*i.e.*, adult retractor muscle)

rudiments. These cavities are the rudiments of the kidneys, which thus arise as very thick walled sacs, *k.v.l.*, *k.v.r.*, figs. 25, 26*a*.

There is no cavity at first in the median cell mass, but subsequent development shows that it represents a solid pericardial rudiment. Soon after its formation the edges of the cell mass grow out and round so as partially to enclose a cup-like portion of the primary body cavity, which is the rudiment of the heart, *h.r.*, figs. 22*b*, 26*c*. The pericardial rudiment is not situated immediately below the rectum, but owing, no doubt, to the crowded condition of the larval anatomy, it is to be found a little to the left of the anal cell, at the side of, or slightly ventral to the mantle cavity.

Before the larva begins to metamorphose, it lives for about a week without undergoing a great amount of change in development, so that when metamorphosis starts the larva is in most respects similar to the stage already described. During this

period the larva is able to swim by means of the still powerful velum, but at irregular intervals it settles down on the substratum and glides along by means of its ciliated foot. When disturbed it is able to retract both foot and velum into the shell, which is then closed by means of the operculum.

At the beginning of this period the buccal ganglion is formed. During development of the radula sac and the stomodaeum, a mass of cells, stomodaeal in origin, appears between the radula sac and the gullet. It is from these cells that the buccal ganglia are differentiated, with fibres extending to the cerebral ganglia, *b.g.* fig. 28.

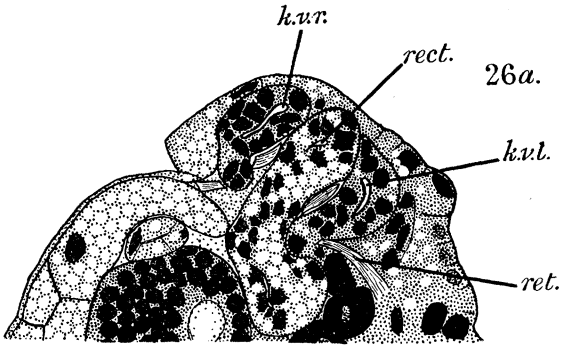

FIG. 26*a*

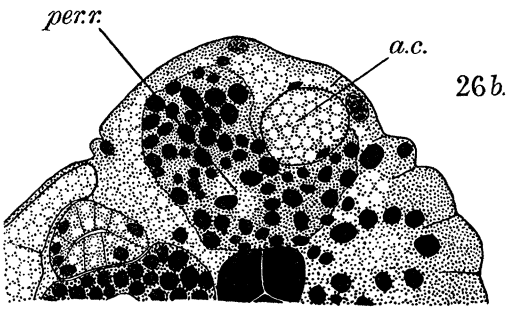

FIG. 26*b*

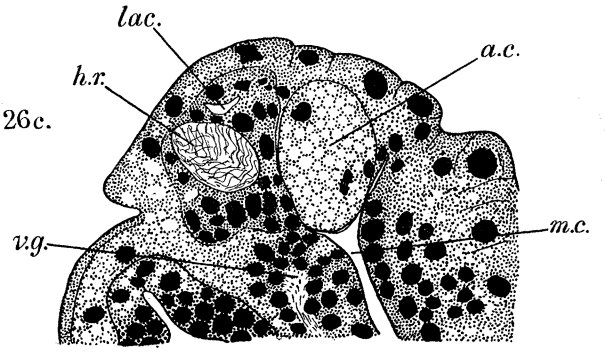

FIG. 26*c*

Three consecutive sections showing origin of kidneys and pericardium (more highly magnified than fig. 25). Abbreviation as in previous figures. In addition: *a.c.*, anal cell; *lac.*, lacuna (first trace of pericardial cavity); *per.r.*, rudiment of pericardium; *v.g.*, right visceral ganglion

Along the sides of the foot there appear two folds in the ectoderm. These persist until late in the post-larval period of development, and are identical with the lateral folds mentioned by DAVIS and FLEURE (1903) in their memoir on *Patella*. At this stage there is no trace of any glandular tissue associated with them. They are simple folds.

The adult shell retractors are of entirely different origin to the retractor muscles of the larva, already mentioned, and they begin to develop at about 6 days, *sh. musc.*, figs. 24*a*, 25. They are muscular cells which become drawn out into spindle-like fibres extending from the edges of the shell and mantle into the foot. At first they

are only developed along a small area in the ventral region, fig. 22*a*. In the larva they function as foot retractors acting with the dorsal and ventral larval retractors when the larva is withdrawn into the shell. The larval retractor muscles do not persist in the adult and are probably equivalent to the columellar muscles of the spirally twisted Gastropods.

The tentacles also develop early, and during the larval period between torsion and the beginning of metamorphosis they become prominent outgrowths.

The radula is provided with teeth at an early stage, but the arrangement differs

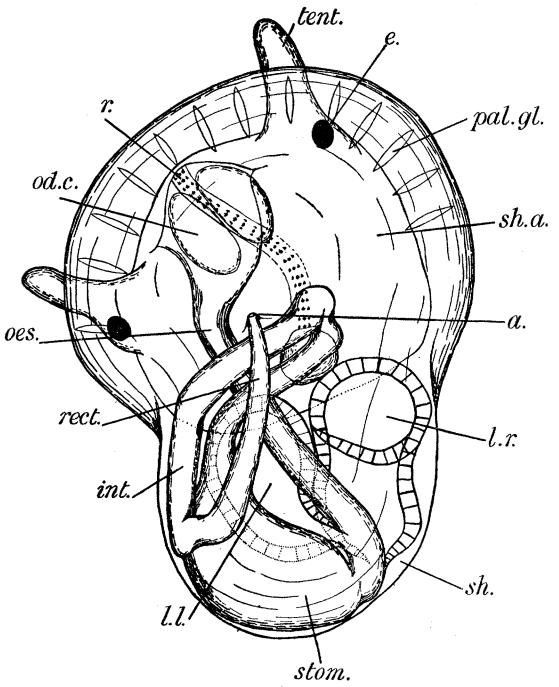

Fig. 27—Dorsal view of larva during metamorphosis. *a.*, anus; *e.*, eye; *int.*, intestine; *l.l.*, *l.r.*, left and right lobes of liver; *oes.*, oesophagus; *od.c.*, cartillages of odontophore; *pal.gl.*, gland of mantle edge; *r.*, radula; *rect.*, rectum; *sh.*, larval shell; *sh.a.*, adult shell; *stom.*, stomach; *tent.*, tentacle

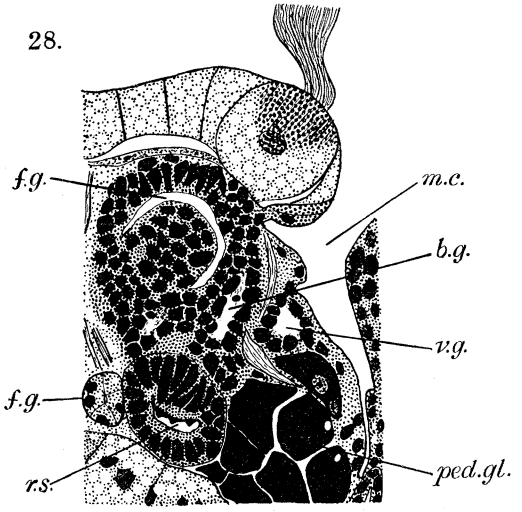

Fig. 28—Frontal section of 196-hour larva seen from above. *b.g.*, buccal ganglion; *f.g.*, fore-gut: *m.c.*, mantle cavity; *ped.gl.*, pedal gland; *r.s.*, radula sac showing teeth inside; *v.g.*, visceral ganglion

very much from that of the adult. From about seven days onwards, each row consists of three teeth. The median tooth is three-clawed, fig. 30, and the two lateral teeth are conical structures with a small lateral claw projecting inwards. This arrangement persists until a late post-larval stage.

Further small changes taking place in the pre-metamorphic stages are the appearance of a small cavity in the pericardial rudiment, *lac.*, fig. 26*c*, and the slow growth of the shell. A slight bending takes place in the intestine owing to its increase in length, so that it now has a single loop. The mantle cavity undergoes a slight increase in depth.

V—Metamorphosis

Metamorphosis of the larva takes place by the rapid growth of the shell border until there is a rim or peristome attached to it, which is the beginning of the adult shell. This is essentially the same process as in *Acmea*, described by Boutan (1899).

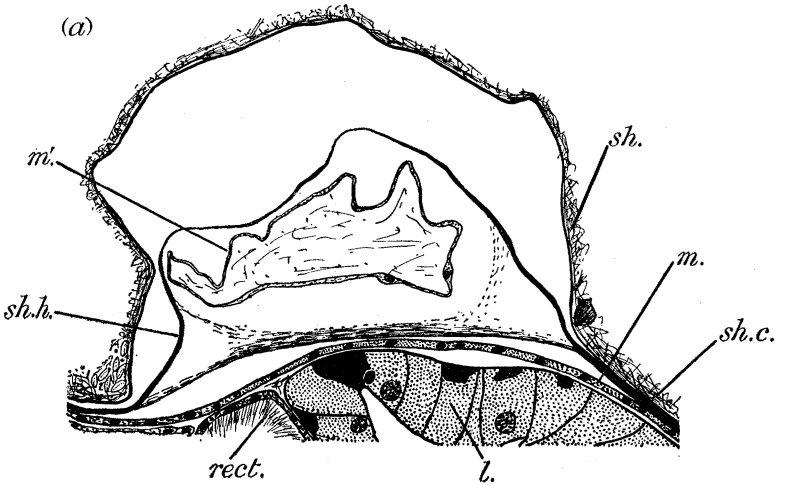

Fig. 29*a*

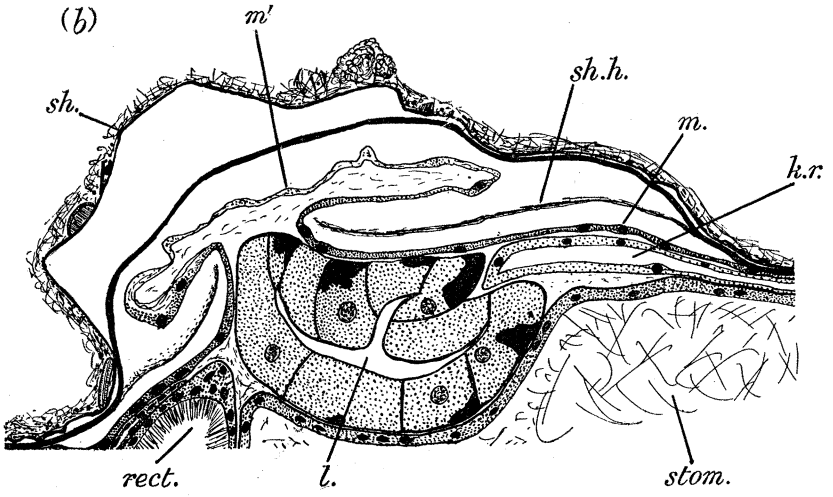

Fig. 29*b*

Transverse sections through post-larva showing loss of larval shell, (*a*) showing a portion of the larval mantle entirely separated from the adult mantle, (*b*) showing the larval mantle in continuity with the adult mantle. *kr.*, right kidney of the adult (*i.e.*, the left kidney of the pre-torsional larva) ; *l.*, lobe of liver ; *m.*, adult mantle ; *m'.*, larval mantle ; *rect.*, rectum ; *sh.*, larval shell ; *sh.c.*, calcareous layer of adult shell ; *sh.h.*, horny layer of adult shell

The development of the rim continues until the shell resembles that of a grown adult, but with the larval shell still attached to its apex. After a while the larval shell is rubbed off, or is cast off, and the post-larva becomes a young and very small adult.

At the beginning of the process, before the peristome of the shell can be seen, the velar cells lose their cilia and the yolk vacuoles enlarge. These vacuoles eventually run together so as to form large irregular spaces in the cells, whose nuclei in many cases disappear. At the same time, the velar support cells in front and behind grow in between the velar cells and the remainder of the velar area. Finally the velar cells disappear completely. It seems highly probable that they are cast off owing to the ingrowth of the support cells, but this has not actually been observed.

The anal cell loses its nucleus during the free-swimming period after torsion, and begins to degenerate. At the end of this period, the cell becomes completely degenerate and is either absorbed or is cast off. The rectum then opens by a ciliated anus.

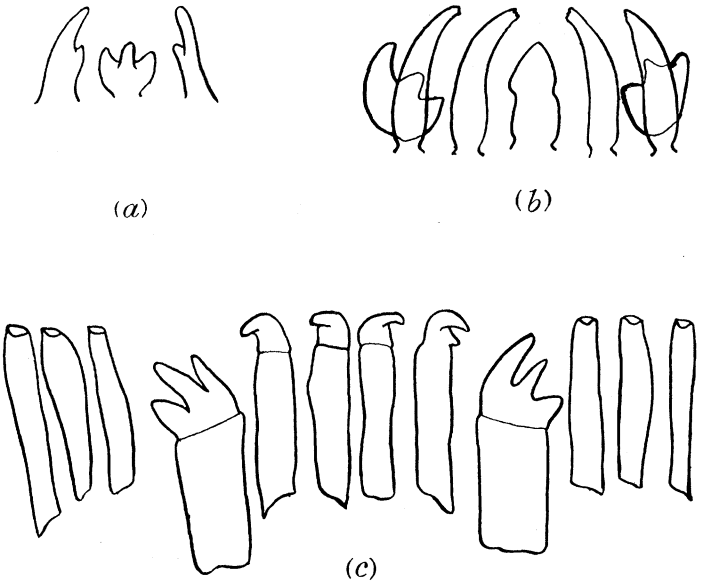

FIG. 30—Arrangement of radular teeth at various stages of development, (*a*) 8 days old to beginning of metamorphosis, (*b*) near the end of metamorphosis, (*c*) post-larva and adult

The larva, as soon as it ceases to swim and settles down to a creeping existence, is no longer able to retract itself into the shell because of the gradual loss of the larval retractors. At the same time the adult shell retractors spread to the rim of the shell and no longer serve to pull the foot into the shell cap, but rather to pull the shell rim down over the enlarged foot. At the same time the operculum is lost, and with the growth of the retractors along the shell rim they can be recognized as the horse-shoe shaped shell-muscle of the adult. During this period also the glands of the foot enlarge, that on the right-hand side developing to a much greater extent than that on the left. This enlargement is no doubt due to the increased needs of the larva as it adopts a crawling mode of life, and larger supplies of mucous secretion are needed for the foot. The branches of the pedal gland, as well as the main body of it are filled with a secretion staining uniformly with haematoxylin. The branches terminate among the ectoderm cells of the sole of the foot.

The median gland, as already mentioned, disappears, leaving no trace in the post-larva, at about the same time as the operculum is lost.

The differentiation of the mid-gut into gullet, liver lobes, and stomach is completed. The cavities of the kidneys and the pericardium become enlarged as the larva grows and as more room exists for their development, and the walls of the sacs become thinner. No trace can be seen during development of the reno-pericardial ducts, which are probably represented by the solid cell masses connecting these organs. At the beginning of metamorphosis, the mantle cavity grows so as to occupy the whole of the anterior dorsal region. Owing to this growth the kidney and pericardial rudiments are, at their anterior ends, carried to the dorsal part of the mantle cavity, together with the anus.

Fig. 27 shows the larva at a stage some time after metamorphosis has begun. It will be seen that the new shell, or peristome to the larval shell, has not been added on the same bilateral axis, but on a new axis inclined downwards and to the left of the original larval axis. This gives to the metamorphosing larva a spiral twist, which though small is quite definite. The mantle cavity continues to enlarge, and at the anterior and lateral edges appear the mantle glands. The first traces of the mantle papillae also appear at this stage.

It is towards the end of this period that the kidneys become open to the exterior (*i.e.*, the mantle cavity), but it is not possible to say whether or not the ducts are ectodermal in origin, although the epithelium lining them is ciliated and continuous with that of the mantle cavity, and the openings are at first very wide.

The odontophore cartilages develop from the mesoderm covering the gullet, between the mouth and the radula sac. In the metamorphosing larva there is only one pair of these.

The radular teeth still have the same arrangement as they had before metamorphosis, consisting of three teeth in each row (1.1.1.), but with the increased size of the larva and the active feeding the teeth become progressively larger in size as the radula grows.

At the end of the metamorphosis, before the larval shell is lost, a change takes place in the radular formula. The lateral teeth increase in number to two on each side, and a new three-clawed tooth appears at the outer edge of each row, slightly behind the others (1.2.1.2.1.). The central tooth is still retained. After the larval shell has been lost the radular formula becomes the same as the adult (3.1.2.0.2.1.3.), by the disappearance of the median tooth, and the appearance of three marginals, fig. 30 *a*, *b*, and *c*.

The shell cap of the post-larva is cut off eventually when the animal is about 0·5 mm long, by the secretion of a shell plate across the base of it, figs. 29*a*, 29*b*. Where the larval and adult shells are joined, the mantle begins to secrete a new horny layer as an annular projection into the underlying tissue. This forces back the mantle itself, thus constricting the organs—mainly lobes of the liver—contained in the region between the two parts of the shell, and eventually squeezing them out of the larval portion. In this way the larval shell and a portion of the mantle are

cut off by the plate, which eventually closes right across the base of the shell cap. Cut off from nerve and blood supplies, the larval cap dies and either drops off or is eventually rubbed off.

At an early stage in metamorphosis the two liver lobes begin to grow out, so that in the early post-larva the greater part of the body cavity is filled by the branches of the liver. In the post-larva of over 1 mm in length the development of the right kidney becomes more pronounced and it grows out over the visceral hump, eventually reaching the ventral surface. No trace of the origin of the gonads has been observed.

The pallial gills appear during metamorphosis and are in all respects similar to those of the adult. At no stage in development are there to be seen any traces of ctenidia on the wall of the mantle cavity. The osphradia, which are sometimes said to represent the ctenidia, develop from ectodermal thickenings of the mantle floor at the right and left of the mantle cavity at its anterior end.

VI—Summary of New Points

The mesoderm rudiments are a pair of cells formed by the division of 4D, figs. 2*a*, 2*b*, into right and left daughter cells. Endoderm is formed from the macromeres in quadrants A, B, and C alone.

Patten (1885) is shown to be mistaken in ascribing to the foot a bilobed origin.

The radula sac is formed as a ventral pouch of the stomodaeum. The radular teeth are present in the larva, and the radular formula undergoes two changes before it acquires the adult arrangement.

The larva possesses a dorsal and a ventral retractor muscle. The first named is present in the larva before torsion takes place and is asymmetrically situated.

Torsion is shown to take place under the action of this asymmetrically placed dorsal retractor, and to be caused partly by the antagonistic (*i.e.*, mutually interfering) growth of the foot and the shell.

The cerebral ganglia arise as ectodermal proliferations from the base of pits in the apical plate, and the commissures from the base of a groove connecting them. Pedal, pleural, and visceral ganglia arise from the cells of ectodermal thickenings at the front of the foot, the sides of the larva above the foot and the floor of the mantle cavity respectively. The buccal ganglia arise from a cell mass derived from the stomodaeum.

A constriction separates the adult stomach and the posterior part of the gullet from the larval stomach, the remainder of which forms a bilobed liver, which branches profusely to form the adult liver.

The otocysts are formed from invaginations at the posterior angles of the mantle cavity which sink into the foot.

The original mesoderm mother cells, after giving rise to the lateral mesoderm bands, form two compact masses of cells at the sides of the rectum, which are the coelomic wall rudiments. These fuse below the rectum to form a solid pericardial rudiment, and the paired masses acquire cavities which later form the kidneys.

The heart arises as a portion of the primary body cavity enclosed by an outgrowth of the solid pericardial rudiment.

After undergoing progressive degeneration the velum is cut off from the rest of the head by the ingrowth of the support cells and is finally cast.

The adult shell arises as a peristome or rim to the larval shell, which is afterwards cut off by a shell plate secreted by the mantle and is then cast off.

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