

# THE EMBRYOLOGY OF THE INDIAN APPLE-SNAIL, *PILA GLOBOSA* (SWAINSON) [MOLLUSCA, GASTROPODA].

By A. R. RANJAH, D.Sc., *Department of Zoology, University of Lucknow.*

## CONTENTS.

	PAGE.
I. Introduction .. .. .	217
II. Oviposition .. .. .	219
III. The Fertilised Ovum .. .. .	222
IV. The Cleavage and the Cleavage-Cavity .. .. .	223
V. Gastrulation .. .. .	228
VI. The Trochophore .. .. .	229
VII. The Development of the Mesoderm .. .. .	229
VIII. The General Outline of Development beyond the Trochophore Stage .. .. .	232
IX. The Development of the Organ-Systems .. .. .	249
(A) The Alimentary Canal .. .. .	249
(B) The Differentiation of the Common Rudiment of the Kidney, the Pericardium, the Heart and the Gonad .. .. .	261
(C) The Kidneys and the Ureter .. .. .	268
(D) The Pericardium, the Heart and the Blood-Vessels .. .. .	276
(E) The Gonad and the Genital Duct .. .. .	280
(F) The Respiratory Organs .. .. .	282
(G) The Nervous System .. .. .	287
(H) The Receptor Organs .. .. .	298
X. The Larval Organs .. .. .	304
XI. The Foot .. .. .	307
XII. The Shell Gland and the Mantle .. .. .	311
XIII. The Mantle-Cavity .. .. .	314
XIV. Summary .. .. .	315
XV. Explanation of Lettering in Text-figures .. .. .	318
XVI. References .. .. .	319

## I. INTRODUCTION.

### *General.*

Our knowledge of the embryology of the Gastropoda dates from the latter part of the 19th Century, and a considerable amount of work has been published on the subject. A perusal of the literature shows that while a great deal of attention has been paid to the phenomena of cleavage and cell-lineage, equal justice has not been done to organogenesis. Most of the investigations are confined to the early development of the embryo up to the gastrula or the trochophore stage. Only a few authors have described the origin of the rudiments of the various organ-systems and their subsequent development.

The Indian Apple-Snail, *Pila globosa* (Swainson), is studied as a type of the Gastropoda in many of the Indian Universities, and an account

of its anatomy was published by Prashad (1932). In order to complete our knowledge of this form I took up at Professor K. N. Bahl's suggestion, the study of its embryology.

This investigation was carried out in the Zoology Department of the University of Lucknow under the guidance of Professor K. N. Bahl, D.Sc. (Oxon.), to whom I am deeply indebted for advice and constant encouragement. He made valuable suggestions and took great pains in revising the manuscript. I am also indebted to Dr. M. L. Bhatia and Dr. S. M. Das for their helpful criticisms and assistance in the preparation of the figures. My thanks are also due to Dr. S. M. Sane and Dr. A. C. Chatterjee of the Chemistry Department for translating the bulk of the German references for me ; and to Mr. Natu for help in the preparation of some of the drawings. Finally, to Professor E. S. Goodrich, F.R.S., of Oxford, I am deeply indebted for examining the manuscript critically.

#### *Material and Technique.*

The material for the present investigation was obtained during the monsoon months of July and August. Freshly laid spawn was obtained both from Chinhat and Marayaon Lakes in the vicinity of Lucknow, as well as from the aquarium of the University Zoological Laboratory at Lucknow, where copulating pairs were brought and kept for the study of oviposition. The time taken for oviposition was recorded. The egg-masses were kept on moist earth in large glass dishes in as natural surroundings as possible in the laboratory. The earliest embryonic stages were dissected out of the eggs in normal salt solution by removing a part of the egg-shell and incising the thick solid layer of albumen with a needle, thus allowing the albuminous fluid within to flow out of the egg along with the contained embryo. The embryo was then separated from the albumen with fine needles.

The material was fixed variously. For a study of the cleavage, the segmenting ova were fixed in Gilson's mixture or 5 per cent. formalin, or according to Wilson's method (1904)—fixing in acetic acid to which drops of glycerine were added to render the ova transparent. Gilson's mixture was found quite satisfactory for the study of whole embryos. For advanced stages of the embryos, Kleinenberg's picro-sulphuric acid, Gilson's mixture, the Petrunkevitch's mixture, 5 per cent. formalin, Zenker's fluid, and hot corrosive sublimate were employed. Of these Gilson's mixture and the Petrunkevitch's mixture gave the best results.

The time of fixation varied with the different fixatives and with the different stages of development. Gilson's and Petrunkevitch's mixtures required 5 to 20 minutes.

In advanced stages when the shell is well developed and the embryos possess an adult appearance, it is always essential to fix them in their fully extended condition in order to avoid complications caused by the retraction of the embryo within the shell. For this purpose, the embryos were narcotised either with sulphuric-ether or with chloroform. The shell was then removed with fine needles.

The fixed material was stored in 75 per cent. alcohol. Some of the material was preserved in the alcohol-glycerine mixture. It was found,

however, that the glycerine penetrated the tissue of the material so thoroughly that it could not be completely removed subsequently, with the result that good sections could not be obtained.

It may be emphasised that material should preferably be kept in glass-stoppered tubes rather than in corked tubes. In the latter case, presumably on account of some chemical change produced by the action of alcohol on the cork, the material becomes discoloured, and does not yield good sections, as the cells of the outer or epidermal layer become vacuolated, shrunken or distorted. On the other hand, the material kept in glass-stoppered tubes retains its natural colour for a long time and the tissues remain in good condition.

For whole mounts, the best results were obtained by first keeping the embryos in the alcohol-glycerine mixture for some time. This treatment renders them more transparent than is otherwise possible. Embryos as well as the early eggs in cleavage-stages were cleared in clove oil and mounted in cavity slides—which procedure has the advantage that, on rolling the cover-slips, the ova and the embryos can be examined on all sides without being injured.

For sectioning, the embryos were cleared in cedar-wood oil and embedded in paraffin. For the orientation and the block-making of very early stages, the following method proved to be satisfactory. The embryos were transferred from the clearing medium on to a piece of blotting paper, whence they were transferred by touching the embryos with a needle dipped in melted paraffin, when the embryos adhere to the paraffin. They were then transferred to watch glasses containing molten paraffin inside the bath. At the time of block-making, the embryos were orientated under the binocular-microscope and the paraffin allowed to cool. If the material is lightly stained with Borax Carmine while it is still in alcohol, the orientation in paraffin is very much facilitated.

Sections were cut in three planes, *viz.*, transverse, sagittal, and horizontal. Sections of the early stages were cut  $5\mu$  thick, but those of some of the advanced stages were cut up to  $8\mu$ .

Whole mounts were stained in alcoholic borax-carmine which was also used as a bulk stain for sectioning purposes. Delafield's Haematoxylin, either alone or with eosin as a counter-stain, and Heidenhain's haematoxylin were employed for staining the sections.

All figures were drawn with the help of the camera lucida. The figures of the whole mounts were drawn in outline with a camera lucida, but the outlines of the internal organs, though first traced from whole mounts, were checked later by a study of the sections. The figures have been so drawn that the dorsal side of the animal is the upper side of the section, and thus the direction of the foot is always kept ventral. In figures of transverse sections, the right and left sides of the figures correspond to the right and left sides of the animal.

## II. OVIPOSITION.

### *The General Process.*

Like most Gastropods, *Pila globosa* is oviparous. It breeds during the rainy season, which in Northern and Eastern India extends over a period of about four months, from June to September. In the United

Provinces, the period extends from the beginning of July to about the first week of September, but the middle of July, when the monsoon is in full swing, is the most active period. In Bengal, where the monsoon is early, breeding takes place in May and June (Prashad, 1925). Breeding depends not only on the onset of the rains but also on their continuity. If there is a break in this continuity for several days on end, breeding ceases altogether, except in rare cases.

As soon as the rains set in, the snails, which have been aestivating underground upto a depth of four feet (Bahl, 1928), come out of the ground and enter small ponds and puddles formed by the rain. They feed voraciously on succulent aquatic plants such as *Vallisneria* and *Pistia*, and later copulate. Copulation may take place either in water while the snails are floating, or near the edge of water amongst water-weeds.

Eggs are laid two to three days after copulation, and generally about two weeks elapse after the onset of the rains before the females are seen laying their eggs. According to Bahl (1928), oviposition can be delayed by preventing impregnated females from going out of water on to the land, but when this check is removed, oviposition begins. He writes, "after keeping several fertilised females in water in a glass aquarium for a week, I took them out and placed them on moist ground in the frog-pond with the result that there was, so to speak, an 'epidemic' of oviposition, since all of them had been waiting to lay eggs" I observed also that females, which were disturbed in the act of oviposition, if kept on moist soil, started laying again after about three hours, so that the interrupted oviposition was resumed.

Generally, *Pila* lays its eggs in the early hours of the morning from about 5 to 9 A.M., though sometimes eggs are laid at other hours of the day as well.

For oviposition, *Pila* leaves the water and comes out on the land. It lays its egg-mass in a suitable place, generally a few inches to a few feet above the water-margin in a natural depression in the ground or a special pit made for the purpose in soft ground with the help of its shell and foot. When the ground is hard, no pit or depression is formed and the eggs remain quite exposed and unprotected, but they are usually concealed amongst water-weeds or grass close to the water-margin.

There are two distinct advantages to the snail in laying the egg-masses amongst water-weeds rather than on open ground. In the first place, the eggs are protected from desiccation; they retain their moisture for a long time, and even the outermost eggs in the mass do not dry up; the number of embryos which hatch out is much larger than in the case of those egg-masses which are laid out in the open. In the latter case, there is exposure to direct sunlight and excessive evaporation with the result that the outer layers of eggs in the masses dry up and the embryos die.

When *Pila* comes out of water for oviposition, a process which takes four to six hours, it is exposed to its enemies, such as water-birds, specially the herons, which are always on the look-out for and pounce upon the snail, which has protruded its foot and head out of its shell and lies in a relaxed condition during oviposition. These birds tear the snail

out of its shell and incidentally destroy the eggs, trampling them under their feet.

During oviposition, the head region protrudes out of the shell along with the fully extended foot, the latter functioning as an 'ovipositor' (Bahl, 1928) by being transformed at its base into a cup-shaped structure with the concavity towards the outside. By its muscular action, the foot helps in the deposition and collection of the eggs into a mass as soon as they pass out of the vagina, one after another. The cup-shaped foot envelopes the eggs as they are laid and keeps them together till the last egg is laid, after which the female withdraws its foot from the egg-mass and creeps back to the water, leaving the egg-mass outside. It was probably after seeing *Pila* in such a state, *i.e.*, when the female was laying its eggs and the eggs were partially covered by the dome-shaped sole of the foot, that Ramanan formed a wrong idea that eggs were being incubated by the female; he also wrongly considered the upper part of the cup-shaped foot enveloping the egg-mass as a part of the mantle. Bahl (1928) first showed that Ramanan was mistaken; and I agree with him. Bahl's statement that "the foot is attached to the egg-mass for kneading together and adding new eggs as they are being laid to the pre-existing egg-mass and not for the purpose of incubation" is corroborated by my own observations.

#### *The Egg-Mass.*

The eggs are laid in more or less irregular masses which differ in size, weight, and shape, and also in the number of eggs they contain. The size of the egg-mass varies from  $2 \times 2.5$  cm. to  $5 \times 9$  cm., averaging about  $3.5 \times 5$  cm.; the eggs are placed 5 to 6 layers deep. The weight of the egg-masses varies from 5 to 34 grms., average 15 to 25 grms. The number of eggs in each mass varies from 100 to 850; in masses which are about  $3.5 \times 5$  cm. in size and 15 to 25 grms. in weight, the number is from 400 to 500.

The shape of the egg-masses varies. If the eggs are laid in a hollow, the mass assumes a convexity on the side lying against the ground. On flat ground, the mass is flat on the lower side. The upper surface of the mass may be either flat or convex. The mass is more or less circular or oblong in outline and may show a depression in the centre.

During oviposition, as the eggs are laid in succession and are sticky in the fresh condition, they get pressed and glued together due to the muscular action of the foot. The eggs thus become slightly flattened at their places of contact.

#### *The Egg.*

The eggs of different masses may vary slightly in size, but the eggs of the same mass are of the same size, though in a few cases one or more eggs in the same mass may be larger than the rest. The egg is either rounded or oval in shape, varying in diameter from 4 to 7 mm., the latter size being rare. The average diameter is about 5 mm.

The egg is composed of a shell; two thin membranes, the shell-membrane and the albumen-membrane; a solid albuminous sphere; and the albuminous fluid in which the embryo floats.

The shell forms the outermost covering of the egg. It is calcareous and is milk-white in colour; when fresh it is soft and sticky. Shortly after oviposition, the shell dries up and becomes hard and brittle. The shell is also porous. The outer surface of the shell is rough and is studded with minute tubercles. The shell is about 0.5 mm. thick and is lined internally with a very thin and delicate transparent membrane, the shell membrane. On removing the shell with the shell membrane, a thick solid globular mass, the albuminous sphere, is seen. It is about 4 mm. in diameter and 1.2 mm. thick, and fills the entire space within the shell. Closely investing the albuminous sphere, is another delicate membrane, the albumen-membrane. The albuminous sphere is solid in texture, and is greyish-white in colour; inside, it is hollow and the cavity is filled with a transparent albuminous fluid in the centre of which floats the yellowish embryo. The area covered by the albuminous fluid is about 1.7 mm. in diameter.

The growing embryo ingests the entire albuminous fluid and then the solid albumen, so that at the time of hatching, only the albumen and shell-membranes are left within the shell; the latter ruptures to allow the embryo to come out.

Development evidently begins as soon as the eggs are laid. Eggs taken from different regions of an egg-mass contain embryos at different stages of development. In a freshly laid egg-mass, the eggs laid last show no cleavage of the fertilised ovum at all; the eggs from the middle layers of the egg-mass are in the two-cell stage; and the eggs that were the first to be laid, *i.e.*, those at the lowermost layer of the egg-mass, are already in the four to eight-cell stages.

Apparently, all the eggs are fertilised, and are capable of development under favourable conditions; no sterile eggs were met with<sup>1</sup>.

The incubation period varies with the nature of the environment. Brighter sunshine (higher temperature) and suitable humidity accelerate development. I found that in one year when the temperature in the shade varied between 90 to 100°F the incubation period was 10-14 days; while in another year, during the corresponding period when, due to constant and heavy rainfall, the temperature had come down to 70-80°F. the incubation period was about three weeks<sup>2</sup>.

### III. THE FERTILISED OVUM.

During copulation the spermatozoa are known to make their way to and impregnate the ova while the latter are still within the oviduct. The thick solid layer of albumen and the egg-shell are formed later and surround the fertilised ovum. Careful examination of a freshly laid egg, on the removal of the egg-shell and the solid albumen-sphere, shows a large number of spermatozoa floating in a more or less disintegrated condition in the liquid albumen (*cf.* Semper, 1862; Scott, 1934).

---

<sup>1</sup> According to Annandale (1920) a covering of the sterile eggs surrounding the fertile ones is met with in the case of the Siamese species, *Pila turbinis*.

<sup>2</sup> Prashad (1925) gave the incubation period in *Pila globosa* as about a month. In *Ampullaria polita*, according to Semper (1862), 14 days was the maximum period required by the young ones to hatch out. Scott (1934) gives this period as about one month in the case of *Ampullaria canaliculata* but this, she says, is the case with eggs produced at the end of the season and kept in shade.

The fertilised ovum (Text-fig. 1a) is spherical, and is a bright lemon-yellow due to the presence of the coloured food-yolk which renders the egg quite opaque. Each ovum in the living state is about  $160\ \mu$  in diameter<sup>1</sup>. In it the yolk is uniformly distributed and there is no demarcation between the protoplasmic and deutero-plasmic portions. The first indication of a polar differentiation is noticed when the two polar bodies are extruded, one after the other, at one end of the egg, which forms the animal or formative pole of the egg. In *Pila globosa*, as in *Paludina* (Tönninges, 1896) and *Crepidula* (Conklin, 1897) the polar bodies do not remain attached to the egg for a very long time, but soon get detached and disappear<sup>2</sup>. On examining the ovum immediately before the first cleavage, we find that just beneath the polar bodies there lies a small comparatively lighter area, while the yolk is concentrated at the opposite end of the egg; the nucleus has moved from its middle position into the animal pole while the polar bodies are being extruded. Thus a polar differentiation is clearly established in the egg just before cleavage starts, as is generally the case in all Gastropoda. In *Crepidula*, however, Conklin (1897) believes that this polarity is established at a very early stage when the egg is still within the ovary.

#### IV CLEAVAGE AND THE CLEAVAGE=CAVITY.

##### *The First Cleavage.*

Cleavage begins about  $2\frac{1}{2}$  to 3 hours after the eggs are laid. The first dividing furrow appears at the animal pole where the polar bodies were extruded, and gradually extends along both sides in a meridional direction to the vegetative pole. The first cleavage is completed in about 15 minutes and divides the egg into two equal halves, the blastomeres. Each hemispherical blastomere soon becomes almost spherical again, the two being joined along a small area in the middle of each sphere. But this spherical shape does not persist, as the blastomeres again become flattened against each other (Text-fig. 1b), so that the two together look like a single undivided sphere (cf. *Paludina*, *Littorina*, *Succinea*, *Crepidula*, *Limax*, *Planorbis*, *Physa*, etc.).

At the junction of the two blastomeres a lenticular cavity makes its appearance and after reaching its maximum size, suddenly disappears before the commencement of the next cleavage.

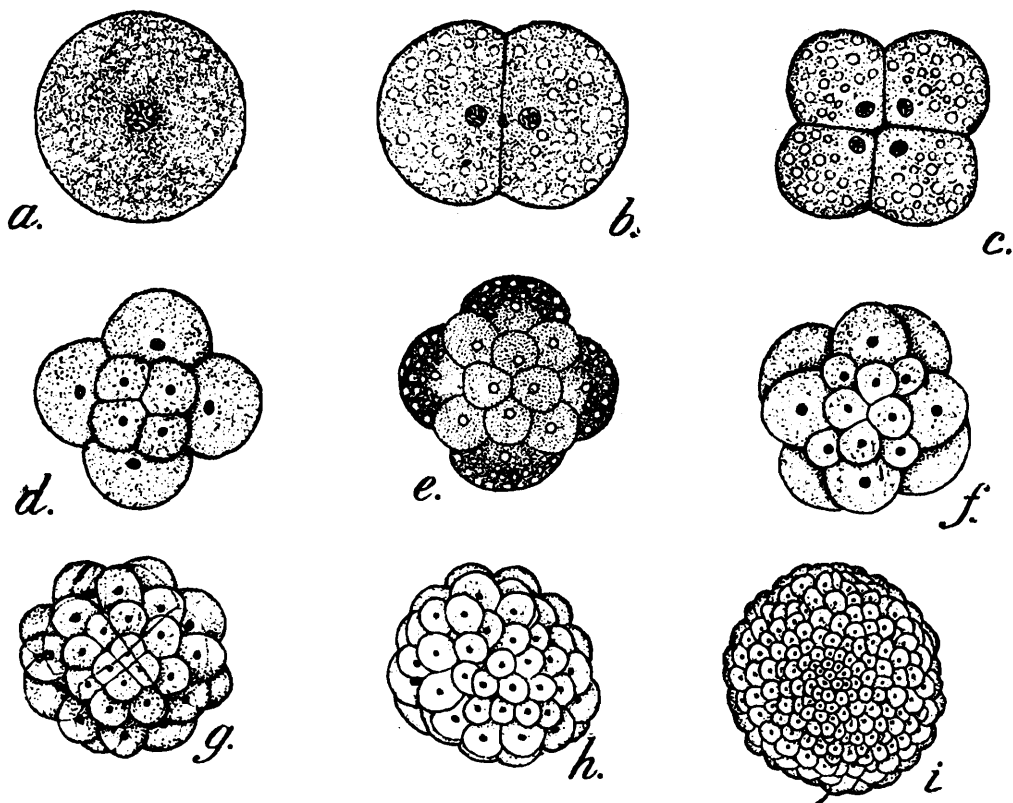
##### *The Second Cleavage.*

The second cleavage occurs about two hours after the completion of the first. Just before the appearance of the second cleavage-furrow, the two hemispherical blastomeres show the same arrangement of their contents as in the unsegmented ovum, *i.e.*, the clear protoplasmic area with the nucleus lies towards the animal pole, while the yolk is concentrated towards the vegetative pole. This cleavage is also meridional.

<sup>1</sup> I have observed that on fixation the ovum shrinks in size. Thus, for example, a living fertilised ovum about  $160\ \mu$  in diameter became reduced to  $144\ \mu$  in diameter, after it was fixed, dehydrated and cleared.

<sup>2</sup> Conklin (1897) has traced these polar bodies in *Crepidula* inside the mesenteron, where they had "been drawn in with the nutrient fluid (albumen) surrounding the embryo".

Beginning from the animal pole, it cuts across the first furrow at right angles, and gives rise to four blastomeres of equal size, designated as A, B, C and D. The division of the two blastomeres occurs simultaneously and I never came across any stage where the division of one of the two blastomeres preceded even slightly that of the other. At the time of their formation, the blastomeres are, as in the preceding stage, spherical but later get pressed against one another (Text-fig 1c). The second cleavage is the first indication of the occurrence of the 'spiral' cleavage and is *laevotropic* in direction. It occurs in such a manner that 'cross' or 'polar' furrows are formed at the two poles of the segmenting egg, as a result of which two of the blastomeres come to lie against each other at one pole and prevent the other two from coming in contact with each other; the latter two however, meet at the opposite pole (Text-fig. 1c) where the first two remain apart. The polar furrow is of great practical and theoretical importance. It has been stated to bear a constant relation, in all cases of spiral cleavage, to the first and second cleavages. If an egg at the 4-cell stage is seen from the animal pole, the polar furrow is seen bending to the right if the first cleavage-furrow be kept in the line of vision, and in the reverse direction, *i.e.*, towards the left, if the second furrow is kept in the line of vision. This order is reversed if we see the segmenting egg from the vegetative pole. According to Conklin (1897) and others, the segmenting egg can be easily and rightly orientated, even in its later stages, by keeping this early position and condition of the polar-furrows in mind.



TEXT-FIG. 1. Early cleavage stages up to the formation of the blastula:  $\times 200$

a. Fertilised ovum as seen from the animal pole:  $\times 200$ .

b. 2-cell stage. c. 4-cell stage. d. 8-cell stage. e. 12-cell stage. f. 16-cell stage. g. 32-cell stage. h. 48-cell stage. i. Surface view of a fully developed blastula as seen from the animal pole:  $\times 200$ .

At this stage again, a quadrilateral cavity makes its appearance in the centre of the egg where the four blastomeres meet, and, after reaching its maximum size, suddenly disappears just before the formation of the third cleavage, in exactly the same manner as in the preceding stage. It reappears again after the next cleavage is completed and disappears after a short pause, to repeat the process in the following cleavages. It is the rudiment of the cleavage-cavity, and has been rightly described by Kofoid (1895) as "an ephemeral recurrent cleavage-cavity"

*The third cleavage or the formation of the first quartette of micromeres.*

Unlike the first two, the third cleavage-furrow, formed after an interval of 1 to 1½ hours, is equatorial in direction and lies more towards the animal pole. It cuts off four small protoplasmic micromeres from the four large macromeres in which the yolk-material is now concentrated, thus giving rise to the 8-cell stage (Text-fig. 1d). The four micromeres, designated as *a*, *b*, *c* and *d*, form the first quartette<sup>1</sup> of micromeres or the ectoblast. The spiral cleavage is more pronounced at this stage and the actual rotation of the cells, *1a*, *1b*, *1c* and *1d*, towards the right side can be easily seen; this cleavage is *dexiotropic* in contrast to the preceding *laevotropic* one. The micromeres rotating approximately through 45° come to lie finally on the furrows between the macromeres. After the rotation, the micromeres draw together, get pressed against one another and lose their spherical shape. A "secondary" polar furrow is formed in the centre where the cells meet. A central cleavage-cavity also makes its appearance at this stage but after attaining its maximum size disappears before the commencement of the next cleavage.

*The formation of the second quartette of micromeres.*

The next cleavage results in the formation of a 12-cell stage (Text-fig. 1e). The four micromeres of the second quartette are cut off from the macromeres; these micromeres, *2a*, *2b*, *2c* and *2d*, are smaller than the macromeres *2A-2D*, but are distinctly larger than the micromeres of the first quartette (*1a-1d*). The 12-cell stage is not a "transitory" stage but a "resting stage" in *Pila*. After the cleavage is completed the blastomeres draw together and give the segmenting egg a spherical form. An "ephemeral recurrent" cleavage-cavity again makes its appearance to disappear again at the time of the next cleavage.

*The division of the first quartette of micromeres.*

After a pause of about half an hour the cells of the first quartette (*1a-1d*) divide unequally into double their number. This cleavage is again anti-clockwise or *laevotropic*. The daughter-cells which are cut

<sup>1</sup> The term 'quartette' is used to designate the products of one 'generation' of the egg, which are cut off at the animal pole from the four cells lying below. The subdivisions of these quartettes will be described as "tiers"—those which are cut off towards the animal pole form the 'upper tier', while those towards the vegetative pole form the 'lower tier'.

Similarly, the quartettes of the different 'generations' are designated by co-efficients (*1a*, *2a*, *3a*, etc.), while the geneology of the cells of each quartette is indicated by exponents (*1a*<sup>1</sup>, *1a*<sup>2</sup>, *1a*<sup>11</sup>, *1a*<sup>21</sup>, *1a*<sup>111</sup>, *1a*<sup>112</sup>, *1a*<sup>211</sup>, *1a*<sup>212</sup>, etc.).

off towards the animal pole form the "upper tier" of micromeres, namely,  $1a^1$ ,  $1b^1$ ,  $1c^1$ ,  $1d^1$  and are pushed towards the periphery. Conklin (1897) calls these peripheral cells ( $1a^2-1d^2$ ) "the turret-cells". Thus a 16-cell stage is reached (Text-fig. 1f).

*The formation of the third quartette of micromeres.*

The formation of the third and last quartette of micromeres ( $3a-3d$ ) occurs almost simultaneously with the first division of the second quartette of micromeres, with the result that a 24-cell stage is obtained. At first, four micromeres ( $3a-3d$ ) are separated off from the macromeres ( $3A-3D$ ) in a dextrotropic direction, and this is immediately followed by the dextrotropic division of the micromeres of the second quartette ( $2a-2d$ ), so that a 20-cell resting stage never results, and we get directly to the 24-cell resting stage. The micromeres of the third generation are fairly large but are smaller than the macromeres, and are distinguished from the latter by their clear protoplasm. At this stage the first quartette consists of 8 cells in two tiers: (1) the upper tier ( $1a^1-1d^1$ ), in the centre looking more or less like a cross; and (2) the lower tier ( $1a^2-1d^2$ ) (turret-cells or trochoblasts) lying in the angles of the arms of the cross of the upper tier. The second quartette of micromeres also consists of 8 cells ( $2a^1-2d^1$  and  $2a^2-2d^2$ ). Beneath these lie the cells of the third quartette which are only 4 in number, and lie in the furrows of the four macromeres still laden with yolk and forming the vegetative pole of the embryo. A cleavage-cavity now appears which, unlike the preceding ones, does not disappear but persists throughout the later stages, and forms the cleavage-cavity of the blastula.

These three quartettes represent the ectoderm and mark the complete separation of the ectoderm from the endoderm and mesoderm, both of which are represented by the four macromeres.

In the 32-, 48- and 64-cell stages (Text-figs. 1g, h) as in the earlier ones the cells of the segmenting egg come together after the completion of the divisions, and get pressed against one another so that the egg assumes a spherical shape and the outlines of the cells are lost, making it difficult to count their number. In these stages, neither sections of embryos nor whole mounts show any indication of the differentiation of the primary mesoderm cell which has been traced in many Gastropoda to the 24-cell stage, as in *Crepidula*, *Planorbis*, and others. The earliest trace of the mesoderm in *Pila*, as in *Paludina*, appears much later, viz., in the gastrula stage.

The micro- and macromeres increase in number and the cleavage-cavity expands till a blastula with a distinctly large blastocoele is formed. A fully developed blastula (Text-fig. 1i) is a spherical structure, about 0.16 mm. in diameter, with the micromeres at the animal pole and the macromeres at the vegetative pole. A blastula is formed in about 19 to 21 hours after the eggs are laid.

I have not been able to ascertain with certainty whether the albumen is deposited first in the micromeres or in the macromeres, or simultaneously in both. I observed it only at a stage when it was already present in all the blastomeres. After the gastrula stage, however, the albumen is absorbed by the endodermal cells alone.

*Discussion.*

The study of the cleavage in *Pila* shows that it resembles other Gastropods in which the eggs do not contain a large amount of yolk, as in *Ampullaria canaliculata* (Scott, 1934). The spiral cleavage in *Pila* at the 4-cell stage is characteristic of all Gastropods. Again, in the direction of its polar furrow, *Pila* resembles all other dextral Gastropoda such as *Crepidula* (Conklin, 1897) and *Umbrella* (Heymons, 1893). In sinistral Gastropoda such as *Planorbis* (Rabl, 1879; Holmes, 1900) and *Physa* (Crampton, 1894; Wierzejski, 1905) the direction of the polar furrow is the reverse.

In most Gastropods the blastomeres up to the 4-cell stage are, as in *Pila*, nearly of equal size, and inequality first becomes evident in subsequent cleavage stages. In *Aplysia* and *Acreea*, however, the blastomeres are unequal already in the 4-cell stage.

Among Gastropods the amount of yolk seems to play an important rôle in regard to the relative size of the micro- and macromeres. The greater the amount of yolk in the egg, the smaller are the micromeres in relation to the macromeres, and this relation is maintained in the later stages of cleavage. In eggs with little yolk this difference is ill-marked, and later all the blastomeres apparently become equal in size.

In *Pila globosa* the micromeres of the 3rd quartette (3a-3d) are distinctly smaller than the macromeres, though the difference is not considerable.

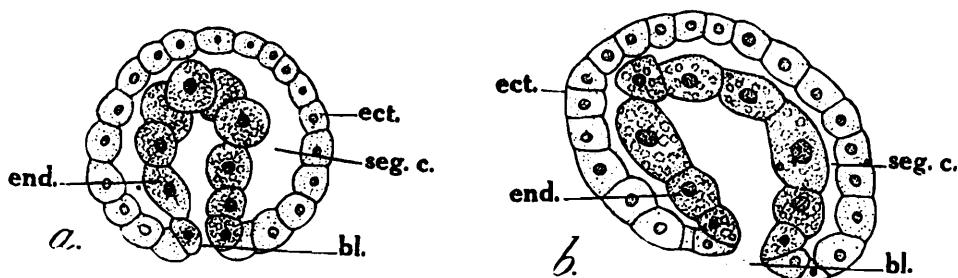
I agree with Delsman's view (1914) that the diameter of the egg is no measure for gauging the amount of food-yolk present in them. He has shown that though in *Littorina obtusata* the egg is 200  $\mu$  in diameter as compared with 112  $\mu$  in *Crepidula fornicata* (Conklin, 1897), the inequality of cleavage is much more marked in the latter than in the former, as shown by the micro- and macromeres of the 3rd quartette. In *Pila globosa* the egg-diameter is 160  $\mu$ , yet there is great inequality in the size of the micro- and macromeres, though this is not so pronounced as in *Crepidula*.

*The cleavage-cavity.*

The cleavage-cavity first appears as a small lenticular cavity in the 2-cell stage at the region where the two blastomeres meet; after attaining its maximum size, it suddenly disappears, the blastomeres get closely pressed against each other and are ready for the next cleavage. It reappears in the 4-cell stage as a central quadrilateral cavity formed at the meeting-place of the inner ends of the four blastomeres. The cavity gradually enlarges till the blastomeres get flattened along their inner surfaces and give the segmenting egg a spherical shape just before the third cleavage. Now the cavity disappears to appear again at the 8-cell stage. This process of appearance and disappearance continues till the 24-cell stage is reached, when the cavity no longer disappears but persists, and gradually enlarges till it assumes a considerable size within the fully developed blastula. In its early stages the cleavage-cavity lies more towards the animal pole of the developing egg.

## V GASTRULATION.

The fully developed blastula becomes flattened at its thickened vegetative pole. The macromeres at this thickened region become elongated and extend into the segmentation-cavity. Gradually a depression appears at this thickened region, the depression being deeper towards the centre than towards the periphery. As development proceeds the depression deepens, thereby pushing the elongated cells of the flattened region deeper into the segmentation-cavity and leading to the invagination of all the macromeres at the vegetative pole to form the future endoderm. Thus arises the gastrula (Text-figs. 2a, b) and the place of invagination forms the blastopore. Gastrulation in *Pila* is, therefore, embolic. The edge of the blastopore marks the boundary between the micromeres (ectoderm) and the yolk-laden macromeres (endoderm). The endoderm cells, in addition to their larger size, can be easily distinguished from the ectoderm cells in taking a lighter stain because of the presence of yolk within them.



TEXT-FIG. 2. Gastrulation.

a. Horizontal section of an early gastrula:  $\times 280$ .

b. Optical horizontal section of an older gastrula:  $\times 487$ .

Unlike some other Gastropoda such as *Paludina*, the segmentation-cavity in *Pila* is never obliterated completely at gastrulation, but is merely reduced and later again enlarges.

When invagination is complete, it is seen that the invaginated endoderm or archenteron is enclosed on two sides by the two arms of the mesodermal bands which, though separate from each other anteriorly, converge posteriorly at their place of origin from the mesodermal teloblasts.

In the early stages of invagination the gastrula appears more or less kidney-shaped in a lateral view. On further growth, however, it reassumes the spherical shape of the blastula. The blastopore is large in its earlier stages, but becomes smaller and smaller until finally it becomes greatly reduced.

The lumen of the archenteron is not simple, but right from the time of the invagination the endodermal wall becomes folded and the archenteric cavity gives rise to either two or more accessory chambers of different sizes and shapes. These chambers communicate with the central cavity of the archenteron through narrow or wide passages. Thus, in some cases one gets the impression that the accessory chambers are evaginations from the main central lumen of the archenteron. *These evaginations simulate archenteric pouches which are liable to be mistaken for coelomic pouches.* The infoldings of the archenteric wall provide a large surface evidently for the assimilation of the ingested albumen.

## VI. THE TROCHOPHORE.

As the gastrula assumes its final, more or less bell-shaped, form, the blastopore becomes narrowed to such an extent that it is difficult to locate it in sections which are even slightly oblique. In the living condition, the presence of yolk within the endoderm cells renders the gastrula quite opaque and yellow, so that the blastopore cannot be seen. The invaginated archenteron becomes more or less flask-shaped—the part around the blastopore is narrow, while that at the opposite end is broad and has a wider lumen. The part of the embryo away from the blastoporal end is the anterior one while the blastoporal end forms the posterior end. The blind end of the archenteron widens out and, consequently, the cleavage cavity becomes reduced in that part of the embryo, while in its posterior part, it remains quite spacious. A further differentiation takes place in the embryo at this stage; an equatorial band of two rows of cells, situated more towards the anterior end than in the middle, becomes differentiated by an increase in size of these cells and encircles the embryo. As development proceeds, the cells of this band grow in size and become fairly prominent. Vacuoles appear within these cells giving the latter a more or less transparent appearance. Later, fine cilia develop on these cells and the ciliated band forms the velum; and thus the embryo passes into the trochophore stage. At this stage neither the rudiment of the stomodaeum nor that of the foot has differentiated. The blastopore, however, persists as a narrow aperture and forms the anus, while the mouth is a new formation. At the posterior end, on that side of the embryo which in later stages becomes the dorsal side, a flattening of the ectodermal wall takes place; this marks the area where, after a short time, the thickened rudiment of the shell-gland makes its appearance.

VII. THE DEVELOPMENT OF THE MESODERM (TEXT-FIGS. 3*a-e*).*The Development.*

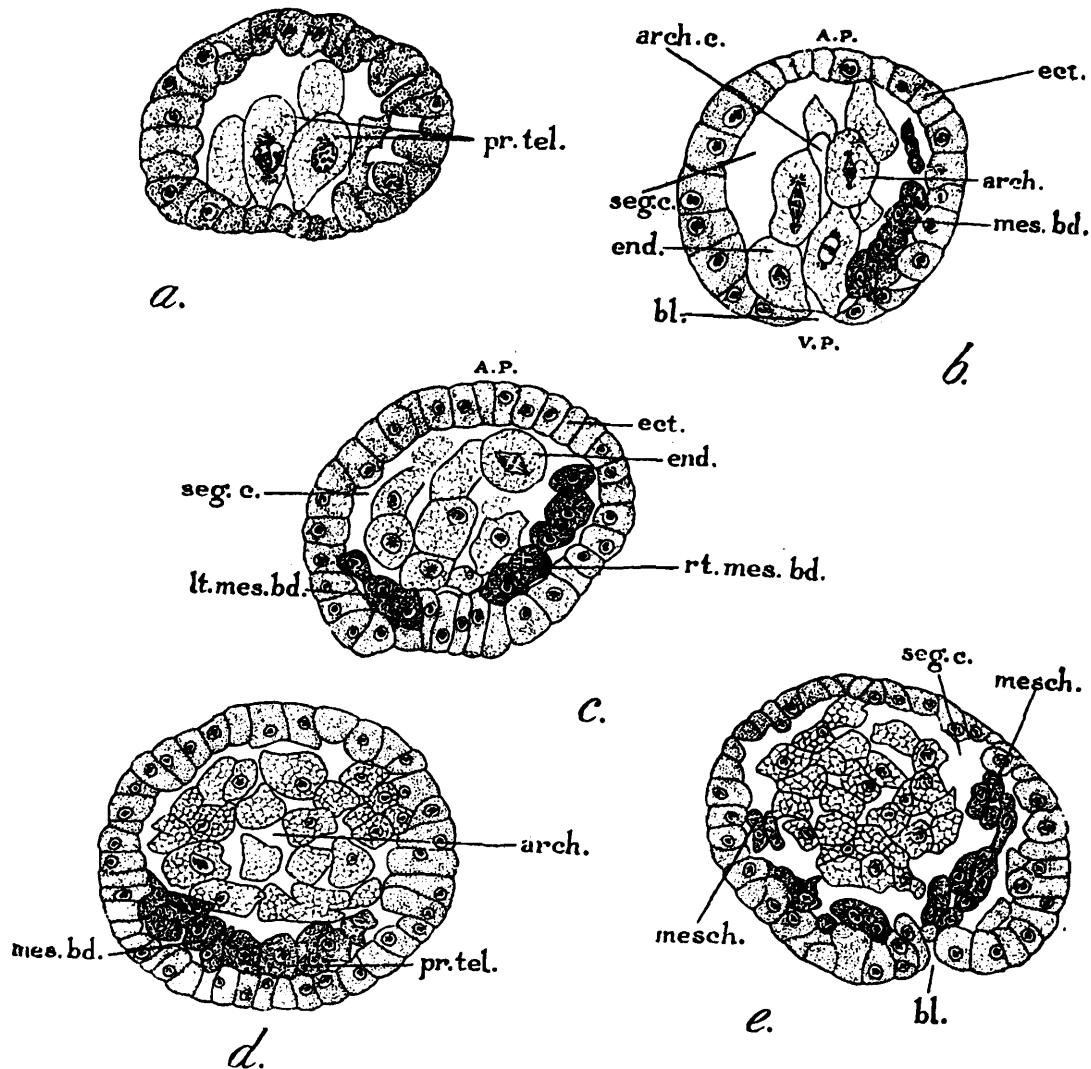
The earliest stage examined by me regarding the origin of the mesoderm is an early gastrula as shown in text-fig. 3*a*. Here the mesoderm is represented by two large bilaterally arranged cells, the mesodermal teloblasts. These two cells, which lie side by side, are slightly larger than the endoderm cells and have distinct and large nuclei. They lie close to and immediately posterior to the position of the blastopore. From their position, structure and size I conclude that they (teloblasts) are derivatives of one of the posterior macromeres. The mother mesoderm cell presumably migrates inwards and comes to lie within the segmentation-cavity where, in the stage available to me, that cell has already divided into two.<sup>1</sup>

As development proceeds these two teloblasts divide in such a manner that the daughter cells lie on the right and left sides of the median axis passing through the blastopore. They thus form paired bands, each

---

<sup>1</sup> Unfortunately all the well-fixed embryos I have sectioned represent stages later than the stage in which the primary or mother mesoderm cell might be seen either migrating into the segmentation-cavity (after its abstraction from one of the macromeres) or lying free within the cavity after migration.

consisting of a row of cells in single file. These are the mesoblastic bands. At first each band consists of a few cells only, but as development pro-



TEXT-FIG. 3. Development of the mesoderm.

*a.* Horizontal section of an early gastrula, passing through the two primary mesoderm cells or teloblasts:  $\times 264$ ; *b.* Oblique horizontal section of an embryo slightly older than in (*a*), passing through the blastopore and showing the mesoblastic band on the right side:  $\times 256$ ; *c.* Horizontal section of a gastrula, showing the mesoblastic bands in the form of a single row of cells:  $\times 256$ ; *d.* Horizontal section of a gastrula older than in (*c*), showing the thickening of the mesoblastic bands. The primary teloblasts are still distinct and are distinguishable by their large size:  $\times 260$ ; *e.* Horizontal section of an embryo about the trochophore stage, showing the differentiation and detachment of the spindle-shaped mesenchyme cells from the mesoblastic bands:  $\times 268$ .

ceeds, the bands increase in length and in the number of their constituent cells. The growth of each band proceeds towards the anterior end of the embryo. In early stages the primary teloblasts, from which the bands are formed, remain quite distinct and are larger than the rest of the mesodermal cells in the bands, and they remain at the posterior end of the embryo; but in later stages the teloblasts cannot be distinguished from the other cells of the mesoderm bands. The cells of the mesoblastic bands can be easily differentiated from the endoderm cells: firstly, by their more or less cubical shape and smaller size as compared with the rounded or oblong shape and larger size of the endoderm cells, and secondly, by their taking a deeper stain than the endoderm cells, because of the absence of yolk and other fatty substances in the contents of their cytoplasm.

As development proceeds the mesodermal bands become thickened by an increase in the number of their cells. The shape of the mesodermal cells also undergoes a change ; they gradually lose their cubical form and become stellate in appearance.

With further development the mesodermal cells at the anterior ends of the bands become detached and come to lie scattered within the segmentation-cavity as spindle-shaped mesenchyme cells.

#### *Discussion.*

The origin of the mesoderm in *Pila* is teloblastic, thus resembling the condition most commonly met with in a large majority of Gastropods in particular, and in the Mollusca in general. In all those cases where particular attention has been paid to the study of cell-lineage, it has been found that the mother mesoderm cell can be traced to one of the posterior macromeres, namely 3D the left posterior one in dextral and the right posterior one in sinistral forms (Crampton, 1896).

A comparative study of the earlier accounts of the origin of mesoderm in Gastropoda shows that besides the abovementioned teloblastic type, two other types have been described, viz., ectodermal and enterocoelic.

In the first type, the mesoderm is said to arise directly from the ectoderm, as in *Fusus* (Bobretzky, 1877), *Vermetus* (Salensky, 1885), and *Paludina* (Tönniges, 1896 ; Dautert, 1929). According to Tönniges (1896) and Otto and Tönniges (1905) the mesoderm in *Paludina* originates by a wandering in of the ectodermal cells into the segmentation-cavity from the place where the blastopore has closed. MacBride (1914) denies the accuracy of Tönniges's conclusions. Dautert (1929), while confirming the ectodermal origin of the mesoderm, holds that the mesoderm originates anteriorly from the ectodermal wall just below the velum and not in the region of the blastopore.

The second or enterocoelic type of mesoderm formation has so far been described only in *Paludina* (Erlanger, 1891 ; Fernando, 1931) which was the form also studied by Tönniges. Erlanger (1891) found that at first a ventral pouch-like outpushing of the archenteric wall takes place close to the blastoporal end, which, on further development, expands laterally into a bilobed outgrowth which later becomes completely detached from the archenteron. This closed bilobed outgrowth represents, according to him, the primary coelomic sacs, the cells of the walls of which break up in later stages to wander into the segmentation-cavity as mesenchyme cells. It was this description of mesoderm formation in *Paludina* which later led to the investigations of Tönniges (1896), Otto and Tönniges (1905) and Dautert (1929) who contradicted Erlanger's statement and expounded, instead, the abovementioned ectodermal origin of the mesoderm. Fernando (1931) recently has confirmed Erlanger's view. He, however, points out that different fixatives lead to different results : thus, Kleinenberg's fixative (which Dautert employed in his investigations) " produces a general loosening of the cells " and " in fact if all the material had been fixed in Kleinenberg's alone, there is little doubt that the result of present investigations would have been to confirm Dautert's view "

In the present investigation I tried different fixatives like formalin, Flemming's chromo-osmic, Perenyi's fluid, Gilson and Petrunkevitch's mixtures and Kleinenberg's fixative, and found that the different fixatives do act differently on the various germ-layers. I obtained satisfactory results with Kleinenberg's picro-sulphuric fluid, and found that no loosening of cells occurred with this fixative and that the mesoderm could be well differentiated from the other germ-layers. On the other hand, fixatives like Gilson's mixture, Flemming's fluid and others led to a loosening of the cells, with the result that the mesoderm was not well differentiated in its earlier stages of formation. This difficulty in the differentiation of the mesoderm, in fact, misled me, and I at first mistook the pouches formed by the infolding of the archenteric walls (described in section V) as the coelomic pouches described by Erlanger and by Fernando in *Paludina*.

#### VIII. THE GENERAL OUTLINE OF DEVELOPMENT BEYOND THE TROCHOPHORE STAGE.

Before describing the development of the various organ-systems, it will be useful to give a general outline of the development which can roughly be divided into 12 stages.

##### *Stage 1 (Text-fig. 4a).*

The embryo at this stage is about 36 hours old and about 0.192 mm. in length. It is more or less pear-shaped in outline, slightly elongated antero-posteriorly, the anterior end being spherical while the posterior end is tapering and conical. It is bilaterally symmetrical both externally as well as internally. Externally, the embryo is characterised by the presence of the velum, the foot rudiment, and the shell gland. The velum runs as a transverse ciliated band and delimits the pre-oral (cephalic or velar) area from the post-oral part of the embryo. The cells forming the velum are large, and in the living condition exhibit a glassy transparency on account of the hyaline character of their cytoplasm.

The foot rudiment is a conical outpushing of the mid-ventral area just behind the velum.

On the aboral (dorsal) surface of the embryo, the ectoderm cells behind the velum become columnar and form a circular plate which appears darker in colour on account of the thickly granular character of its cells. It is the rudiment of the shell gland, and is thickest in the middle.

The archenteron occupies a considerable space within the embryo but no differentiation of regions has so far taken place in it. Its anterior spherical end is much broader than the posterior one, which is narrow and forms a straight tube opening to the exterior through the anus formed by the blastopore (Text-fig. 6a). This narrow tube is the rudiment of the intestine. The cells lining the archenteron are full of yolk and thus impart a yellow colour to the embryo.

The ectodermal cells on the ventral surface lying immediately behind the velum multiply rapidly to form a thick plate which sinks below the surface and is pushed inwards. This invagination between the velum and the foot is the rudiment of the stomodaeum.

The mesenchyme cells (Text-fig. 6a) are stellate and lie scattered in the body-cavity between the ectoderm and the endoderm, which is more extensive in the posterior half of the embryo than in the anterior.

*Stage 2 (Text-fig. 4b).*

The embryo at this stage is about 40 to 45 hours old, and is 0.208 mm. in length. There is no marked change from Stage 1 in the general shape of the embryo, except that the foot-rudiment has become more prominent, although there is as yet no line of demarcation between the foot and the rest of the body either at the anterior end of the foot or along its lateral borders. In fact the walls of the foot pass imperceptibly into those of the head-vesicle (the part of the embryo above as well as anterior to the foot).<sup>1</sup> At the posterior end of the foot, however, there appears a slight depression which, together with the shell-gland on the dorsal side, marks off the posterior part of the embryo, the rudiment of the visceral-sac. On the ventral surface the velum forms an arched lobe just above the stomodaeal opening, thus giving rise to two velar lobes, one on either side of the stomodaeal opening.

The stomodaeum has become deeply invaginated but has not yet opened into the anterior end of the archenteron or primitive stomach (Text-fig. 6b).

A few large, glassy and transparent cells are visible at the extreme anterior end of the dorsal surface just in front of the velar cells: these constitute the *apical cell-plate*. A few ectodermal cells at the ventral conical end of the foot become large in size and develop cilia; Conklin (1897) observed these cells in *Crepidula*, and finding their close resemblance with the cells of the apical cell-plate called them the "*pedal cell-plate*"—the name adopted by me. Similarly, another group of large transparent ciliated cells with yellowish nuclei appears at the postero-ventral end around the anus. This may be called the *anal cell-plate*.

Haddon (1882) writes "In all the Gastropods I have examined, I have found a patch of cilia either around the anus or at that spot where the anus will appear". Rabl (1879) and Meisenheimer (1898) make no mention of these cells in *Planorbis* and *Limax*, respectively. Casteel (1904) observed two such cells in very early stages in *Fiona* and compared them with the anal cells of other Molluscs. Wierzejski (1905) observed them in *Physa*, while Delsman (1914) has described their origin in *Littorina*.

At the anterior end of the embryo, the ectoderm cells, lying between the median apical cell-plate and the velar lobe on either side, become thickened to form two plate-like structures, one on each side, from which, at a later stage, arise the rudiments of the cerebral ganglia, the tentacles and the eyes. The protoplasm of these cells is thickly granulated and can thus be easily distinguished from the surrounding ectoderm cells. These two ectoderm plates correspond to what has been described as the "*Scheitelplatte*" by Meisenheimer (1898) in *Limax*, Wierzejski (1905) in *Physa*, and others, and as "*Sinnesplatte*" by Schmidt (1891).

In the posterior region of the embryo, just beneath the anterior part of the intestine are found two compact masses of mesenchyme cells,

---

<sup>1</sup> Both the head-vesicle and the foot merge into each other in *Pila globosa*, as is the case in *Littorina* and *Paludina* in which these regions together have been named as the "*Kopffuss*" (head-foot) by Delsman (1914) and Anderson (1924), a name which holds good for these regions in *Pila globosa*.

one on either side: these are the right and left rudiments of the pericardium, in each of which a lumen appears as growth proceeds (Text-fig. 16a).

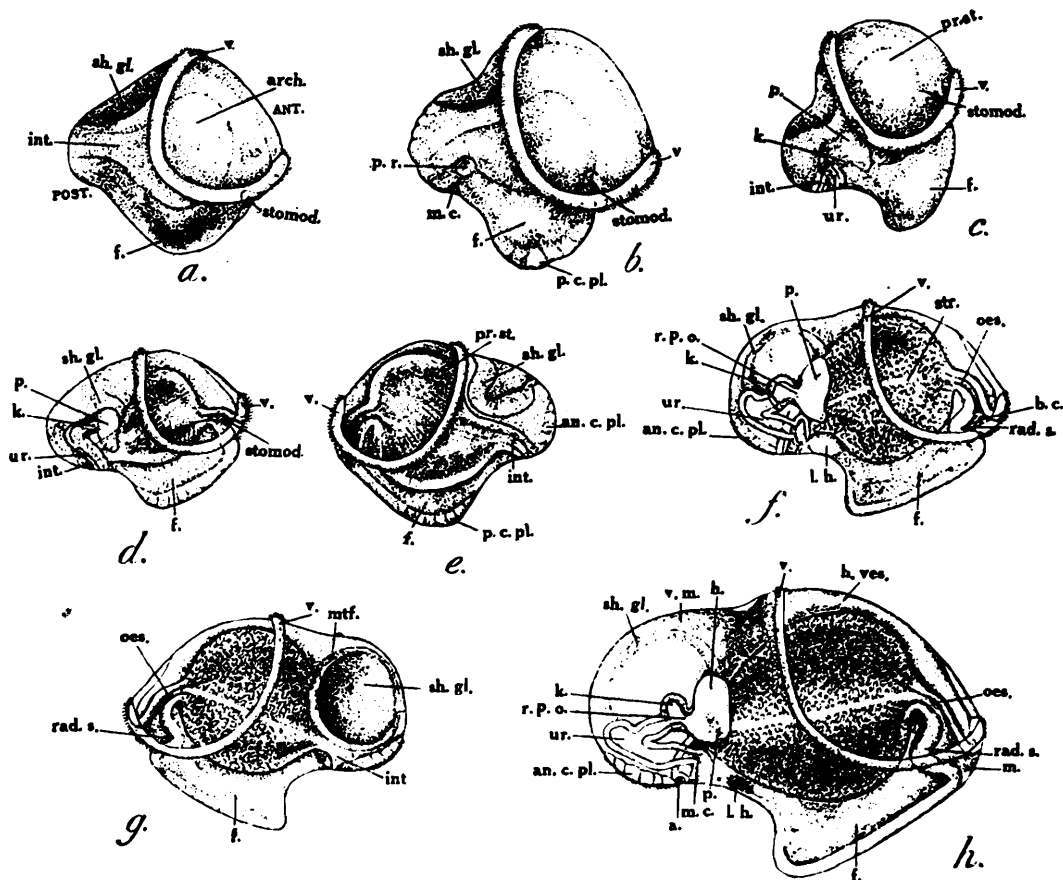
Immediately beneath these pericardial rudiments, the mid-ventral ectodermal body-wall becomes invaginated into a small depression: this is the rudiment of the mantle-cavity.

### Stage 3.

This stage is more or less of the same age and size as Stage 2, but the ureter rudiment has now appeared, and a few other changes have also taken place.

The shell-gland has increased in dimensions and has invaginated. A transverse section through the visceral-sac rudiment in the region of the shell-gland is U-shaped (Text-fig. 16b); the dorsal wall consists of tall columnar cells constituting the shell-gland, while the side-walls and floor are composed of small ectodermal cells. The "Kopffuss" (head-foot)<sup>1</sup> region is oval or egg-shaped, its dorso-ventral axis being longer than its transverse axis.

The foot in a lateral view appears triangular with its apex directed downwards and a little backwards. It is broad and thick at its base but



TEXT-FIG. 4. Figures of whole embryos, showing Stages 1-7.

a. Right view of an embryo in Stage 1:  $\times 142$ ; b. Right view of an embryo in Stage 2:  $\times 148$ ; c. Right view of an embryo in Stage 4:  $\times 107$ ; d. Right view of an embryo in Stage 5:  $\times 83$ ; e. Left view of an embryo in Stage 5:  $\times 91$ ; f. Right view of an embryo in Stage 6:  $\times 88$ ; g. Left view of an embryo in Stage 6:  $\times 88$ ; h. Right view of an embryo in Stage 7:  $\times 78$ .

<sup>1</sup>Both the head-vesicle and the foot merge into each other in *Pila globosa*, as is the case in *Littorina* and *Paludina* in which these regions together have been named as the "Kopffuss" (head-foot) by Delsman (1914) and Anderson (1924), a name which holds good for these regions in *Pila globosa*.

tapers towards the apex. The pedal cell-plate now consists of two median longitudinal rows of cells (Text-fig. 15a) which extend along the whole of the anterior surface of the foot (Text-fig. 6c).

The stomodaeum has opened into the archenteron (Text-fig. 6c).

The right and left pericardial rudiments have grown towards each other but are still separated by a septum which is many cells in thickness. As development proceeds, they shift towards the right side of the intestine and the primitive stomach.

The rudiments of the right and left kidneys have made their appearance, that of the right kidney being an evagination of the postero-ventral wall of the right pericardium, and that of the left being only a thickening of the left pericardium.

The invagination of the mantle-cavity has deepened, and an evagination taken place from its right inner surface—this is the rudiment of the ureter (Text-fig. 16f). It arises in such a manner that a demarcation between it and the mantle-cavity cannot be made out. It grows obliquely backwards and upwards as a tubular structure, to end blindly against the posterior end of the right kidney.

#### *Stage 4 (Text-fig. 4c).*

The embryo at this stage is about 52 to 56 hours old and about 0.232 mm. in length. It has grown more in length than in breadth, the growth being more marked in the "Kopffuss" (head-foot) region than in the region of the visceral-sac rudiment.

The invagination of the shell-gland at this stage (Text-fig. 6d) is deeper at its anterior than at its posterior end where it is almost flush with the dorsal surface of the embryo. The walls on the sides of this invagination become raised to form a kind of rudimentary ridge round the shell-gland; called the mantle ridge or mantle fold because the edge of the mantle is formed from it.

The foot has become more prominent and tapers antero-posteriorly as in Stage 3. A transverse section passing through the middle of the foot is V-shaped, the two arms of the V representing the two lateral walls of the foot (Text-fig. 24c). The mesenchyme cells of the foot are more closely packed than they are at any other place in the embryo. The embryo at this stage begins to rotate (within the egg-shell) with the help of the cilia on the foot.

The mouth is still situated ventrally. It faces downwards, and leads into the primitive stomach which is broad anteriorly but tapers posteriorly to the beginning of the intestine. The cells lining the primitive stomach become large and vacuolated and thus form the rudiment of the anterior lobe of the digestive gland (Text-fig. 6d). The coelomic cavity between the primitive stomach (digestive-gland) and the body-wall (that is, in the cephalic or head-vesicle region) is small, but that between the intestine and the body-wall (that is, within the region of the visceral-sac rudiment) is large and contains the rudiments of the pericardium and the kidneys. The intestine, as a result of the shifting of the pericardial rudiments towards the right and their gradual increase in size, is pushed towards the left side of the embryo where it opens to the exterior through the anus which has also now shifted towards the left side and lies posteriorly to the left of the invagination of the mantle-cavity.

The mantle-cavity is now much larger and appears as a tubular structure with its external opening in the mid-ventral line. The rudiment of the ureter ascends obliquely on the right side of the embryo and grows backwards and upwards to open at the posterior end of the right kidney.

*Comparison of Stages 1-4 with those of Semper and others:*—On comparing these four stages with those described by Semper (1862) in *Ampullaria (Pila) polita*, I find (from his diagrams in Pl. I), that Semper could not quite correctly orientate the various regions of the embryo. His mistake was probably due to the fact that he did not confirm his observations with the help of sections; and since embryos are almost opaque on account of the presence of a large amount of yolk, one is easily liable to make a mistake in their orientation. Taking, for instance, his fig. 6, Pl. I, comparable to my Stage 1, and bearing in mind the position *b* where according to Semper, the larval heart will develop, I think it would have been correct if *a*, *c* and *d*, instead of representing the foot, the head and the posterior end, as Semper has shown, had denoted the posterior end, the dorsal side and the foot respectively, a view confirmed by the observations of Maria Scott (1934) on *Ampullaria canaliculata*. As regards his fig. 8, Pl. I, its orientation is correct, but Semper could not distinguish the formation of the shell-gland on the postero-dorsal surface of the embryo at this stage. He observed it for the first time, as shown in his fig. 10, Pl. I, *e*, at a much later stage, when the shell-gland area has already shifted down towards the left side. Fernando (1931) and Scott (1934) observed the shell-gland formation in the earlier stages of *Ampullaria gigas* and *A. canaliculata* respectively, and confirm my observations.

In *Pila globosa* the velum forms a continuous circular band around the cephalic (velar) area and it persists as such till a very late stage. According to Scott (1934), the velar cells cease half way along the body and the remaining velar cells protrude in the dorsal region, though they form a continuous band in the earlier stages (see her figs. 9 and 13, Pl. II). Brooks and McGlone (1908) described and sketched the velum as a complete band even at a stage when the gill has started making its appearance (*cf.* their fig. 7, Pl. I).

#### Stage 5 (Text-figs. 4*d*, *e*).

The embryo at this stage is about 60 hours old and about 0.304 mm. in length. Its dorso-ventral axis is longer than the horizontal axis in the "Kopffuss" region, but it is almost circular in section immediately behind the posterior region of the primitive stomach, as well as in the region covered by the anterior end of the shell-gland, that is, at the junction of the "Kopffuss" and the visceral-sac rudiment.

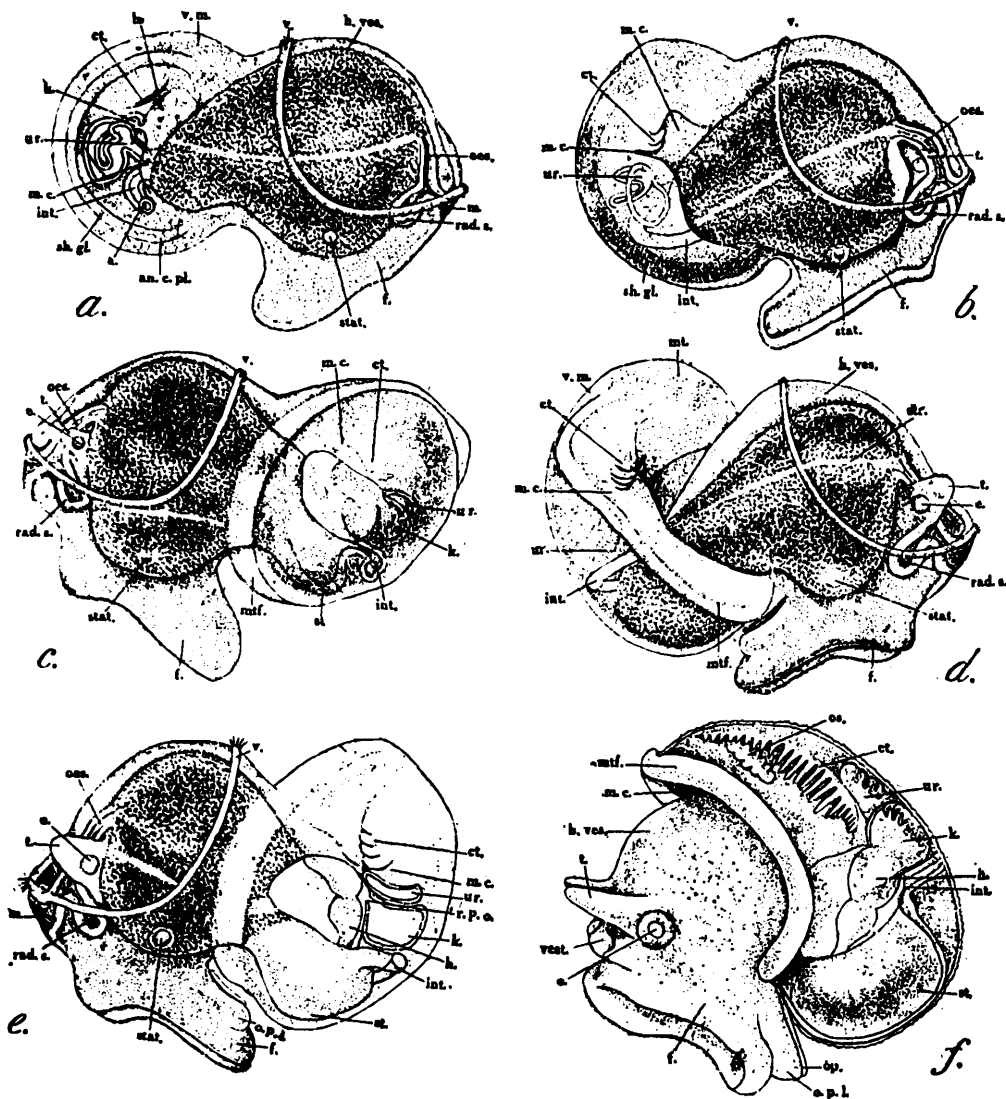
The shell-gland has shifted from its original dorsal position to the left side of the embryo and forms a cup-shaped depression opening widely to the exterior. On account of the invagination growing deeper, the floor of the shell-gland comes to lie anteriorly almost against the postero-dorsal wall of the primitive stomach.

The intestine at first leads straight backwards, but towards the posterior end it curves downwards and opens on the mid-ventral line posterior to and on the left of the outer opening of the mantle-cavity.

The pericardium is now a single, fairly large, sac-like structure situated on the right side of the junction of the primitive stomach and the intestine, the septum between the right and the left pericardial cavity having disappeared.

With the shifting of the shell-gland to the left side, the opening of the mantle-cavity is shifted to the right of its former mid-ventral position. The rudiment of the right ureter has increased in length and leads backwards along the right side, and then upwards to open into the posterior end of the right kidney. This tubular rudiment which cannot yet be differentiated easily from the mantle-cavity, on account of the uniform character of the lumen in both, develops subsequently into the so-called anterior kidney (=ureter) of the adult.

The rudiment of the right kidney has already become a distinct structure and encloses a cavity which communicates widely with the



TEXT-FIG. 5. Figures of whole embryos showing Stages 8-12.

*a.* Right view of an embryo in Stage 8:  $\times 64$ ; *b.* Right view of an embryo in Stage 10:  $\times 59$ ; *c.* Left view of an embryo in Stage 10:  $\times 66$ ; *d.* Right view of an embryo in Stage 11:  $\times 52$ ; *e.* Left view of an embryo in Stage 11:  $\times 50$ ; *f.* Left view of an embryo in Stage 12:  $\times 34$ .

pericardial cavity from which it has evaginated. The kidney can be easily distinguished by its colour which is darker than that of the pericardium, and by the uniform thickness of its walls.

Judging from Semper's figs. 9 and 10, Pl. I, my Stage 5 is approximately the same as his 'third' stage; while his orientation of fig. 10 is correct, fig. 9 is again wrongly orientated. In order that the figure be correctly orientated, letters, *a*, *c* and *d* should represent the posterior end, the dorsal side and the foot respectively of the embryo. Only in this way will it show proper resemblance to fig. 10; *a* would then represent the buccal cavity, instead of an aperture related to respiratory organs<sup>1</sup>. Semper is wrong about the opening of the respiratory organs, as, in fact, the respiratory organs in *Pila* appear much later and there can be no aperture at this stage (cf. Brooks and McGlone, 1908, Fernando, 1931, and Scott, 1934).

Up to this stage Semper does not make any mention of the formation of either the pericardium or the mantle-cavity or the kidneys which, as is quite evident from my preceding account, are already in an advanced stage of development.

<sup>1</sup> It was at this stage that Semper first noticed the appearance of the shell-gland, which had already shifted to the left side of the embryo.

*Stage 6 (Text-figs. 4f, g).*

The embryo at this stage is about 62 to 68 hours old and 0.392 mm. in length. Considerable increase has taken place in the size of the embryo, specially in the region of the visceral-sac rudiment. The yellow colour of the embryo present in the preceding stages is now restricted to the alimentary canal, with the result that the embryo becomes slightly transparent. Growth has taken place chiefly in the dorso-ventral direction, as can very well be seen by examining the embryo from the side. Dorso-ventrally the embryo is still broader in the anterior or "Kopffuss" region than in the posterior or visceral-sac rudiment region.

A transverse section passing through the "Kopffuss" shows that the convexity of the side walls has decreased and the part lying dorsal to the velum appears horse-shoe shaped, while the part below the velum gradually tapers downwards and inwards to meet at a conical point thus forming the V-shaped foot. A section passing immediately behind this region shows an oval outline, the rounded appearance of the preceding stage being lost. A transverse section passing through the middle of the visceral-sac rudiment shows a convexity of the body-wall on the right side, while there is a concavity on the left side due to the shell-gland invagination.

So far, the longitudinal axis of the "Kopffuss" and the visceral-sac rudiment is in a straight line.

The shell-gland develops a cuticle, the rudiment of the shell, surrounded by a raised ridge of the mantle-fold (Text-fig. 4f) occupying the greater part of the left surface of the visceral-sac rudiment. A deep groove, deeper anteriorly than posteriorly, runs all round the shell-gland just beneath the mantle-fold: this is the mantle groove. Though the extreme anterior end of the shell-gland is still deeply invaginated and lies against the postero-dorsal wall of the primitive stomach (Text-fig. 7b), evagination has already set in a little behind it, and towards the posterior part of the shell-gland this eversion or evagination is complete, and a flat surface of uniform thickness is obtained extending right up to the posterior end of the embryo.

The foot is now, for the first time, marked off anteriorly from the head-vesicle of the embryo. In a transverse section, the side-walls of the foot lying just beneath the velum are seen to be pressed inwards to become almost parallel to each other, after which they bend at an approximate angle of  $45^\circ$  and pass downwards and inwards to meet in the mid-ventral line. The anterior surface of the conical foot begins to flatten and marks the beginning of the formation of the flat creeping sole of the foot, the pedal cell-plate now forming a ridge on the mid-ventral surface of the foot. The sole is restricted to the anterior surface of the foot, the part lying behind appearing almost similar to that in the preceding stage.

In the stomodaeum, just behind the mouth, the floor of the fore-gut sends out a ventral outpushing, the rudiment of the radular sac (Text-fig. 7b), which marks the posterior boundary of the buccal cavity. The part of the gut between this ventral outpushing and the primitive stomach is the oesophagus.

The primitive stomach (Text-fig. 7b) has grown considerably in size and has a spacious lumen, so that the cavity lying between the primitive

stomach and the body-wall is very much reduced. This stomach is broad anteriorly but narrows gradually towards its posterior end, where it projects into the antero-ventral part of the visceral-sac rudiment, and bends slightly ventrally to open into the intestine. The cells of the wall of the primitive stomach (the rudiment of the digestive-gland) have grown much longer in size and have also become more vacuolated.

The darkly staining postero-ventral part of the primitive stomach which now lies within the visceral-sac rudiment and appears cone-like in a lateral view, becomes differentiated from the rest of the primitive stomach; this is the first appearance of the true or adult stomach which lies towards the right ventro-lateral side. In transverse (Text-fig. 15*b*) and sagittal sections (Text-fig. 7*b*), its wall is seen to be composed of regularly arranged, thickly granular, columnar cells.

The intestine leads backwards and, after making a sharp bend downwards of about 90°, opens to the exterior on the mid-ventral line (Text-fig. 7*b*), a little behind and to the left of the external opening of the mantle-cavity (Text-figs. 4*f*, *g*). The anus is situated at the anterior end of a group of large ciliated cells (anal cell-plate) which extends right up to the posterior end of the embryo.

The thin-walled, sac-like pericardium, which is elongated dorso-ventrally, is situated midway on the right side of the embryo, lying antero-dorsally to the junction of the intestine and the primitive stomach (Text-fig. 4*f*). Anteriorly it occupies nearly the whole of the space between the primitive-stomach and the body-wall. The heart, which is quite prominent at this stage, is situated in the pericardium near its postero-dorsal wall.

The mantle-cavity is marked off internally from the ureter by the appearance of a constriction in its inner wall.

The right kidney still occupies a postero-ventral position and, therefore, the reno-pericardial aperture (*i.e.*, the aperture through which the kidney and the pericardium communicate with each other) is also ventral in position.

At this stage a part of the integument lying between the foot and the anus just below the intestine, with its underlying mesenchyme cells, begins to pulsate. This is the embryonic or larval heart.<sup>1</sup> But whether it is at this stage that the larval heart pulsates for the first time or whether it was pulsating earlier I cannot definitely say, as the earlier stages are opaque and make observation difficult.

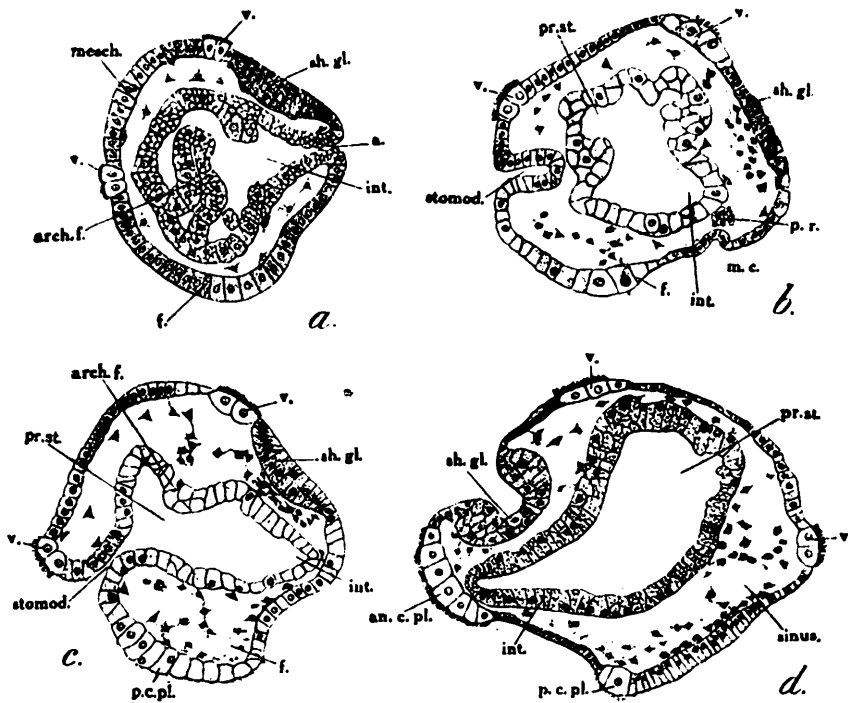
#### *Stage 7 (Text-fig. 4*h*).*

The embryo at this stage is about 72 to 78 hours old and about 0.616 mm. in length. It is of a dull white colour and more or less transparent, so that the outlines of the various organs can be clearly seen. The rudiment of the visceral-sac has grown more in length and height than any other part of the embryo. The rotation of the organs towards the right side seen in the earlier stages as having brought about the

---

<sup>1</sup> Semper (1862) and Scott (1934) observed it in a stage almost the same as that of mine (Text-figs. 4*f*, *g*), as is shown in figs. 12 and 13, Pl. II of the former author, and fig. 17, Pl. III of the latter.

asymmetry of the embryo, becomes well-marked now. The rotation being from left to right, *Pila* exhibits dextral torsion.



TEXT-FIG. 6. Sections of embryos in Stages 1-4, showing early stages in the development of the archenteric folds, the pericardial rudiment, the stomodaeum and the shell-gland.

*a.* Sagittal section of an embryo in Stage 1, passing through the anus and showing the archenteric folds:  $\times 332$ ; *b.* Sagittal section of an embryo in Stage 2, passing through the rudiment of the mantle-cavity and the inner end of the right pericardial rudiment:  $\times 246$ ; *c.* Sagittal section of an embryo in Stage 3, showing the opening of the stomodaeum into the primitive stomach:  $\times 246$ ; *d.* Sagittal section of an embryo in Stage 4, passing through the invaginated shell-gland:  $\times 204$ .

The shell-gland at its anterior end is now almost completely flattened out and the shell cuticle forms a thin membrane over it. On account of the transparency of the embryo, the pulsations of the definitive heart are seen for the first time. These pulsations, unlike the irregular beats of the larval heart, are rhythmical. The foot begins to show movements, which are well marked towards its posterior end. When a contraction occurs, the posterior part of the foot is pulled upwards and almost touches the antero-ventral surface of the visceral-sac rudiment.

The radular sac has become well-marked. The anus no longer lies on the mid-ventral line but has shifted to the right, although it still maintains its original relation to the opening of the mantle-cavity, and lies behind and to the left of the external opening of the mantle-cavity.

The pericardium now comes to lie dorsally to the intestine, and the pulsating heart lies within it. If the embryo is taken out of the egg-shell, separated from the surrounding albumen and placed in normal saline solution, the pulsations can be seen for a few minutes, after which the rate of pulsation gradually decreases till it finally ceases.

The opening of the mantle-cavity has, as a result of further torsion, been displaced upwards and comes to lie near the middle of the right side.

The right kidney, though retaining its connection with the pericardium through the postero-ventral reno-pericardial aperture, begins to shift towards the dorsal side (Text-fig. 8a).

*Stage 8 (Text-fig. 5a).*

The embryo at this stage is about  $3\frac{1}{2}$  days old and 0.736 mm. in length. The dextral torsion has become still more prominent, the left postero-ventral part of the visceral-sac rudiment having rotated towards the right and showing a tendency to grow upwards and forwards. The visceral-sac rudiment, as a whole, has grown as large as the "Kopffuss" and appears now as a saucer-shaped structure with a shallow depression on its right side. Its longitudinal axis, as a result of torsion, lies at an angle of about  $45^\circ$  to that of the "Kopffuss". On its dorsal side, just behind the velar cells, appears a depression which marks the anterior boundary of the visceral-sac. In a lateral view the embryo looks like two incomplete spheres joined end to end. The visceral-sac rudiment gradually diminishes in thickness antero-posteriorly. On examining a series of transverse sections of the visceral-sac rudiment from the anterior to the posterior end, it is observed that in the region of the stomach the body-wall of the right side bulges outwards, while it curves in dorsally in front of the pericardium. The left body-wall, however, is almost flat, consisting of the shell-gland bounded by the mantle folds. A transverse section passing through the pericardium shows that the flattened part of the shell-gland has started bulging out, and that the part of the embryo lying dorsally to the pericardium is laterally compressed and is narrower than the ventral part. It is the rudiment of the mantle. The bulging on the left side, where the cells of the shell-gland have flattened, lies against the posterior region of the stomach and is the rudiment of the visceral hump. The peripheral part of the everted shell-gland represents the shell-gland of the adult. The shell-gland is still circular in outline and covers the entire left side of the visceral-sac.

In sections, as well as in whole mounts (Text-fig. 5a), the foot is seen to have developed ventrally a flat, creeping sole, broader anteriorly than posteriorly. Anteriorly, the lateral walls of the foot run parallel to each other and at right angles to the flat sole, and the foot is marked off from the head-vesicle as its anterior wall forms a curve backwards and downwards to continue into its ventral wall. At about its middle, the side walls of the foot still imperceptibly merge into those of the head-vesicle. The height of the foot is greatest about the middle of its length but diminishes anteriorly. Viewed from the ventral side, the anterior border of the foot appears rounded while the lateral walls look curved slightly inwards. The postero-dorsal wall of the foot forms an acute angle with the longitudinal axis of the embryo.

The radular sac evagination of the floor of the fore-gut has sunk deep, and is directed downwards and backwards. The stomach no longer lies in the mid-ventral line but has shifted towards the left side, and it is here that the rudiment of the visceral hump lies against it. The part of the primitive stomach from which the adult stomach differentiates moves to the right-dorsal side, but the two remain communicating widely with each other. The bulging on the right side lies opposite to it. The

intestine has elongated considerably and runs backwards and downwards and then bends forwards to open on the right side almost in the same vertical plane as that of the external opening of the mantle-cavity, but slightly ventral to it. The anus which lies at the anterior end of the anal cell-plate is placed to the right of the dorso-ventral axis of the visceral-sac rudiment (Text-fig. 23a);

The mesenchymatous tissue within the body is very much concentrated in the foot.

The pericardium now lies completely on the dorsal side of the gastrointestinal junction.

The tubular mantle-cavity is constricted off from the ureter, which runs postero-dorsally along the body-wall of the right side of the embryo, and then bends forwards to open into the kidney.

The kidney lies against the posterior wall of the pericardium and is now directed dorsally.

A depression appears on either side of the body-wall, posteriorly to the velum at about the middle length of the "Kopffuss", where the foot merges into the head-vesicle. This is the rudiment of the statocyst.

A part of the right wall of the visceral-sac rudiment situated dorsally to the level of the pericardium becomes thickened to form a common ridge-like area from which the rudiments of the gill, the osphradium, and the lung are formed (Text-figs. 5a and 23a).

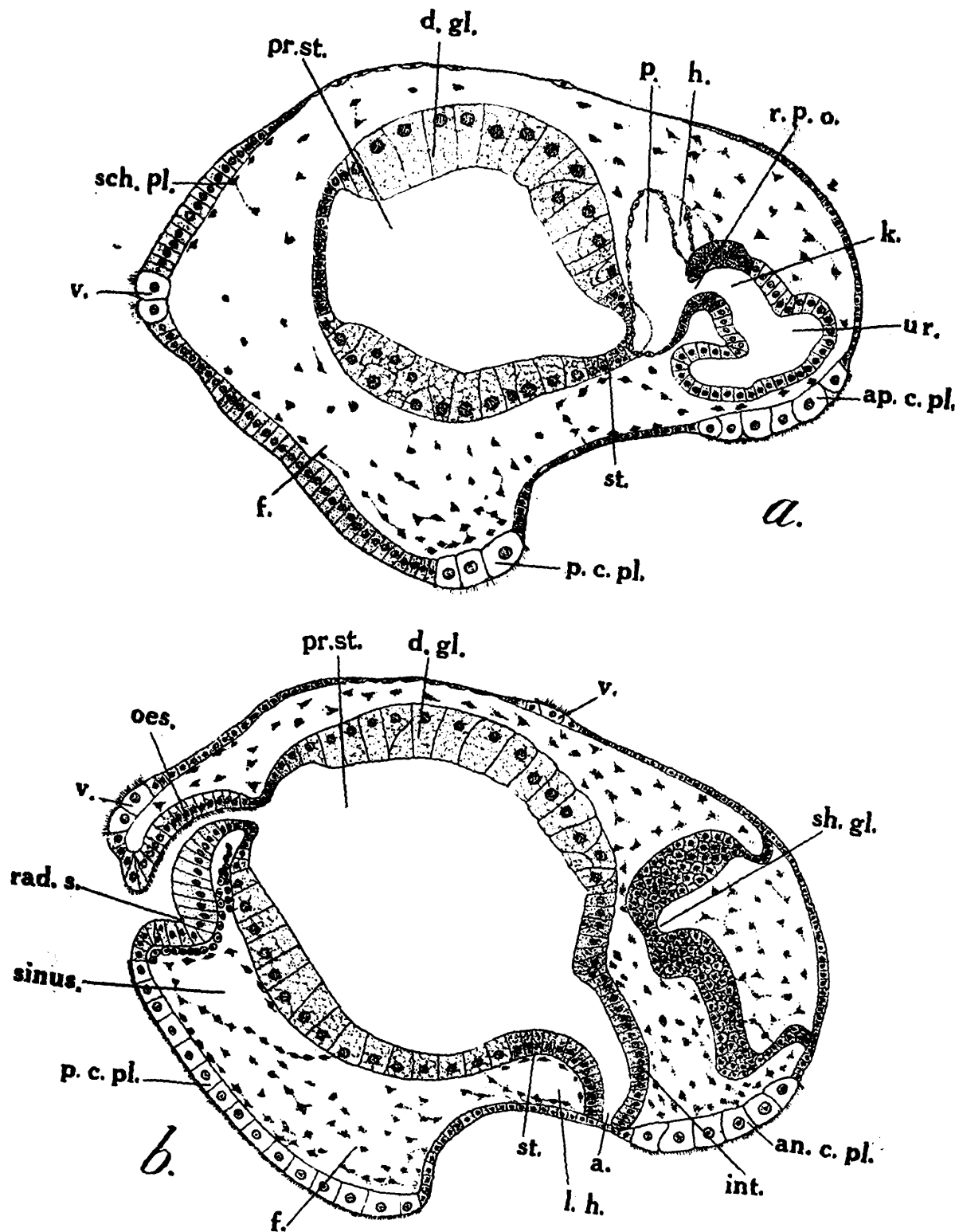
#### *Stage 9 (Text-fig 8b).*

The embryo at this stage is about 4 days old and 0.756 mm. in length. The shell-gland, along with the shell cuticle, has increased in size, and has grown further towards the ventral side as a result of further torsion of the visceral-sac rudiment. The bulge of the rudimentary visceral hump has increased in size and now extends right up to the posterior end, the direction of the bulge being downwards and outwards. Similarly, the bulge on the right side has also become well developed and has grown upwards in the posterior region behind the external opening of the mantle-cavity. The concavity of the visceral-sac on its right side has deepened posteriorly and appears bowl-shaped. The rudiments of the gill, the osphradium, and the lung are already formed from the thickened ridge-like area which bounds the concavity on the right side. Of these, the first two develop as outpushings, while the last forms as an inpushing between them.

On the postero-dorsal surface of the foot, a thin cuticular membrane has already been secreted by the ectodermal cells of that region; this forms the rudiment of the operculum.

The postero-dorsal part of the primitive stomach lying opposite the adult stomach-rudiment grows dorso-laterally on the right side, and forms the abovementioned bulge on that side. This outgrowth, which communicates widely both with the anterior rudiment of the digestive-gland and the true stomach, is the second and posterior rudiment of the digestive-gland, which lies completely within the visceral-sac rudiment. Subsequently, it develops into the digestive-gland of the adult, the anterior rudiment having been absorbed at a later stage.

A part of the pericardium has already shifted downwards to the left side of the intestine. As the pericardium is thus displaced, the reno-



TEXT-FIG. 7. Early development of the kidney, pericardium, ureter and radula sac.

*a.* Reconstruction of a few sagittal sections of the right side of an embryo in Stage 6, showing the opening of the right kidney into the pericardium and the ureter:  $\times 195$ ;  
*b.* Reconstruction of a few sections of the same embryo as in (*a*), showing the evagination of the radular sac:  $\times 195$ .

pericardial aperture, which was ventral in position, now comes to lie in a postero-dorsal position. The kidney has grown in size and is now a sac-like structure situated posterior to, and a little to the right of the pericardium.

The mantle-cavity is now large and spacious.

The statocysts are now in the form of deep invaginations but still open to the exterior.

*Stage 10* (Text-figs. 5*b* and 9).

The embryo at this stage is about 4½ to 5 days old and 0.776 mm. in length. The torsion is complete. The postero-ventral part of the visceral-sac has moved on to the right side, and has come to lie upwards and forwards, its former longitudinal axis now lying nearly at right angles to that of the "Kopffuss" (*cf.* Text-figs. 19*a, b*; 21*d*; and 24*o*). The visceral hump is now directed downwards, outwards and backwards. The concavity on its right side, seen facing outwards in the preceding stage, now lies on the dorsal side and faces antero-dorsally. The lower (ventral) part of the mantle fold, lying on the left side in the earlier stages, has now come to lie on the right, and is nearly at right angles to its former position (Text-figs. 21*d* and 24*o*).

The foot has developed further; in its anterior part the side walls have curved in towards each other to join below at the base of the head-vesicle (Text-fig. 24*j*), which at its extreme antero-dorsal end is now developing into the characteristic head of the adult. The ventral surface of the foot is quite flat and the two longitudinal rows of cells of the pedal cell-plate are still present along the entire length of the foot.

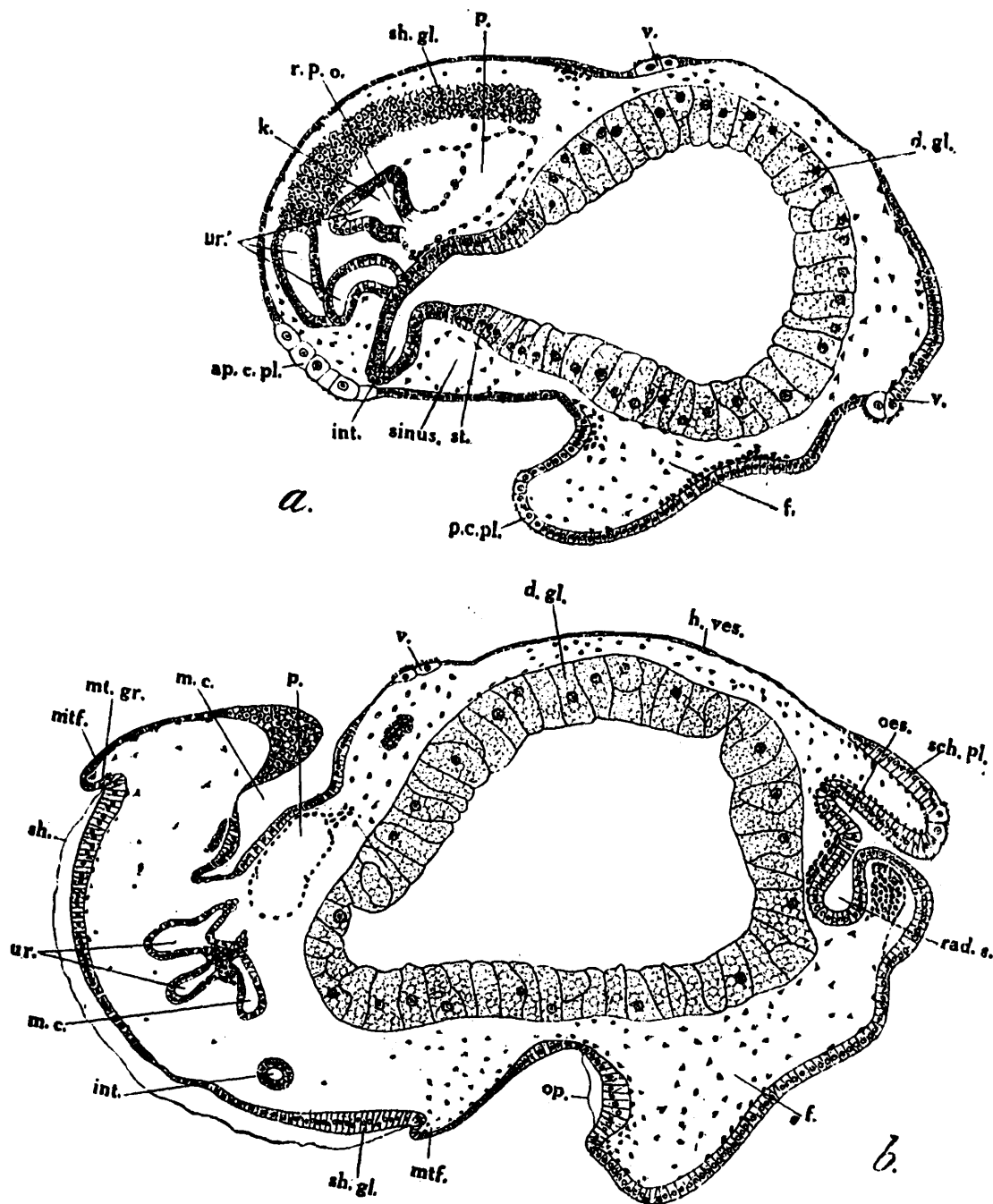
On each of the dorso-lateral walls of the oesophagus, opposite the opening of the radular sac, arises an outpushing; these two outpushings are the rudiments of the salivary glands, and arise simultaneously with the oesophageal pouches, which are similar outpushings situated a little anteriorly. In the mid-gut, the second rudiment of the digestive-gland enlarges within the visceral-sac, so that the two appear as diagonally opposed bulgings. The intestine elongates as a result of torsion. Arising from the left postero-dorsal side of the stomach, it runs downwards and backwards and makes a curve to come forward and open anteriorly, for the first time, at the extreme right end into the mantle-cavity, at one end of the anal cell-plate.

The deep and spacious mantle-cavity now occupies its final dorsal position (Text-fig. 21*d*). Posteriorly, by the upward and forward rotation of the postero-ventral part of the embryo, the right and left mantle lobes approach each other, at a later stage, to meet in the centre and form the roof of the cavity.

The ureter (or anterior kidney) has elongated a great deal, but still retains its simple tubular character. A part of it now comes to lie anteriorly to the kidney, which in turn lies posteriorly to and to the right of the pericardium, and forms a wide chamber. The reno-pericardial aperture opens on the postero-dorsal wall of the pericardium which has also grown considerably in size.

The statocysts no longer open to the exterior but form closed vesicles beneath the side walls of the foot, having shifted downwards and inwards, away from their places of origin. They appear circular or oval in a lateral view of the embryo.

The rudiments of the gill, the osphradium and the lung are now well differentiated. I have been able to count rudiments of five to six gill-



TEXT-FIG 8. Embryos in Stages 7 and 9.

a. Sagittal section passing through the right side of an embryo in Stage 7:  $\times 134$ ;  
b. Sagittal section of an embryo in Stage 9:  $\times 114$ .

filaments projecting into the mantle-cavity from the inner wall of the mantle on the left side (Text-fig. 19b).

Fernando (1931) failed to observe the formation of the gill at this stage in *Ampullaria (Pila) gigas*. Brooks and McGlone (1908) described the gill in *A. depressa* at a stage which can be readily compared with my Stage 9 (see their fig. 7, Pl. I). If we examine fig. 21, Pl. III of Semper (1862) in case of *A. polita*, it will be noticed that his stage is just a little more advanced than my Stage 10. He has shown the gill in an advanced state of development, in which the gill-filaments have already developed cavities within them (see his fig. 18). Scott (1934) mentions the formation of the gill at a stage (see her fig. 23, Pl. V) corresponding to that of Semper (fig. 21, Pl. III) in which the shell is still definitively left in position and the lamellae of the gill are developing towards the posterior part of the visceral-sac, taking the form of folds on the wall of the mantle. On making these comparisons I believe that Fernando must have failed to observe the formation of the gill in the earliest stages of its development.

The "head plates" (=the "Scheitelplatte" of some of the German authors, and the "Sinnesplatte" of Schmidt), which lie within the velar area and form the side walls of the anterior end of the head-vesicle (p. ), get flattened and lie parallel to each other (Text-figs. 24j and 28b). A depression appears in the middle of each plate, while its antero-dorsal region is pushed out as a small protuberance. These depressions are the rudiments of the eyes, while the protuberances are the rudiments of the tentacles (Text-fig. 28b), the two rudiments appearing simultaneously in *Pila globosa*.

Semper (1862) did not observe the formation of either the tentacle, or the eye, at this stage in *A. polita*. He mentions their appearance at a much later stage (see his figs. 24 and 25, Pl. IV) when the embryo is about to hatch. He mentions that the rudiments of the eye and tentacle are absent at Stage 10. Moreover, he describes the appearance of the eyes to be earlier than that of the tentacles. Brooks and McGlone (1908) do not make any mention of the appearance of the eye or the tentacle rudiments at the stage at which the gill appears. Fernando (1931) has also made no mention of their formation. Scott (1934) describes the formation of the tentacles at approximately the same stage as mine. She makes no mention, however, of the eyes at this stage, but describes them at a later stage (cf. her fig. 24, Pl. V).

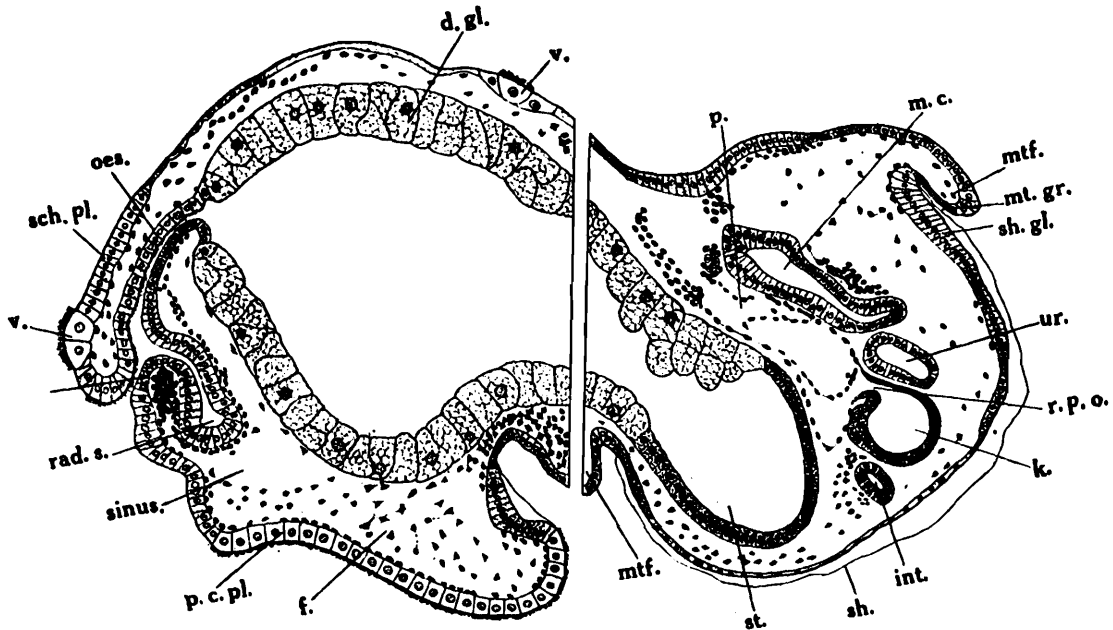
#### Stage 11 (Text-figs. 5d, e, and 10).

The embryo at this stage is about  $5\frac{1}{2}$  to  $6\frac{1}{2}$  days old and 0.960 mm. in length, and looks perfectly snail-like in appearance. The visceral-sac has become dome-shaped as a result of rapid growth, and now lies much forwards, the apex of the dome being directed backwards and downwards. The entire surface of the visceral-sac is covered over by the thin transparent shell which forms a cap over the dome. The thick mantle fold is strongly developed and is directed backwards, with the mantle groove running beneath it (Text-fig. 10). A transverse section of the visceral-sac passing through the anus and the osphradium shows that the dorso-ventral axis of the embryo, in this region, is shorter than its horizontal axis; in other words, the breadth of the embryo, in this region, is greater than its height. This relation is reversed as we go towards the posterior end, *i.e.*, in the region of the kidney.

The postero-dorsal surface of the foot has become thickened and raised to form beneath the operculum a platform, the operculigenous lobe.

The radula begins to be differentiated within the radular sac, which has grown large in size and is directed downwards and backwards. A constriction appears at about the junction of the head-vesicle with the visceral-sac, due to the deepening of the mantle-cavity on the right side (Text-fig. 25e). The anterior part of the digestive-gland lying within the head-vesicle is still large, but hereafter it shows indications of diminution in size. The oesophagus has grown in length and opens into the primitive stomach. The posterior part of the digestive-gland has grown in dimensions and occupies the greater part of ventral half of the visceral-sac. By a rapid growth and expansion of this posterior part, the rest of the organs are displaced towards the latero-dorsal side. The intestine, arising from the left postero-dorsal side of the stomach, extends backwards and, bending round the posterior end of the digestive-gland, turns forwards to run obliquely upwards and opens anteriorly through the anus. The anus is now situated at the extreme right end of the mantle cavity, lying postero-ventrally to the anal cell-plate which is still present.

The pericardium, with the heart, occupies a very large space on the left side of the embryo, and is covered by the epithelium of the visceral-sac which, in turn, is protected by the shell.



TEXT-FIG 9. Diagram showing two sections of an embryo in Stage 10. The left half is a median longitudinal section, while the right half passes on the left of the median line:  $\times 114$ .

The kidney has also grown into a spacious chamber; it is no longer a simple sac but has finger-like folds projecting into its cavity from its walls. It is situated posteriorly to and on the right side of the pericardium and can be readily recognised from the latter by its darker colour. The ureter (=anterior kidney) lies to the right of and dorsally to the kidney, while a part of it lies above the mantle-cavity on its extreme right. The dorsal wall of the ureter is already produced into folds. The kidney proper lies posteriorly to the ureter on its left side.

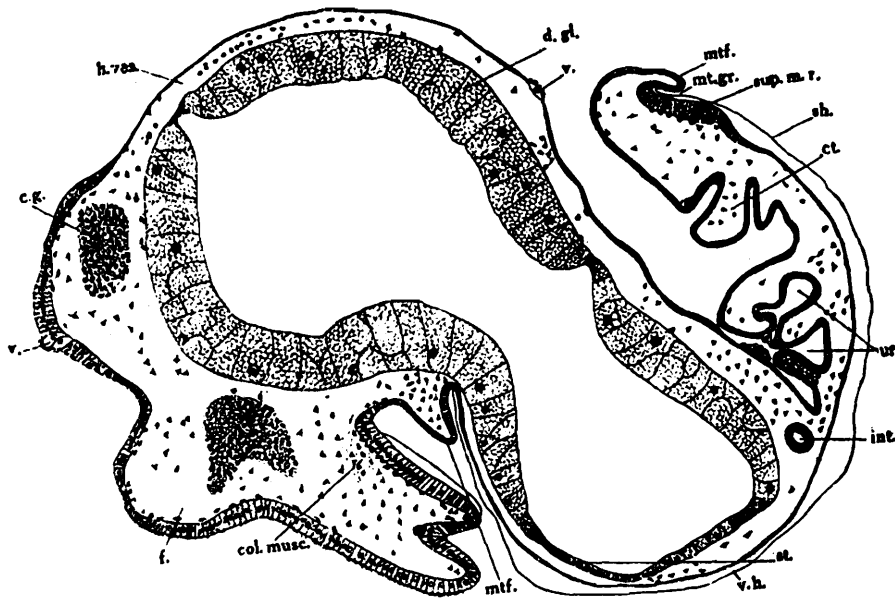
The spacious mantle-cavity has already passed over to the left side of the embryo, and is bounded ventrally by the body-wall covering the stomach, digestive-gland, and the pericardium, while laterally as well as dorsally it is bounded by the thick mantle itself (Text-fig. 23c). The gill lies obliquely inside the mantle-cavity and extends from its left posterior end to its right anterior end. The osphradium is seen suspended from the wall of the mantle towards the left anterior end of the mantle-cavity.

The eyes now form closed vesicles, but no further differentiation of their parts has yet taken place. They lie on the outer side of the bases of the tentacles which form peg-like conical projections, broad at the base and tapering towards their distal end. They are directed outwards, forwards and upwards. The region of the head-vesicle lying between and anterior to the eyes and tentacles is distinctly marked off from the region lying posteriorly to it. It has become broader but shorter in height, a differentiation leading towards the formation of the head proper.

#### *Stage 12 (Text-figs. 5f and 11).*

The embryo in this stage is about  $7\frac{1}{2}$  to  $8\frac{1}{2}$  days old and 1.36 mm. in length. The visceral-sac, covered all over by the shell, gradually travels forwards towards the anterior end, and encloses the head-vesicle of the

embryo. The mantle surrounds the head-vesicle like a cloak, and the shell follows the progress of the mantle and thus reaches its adult shape and position. On account of the deposition of lime salts, the shell loses its transparency and becomes gradually opaque. The visceral hump has gone through one complete spiral on the right side (dextral). A pair of cartilages have already formed anteriorly to the radular sac.



TEXT-FIG. 10. Sagittal section of an embryo in Stage 11, passing through the opening of the ureter into the mantle cavity:  $\times 124$ .

The anterior lobe of the digestive-gland lying within the head-vesicle is now very much reduced and appears as a tubular structure at the anterior end of which opens the oesophagus (Text-fig. 11). As development proceeds, this lobe of the digestive-gland is reduced still further, till it completely recedes from the head-vesicle. The posterior lobe of the digestive-gland, on the other hand, grows in size, becomes spherical and fills a very large part of the visceral-sac.

The oesophagus is very much elongated and, on account of the complete recession of the anterior lobe of the digestive-gland, traverses the whole length of the head-vesicle to enter the left side of the stomach within the visceral-sac (Text-fig. 26g). This can be easily understood by a comparison of Text-figs. 11 and 26a, b, g. In Text-fig. 11 the anterior rudiment of the digestive-gland is present in the form of a tubular structure, but in Text-fig. 26g it has completely disappeared to give place to the oesophagus.

The operculum is now well formed and the columellar muscle is strongly developed (Text-fig. 11). The foot has now assumed its final shape.

The eyes at this stage are well formed, each having developed a lens and a retina (Text-fig. 28e). They are raised on small elevations, the rudiments of the ommatophores. The tentacles have grown in length and, instead of being conical or peg-like, are now elongated and taper towards their apices and show their characteristic movements. The head region has become further differentiated with the formation of the rudiment of the labial palps arising at the extreme anterior end of the head.

No new structures appear after this stage and the embryo now resembles the adult in all respects except in size. The apical cell-plate, the pedal cell-plate and the anal cell-plate have all disappeared, and so also has the velum.

The shell has now all the characteristics of the adult shell and is of a dull yellowish colour. The outer wall of the mantle, lying a short distance behind the edge of the mantle has also become pigmented.

The embryo is now capable of being fully retracted within the shell. The operculum can completely close the mouth of the shell holding the retracted animal. With further development, the shell, along with the visceral mass, develops a second whorl. The embryo, which is now a miniature of the adult, grows in size until it hatches out of the egg-shell to lead a free existence.

## IX.—THE DEVELOPMENT OF THE ORGAN-SYSTEMS.

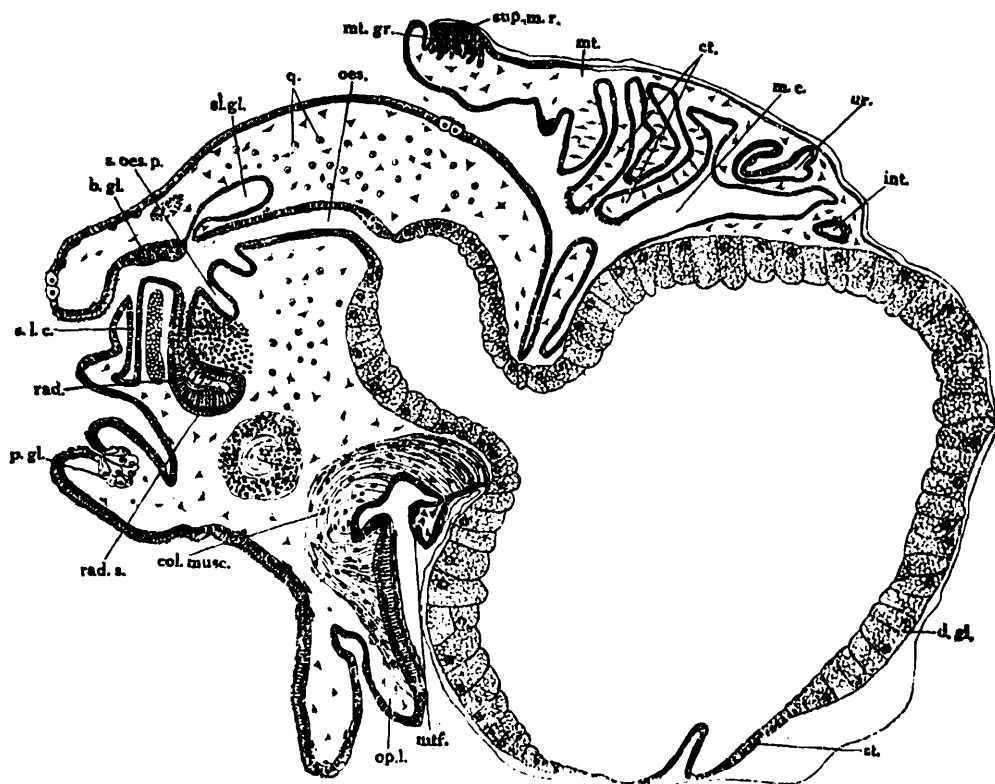
### A. *The Alimentary Canal.*

It has already been described in Section VII that the alimentary canal is laid down in the gastrula as the archenteron, that the original blastopore forms the anus, and that the stomodaeum is a new ectodermal invagination.

The further development of the alimentary canal may now be described. The canal consists of two parts, an ectodermal and an endodermal, which are described below:—

1. The ectodermal fore-gut or stomodaeum, consisting of the radular sac, the salivary glands, and the oesophageal pouches, are all differentiated from the stomodaeum.

2. The endodermal part or mesenteron, which is itself further divisible into two parts: (i) the stomach with its associated digestive-gland and (ii) the intestine.



TEXT-FIG 11. Reconstruction of a few sagittal sections of an embryo in Stage 12;  $\times 80$ ,

*The Stomodaeum.*

The rudiment of the stomodaeum is first recognised in an embryo slightly younger than Stage 1 (Text-fig. 4a). A few ectodermal cells of the body-wall, lying immediately behind and ventrally to the velum, become tall and columnar, and form a thick plate of cylindrical cells. They can also be distinguished from the adjacent epithelial cells by their larger size, and by their capacity to take up a deep stain. The cells of this plate divide rapidly and multiply and are pushed inwards to form the stomodaeal invagination (Text-fig. 4a). With further growth, this invagination deepens (Text-figs. 4b and 6b) and grows inwards and posteriorly to fuse with the anterior wall of the archenteron, and opens into the latter (*cf.* Stage 3, Text-fig. 6c). Thus, the digestive tract at this stage of development communicates with the outside at both ends, the anterior end forming the mouth and the posterior forming the anus (Text-fig. 4c), the whole of the rudimentary alimentary canal lying in the median plane of the embryo. The mouth is oval in form and faces downwards; it leads into the stomodaeal invagination which bends slightly dorsalwards to open into the archenteron. The limits of the stomodaeum and the archenteron can be easily distinguished by their staining reactions, the yolky endoderm cells do not take as deep a stain as do the stomodaeal cells. Further, the stomodaeal cells are ciliated, the cilia helping to carry the albumen surrounding the embryo through the stomodaeum into the archenteric cavity.

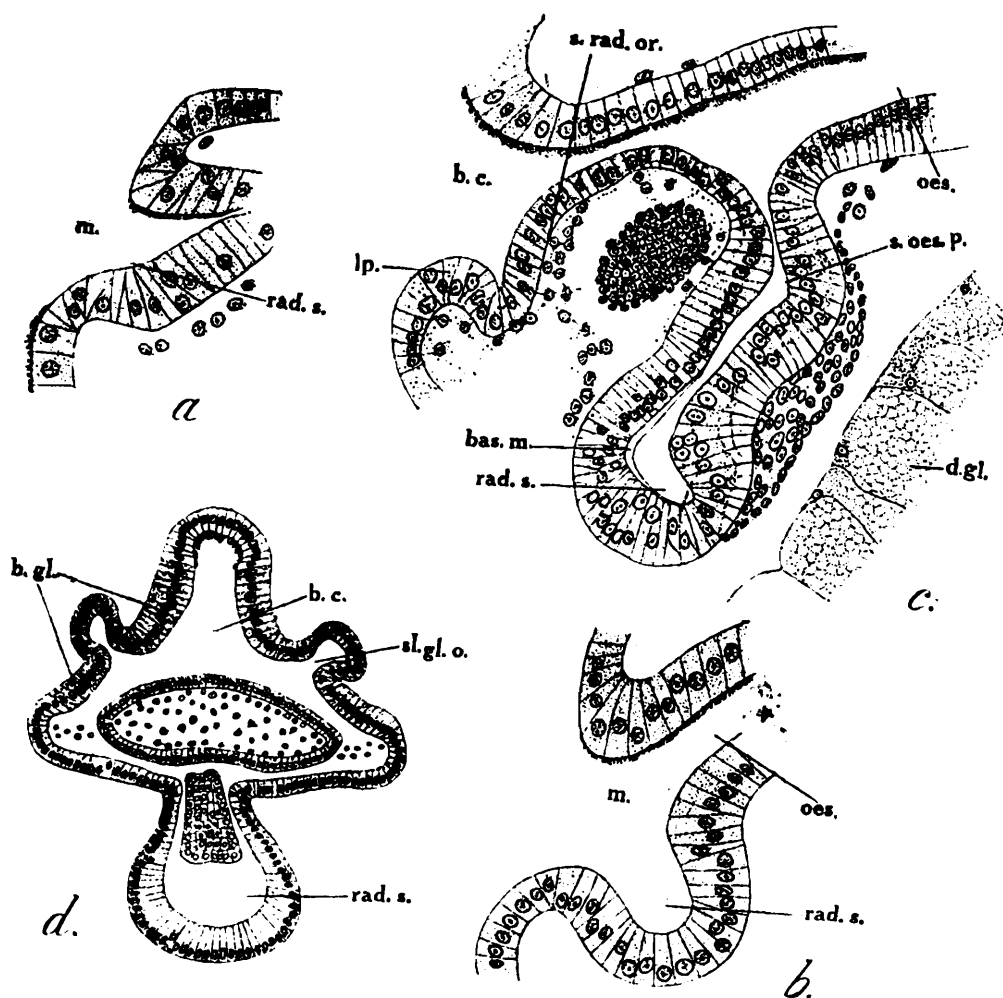
In the earlier stages, the stomodaeum opens into the ventral side (Text-fig. 6c) of the archenteron, but gradually this opening of the foregut first comes to lie at about the middle of the anterior surface of the primitive stomach (Text-fig. 4d), and finally at its antero-dorsal end (Text-figs. 5a, b, d).

The stomodaeum, in its earliest stages, is a simple tubular structure consisting of cells of equal size and similar shape (Text-fig. 6b, c). But in about Stage 6 the cells of its floor, lying a little behind the triangular mouth (now facing forward), form a thick plate of tall cylindrical cells (Text-fig. 12a), and the lumen of the stomodaeum, in the region of this plate as well as anterior to it, grows wider than it is behind. I call this thickened ectodermal plate the rudiment of the radular sac (Text-fig. 12a), since it immediately precedes the radular sac depression, which forms at this place in the next stage (Text-figs. 4f, g, and 7b). Just at this time a few scattered mesenchyme cells aggregate together to form a kind of "string of beads" all along the ventral surface of the stomodaeal wall, extending from the mouth to a little behind the radular sac thickening (Text-fig. 7b). These cells form the early representatives of the radular cartilages and their muscles.

The radular sac depression deepens and forms an evagination of the floor, directed downward and slightly backward (Text-fig. 7b). This evagination divides the stomodaeum at this stage into two parts—the part lying anterior to it represents the buccal or pharyngeal region, while the posterior part forms the oesophagus. In its earlier stages, the lumen of this evagination (radular-sac rudiment), as seen in a lateral view, appears wider anteriorly, *i.e.*, at the place of its opening into the buccal cavity, but narrower postero-ventrally, *i.e.*, towards its blind end

(Text-figs. 4*b*, *h*; and 7*b*). As development proceeds, the sac grows deeper and its lumen widens, its opening into the buccal cavity being directed antero-dorsally (Text-fig. 12*b*). The posterior wall of the radular sac is continuous with the ventral wall of the oesophagus which bends sharply upwards and then obliquely backwards to open into the primitive stomach.

Along with the increase in length of the whole of the fore-gut, the radular sac also grows in size. In the roof of the fore-gut the outer parts of the cells become hyaline, while their inner ciliated halves retain their original character (Text-fig. 8*b*). The cells forming the floor show no such change. The radular sac increases in length and curves backwards in such a way that the posterior wall of the sac now becomes the roof while the anterior wall becomes the floor of the sac. There is also a dorso-ventral flattening beginning from the opening of the radular sac into the buccal cavity, which consequently becomes narrow (Text-figs. 5*a* and 8*b*). The floor of the buccal cavity, lying immediately in front of the opening of the radular sac, becomes slightly raised upwards, and the mesenchyme cells (Text-figs. 5*a* and 8*b*) beneath this raised epithelium increase in number and form a compact mass. This mass of cells, together with the raised buccal floor, form the beginning of



TEXT-FIG. 12. Stages in the development of the radular sac and the salivary glands.

*a.* Sagittal section through the radular sac rudiment of an embryo in Stage 6:  $\times 390$ ; *b.* Sagittal section passing through the radular sac of an embryo in Stage 7:  $\times 390$ ; *(c).* Sagittal section of an embryo in Stage 11, passing through the radular sac and the fore-gut; *d.* Transverse section of the buccal cavity of an embryo in Stage 11, passing through the opening of the salivary glands:  $\times 228$ .

the odontophoral mass or tongue-mass. This compact mass of mesenchyme cells gives rise to the so-called "cartilages" and their associated muscles.

The dorso-ventral flattening of the radular sac continues except at its extreme posterior end, which becomes knob-like, giving the whole structure a club-shaped appearance, the posterior end forming the knob, and the anterior part forming the body of the club (Text-fig. 9). Due to this flattening, the lumen of the sac in transverse sections loses its circular character and appears half-moon shaped (Text-fig. 24j). In the knob-like posterior end, however, the lumen remains wide.

The odontophoral mass develops further, and a transverse section passing through this region shows that, on account of the growth of the odontophore into the floor of the buccal cavity, the latter now consists of two narrow laterally-compressed side-alleys with the odontophoral mass situated in the middle (Text-fig. 24j). Thus, while the buccal cavity in front of the odontophoral region has a wide lumen, it forms two narrow chinks in the region of the odontophore, and then widens out into a triangular cavity just above the opening of the radular sac in front of the oesophagus (Text-fig. 25f).

Vacuoles make their appearance in the cells composing the dorso-lateral walls of the oesophagus, which is ciliated all along its length up to its opening into the primitive stomach.

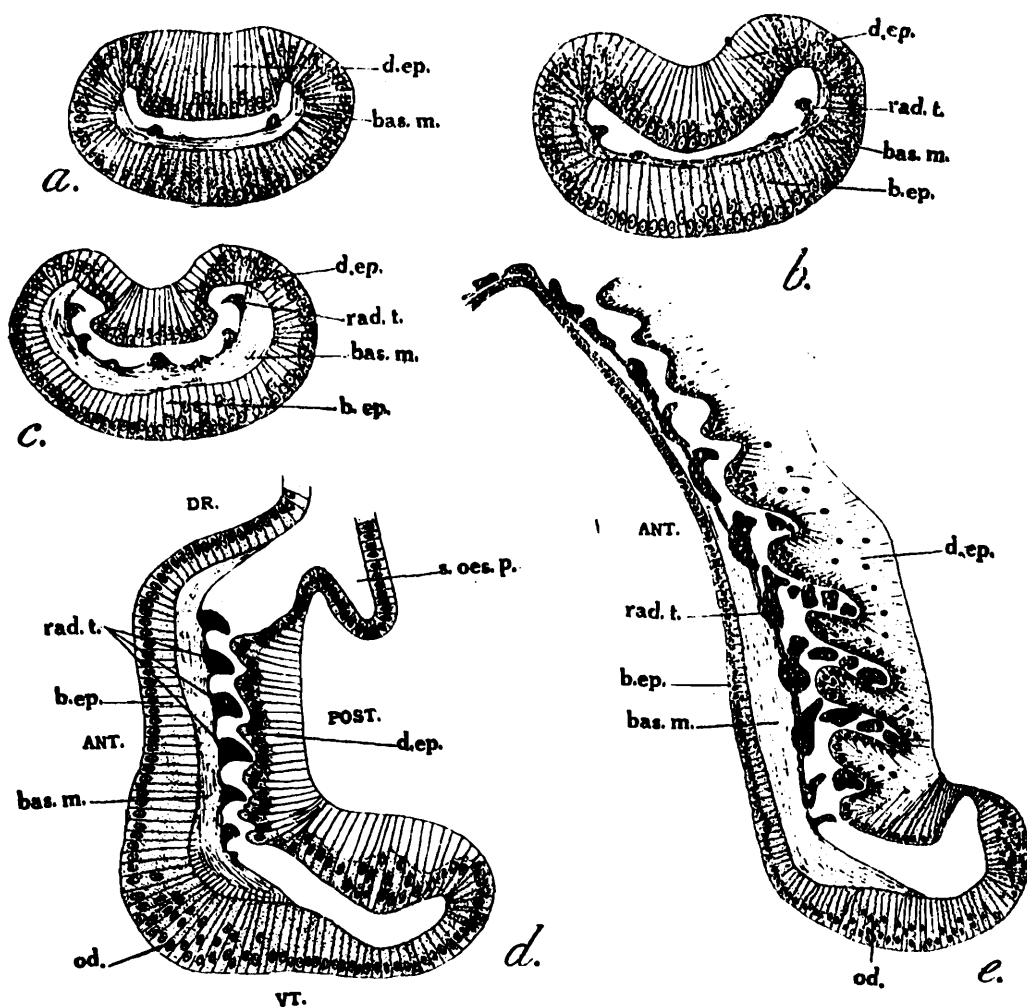
By the time torsion is complete (*cf.* Stage 10), the margins of the slit-like mouth have thickened to form "secondary lips" (Text-fig. 12c), and the buccal cavity has differentiated into an anterior vestibule and a posterior odontophoral region extending up to the oesophagus. The vestibule is lined dorso-laterally with a single row of half-hyaline ciliated cells. The arched roof of the odontophore extending anteriorly makes an abrupt bend downwards to be continued into the floor of the vestibule. The area where the downward bend occurs, forms the rudiment of the sub-radular organ.

The dorso-lateral walls of the buccal cavity above the opening of the radular sac become glandular on each side—these glandular areas form the rudiments of the buccal glands (Text-fig. 12d). At the middle of each gland the buccal wall forms an outpushing, the rudiment of the salivary gland. The openings of these glands into the buccal cavity lie a little anteriorly to the transverse plane of the eye-rudiments, as seen in Text-fig. 28b (Stage 10). The rudiments of the salivary glands develop into simple tubular glands extending posteriorly along the walls of the oesophagus. At the time of their first appearance, the openings of the glands into the buccal cavity are wide, and remain so even when the glands become tubular. Text-fig. 12d is an obliquely transverse section of a later stage (Stage 11) passing through the openings of the salivary glands into the buccal cavity. It also shows the rudiments of the buccal glands which are easily distinguished by their deeply staining capacity, and by the absence of cilia on their cells.

By this time the radular sac has increased in size, and its proximal portion shows a curvature which can be well seen in Text-fig. 12c. Moreover, the anterior part of its roof (upper part of the posterior wall in Text-fig. 12c) shows a slight outpushing directed posteriorly—this

is the rudiment of the sub-oesophageal pouch (*cf. Limax*, Meisenheimer, 1898). Text-fig. 12c is a sagittal section of the anterior part of the stomodaeum showing the vestibule, the odontophore and the radular sac along with the anterior part of the oesophagus. At this stage a thin transparent membrane is secreted by the basal epithelium which becomes thicker posteriorly—this membrane is the rudiment of the basal membrane. The cells which line the “knob” of the radular sac are the odontoblasts or the teeth-secreting cells of the radular sac. No teeth of the radula are yet visible, but changes leading to their formation have set in. The cells of roof of the radular sac increase in height. Their outer parts become hyaline and do not take up any stain, while their nuclei migrate inwards, *i.e.*, towards the lumen of the sac. In the region of the odontoblasts, the nuclei are seen at different levels in the cells, thus apparently imparting to the latter a multi-nucleate appearance. But as has already been pointed out by Schnabel (1903) in the case of *Paludina*, this is due to the apparent multiplicity of layers and not to the multi-nucleate character of the cells.

Text-figs. 13a, b, c are three transverse sections of the radular sac of slightly older embryos showing the development of the different rows



TEXT-FIG. 13. Stages in the development of the radular teeth.

a. Transverse section of the radular sac of an embryo in Stage 11, showing the formation of the lateral pair of radular teeth:  $\times 260$ ; b. Transverse section of the radular sac of an embryo in Stage 11, showing the formation of the inner pair of the marginal row of radular teeth:  $\times 260$ ; c. Transverse section of the radular sac of an embryo in Stage 12, showing all the seven rows of radular teeth:  $\times 260$ ; d. Sagittal section of the radular sac of an embryo in Stage 12:  $\times 320$ ; e. Sagittal section of the radular sac of a newly hatched embryo:  $\times 136$ .

of radular teeth secreted by the activity of the odontoblasts. Just before the formation of these teeth the basal membrane forms small projection over which lies the chitin secreted by the odontoblasts. In *Pila*, only seven teeth are laid in each transverse row, that is, two marginals and one lateral on each side of a median tooth (*cf. Paludina*, Bloch, 1896; and Schnabel, 1903). The rudiments of the lateral pair of teeth are differentiated first (Text-fig. 13*a*), followed by those of the inner pair of marginals (Text-fig. 13*b*). Those of the outer pair of marginals and the median tooth are differentiated last and arise almost simultaneously (Text-fig. 13*c*).

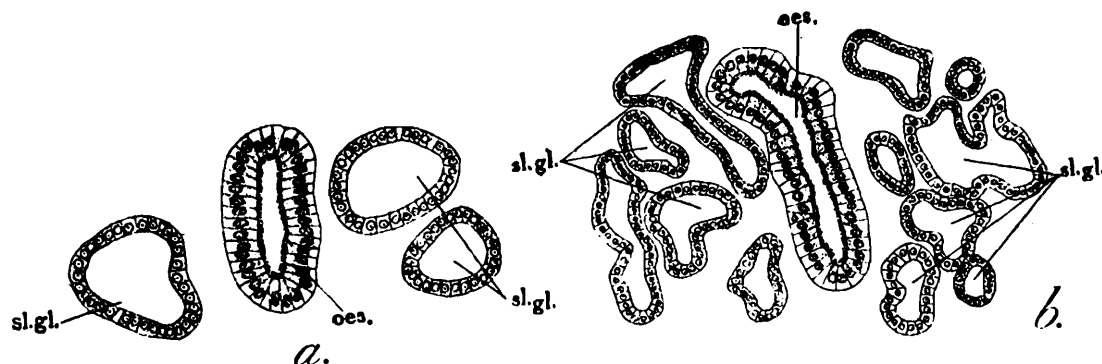
As development proceeds, the buccal cavity in the region of the sub-radular organ gives off two outpushings directed ventrally, one on each side: these are the rudiments of the sub-lingual cavity and are lined with a row of non-ciliated cells. Text-fig. 28*f* is a transverse section through the region of the sub-radular organ of an embryo belonging to Stage 12, and shows well developed sub-lingual cavities projecting as narrow chink-like prolongations of the buccal cavity.

In this stage, as well as in later stages, the ciliated cells of the buccal cavity extending from the sub-radular organ to its posterior limit, look completely hyaline, only the chromatin of the nuclei taking up the stain. Moreover, the cells are so arranged as to form a median groove running all along the roof of the buccal cavity up to its opening into the oesophagus (Text-figs. 26*d, e*). By this time the rudiments of the so-called cartilages have appeared by the modification of the closely packed mesenchyme cells lying ventrally to the arched odontophore. Text-fig. 11 is a sagittal section of an embryo of Stage 12, and shows the dorso-ventrally elongated cartilage of one side. I have not described the further development of these cartilages along with their muscles, as this has already been exhaustively dealt with by Delsman (1914) in *Littorina*. *Pila* agrees with *Littorina* as regards the origin and development of these cartilages and their associated muscles.

As development proceeds, all the structures described so far increase in size and become more defined. The jaw-rudiments make their appearance at a comparatively late stage, and lie dorso-laterally to the sub-radular organ, one on each side.

In the case of the radular sac, the roof epithelium loses its inner smooth surface on further growth, but forms cell-complexes instead, with nuclei lying at the inner ends of the cells which project in between the developing radular teeth on the opposite surface (Text-fig. 13*e*). The basal membrane forms a thick layer and extends all along the inner surface of the basal epithelium, while the roof epithelium projects in the form of cell-groups or complexes into the spaces in between the teeth which now show a hooked appearance. The roof epithelium takes no part in the formation of the teeth. The sub-oesophageal pouch is well developed. Text-fig. 14*a* is a sagittal section of the radular sac of a newly hatched embryo, and shows the odontoblasts retaining their original position at the blind end of the radular sac; the lumen of the radular sac persists and so does its communication with the buccal cavity, the lumen being wider, as in earlier stages, at the posterior end of the

radular sac than at its anterior. The radula appears as a long continuous ribbon passing over the odontophore and extending as far anteriorly as the radular organ. The roof epithelium of the sac now completely fills in the interspaces between the teeth which are now completely developed and have assumed their adult form. By this time the musculature of the buccal mass is also fully developed.



TEXT-FIG. 14. Development of the salivary glands.

- a. Transverse section of the salivary glands of an embryo in Stage 11:  $\times 244$ .  
 b. Transverse section of the salivary glands of an embryo in Stage 12:  $\times 244$ .

The salivary glands increase in size and become enlarged at their posterior ends into sac-like structures, while their anterior ends remain narrow and eventually form the ducts of these glands (Text-fig. 11). The glands are lined with a single layer of non-ciliated cubical cells with a rounded nucleus lying in the middle of each cell. Each nucleus has a distinct nucleolus in its centre which takes up a deeper stain than the chromatin of the nucleus itself. In the next stage of development the sac-like portions of the salivary glands begin to branch. Text-fig. 14a represents a transverse section passing through these sac-like portions, one on either side of the now laterally compressed oesophagus. The gland on the right shows two rounded sacs, the lower being a branch of the upper original sac. The left salivary gland shows the beginnings of a branch from the original sac. With further growth, these branches give out secondary branches, which in their turn branch again, so that by the time the embryo is ready to hatch, each salivary gland is composed of a large number of branching tubules extending in all directions (Text-fig. 14b). The salivary glands increase considerably in size and lie dorso-laterally on either side of the oesophagus. Each salivary gland has a common duct which runs anteriorly and then bends a little downwards to open into the lateral side of the buccal cavity, almost in the middle of the buccal gland of each side.

The oesophagus becomes considerably elongated (Stage 12) and runs backwards towards the visceral mass, opening at the anterior end of the primitive stomach now lined by the cells of the digestive-gland. This gland, though still lying in the head-vesicle, is very much reduced in size (Text-fig. 11), as compared with its earlier condition (Text-figs. 9 and 10). So far, the oesophagus has maintained its median position, but in stages subsequent to that shown in Text-fig. 11, in which the digestive-gland lies completely within the visceral mass, the oesophagus stretches throughout the length of the head-vesicle and deviates posteriorly from its median course towards the left (Text-fig. 26f), to open

into the stomach which is situated in the left ventral part of the visceral-sac. In these stages it is noticed that the oesophagus, after arising from the postero-dorsal end of the buccal cavity, runs obliquely downwards to enter the visceral sac. The connection of the head-vesicle with the visceral-sac has, due to a constriction and deep indentation of the mantle-cavity on the right side of the embryo, become so narrow that only the oesophagus and the columellar muscle can pass through. The oesophagus lies to the left of this muscle.

*Discussion.*—In some Gastropoda, e.g., *Littorina* (Delsman, 1914), *Limax* (Meisenheimer, 1898), *Physa* (Wierzejski, 1895), etc., when the embryo is passing from the gastrula to the trochophore stage, the blastopore shifts anteriorly from its original position and comes to lie almost immediately behind the velum. But the blastopore remains open and gives rise directly to the mouth, while the anus is a new formation. In some other Gastropoda, e.g., *Patella*, *Bythinia* and *Crepidula*, the blastopore, having shifted anteriorly, closes up; but, in spite of the closure, the stomodaeal invagination is formed at this very place.

In *Pila*, as in *Paludina* (Lankester, Erlanger, Fernando, etc.), on the other hand, the blastopore does not shift anteriorly but retains its original position; there it remains open and forms the anus. In *Pila*, therefore, the mouth is a new formation quite independent of the blastopore. The position of the mouth, however, corresponds exactly to that of other Gastropoda, i.e., the stomodaeal invagination is formed anteriorly on the ventral surface immediately behind the velum.

The buccal mass and the oesophagus, with all their accessory apparatus, i.e., sub-lingual cavities, oesophageal pouches, radular sac, and the salivary glands, which develop from the stomodaeum, are therefore ectodermal in origin. In *Limax*, however, Meisenheimer (1898) holds that the floor of the oesophagus is partly ectodermal and partly endodermal; the roof is completely ectodermal, while the side-walls show a transition from one layer to the other.

In all other Gastropods, e.g., *Paludina*, *Littorina* (Delsman, 1914), *Physa* (Wierzejski, 1905), Heteropoda (Fol, 1876) the entire fore-gut is ectodermal in origin.

As regards the radular sac, its origin and formation, as an evagination from the floor of the stomodaeum, are similar to those of other Gastropods. In almost all Gastropoda this evagination occurs after the stomodaeum has opened into the primitive stomach. In *Helix* (Fol, 1880), however, the rudiment of the radular sac appears even earlier than the joining of the stomodaeum with the archenteron.

In *Physa* (Wierzejski, 1905) the radular sac arises as a pair of structures which unite later to form a single-chambered sac. In this respect *Physa* is unique, as in no other Gastropod so far investigated, has a double origin of the radular sac been observed. In *Pila* the lumen of the radular sac does not completely disappear, but remains in constant communication with the buccal cavity. Schnabel (1903) describes a similar condition in *Paludina*. But in the Pulmonata (e.g., *Succinea*, *Helix*, *Planorbis*) this lumen disappears at the time of teeth formation. The Pulmonata and the Opisthobranchs (Rossler, 1885) are further characterised by a fixed number of odontoblasts, i.e., four to five. In

*Pila*, on the other hand, as in *Paludina* (Schnabel, 1903) and other Proso-branches, and in Heteropoda (Rössler, 1885), there is no fixed number of odontoblasts; in fact, they are many, and they retain their terminal position to the last.

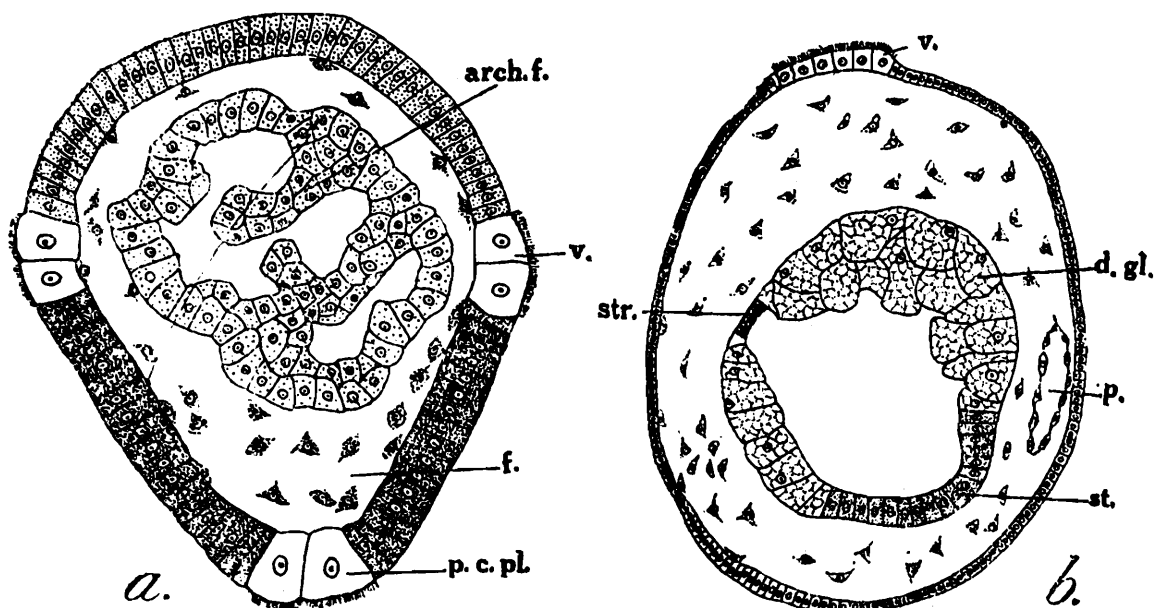
In *Littorina*, as in *Turbo* and *Patella*, the radular sac increases to such a length (Delsman, 1914) that, finding no place to accommodate itself, it deviates from its middle course towards the right (while the oesophagus lies on its left), and makes a spiral with the club-shaped end lying in the centre of the spiral.

#### *The Mesenteron.*

It has already been described that the archenteron gives rise anteriorly to the stomach and its digestive-gland and posteriorly to the intestine. I shall now first describe the development of the stomach and the digestive-gland, and then of the intestine.

#### *The Stomach and the digestive-gland.*

The cells forming the wall of the archenteron are laden with yolk which imparts to them a yellow tinge and also does not allow the cytoplasm to take up a deep stain; the nuclei, however, stain deeply. At an early stage, the boundary between the primitive stomach and the intestine cannot be easily made out and the two imperceptibly merge into each other, as all the cells are alike in size and shape (Text-figs. 6a, c). In the trochophore stage, just before the formation of the stomodaeum, however, the walls of the primitive stomach show internal folds (Text-figs. 6a and 15a). These folds are of various sizes in the different embryos of the same stage, and project into the lumen of the primitive stomach; that lumen is consequently greatly reduced. The wall of the primitive stomach thus provides an extensive surface for the absorption of the albuminous food. As development proceeds, the primitive stomach occupies greater and greater area within the



TEXT-FIG. 15. Development of the archenteric folds and the adult stomach.

a. Transverse section through the "Kopffuss" region of an embryo in Stage 3, showing the archenteric folds within the primitive stomach:  $\times 390$ ; b. Transverse section of an embryo in Stage 6, showing the differentiation of the adult stomach:  $\times 225$ .

“Kopffuss” region by the extension of its lumen, while the folds gradually dwindle away antero-posteriorly. The growth of the primitive stomach is due not so much to the multiplication of its cells as to the increase in their size.

As a result of the absorption and deposition of the ingested albumen, food-vacuoles appear at the inner ends of the large columnar cells of the primitive stomach. The vacuoles are very small to begin with, but gradually increase in size along with the growth in size of the cells; consequently, the nuclei are displaced from their middle position and are pushed to the outside where they come to lie at odd places. This indicates the first appearance of the digestive-gland<sup>1</sup>. The cells remain columnar and cylindrical but reach an enormous size. All the cells of the primitive stomach, become differentiated except at two places approximately opposite to each other. At these two places, the cells remain small and undifferentiated. The transition from undifferentiated cubical cells to the large vacuolated cells is gradual. In a whole embryo the two places appear as two clear zones or streaks amongst the hypertrophied cells of the digestive-gland rudiment. One of these streaks begins on the dorsal side at the junction of the oesophagus and the primitive stomach, and runs posteriorly, in an oblique direction, to a point where the intestine leaves the primitive stomach, keeping all along towards the right of the median line. The second streak lies imbedded in the left ventral part of the digestive-gland and leads posteriorly. The position of this streak is not constant but changes at different stages of development. In its earlier stages it is seen on the right of the median line (Text-fig. 15*b*), whence it changes in later stages to a mid-ventral position and finally comes to lie on the left wall of the primitive stomach. These clear streaks are the rudiments of the alimentary canal proper while the vacuolated lateral walls represent the digestive-gland. The ventral streak leads straight into the floor of the adult stomach (when formed), while the dorsal streak joins its roof (Stage 6).

Semper (1862) describes only one such streak in *Ampullaria polita* at a much later stage (cf. his fig. 15), which corresponds to the dorsal streak of *Pila*. Semper regards this streak as an independent structure which is only embedded within the wall of the digestive-gland, but I regard both the streaks as mere undifferentiated regions in the wall of the gland. Describing the appearance of the liver rudiment (digestive-gland), he says that it is hollow and the cavity is mostly full of bile-secretion. He apparently mistook the ingested albumen for bile-secretion. Scott (1934) also mentions only one streak in *Ampullaria canaliculata*. I am of the opinion that if these workers had cut sections of the embryos they would have seen the second streak of clear zone on the floor of the digestive-gland almost concealed by the large vacuolated cells.

The cells lining the posterior part of the floor of the primitive stomach on the right of the mid-ventral line, can be distinguished from the adjacent highly vacuolated cells; they are small and columnar, with a granular cytoplasm and a centrally situated nucleus (Text-fig. 16*a*). These cells represent the true stomach. The lumen of the primitive stomach enlarges to such an extent in the anterior part of

<sup>1</sup> This gland has been described as “liver” or has been given other equivalent names (e.g., Leber, foie, etc.). But as Pelsener (1905) and Prashad (1925) point out, the name “liver” is quite unsuitable, “for the gland combines the functions of the various digestive glands of the vertebrates and is, in addition, the chief organ for the absorption of the digested food”.

the head-vesicle, that the dorsal wall of the latter becomes very much arched with a sharp anterior declivity in the region of the fore-gut, and a posterior gradual slope, where the stomach enters the visceral-sac rudiment (Text-fig. 5*d*).

With further growth the stomach first shifts completely to the mid-ventral line, but later comes to occupy its adult left-ventral position and lies completely within the visceral-sac (Text-figs. 9 ; 24*n* ; and 25*h*).

After the true stomach comes to lie within the visceral-sac, the posterior rudiment of the digestive-gland lying dorsally to it (Text-fig. 24*n*) increases in size and extends posteriorly beyond the stomach up to the posterior end of the embryo. It thus forms a large sac-like structure with its lumen full of albumen (Text-figs. 10 ; 11 ; and 26*a*, *b*). As development proceeds, the digestive-gland follows the course of the visceral-sac which, on account of the lateral torsion round a horizontal axis, passes from right to left, so as to produce an endogastric spiral—a characteristic of the visceral-sac of most Gastropoda.

With further development, while the posterior lobe of the digestive-gland shows a considerable increase in size and fills almost completely the cavity of the visceral-sac, displacing the other organs dorsally, the anterior lobe is reduced in size (Stage 12 ; Text-fig. 11).

By this time the cavity of the digestive-gland is almost completely filled up with the ingested albumen which is completely absorbed by the time the embryo hatches. As there is no more space available for the digestive-gland to expand, lobes develop on its inner surface which increase in number and completely obliterate the lumen. Thus the lobed character of the gland, consisting of long follicles characteristic of adult *Pila*, is obtained.

*Previous work and discussion.*—Thus, in *Pila*, the adult stomach arises from the right side of the floor of the posterior end of the stomach. In *Bythinia* (Erlanger, 1892) the stomach is differentiated at the postero-dorsal part of the endodermal sac. In *Littorina* also, the posterior part forms the stomach. In *Paludina* (Bütschli, 1877 ; and others), on the other hand, it is the dorsal and the anterior part of the primitive stomach which gives rise to the adult stomach.

On comparing the origin of the digestive-gland in *Pila* with that of other Gastropoda, we find that there is a great variation, apparently due to the different positions of the areas of storage of the nutritive material within the endoderm.

In *Paludina* (Erlanger, 1891 ; Drummond 1903 ; and Otto and Tönniges, 1905), the “liver” (digestive-gland) arises as a single rudiment from the floor of the endodermal sac, the cells of which increase in size and become vacuolated. The dorsal anterior part follows suit, but it is always on the ventral side that there is greater accumulation of nutritive substances and consequent formation of the “liver” rudiment.

In *Bythinia* (Erlanger, 1892), the “liver” arises in the form of two outgrowths—an anterior, which is larger, wider and dorsal in position ; and a posterior which is smaller and ventral in position.

In *Littorina* (Delsman, 1914), the “liver” arises as an unpaired structure from the left wall of the endodermal sac, and is directed dorsally. But it is divided later into two lobes of unequal size ; the larger

one lies anteriorly to the stomach, while the posterior one, which is smaller, lies to the right of the stomach.

In *Planorbis* (Rabl, 1879) and other Pulmonates (Fol, 1880; Jourdain, 1884), the "liver" arises from the antero-dorsal vacuolated part of the endodermal sac, the stomach originating from the posterior part. These rudiments of the liver and stomach later become separated by the appearance of a partition between them. In *Limax* (Meisenheimer, 1898), the "liver" arises as two rudiments, the anterior left, and the posterior right, arising chiefly from the anterior part of the primitive stomach. Only the left rudiment, which consists of two parts, forms the "liver" of the adult.

### *The Intestine.*

To begin with, the cells lining the intestine are of the same shape and size as those of the primitive stomach (Text-figs. 6a-d). In later stages, however, while the cells of the primitive stomach become large and vacuolated and give rise to the rudiments of the digestive-gland, those of the intestine become columnar and thickly granulated, and remain non-vacuolated, and thus the boundary between the two structures can be easily made out (Text-fig. 7b). In the earliest stages the intestine possesses a broader lumen anteriorly near its junction with the primitive stomach than it does posteriorly (Text-figs. 6a-d); in later stages, however, there is a uniform rounded lumen along its entire length (Text-fig. 7b).

In its earliest stages, when the embryo is bilaterally symmetrical, the intestine runs a straight median course extending from the posterior end of the primitive stomach to the anus (Text-fig. 6a). But later, it is displaced out of its median course and is pushed towards the left (Stage 4; Text-fig. 16b). At a still later stage, as a result of torsion, a ventral flexure appears in the intestine (Stage 5) and it elongates and becomes displaced towards the right side (Stage 7). By the time torsion is complete (Stage 10), the intestine arises from the left postero-dorsal side of the stomach and courses round the hinder end of the digestive-gland towards the right; it then turns upwards and reaches the anterior face of the visceral-sac to open into the mantle-cavity through the anus.

The blastopore persists and forms the anus, there being no trace of a proctodaeum.

*Previous work and discussion*:—Semper (1862) and Scott (1934) do not make any mention of the persistence of the blastopore and the formation of the anus in *Ampullaria polita* and *A. canaliculata*. But from Semper's description it appears that the rudiment of the intestine arises from the primitive stomach to open out through the anus (his fig. 7, Pl. I). Similarly, according to Fernando's description of a stage in *Pila gigas* where he says "it is a trochophore in which the intestine has not yet opened to the outside", it appears that the blastopore does not persist to form the anus, but that the latter is a new formation. The failure of Semper and of Scott to notice the opening of the intestine to the outside was due to the fact that they worked on whole

embryos, which are full of yolk and consequently opaque at this stage, and it is almost impossible to observe internal structures correctly. The only explanation for Fernando's mistake can be that his sections were probably not properly orientated or that Fernando might have cut sections more than  $4\ \mu$  thick, in which case there is every possibility of his having missed the anal opening. These suppositions are strengthened by the fact that Fernando, in his fig. 1, Pl. I, shows the shell-gland as 7 to 8 cells thick, while in reality at this stage it consists of a single layer of cells. It can only appear many-layered if the sections are oblique.

*Paludina* (Erlanger, 1891; Fernando, 1931) resembles *Pila* in the fact that the blastopore persists and forms the anus, and there is no trace of an ectodermal invagination.

In the majority of Gastropoda, the intestine arises as a posterior prolongation of the endodermal "primitive stomach", which later meets with the ectoderm of the body-wall, and, breaking through it, opens to the outside through the anus. Thus it is held that a very small proctodaeum is formed.

In *Lymnaea* (Lankester, 1874) a "pedicle of invagination" is formed where the blastopore closes "which grows up against the primitive alimentary cavity and finally unites with it"

In *Planorbis* (Rabl, 1879), *Umbrella* (Heymons, 1893), and *Physa* (Wierzejski, 1905), the intestine originates as a solid band of cells, in which a lumen is formed later. In *Physa*, towards the posterior end of this band, two large ectodermal cells appear in the body-wall of the embryo; here a shallow ectodermal invagination occurs, but it does not give rise to any part of the actual or true intestine.

In *Limax* (Meisenheimer, 1898), the entire intestine right up to its opening into the stomach is ectodermal in origin. According to him, the ectodermal invagination is formed with a very narrow lumen. In later stages, the lumen becomes rounded and the invagination becomes cut off from the outer ectoderm and fuses with the endoderm so that it can easily be mistaken for an evagination from the latter. Later it elongates and opens again to the outside through the anus which is thus a secondary formation. This point, in my opinion, needs confirmation.

#### B. *The Differentiation of the Common Rudiment of the Kidney, the Pericardium, the Heart and the Gonad.*

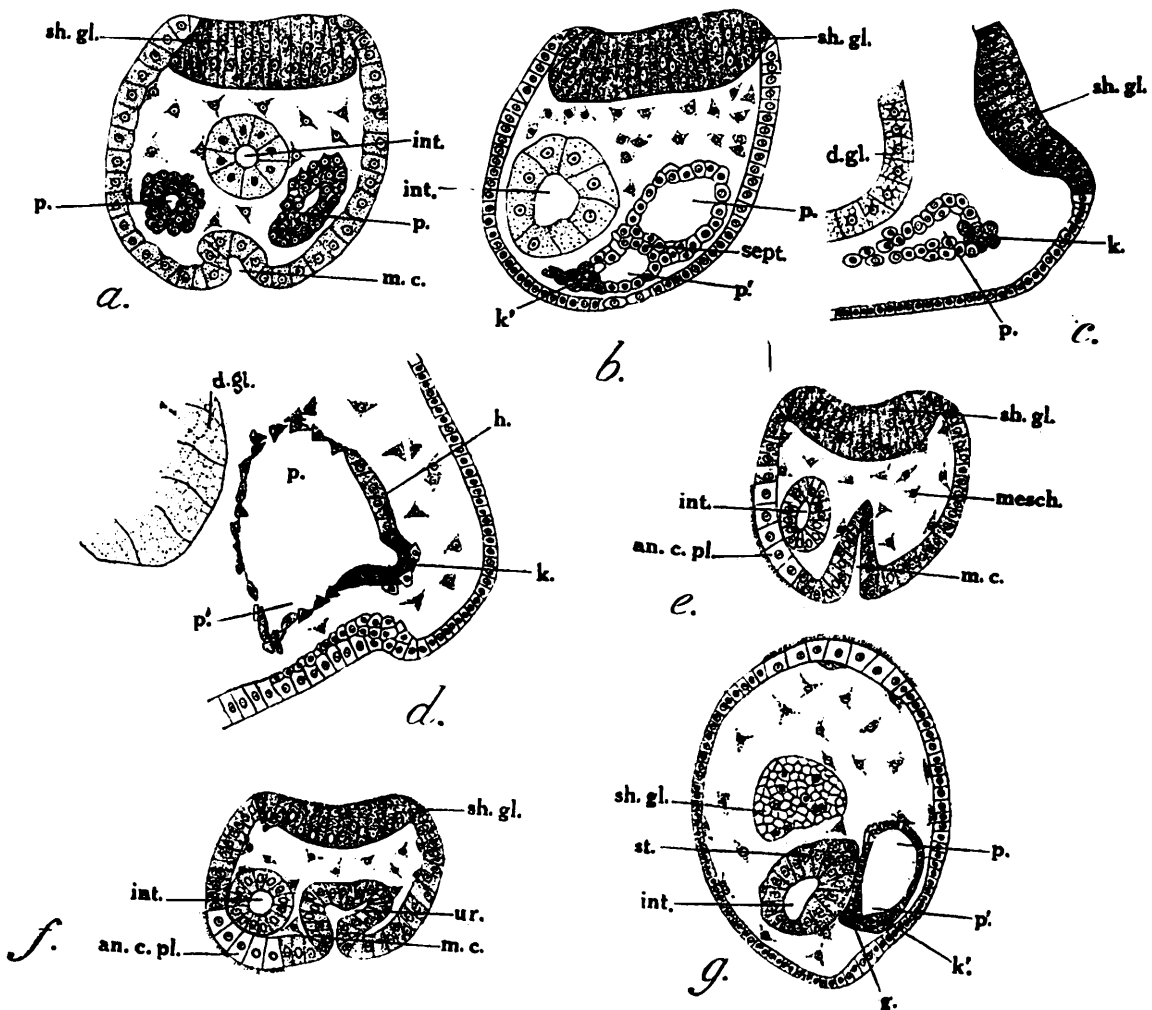
The appearance of two small compact masses of mesenchyme cells at the postero-ventral part of the embryo has already been described in Stage 2 above. These cell-masses are formed below the level of the intestine, one on either side by the aggregation of mesenchyme cells (Text-fig. 6b). They are irregular in shape and of unequal size, the right mass being larger than the left.

Soon a small cavity appears in the cell-mass of the right side; it is immediately followed by the formation of a similar cavity in the left one. Each cavity is formed by the separation of mesenchyme cells from one another in the centre of each cell-mass, but is not delimited by a regular epithelium. The two cavities, like the cell-masses, are

unequal in size, the right cavity being always larger than the left (Text-fig. 16a). These two cell-masses with their cavities form the rudiments of the pericardium.

As development proceeds, the pericardial rudiments increase in size and move towards each other and meet beneath the intestine, the cavity of the right pericardial rudiment still remaining larger than that of the left. The growth of the two rudiments occurs in such a manner that, while the cavity on the right side develops in all directions, the left cavity develops more towards the median plane. Although the two rudiments lie against each other, their cavities are separated by a septum many cells in thickness.

With further development, the two pericardial rudiments shift from their ventral position to the right side of the intestine. On account of greater increase in the size of the right cavity, its wall becomes thinned to a single layer of cells. Gradually, the other cavity also



TEXT-FIG. 16. Early stages in the differentiation of the rudiment of the kidneys, the pericardium, the heart and the gonad.

a. Transverse section of an embryo in Stage 2, passing through the right and left pericardial rudiments and the invagination of the mantle-cavity rudiment:  $\times 320$ ; b. Transverse section of an embryo in Stage 4, passing through the rudiment of the left kidney and the septum between the two pericardial rudiments:  $\times 320$ ; c. Sagittal section passing through the right pericardium, showing the formation of the right kidney rudiment:  $\times 304$ ; d. Sagittal section passing through the pericardium, and showing the differentiation of the heart rudiment:  $\times 292$ ; e. Transverse section of the embryo in Stage 3, passing through the mantle-cavity rudiment:  $\times 264$ ; f. Transverse section of an embryo in Stage 4, showing the evagination of the right ureter from the mantle-cavity:  $\times 252$ ; g. Transverse section of an embryo in Stage 5, passing through the gonad and the left kidney rudiment:  $\times 204$ ,

become bounded by a single layer of cells. The two cavities are still separated from each other by the septum which has now become thin and consists of one or two layers of cells. It is from these two mesenchymatous rudiments of the pericardium that the kidneys, the pericardium, the heart and the gonads arise.

*Discussion* :—Fernando's (1931) observations regarding the formation of the mesenchymatous rudiments of the pericardium in *Pila gigas* differ in some details from those of mine. According to him, the left pericardial rudiment is formed later when the rudiment of the right pericardium, leaving its ventral position, "has come to lie on the right of the intestine, and the cells are so arranged as to enclose a lumen—the right pericardium" It is at this stage that the left pericardial rudiment is formed as "a very small cavity" I think Fernando failed to observe the formation of the left pericardial rudiment when it still lies ventrally to the intestine as a solid compact mass, like that of the right pericardial rudiment, as is generally the case in all Gastropods which have paired rudiments of the pericardium, as in *Paludina* and *Physa*. Again, though the left pericardial rudiment arises, according to Fernando's description, as well as his fig. 2, Pl. I, later than that of the right pericardium, and its lumen is also much smaller than that of the latter. Yet in his fig. 4, Pl. I, Fernando has shown the left pericardium to be almost as large as the right one, which can only happen if it shows a quicker and stronger development than that of the right pericardium. But this is contrary to the actual facts, since the lumen of the right pericardium, besides appearing earlier than that of the left pericardium, as Fernando himself points out, remains much larger than that of the left. Another difference consists in regard to the positions of the two pericardial rudiments in relation to each other. As seen in fig. 4, Pl. I, of Fernando, the rudiment of the left pericardium lies between the rudiment of the right pericardium and the intestine, while according to my observations, at a stage corresponding to the one represented by fig. 4, Pl. I, of Fernando, the rudiment of the left pericardium, though it has shifted to the right of the intestine, yet lies ventrally to the right pericardium but closely applied to it (Text-fig. 16b). I cut horizontal sections also of such a stage and they confirmed my observations. My observations are in complete agreement with those of Erlanger (1891), Drummond (1903), and Otto and Töniges (1905) in *Paludina* (= *Viviparus*). Hence I consider that the observations of Fernando in *Pila gigas* require revision.

### *The kidneys.*

The rudiments of the kidneys make their appearance at a stage when the rudiments of the right and left pericardium, separated from each other by a median septum, still lie below the level of the intestine (Stage 3). A thickening appears at the postero-ventral end of the right pericardium (Text-fig. 16c), which is followed immediately by the formation of another but smaller thickening in the wall of the corresponding region of the left pericardium. These two thickenings are the rudiments of the right and left kidneys respectively. With further development an evagination takes place in the right kidney rudiment

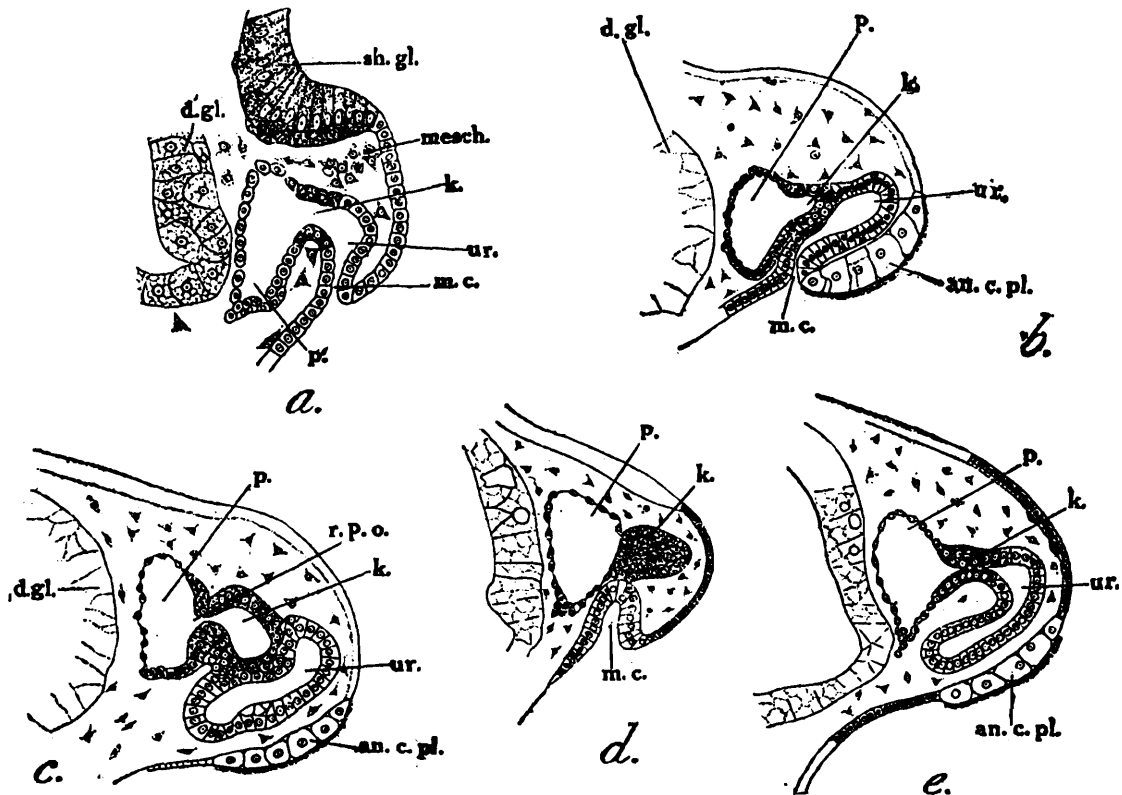
which deepens and gives it a more or less vesicular appearance (Text-fig. 16*d*). Meanwhile the left kidney rudiment continues as a mere thickening (Text-fig. 16*c*). The right kidney rudiment is distinguished by the thickly granulated character of its cells which, by this time, assume a columnar shape and form a regular epithelium for the kidney.

While the right kidney rudiment has developed into an open vesicle lined by a regular epithelium, another structure, which comes into relation with it later, makes its appearance; it is the rudiment of the ureter (=anterior kidney). The rudiment of the mantle-cavity (Stage 1) grows deeper and acquires a lumen (Text-fig. 16*e*), and from its inner right end a tubular evagination arises towards the right side (Text-fig. 16*b*)—this is the rudiment of the ureter. While keeping towards the right side within the visceral-sac rudiment, it grows posteriorly and ascends obliquely upwards and comes to lie against the posterior end of the open vesicular rudiment of the right kidney into which it finally opens.

The rudiment of the left kidney still persists as a thickening, its cavity and evagination appearing much later; there is no trace yet of the rudiment of the ureter of the left side.

In *Pila* the rudiments of both the kidneys, that is, the right and the left, are laid in the earliest stages of development and are mesodermal in origin, while the ureter is ectodermal.

*Discussion*.:—Semper (1862) makes no mention of the right and left rudiments, but from his description it is evident that he describes only



TEXT-FIG. 17. Stages in the development of the kidneys and the ureter.

*a.* Oblique sagittal section of the embryo in Stage 4, showing the opening of the right kidney into the ureter:  $\times 312$ ; *b.* Sagittal section of the embryo in Stage 6, showing the wide lumen of the right ureter:  $\times 176$ ; *c.* Sagittal section of the embryo in Stage 6, passing through the reno-pericardial aperture:  $\times 176$ ; *d.* Sagittal section of the embryo in Stage 6, showing the relation of the mantle-cavity with the pericardium and the right kidney:  $\times 180$ ; *e.* Sagittal section, showing the bending forward of the ureter to meet the kidney:  $\times 180$ .

the origin of the functional left kidney (=morphologically right). According to him, the cells which form the rudiment of the kidney "arise by transformation directly out of the epidermis cells", *i.e.*, they are ectodermal in origin. But the stage at which he observed this rudiment of the kidney as well as the position of these cells in his fig. 21, Pl. III, at once make it clear that Semper was unable to observe or locate the origin and the formation of the kidney in *Ampullaria polita*.

Fernando's description of the origin of the kidney in *Pila gigas* (1931) is almost in complete agreement with my observations, with the only exception that he considers the formation of the rudiment of the right kidney to arise first by evagination, which, according to my observations, is the second stage of development, the first being the thickening stage. He has also shown that while the kidney proper is mesodermal in origin, its ureter is ectodermal.

Scott (1934) makes no reference to the left kidney rudiment in *Ampullaria canaliculata*. Similarly, she does not describe the nature and manner of the origin of the kidney. She only describes the presence of a kidney-pericardium complex which she observed for the first time at a pretty late stage when the ureter is already opening to the exterior (*cf.* her fig. 17).

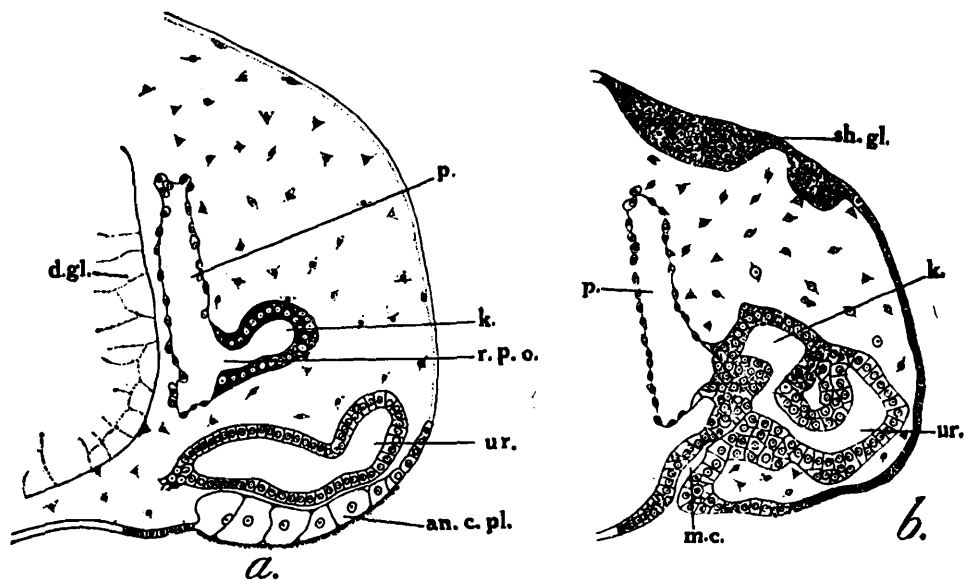
#### *The pericardium and the heart.*

It has already been described that the rudiments of the right and left pericardium, even after they come to lie against each other, are still separated by a septum which is quite thick to begin with, but becomes thinner later on (Text-fig. 16*b*). Even at this stage the difference in the size of the two sacs of the pericardium is well marked, the right one (now dorsal in position) being much larger and broader than the left (now ventral in position) which is very narrow at its inner ventral end. As development proceeds, the septum becomes reduced and is gradually absorbed antero-posteriorly. By the time the embryo reaches Stage 5, it disappears completely by being absorbed into the wall of the pericardium-sacs, which now form a single large thin-walled sac, lying completely on the right side of the gastro-intestinal junction. This sac forms the adult pericardium in which the heart is differentiated later (Text-figs. 4*c-h* ; 5*a-f* ; and 16*d*).

Just when the pericardium comes to lie on the right side of the gastro-intestinal junction as a single chamber, a part of the pericardial wall lying anteriorly but dorsally to the right kidney becomes thickened, and is thus distinguishable from the adjoining area of the pericardial wall. This thickening really lies in the original rudiment of the right pericardium and forms the rudiment of the heart (Text-fig. 16*d*). It invaginates into the pericardial chamber and gives rise to the heart. Thus, unlike the rudiments of the right and left kidney and those of the right and left pericardium, the heart arises as a single unpaired rudiment inside the cavity of the original right pericardium.

Even in later stages of development, it is that part of the pericardium which corresponds to its original right rudiment that grows more and increases in dimensions, while the original left pericardial rudiment

remains small, and corresponds to the narrow part of the pericardium in later stages.



TEXT-FIG. 18. Relation of the right kidney and the ureter.

*a.* Sagittal section of the embryo in Stage 8, passing through the reno-pericardial aperture and showing the dorsal bending of the right kidney:  $\times 225$ ; *b.* Another sagittal section of the same embryo in (*a.*), passing through the opening of the ureter into the right kidney:  $\times 225$ .

### *The gonad.*

After the differentiation of the right and left kidney rudiments as well as that of the heart, another cell-thickening makes its appearance in the roof of the narrower left part of the pericardium, situated ventrally to the gastro-intestinal junction—this is the rudiment of the gonad. Text-fig. 16*g* is a transverse section passing through the gonad-rudiment of an embryo, which is slightly older than the one in which the gonad rudiment is first laid; hence the gonad rudiment, instead of being exactly ventral, lies a little to the right of the gastro-intestinal junction. At the stage at which it is first differentiated, the larger part of the thin-walled pericardium (right pericardial sac) lies on the right side between the gut and the body-wall; while the left pericardial sac still lies partly ventrally to the gastro-intestinal junction. It is on the roof of this narrower part of the pericardium, almost on its extreme left, that the rudiment of the gonad is first laid. It lies dorsally, close to the rudiment of the left kidney, with which it is very closely associated.

The cells of the gonad rudiment proliferate and extend along the wall of the digestive gland in the form of a cord of cells. Text-figs. 16*g* and 20*c* pass through the gonad rudiment and show its relative position with regard to the rudiment of the left kidney and other structures.

*Discussion* :—Semper (1862) mistook an advanced stage of the gonad for its first rudiment (“*erste anlage*”) at a stage at which “the shell has already made one complete spiral and has thus surrounded the liver (=digestive gland), a part of the latter still lying within the head-vesicle” (*cf.* his fig. 25, Pl. IV). At this stage, according to him, the gonad lies at the apex of the spiral as a glandular mass of small greyish cells in contrast to the yellow mass of the liver. But this, as is apparent

from his description, is a much later stage in which the distal end of the gonad has also participated in the spiral of the digestive gland. Semper, however, does not allude to the origin (mesodermal or otherwise) of the rudiment of the genital gland.

Fernando (1931) on the other hand, has overlooked the formation of the gonad rudiment in *Pila gigas* and believes it to be post-embryonic in formation.

A comparison of the origin of these organs, *i.e.*, the kidney, the pericardium, the heart and the gonad in different Molluscs reveals great variations. These variations concern the following main points: (1) whether there is only one common rudiment for all these organs, or each of these organs has an independent origin; (2) whether all these organs are mesodermal or ectodermal in origin or whether some are mesodermal, and others ectodermal; and (3) whether the common rudiment, if present, is paired or unpaired.

I shall discuss below these three points in the order given above.

(1) In nearly all the Lamellibranchs and the following forms amongst the Gastropoda, all the organs, that is the kidney, the pericardium, the heart and the gonad are differentiated from a common rudiment: *Pila*, *Paludina*, (Erlanger, 1891; Otto and Tönniges, 1905), *Littorina* (Delsman, 1914), *Planorbis* (Rabl, 1879), *Physa* (Wierzejski, 1905).

*Paludina* (Erlanger, 1891; Drummond, 1903; Otto and Tönniges, 1905) shows the greatest resemblance to *Pila* with regard to the development of the paired common rudiment.

There are other forms, such as *Calyptraea* and *Vermetus* (Salensky, 1872) and *Bythinia* (Erlanger, 1892) amongst Prosobranchs, and *Arion* (Heyder, 1909) and *Limax* (Hoffmann, 1922) amongst Pulmonates, in which only the kidney, the pericardium and the heart form a common rudiment, while the gonad rudiment arises independently.

(2) Again, there is either an actual variation, or probably only a difference of opinion as to the mesodermal or ectodermal origin of these rudiments. For example, Erlanger (1891) and Fernando (1931) describe the common rudiment as mesodermal in origin, while Tönniges (1896) and Otto and Tönniges (1905) describe it as ectodermal. According to the latter authors, the paired rudiment arises directly, one on each side of the middle line, by the inward growth of the ectodermal cells from the body-wall, and thus gives rise to two irregular compact cell-masses. But this view has been rejected by a great many authors like MacBride (1915), Heyder (1909), Naef (1913), Herbers (1914), Fernando (1931) and others, who have remarked that those authors who believe in the ectodermal origin of the common rudiment are certainly wrong, and that cell-masses forming the common rudiment arise through the division of the primary mesoderm cells. According to these authors, the ectodermal nature of the rudiment is an illusion due to the close proximity of these rudiments to the ectodermal wall.

(3) Regarding the paired or unpaired character of the common rudiment, we find that in majority of cases it is a paired structure lying in the posterior part of the embryo, as in *Paludina*, *Bythinia*, *Physa*, *Arion*, *Planorbis*, *Anodonta*, and *Ostrea*. On the other hand, in *Limax* (1898) and *Dreissensia* (1901), according to Meisenheimer, the common

rudiment is an unpaired structure, but while it is asymmetrical in *Limax*, it is symmetrical in *Dreissensia* as it is mid-ventral in position.

#### *Further Differentiation of the Separate Rudiments.*

##### *The kidney.*

In *Pila*, the kidney originates in two parts. These are: (1) the kidney proper, and (2) the ureter.

While there are differences of opinion regarding the mesodermal or ectodermal origin of the kidney proper, there is more or less unanimity on the ectodermal origin of the ureter, which arises as an invagination from the mantle-cavity.

Erlanger describes the kidney in *Paludina* (1891) and *Bythinia* as arising from the posterior wall of the pericardium (cf. Otto and Tönniges, 1905), and later fusing with the ureter to communicate with the mantle-cavity. In *Littorina* (Delsman, 1914), the kidney does not arise as an evagination from the pericardium, but develops into a vesicle even before the appearance of a lumen in the pericardial part.

##### *The heart.*

With regard to the differentiation of the heart in the Mollusca there is greater agreement amongst the various observers as to the mesodermal origin of the heart and the pericardium, than is the case with the kidney. The exceptions are few and are connected with those cases where the common rudiment for both the kidney and the heart has been taken to be ectodermal in origin.

While according to Bütschli (1877) and Erlanger (1891), the rudiment of the pericardium and the heart in *Paludina* (= *Viviparus*) is mesodermal, Otto and Tönniges (1905) hold it to be ectodermal. Recently, however, Fernando (1931) has proved its mesodermal character. Similarly, Erlanger (1892) in *Bythinia*, Salensky (1872) in *Calyptraea* and *Vermetus*, Delsman (1914) in *Littorina*, Bobretzsky (1877) in *Nassa*, and Fol (1880), Pöetzsch (1904), Wierzejski (1905), Heyder (1909) in the Pulmonates, *Planorbis*, *Physa*, *Arion*, etc., have described the pericardium and the heart to be of mesodermal origin. Meisenheimer, however, describes the heart rudiment in *Limax maximus* (1898), *Cyclas* (1901) and *Dreissensia* (1901) to be ectodermal; but we find that Schalfew (1888) and Ziegler (1885) had already traced the mesodermal character of these in *Limax agrestis* and *Cyclas* respectively.

#### (c) *The Kidneys and the Ureter.*

##### *The Right or Functional Kidney and its Ureter.*

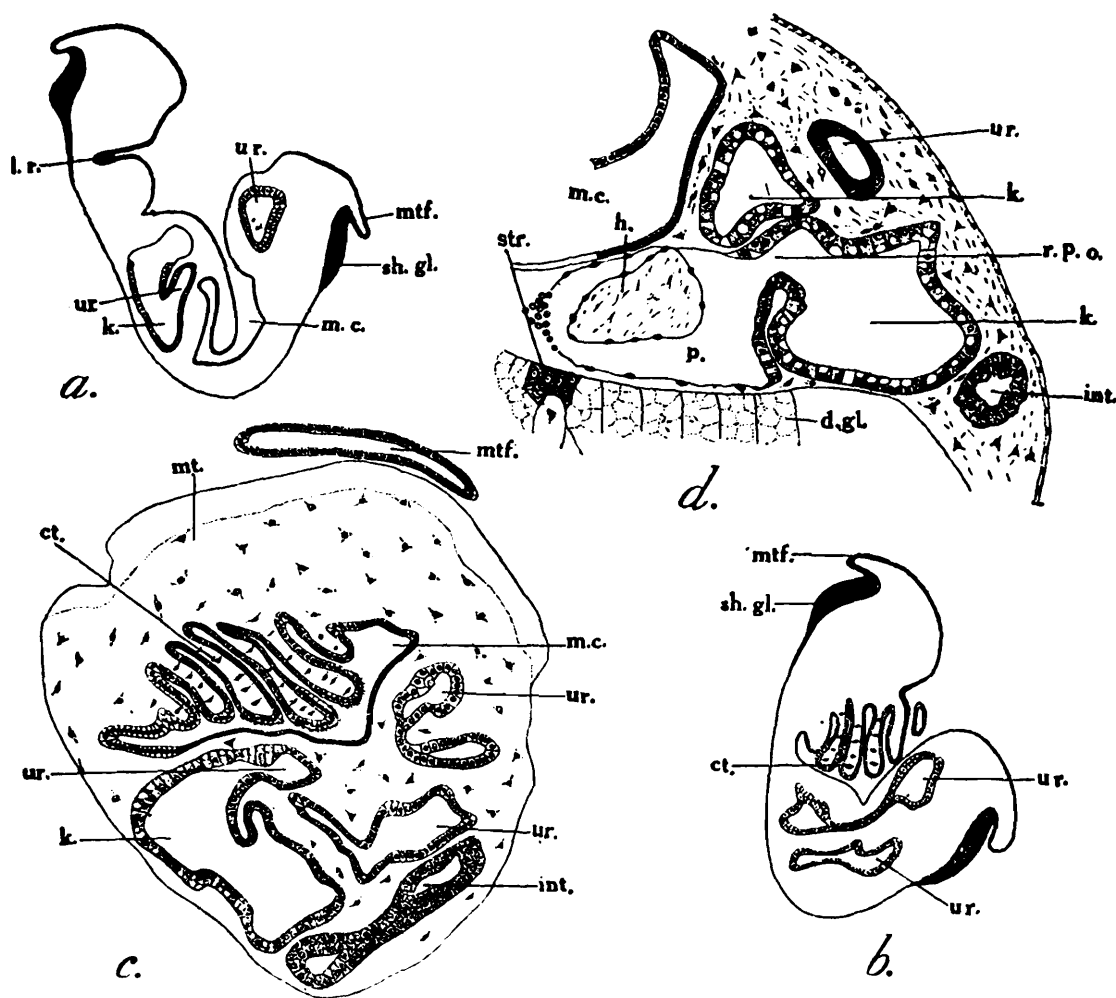
In the preceding part it has been described that the rudiment of the right kidney on evagination from the pericardium forms a small, open pouch communicating widely with the pericardium, and that the ureter, on evagination from the mantle-cavity, forms a tubular structure which, on further development, comes to lie against the posterior end of the right kidney.

As development proceeds (cf. Stage 4, Text-fig. 4 c) a cytolysis of cells, as has already been described and figured by Fernando (1931)

in his fig. 5, Pl. II, takes place at the junction of the right kidney and the ureter with the result that a communication is established between the two (Text-fig. 14 *d*).

In the next stage of development (Stage 5, Text-figs. 4 *d, e*) the kidney has grown in length and appears more or less tubular in shape and is directed backwards where it communicates with the ureter. The ureter, in turn, possesses a lumen uniformly in continuation with that of the mantle-cavity, rendering it difficult to mark off the boundary between the two. Starting from the mantle-cavity, the ureter first leads backwards and then upwards to open into the posterior end of the kidney, but it has a wider lumen at its kidney-end than at its mantle-cavity-end. The wide communication of the kidney with the pericardium (Text-fig. 17 *b*) lies to the right of the opening of the ureter into the kidney. As the tubular ureter increases in length, its kidney-end forms a bend just before opening into the right kidney (Text-fig. 17 *c*).

In the next stage (Stage 6, Text-fig. 4 *b*) the mantle-cavity grows inwards and upwards and comes to lie almost against the postero-ventral pericardial wall, just near the place where the kidney opens into the pericardium (Text-fig. 17 *d*). It is from this inner end of the mantle-



TEXT-FIG. 19. Stages in the development of the kidneys and the ureter.

*a.* Transverse section of an embryo in Stage 10, showing the opening of the ureter into the kidney and the mantle-cavity:  $\times 156$ ; *b.* Transverse section of an embryo in Stage 10, passing through the gill filaments and the ureter:  $\times 156$ ; *c.* Transverse section of an embryo in Stage 11, passing through the opening of the ureter into the kidney, and showing the gill filaments hanging into the mantle-cavity:  $\times 116$ ; *d.* Sagittal section of an embryo in Stage 11, passing through the reno-pericardial aperture, and showing the formation of the vacuoles within the kidney walls:  $\times 176$ .

cavity that the ureter arises and runs backwards and upwards, reaching as far back as the middle of the posterior wall of the embryo (Text-figs. 4 *c* and 17 *a*). The right kidney, arising from the postero-ventral wall of the pericardium, leads straight back to open into the now anteriorly directed end of the ureter (Text-figs. 4 *c* and 17 *e*). At this stage the opening of the ureter into the mantle-cavity lies to the left of the outer opening of the mantle-cavity. A study of a series of sagittal sections of the embryo at this stage shows that the opening of the ureter into the kidney lies to the right of the opening of the ureter into the mantle-cavity (Text-fig. 7 *a*), but to the left of the external opening of the mantle-cavity. In fact, the greater part of the ureter lies to the left of the kidney and the pericardium. The left inner wall of the ureter lies in the same plane as the intestine and the inner surface of the invaginated shell-gland.

As development proceeds (Text-fig. 4 *f*), the right kidney grows in size and becomes a more or less rounded sac communicating with the pericardium through a narrow aperture, the reno-pericardial aperture, which lies to the left of the opening of the kidney into the ureter. The kidney already shows indications of turning upwards, (Text-figs. 4 *f* and 7 *a*), to lie against the posterior wall of the pericardium in later stages. The ureter is now a long tubular structure which runs posteriorly and keeps near the floor of the visceral-sac rudiment on the right side.

In the next stage (Stage 7, Text-fig. 4 *h*), the kidney has grown larger in size and shows a bend towards the dorsal side (Text-figs. 4 *h* and 8 *a*), though the reno-pericardial aperture still retains its original position. Moreover, the posterior part of the kidney shows a bulge towards its right, with the result that in sagittal sections the kidney appears to be divided into two chambers, while in reality (as a reconstruction of these sections shows) there is only one chamber with a swelling on the right side. It is into this swollen part that the ureter now opens at its postero-ventral region (Text-fig. 8 *a*). The opening is comparatively narrow and lies posteriorly, and to the right of the reno-pericardial aperture.

The mantle-cavity has become wide and lies, as in the preceding stage, against the postero-ventral pericardial wall. Arising from the mantle-cavity through a narrow opening (Text-fig. 4 *h*), the ureter now leads downwards and backwards and lies against the postero-ventral part of the visceral-sac rudiment (Text-fig. 18 *a*), whence it runs upwards and forward to enter the kidney (Text-fig. 4 *h*). As development proceeds, the ureter describes a wide bend at the postero-ventral end of the visceral-sac rudiment on its right. After reaching about the middle of the height of the visceral-sac rudiment, it bends forward again and opens into the kidney as described above (Text-fig. 18 *b*). Thus, the ureter has got two limbs running almost parallel to each other, one being ventral and communicating anteriorly with the mantle-cavity, while the second is dorsal and opens anteriorly into the kidney. The loop connecting these two limbs lies at the extreme posterior end of the embryo.

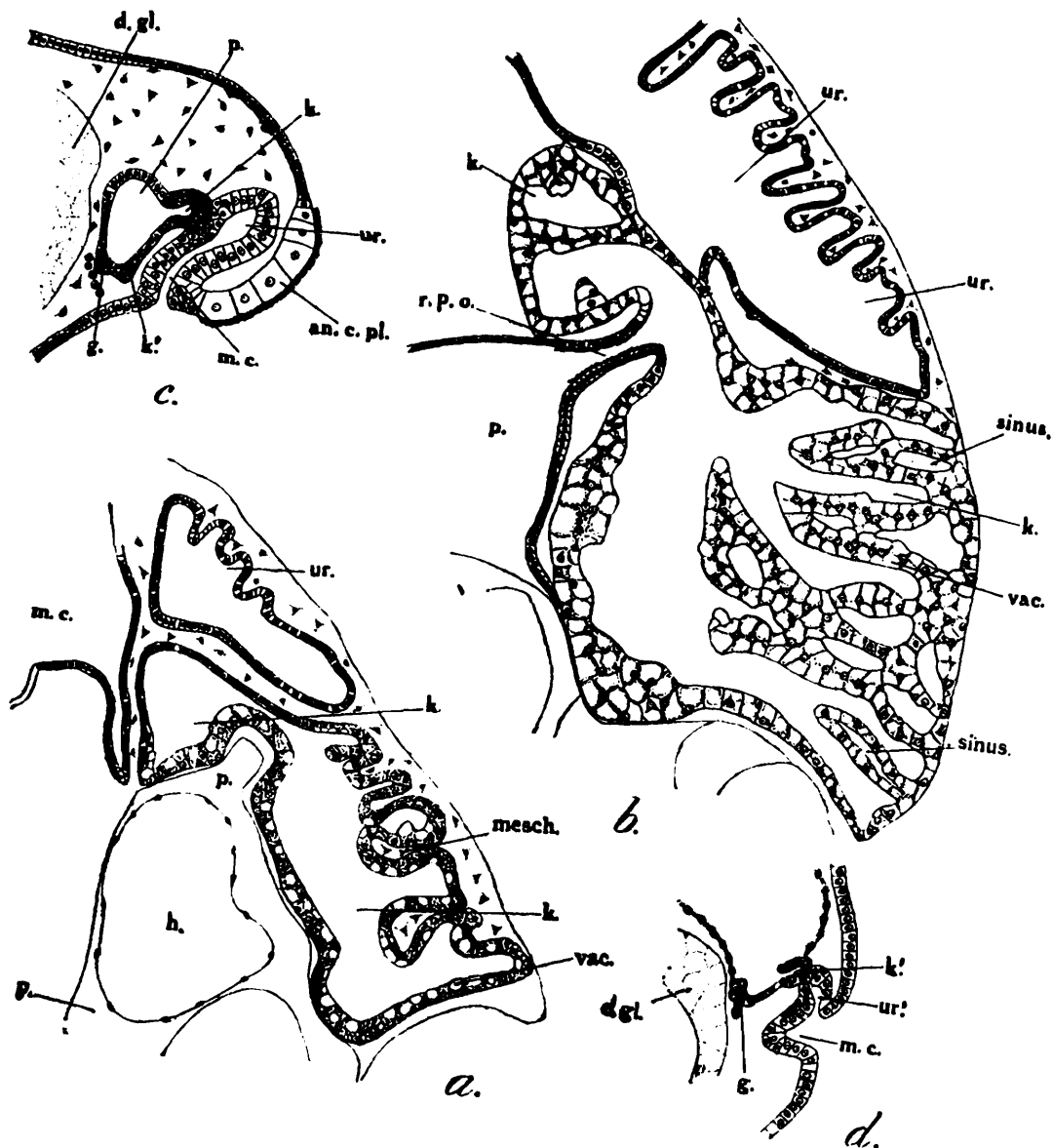
In the next stage of development (Stage 8, Text-fig. 5 *a*) in which the pericardium lies completely on the dorsal side immediately above the gastro-intestinal junction, and the mantle-cavity has grown wider

and lies almost midway between its former ventral and future dorsal position (Text-fig. 5 *a*), the limbs of the ureter no longer remain parallel to each other but form one closed loop. Part of this looped ureter now lies in the postero-ventral part of the embryo, while the remaining part retains its original position. The kidney has grown much in size and has a broad cavity within it. The opening of the kidney into the ureter still lies to the right of but ventrally to the reno-pericardial aperture, which is a clear proof of the fact that the ureter has now come farther forward. The opening of the ureter into the mantle-cavity is a narrow elongated aperture (Text-fig. 5 *a*) situated to the right of and ventrally to the opening of the ureter into the right kidney, so that all the three openings are situated almost in the same line. Text-fig. 8 *b* is a sagittal section of a slightly older embryo (Stage 9) passing through the opening of the ureter into the mantle-cavity, in which a part of the ureter situated dorsally runs to open into the kidney, the actual opening not being seen in the plane of the section.

In Stage 10 (Text-figs. 5 *b, c*) torsion has completed and the wide and spacious pericardium has passed over completely to the left side of the embryo, carrying the kidney along with it. The large mantle-cavity occupies its final and definitive dorsal position and leads ventrally and to the right through a narrow elongated aperture into the loop of the ureter which lies mainly in the right half of the visceral sac (Text-figs. 19 *a, b*). The ureter is now a long tubular structure bent on itself; from the mantle-cavity it runs first posteriorly and then follows a tortuous course to open dorsally into the kidney (Text-fig. 19 *a*). The kidney has enlarged and stretches posteriorly so as to lie against the posterior wall of the pericardium but a little to the right of it (Text-fig. 9). Though the kidney has passed over to the left side of the embryo, the reno-pericardial aperture which was so far ventral in position, now lies, dorsally and to the left of the opening of the ureter into the kidney. Thus, at this stage, the mantle-cavity as well as the ureter come to lie dorsal to the level of the kidney.

In the next stage (Stage 11, Text-figs. 5 *d, e*) the kidney increases considerably in size and appears rhomboidal in transverse sections (Text-fig. 19 *c*). Anteriorly it lies wedged in between the pericardium on the left and the posterior part of the digestive-gland on the right, with the anterior part of the intestine passing beneath it. At this stage, it shows the first indication of folds which project into its lumen from its posterior as well as its left wall. It opens dorsally into the pericardium at its extreme right postero-dorsal end (Text-fig. 19 *d*), close to the opening of the kidney into the ureter. The mantle-cavity communicates with the ureter through a narrow elongated aperture which lies to the right of and posterior to the reno-pericardial aperture. The part of the ureter which extends behind this region lies horizontally extended posteriorly, below the posterior extension of the mantle-cavity. At this stage a part of the loop of the now very much elongated ureter is situated within the wall of the mantle on the right side of the visceral sac, and extends forward to the anterior end of the kidney. Both the openings of the ureter into the mantle-cavity and of the kidney into the ureter lie behind this part of the ureter.

As development proceeds, the kidney grows larger but is so situated that it cannot grow freely in all directions. Anteriorly it is bounded



TEXT-FIG. 20. Further stages in the development of the kidneys and the ureter.

*a.* Sagittal section of an embryo in Stage 12, showing the formation of folds in the kidney and the ureter:  $\times 119$ ; *b.* Sagittal section of an embryo ready to hatch, passing through the reno-pericardial aperture and showing the lamellar condition of the kidney and the ureter:  $\times 96$ ; *c.* Sagittal section of an embryo in Stage 5, passing through the gonad and left kidney rudiments, and showing the differentiation of the left ureter as a depression in the wall of the mantle-cavity:  $\times 206$ ; *d.* Oblique transverse section showing the left ureter evaginating from the mantle-cavity and abutting against the blind end of the evaginating left rudimentary kidney:  $\times 264$ .

by the pericardium, on the left it lies against the wall of the visceral-sac covered over by the shell, on its anterior right lies the posterior end of the digestive-gland, on its posterior right the ureter, while dorsally lie the ureter and the floor of the mantle-cavity. Thus, the kidney is closely pressed on all sides by other structures, and since no more space is available for its expansion, the natural recourse is to form internal folds to provide an increased area for its epithelium which has now become flattened and consists of small squarish cells with finely grained cytoplasm (Text-fig. 20 *a*). Soon, however, a further differentiation occurs in that the cells become vacuolated. These vacuoles are present mostly in those parts of the cells which face the lumen of the

kidney ; and, therefore, the cytoplasm and the nuclei move towards the outer ends.

Further changes that occur in the kidney during its subsequent development are chiefly concerned with the structure of its walls, the relative position of the various organs in relation to the kidney remaining practically the same. The internal folds become deeper and the mesenchyme cells migrate into these folds and form their supporting tissue. The folds penetrate deeply into the interior of the kidney and thus only a small lumen is left which lies towards its right anterior end, where it opens, on the one hand, into the pericardium (through the reno-pericardial aperture) and, on the other, into the ureter.

With further development, there is an increase in the number and size of these folds accompanied by a branching of the folds to form secondary folds (Text-figs. 20 *b* and 25 *h*). These folds now anastomose and, consequently, the kidney presents the appearance of a lamellar gland. When the folds increase in height, the mesenchyme cells which have migrated into them, get pressed into sheet-like structures and thus the two walls of each fold are closely pressed against each other. Sinuses also appear within the folds at the time of their formation and these persist, and are connected with the blood-vessels of the kidney.

By the time the young snail hatches out of the egg, the kidney consists of repeatedly branching and anastomosing folds lined with a flat epithelium of vacuolated and glandular cells. In these cells concretions are now recognisable, which clearly show that the excretory activity of the kidney has already commenced.

As the kidney undergoes these changes, the ureter is not left unaffected, but gradually grows towards its anterior side and comes to lie dorsally to the kidney. Its posterior part which is connected with the kidney no longer remains a tubular structure, but its lumen becomes wider and, like the kidney, it also forms folds from its dorsal wall (Text-fig. 20 *b*). These folds arise almost simultaneously with the formation of the kidney-folds and increase in length till they meet the opposite wall and thus produce a lamellar structure. The cells, however, maintain their original shape and size, and never become vacuolated.

#### *The Left Rudimentary Kidney.*

The origin of the left kidney as a solid thickening on the left postero-ventral wall of the left pericardium has already been described. The rudiment of the left ureter arises as an evagination from the left inner wall of the mantle-cavity at a later stage, but it remains very small as compared with the ureter of the right side.

As the embryo grows, the development of both the left kidney and its ureter (=efferent duct) proceeds so slowly as to give the impression of arrested growth. Text-fig. 16 *g* is a transverse section of the visceral-sac rudiment of an embryo in Stage 5, and passes through the rudiment of the left kidney which is still a solid thickened growth without any indication of an evagination. It lies close to the rudiment of the gonad. Fig. 20 *c* is a sagittal section of the visceral-sac rudiment of an embryo of the same stage, *i.e.*, Stage 5, which shows the rudiment of the left kidney and also a slight depression in the wall of the mantle-cavity

directed towards the left kidney rudiment. This depression is the rudiment of the left ureter.

As development proceeds, the middle of the left kidney-thickening evaginates and the kidney-cells become arranged round its narrow lumen in a single layer (Text-fig. 20 *d*). The lumen retains its communication with the pericardium through an aperture which can be described as the left reno-pericardial aperture. Meanwhile, the ureter rudiment deepens to form a small tubular left ureter which grows inwards and abuts against the blind end of the left kidney rudiment, but has not yet opened into it.

In an embryo in which complete torsion has taken place (Stage 10) the left kidney leaves its former ventral position and comes to lie dorsally. In a transverse section of the visceral-sac rudiment of an embryo in this stage it is seen that the original left kidney, after rotating through  $180^\circ$ , becomes the topographical right kidney. This kidney is now a small vesicular structure opening into the pericardium through a small and narrow left reno-pericardial aperture (Text-fig. 22 *a*) and lies at the right dorsal end of the spacious pericardium. The left ureter has already opened into it and is a long tube with a narrow lumen.

The morphologically left rudimentary kidney and its ureter are closely connected with the genital organs of the animal and so their further fate is discussed along with the development of the reproductive organs. We may note here that this kidney and its ureter persist in *Pila*, but do not function as excretory organs—they function together as the genital duct.

Thus, a close study of the development of the left kidney clearly establishes the fact that it does not “disappear at an early stage” in *Pila*, as stated by Fernando<sup>1</sup> (1931), but that it persists and is closely connected with the development of the reproductive organs.

*Previous work and discussion.*—Numerous accounts of the structure and relations of the Gastropodan kidneys have been written, especially by authors who were interested either in the relationship of the two kidneys in the Diotocardia or in the homology of the single Monotocardian Kidney with either one or the other of the Diotocardia. These accounts are chiefly based on comparative anatomical work and contain a large number of contradictory statements. Three views have been propounded; one set of workers maintain that the single kidney of the Monotocardia is homologous with the left kidney of the Diotocardia (Pelseneer, Lang and Herschler); the second set hold the opposite view, *i.e.*, the Monotocardian Kidney is homologous with the right kidney of Diotocardia (Haller, 1886; Randles, 1905; etc.); while the third view is that the Monotocardian kidney is homologous with both the kidneys of the Diotocardia (Perrier, 1889; Woodward, 1901). Erlanger (1891) was the first to prove, by a study of the development of *Paludina*, that the single kidney of *Paludina* (which is in reality the

---

<sup>1</sup> Fernando (1931) is not very clear in his description of the left kidney. He simply states that the rudiment of the left kidney arises as an evagination from the left pericardium below the intestine, but in his fig. 4, Pl. I, he represents it as a thickened growth rather than as an evagination. Fernando's further statement that the left kidney disappears “without coming in contact with the mantle-cavity”, is not supported by the development of *Pila globosa*.

morphological right, though topographically the left, kidney in the adult corresponds to the left kidney of the Diotocardia. He showed that a rudimentary morphologically left kidney makes its appearance, to disappear later. But Drummond (1903) and Otto and Tönniges (1905) showed that the rudimentary morphologically left kidney does not disappear as Erlanger had thought, but persists and subsequently comes to lie on the right side of the pericardium after undergoing a complete rotation through  $180^\circ$  and becomes connected with the genital system of the animal.

I do not wish to enter into a discussion of the relationship of the two kidneys in the Diotocardia and their homology with the single kidney of the Monotocardia, as this point has already been exhaustively dealt with both by Otto and Tönniges (1905) and Sachwatkin (1920), who, after a survey of all the evidence, reached the conclusion that the kidney of the Monotocardia, whether it consists of a single or a double chamber, is homologous with the left kidney of the Diotocardia. Fernando (1931), on the basis of the development of this organ in *Ampullaria (Pila) gigas*, supports this view. My own investigations, while confirming the conclusions of Fernando, have also revealed the fact that the morphologically left rudimentary kidney does not disappear, as stated by Fernando, but that it persists, as in the case of *Paludina* (Otto and Tönniges 1905), and becomes intimately connected with the genital system of the animal.

Prashad (1925), after giving a brief summary of the earlier works on the kidney of Ampullariidae, describes that "the renal-organ of *Pila globosa*, as of all Ampullariidae, consists of two chambers (i) a right anterior, and (ii) a left posterior, which lies somewhat to the left of and posterior to the anterior chamber. These two parts, though they are homologous with the two chambers in other Taenioglossa, are not similar in structure to that of any form in which the renal organ has been properly investigated" On account of this difference in structure, Prashad refers to them "by the non-committal names of the anterior and the posterior renal chambers" A study of the development of the kidney in *Pila globosa* proves, as has been described by Fernando (1931), that the "Kidney" is the homologue of the "posterior renal chamber" of Prashad in the adult animal, and that his "anterior renal chamber" corresponds to the "ureter" Thus, it is evident that the so-called anterior and posterior renal chambers are quite distinct structures of separate origin—the posterior renal chamber or true kidney is mesodermal in origin, while the anterior renal chamber is ectodermal in origin and constitutes the ureter. Agreeing with Fernando, I have preferred to describe it as the ureter because of its great resemblance to the ureter of *Paludina* and also because of its ectodermal origin.

Prashad (1925) writes : "The mode of excretion in *P. globosa* and other Ampullariidae appears to be similar to that in other Prosobranchs, except that probably both the chambers in this family have an excretory function, and the posterior is not of the nature of a nephridial gland. Owing to the single external opening, excretory products from the posterior chamber are also collected together and poured into the anterior chamber whence they are discharged into the mantle-cavity."

This statement is incorrect because it is only the posterior chamber which is glandular and has a vacuolated epithelium containing excretory granules, while no excretory matter is ever seen stored up within the non-vacuolated cells of the "anterior renal chamber" which corresponds to the ureter and is non-glandular. The ureter, in fact, corresponds to the similar structure in *Paludina*, and simply collects the waste products excreted by the glandular kidney and conveys them to be discharged into the mantle-cavity.

Finally, I must add a few words about the structure of the kidney itself. Haller (1886), Pelseneer (1896) and Randles (1905) describe the Prosobranch kidney as an acinose gland, and that it is the cavities of the acini constituting it which unite with each other and give rise to the principal branches which lead into the urinary chamber. In contrast to this, Perrier (1889) after making a comparative anatomical study of the Prosobranch kidney in different forms, arrived at the conclusion that the kidney consists of a sac-like structure which is divided by a large number of trabeculae lined with glandular cells. Erlanger (1891), after tracing out the full development of the kidney in *Paludina*, confirms the view of Perrier; and my own observations outlined in the preceding pages fully corroborate the fact that the walls of the simple sac-like kidney, which like that of *Paludina* can be compared to the simple kidney of *Halotis*, become folded inwards, and that from these folds secondary folds arise which, on further development, increase in number and after anastomosing with one another give the adult kidney a lamellar or spongy appearance.

#### D. *The Pericardium, the Heart and the Blood-Vessels.*

##### *The Pericardium and the Heart.*

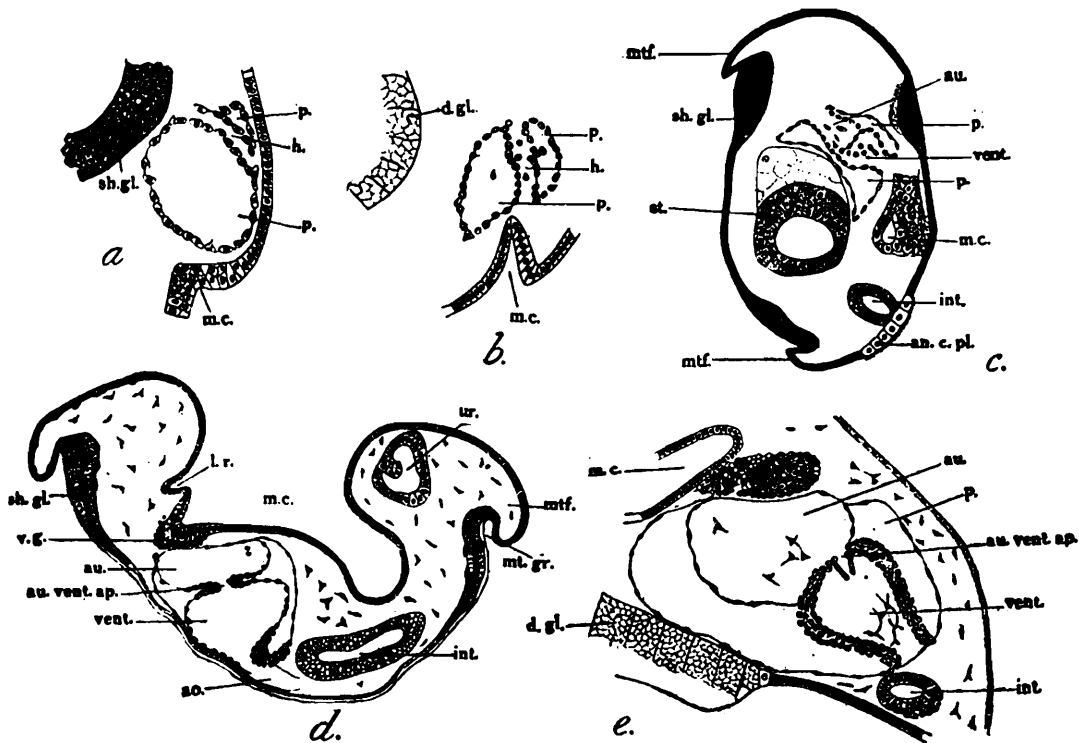
I have already described that the heart first appears as a thickening of the posterior wall of the pericardium just above the right kidney.<sup>1</sup> This thickening develops into a knob-like growth consisting of more or less loosely arranged cells which projects into the cavity of the pericardium. On further growth the thickening forms a small invagination into the pericardial cavity and thus the original knob-like rudiment of the heart develops into a small pouch which hangs down into the cavity of the pericardium and is now lined by a single layer of cells.

With further development, the invaginated pouch reaches the opposite wall of the pericardium with which it joins and fuses. During this period, this rudiment of the heart remains confined to the dorsal part of the pericardium and stretches obliquely across it. In a longitudinal section, the heart appears as a straight tube. Text-fig. 21 *a* is a transverse section of an embryo in Stage 5 in which the heart is seen stretching obliquely across the dorsal part of the pericardium in

---

<sup>1</sup> Fernando (1931) describes the heart at a much later stage. He does not mention as to how or where it originates, but simply states that it "begins to be differentiated within the pericardium". From his description (he has not shown the heart in his figures) it is clear that it is a comparatively late stage in which the heart rudiment has already differentiated into the auricle and the ventricle.

the form of a small, narrow, undifferentiated tubular structure, the lumen of which communicates at both ends with the body-cavity of the embryo.



TEXT-FIG. 21. Stages in the development of the heart and the pericardium.

*a.* Transverse section of an embryo in Stage 5, passing through the pericardium and showing the heart rudiment:  $\times 223$ ; *b.* Sagittal section of an embryo in Stage 6, passing through the pericardium and showing the heart rudiment:  $\times 223$ ; *c.* Transverse section of an embryo in Stage 7, showing the differentiation of the auricle and the ventricle:  $\times 133$ ; *d.* Transverse section of an embryo in Stage 10, passing through the auriculo-ventricular aperture:  $\times 127$ ; *e.* Sagittal section through the pericardium of an embryo in Stage 11, showing the auriculo-ventricular aperture:  $\times 132$ .

At a slightly later stage, the pericardium has increased further in dimensions, and at about the middle of its extent it occupies the entire space between the anterior part of the intestine and the body-wall. Almost in the middle of the tubular heart there appears a thickening which, a little later, develops into a circular constriction gradually dividing the heart into two parts. These two parts develop later into the auricle and the ventricle. Text-fig. 21 *b* is a sagittal section of an embryo in which the pericardium has grown antero-dorsally on the right side of the intestine, and the heart, still situated within its postero-dorsal part, lies to the right of the reno-pericardial aperture. The section shows the thickening of the wall of the heart, which later forms the annular constriction.

In the next stage (Stage 8), a further differentiation of the heart has taken place—it is no longer tubular in shape but has broadened at the region of the constriction. Text-fig. 21 *c* is a transverse section of an embryo in which the pericardium has considerably increased in dimensions and has completely shifted upwards to lie dorsally across the junction of the intestine and the stomach; within the pericardium, the auricular and the ventricular parts of the heart are well differentiated. They are broad at their junction with each other at the constriction, but become narrow towards their outer ends.

Being no longer hampered by the limited space between the gastro-intestinal wall and the body-wall, the pericardium develops into a very wide thin-walled chamber extending almost in all directions. It is bounded on its ventral side by the gastro-intestinal wall, on its anterior and left side by the everted shell-gland, on its right by the body-wall, and posteriorly by the kidney.

As torsion proceeds, the pericardium follows suit, so that by the time torsion is completed, the pericardium, along with the heart contained in it, shifts downwards towards the left side in the visceral mass. Text-fig. 21 *d* is a transverse section of an embryo in which complete torsion has just taken place (Stage 10). At this stage the pericardium is a very wide sac but has an extremely thin wall with flattened cells; it extends anteriorly as far as the digestive-gland, while posteriorly and on the right, it is bounded by the kidney. The posterior limit of the pericardium is almost in the same plane as the anterior end of the origin of the rudiments of the gill-filaments. The pericardium is broader dorsally and, due to lack of space, narrows gradually towards its ventral end. The heart, which has grown considerably in size, still lies obliquely in the posterior part of the pericardium. The auricle is a thin-walled bag full of blood and its wall is lined with flattened cells connected with one another only through their drawn out ends. The ventricle, which is elongated dorso-ventrally and lies in an obliquely antero-posterior direction, is a pear-shaped structure whose cells lie close together and are not flattened, thus forming a thicker wall than that of the auricle. Its upper part which communicates with the auricle is broader, while it tapers towards its ventral end which lies against the intestinal loop to open into the aorta. The auricle and the ventricle have separated almost completely from each other by the deepening of the constriction, although they communicate with each other through the narrow auriculo-ventricular aperture. It is difficult to give the exact outlines of the heart and the pericardium, as these vary greatly due to systole and diastole, and are also greatly affected by the fixatives used.

The pericardium and the heart now lie in their definitive positions on the left side of the visceral sac, so that the heart, which became differentiated from the wall of the pericardium when the latter lay on the right side of the intestine, has rotated to the right through 180° and now lies at its adult definitive position on the left side.

In the next stage (Stage 11), the pericardium has still further increased in size, and the muscle-fibre-forming cells have already developed and can be easily distinguished from the endothelial cells of the ventricle, the ventricular walls having become much thicker. Text-fig. 21 *e* is a sagittal section of an embryo in which the muscle-fibres are already laid, the auriculo-ventricular aperture has considerably narrowed and is protected by two septa which will develop later into valves. These rudimentary septa are here seen hanging into the ventricular chamber, a condition which is met with in the adult stage. They are arranged in such a manner that, on the contraction of the ventricle, the blood cannot flow back from the ventricle into the auricle. This is so on account of the closure of the auriculo-ventricular aperture

by these septa, which act as rudimentary valves. The blood is thus propelled only into the aorta.

Among Gastropods three types of development of the heart and the pericardium have been described. These are: (1) In the first type, comprising the majority of forms, the differentiation of the pericardium takes place first, and the rudiment of the heart appears in its wall afterwards, *i.e.*, it follows the same course as has been described above in *Pila globosa*. (2) In the second type the reverse is the case, *i.e.*, the heart arises first, and is followed by the differentiation of the pericardium. (3) In the third type the pericardium and the heart arise almost simultaneously.

#### *The Blood-Vessels.*

The origin and development of the blood-vessels in *Pila globosa* is similar to that described in other Gastropoda, such as *Paludina* (Erlanger, 1891). I shall not, therefore, give a detailed account of it.

The blood-vessels arise quite independently of the heart. They arise in the form of intercellular spaces or sinuses of indefinite shape and size in between the mesenchyme cells lying scattered within the body-cavity. These sinuses appear in a very early (Stage 1) and are situated at different regions of the body. They are surrounded by flattened mesenchyme cells without any definite walls. One such large sinus is present at the anterior end of the embryo just beneath the stomodaeum, while another is present at the base of the foot beneath the primitive stomach, and similarly there are others in other parts of the body. These have been described by some authors as being capable of pulsating even before the differentiation of the heart (as in *Paludina*), but in *Pila* no such pulsations can be seen in its early stages owing to the opacity of the embryo. The presence of sinuses in the early stages can be made out only on cutting sections, but in later stages, when the food-yolk has been absorbed and the embryos become more or less transparent, these sinuses can be easily made out not only in the living condition but even in the whole mounts after fixation. In the former case they are transparent; in the latter they take a lighter stain than the other parts of the body.

There is one remarkable fact noticed with regard to the position of these sinuses, *viz.*, that they arise exactly at those places where later on (as seen in the adult) the blood-vessels are formed. As development proceeds, these scattered primary sinuses enclosing the primary body-cavity, in contrast to the heart which represents the secondary coelom, become narrower and narrower, until finally they join each other and form blood-vessels. The vessel which joins the open end of the ventricular part of the heart gives rise to the aorta. In Text-fig. 23 c, which represents a transverse section passing through an embryo in Stage 11, the aorta is seen arising from the ventricle. The veins (the pulmonary, the efferent ctenedial and the efferent renal) arise much later than the aorta, and open dorsally into the auricle. Of these, the efferent ctenedial vein differentiates first and is formed from sinuses in the mantle, especially those at the base of the gill-leaflets, when the latter are differentiated on the future inner side of the mantle. These sinuses unite to open into the auricle.

*E. The Gonad and the Genital Duct.*

The origin of the gonad rudiment as a thickening of the roof of the pericardium at its extreme left end, situated dorsally to the left kidney rudiment, has been already described above. To begin with, it consists of a few cells only, but as development proceeds, the rudiment increases in thickness (Text-fig. 16 *g*) by a proliferation of cells next to the digestive-gland, the cells travelling ventral-wards and producing a cord of cells. Subsequently (Stage 6), as a result of torsion of the visceral-sac rudiment, the gonad rudiment is also transposed to the right side of the digestive gland, and comes to lie at the left inner end of the floor of the pericardium. Unlike the evagination of the kidney rudiment and the invagination of the heart rudiment, the gonad rudiment develops in the form of a solid cord of cells. But an important fact to be observed is that, in spite of the torsion, the gonad rudiment, retains its position next to the digestive gland throughout the course of its development.

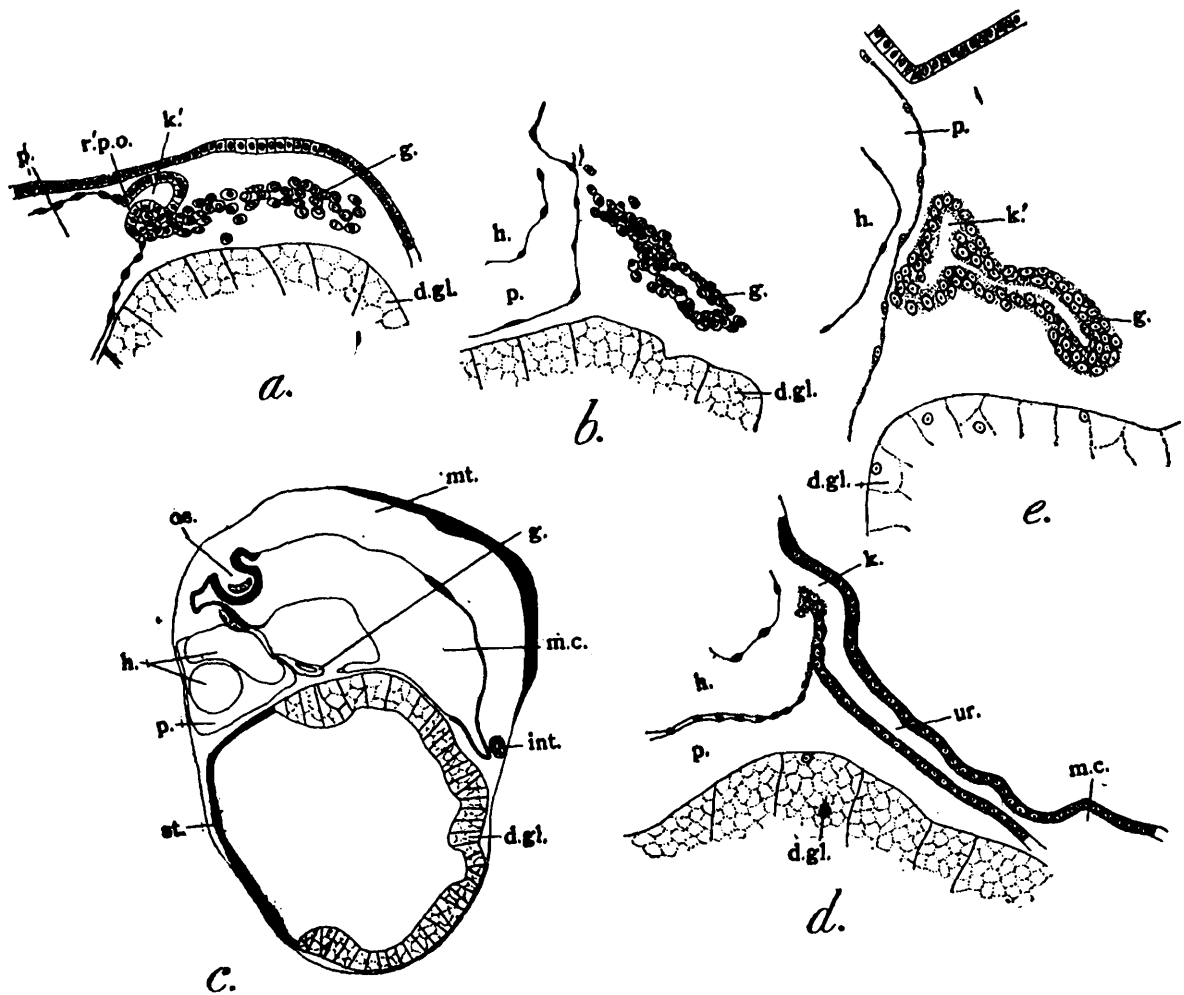
In early stages, the left kidney rudiment lies on the ventral side of the gonad rudiment, but when the pericardium shifts completely towards the right, the gonad comes to lie anteriorly to the left kidney rudiment, though close to it (Text-fig. 20 *c*). This proximity of the two rudiments is due to the narrowed lumen of the rudimentary left pericardial sac, between the left kidney and gonad rudiments.

As the visceral-sac rudiment undergoes a complete rotation (Stage 10), the pericardium along with the gonad and kidney rudiments, passes over to the left side of the visceral sac. Consequently, the kidney and the gonad rudiments come to lie on the extreme right of the pericardium, instead of their former ventral position. Besides, the relative positions of the gonad and the left kidney rudiment are reversed, the left kidney rudiment now lying dorsally to the gonad rudiment, although the two still lie close together. Text-fig. 22 *a* is a transverse section in which the gonad rudiment lies ventrally to the kidney rudiment, but is so close to it that a sharp line of demarcation between the two cannot be drawn. It should be noted here that the left kidney rudiment never becomes differentiated to form a functional kidney but is incorporated into the gonad into which it is assimilated.

At the next stage of development (Stage 11), the distal end of the gonad rudiment lying close to the digestive gland develops a cavity within itself, while its proximal part lying beside the kidney still remains solid (Text-fig. 22 *b*); and the kidney itself communicates with the pericardium through the narrow left reno-pericardial aperture. Text-fig. 22 *c* is a transverse section of the visceral-sac representing the position of the gonad rudiment (shown magnified in text-fig. 22 *b*) in relation to the other organs; while Text-fig. 22 *d*, is a semi-diagrammatic representation of the left ureter of the rudimentary kidney of the same embryo, showing its opening into the latter at its inner end, and into the mantle-cavity at its outer end.

As development proceeds, the gonad rudiment becomes hollow throughout its length, that is to say, the cavity which started first at its distal end now extends right upto its proximal end. This cavity however, does not communicate with that of the rudimentary left kidney.

Hereafter (Stage 12), the gonad rudiment develops rapidly. Proximally, the root of the gonad merges completely into the left kidney rudi-



TEXT-FIG. 22. Stages in the development of the gonad and the ureter.

*a.* Transverse section of an embryo in Stage 10, passing through the left rudimentary kidney, the left reno-pericardial aperture and the gonad:  $\times 264$ ; *b.* Transverse section of an embryo in Stage 11, passing through the gonad and showing the formation of the cavity at its distal end:  $\times 260$ ; *c.* Transverse section of the same embryo as in (*b*), showing the relation of the gonad with the other organs of the body:  $\times 46$ ; *d.* Diagrammatic representation of the left ureter opening into the left kidney and the mantle-cavity, in a transverse section of the embryo in Stage 11:  $\times 260$ ; *e.* Transverse section of the embryo in Stage 12, showing the gonad communicating with the left rudimentary kidney:  $\times 200$ .

ment, with the result that its cavity now communicates with that of this kidney, and, through it, with that of the mantle-cavity *via* the ureter of the left kidney; *i.e.*, the left ureter becomes transformed into the genital duct of the adult. Distally the gonad forms a bend along with that of the digestive-gland. At this stage of development as well as in later stages, I could not observe the reno-pericardial aperture of the rudimentary kidney, and believe that it has disappeared.

By this time all the essential relations between the various parts of the reproductive system are fully established, and further development consists only of the differentiation of this gonad rudiment into either the male or the female genital organs. I have not, however, followed this differentiation.

*Discussion.*—It is evident from the foregoing account that in *Pila*, as in *Paludina* (Erlanger, 1891; Drummond, 1903; Otto and Tonniges, 1905), the gonad rudiment and the rudimentary left kidney with its

ureter are closely connected with each other, and that this rudimentary kidney, after fusing completely with the developing gonad rudiment, takes part, along with its ureter, in the formation of the genital duct. The latter is, therefore, not an independent formation.

Fernando (1931) says that "the genital organs (with which the morphologically left kidney of the embryo is associated in *Viviparus*) do not develop in the embryos of *Ampullaria gigas*, till after they are hatched, so that it is possible that the genital duct may be a new formation. Further, no opening into the mantle-cavity was observed in connection with the morphological left kidney" But, it is clear from my observations that Fernando's conclusions are incorrect. He seems to have overlooked the early stages of the gonad rudiment, and, surprisingly enough, missed it even in the later stages, where it is quite a distinct structure, especially in his Stages 9 and 10, when the torsion is complete, and the embryo has a snail-like appearance.

#### F. *The Respiratory Organs.*

*Pila globosa* is a fresh-water form, but is also adapted to a terrestrial mode of life. In water, respiration is carried on by means of its ctenidium or gill, while on land, aërial respiration comes into play through its pulmonary sac or lung.

Brooks and McGlone (1908) have given a detailed and lucid account of the development of the respiratory organs of *Ampullaria (Pila) depressa*. My own observations on *Pila globosa* are almost in complete agreement with theirs as regards the origin and development of these organs, except for a few differences in minor details in the case of the lung. I consider it unnecessary, therefore, to describe the development of these organs in detail and give here only a brief summary of my observations.

#### *The Gill.*

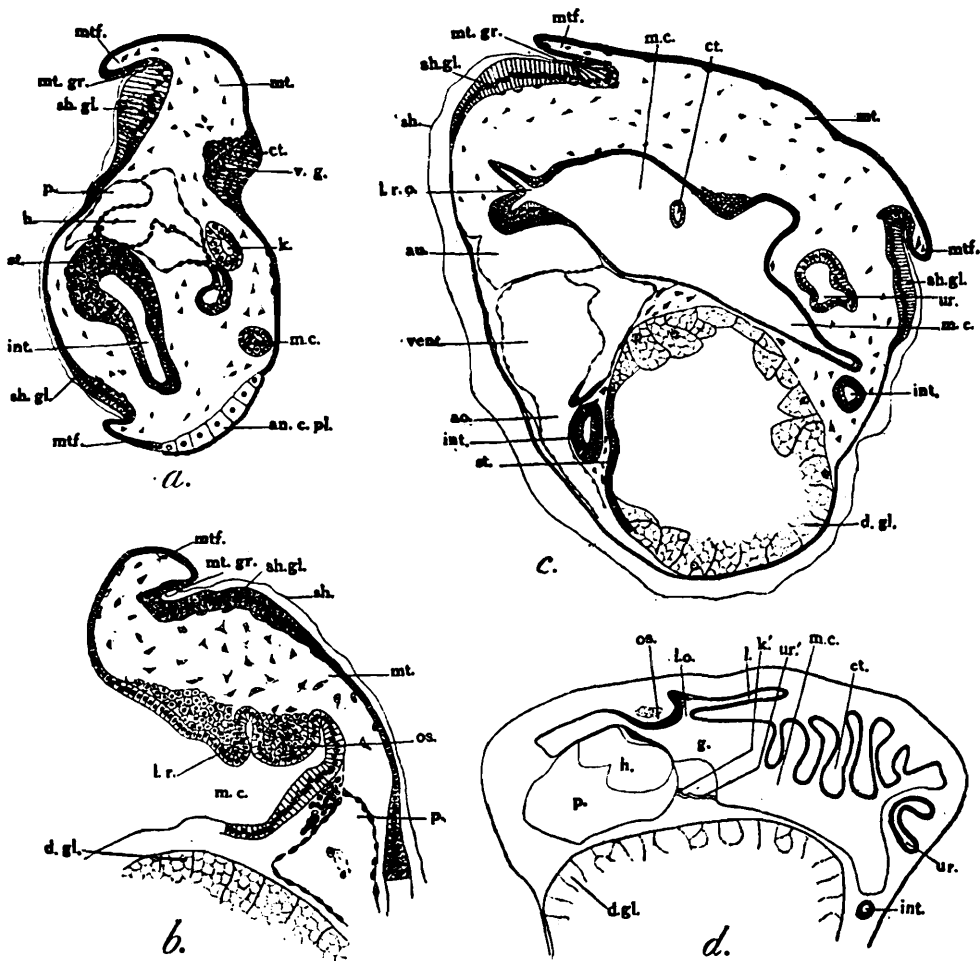
The first traces of the gill rudiment are discerned in Stage 8. Text-fig. 23a is a transverse section (passing through the pericardium and the gastro-intestinal junction) in which the epithelium on the right side is clearly thickened and its cells have become tall and columnar. This thickening is the first formation of the gill rudiment, which lies just dorsally to the concave thickening from which the visceral ganglion is delaminated in earlier stages, but which is now composed of a single layer of tall columnar cells. Text-fig. 23 b is a horizontal section of an embryo in Stage 9, which shows three thickenings or ridges developed from the inner wall of the mantle. Two of these thickenings are separated from the third which lies to their left, by an infolding of the mantle-epithelium.<sup>1</sup> This infolding is the rudiment of the pulmonary sac, while the two thickenings on its right are the rudiments of the gill, and the one on its left is the rudiment of the osphradium. The rudiments of the gill and osphradium are thus seen as parallel ridges and fully confirm the statement of Brooks and McGlone (1908) that "the gill, the lung

---

<sup>1</sup> Text-fig. 23b, unlike other figures of sections, has been drawn in such a way that the left side of the figure is in reality the right side of the embryo, and *vice-versa*.

and the osphradium of *Ampullaria* arise simultaneously or nearly so in the embryo ”

As development proceeds, other thickenings and outpushings representing further rudiments of the gill-filaments arise along the inner dorso-lateral surface of the mantle. Text-fig. 19*b* is a transverse section passing through the gill rudiments of an embryo in which torsion has already been completed and the first rudiments of the eyes have also appeared (Stage 10). The part of the mantle lying dorsally to the pericardium in the preceding stages (Text-fig. 23*a*) has now become dorso-lateral in position, after passing over to the left side of the embryo. Consequently, the rudiments of the gill-filaments, six to eight in number, now lie on the left side and project from the inner wall of the mantle-(Text-fig. 19*b*) into the mantle-cavity. On examining a series of consecutive sections (Text-figs. 19*a, b*), one finds that the ectodermal thickening, from which the visceral ganglion had originated, now lies at the extreme left of the mantle-cavity, just dorsally to the pericardium, and that the gill rudiments lie posteriorly but dorsally to this ectodermal thickening.



TEXT-FIG. 23. Stages in the development of the gill and the lung.

*a.* Transverse section of the visceral-sac rudiment of an embryo in Stage 8, passing through the thickening of the gill rudiment:  $\times 127$ ; *b.* Horizontal section of an embryo in Stage 9, showing the differentiation of the rudiments of the gill-lung and the osphradium:  $\times 157$ ; *c.* Transverse section of an embryo in Stage 11, passing through the opening of the lung into the mantle-cavity.  $\times 90$ ; *d.* Transverse section of an embryo in Stage 11, passing through the opening of the lung into the mantle-cavity;  $\times 65$ .

The rudiments of the gill-filaments first appear as solid outpushings of the epithelial wall completely filled up with compact masses of the

subjacent mesenchyme cells. As development proceeds, these rudiments increase in size, and the mesenchyme mass in each rudiment is detached from the outer epithelium and forms a band-like structure in the middle of each filament. Very soon, however, these mesenchyme bands break up into loose cells in the older filaments (Text-fig. 19c). Each gill-filament thus becomes a long tubular structure with mesenchyme cells lying scattered in its cavity. The row of filaments is attached to the wall of the mantle which forms its ctenidial axis. Each filament consists of a double wall of epithelium composed of cubical cells, each with a rounded nucleus and a distinct nucleolus; the cells are ciliated on their outer surfaces. The elongated space enclosed between the two walls of each filament is traversed here and there by scattered mesenchyme cells, which later develop into muscle-fibrils. These fibrils run transversely across the filament and form a series of septa dividing the space between the two walls into smaller spaces called "lacunae", which communicate with one another and also with the pulmonary sinus, the origin and development of which has been described by Brooks and McGlone (1908).

The gill-filaments continue to increase in size as well as in number, so that, by the time the embryo hatches out, the number of these filaments reaches as many as 35 to 45 in each gill. Moreover, all the lamellae (filaments) are not of equal size, the largest lamellae being in the middle, and their size gradually decreasing towards the two ends.

Like other Pectinibranchiates, *Pila* possesses only one gill which is topographically the left, but morphologically the right. In spite of careful observations, I have never been able to find any trace either of a rudimentary left ctenidium or of a second row of filaments even in the very early stages of its development.

According to Lankester, the primitive gill of Mollusca was a ctenidium—a stalk with plates very much like the gills of *Chiton* and *Fissurella*. Spengel derives the prosobranch gill from the gill of some primitive form like *Chiton*, and according to him, the present form of the prosobranch gill is formed by a gradual coalescence of the free distal portion of the gill with the mantle-wall. The embryological history of the gill in *Pila* does not confirm the hypothesis of Spengel. The course of development throughout the Pectinibranchiata (Monotocardia) is similar to what I have found in *Pila*. Therefore, in the words of Osborn (1886), "it does not seem safe to accept the conclusions of Spengel and Lankester, that the Ctenobranch (=Monotocardian) gill is derived from a feather-form gill, like that of *Fissurella*, by the fusion of one side with the body-wall".

### *The Lung.*

The lung, as has already been pointed out, arises as an infolding of the mantle epithelium almost simultaneously with the formation of the gill and osphradial rudiments. Text-fig. 23b is a horizontal section passing through the gill and osphradial rudiments, between which lies the lung rudiment, a position which is maintained to the last. Text-fig. 21d is a transverse section of an embryo in Stage 10, passing through the lung rudiment which appears as an invagination, lying a little posteriorly to and to the right of the osphradial rudiment. This lung rudiment is well marked in Text-fig. 19a which is a transverse section of a slightly older embryo.

In the next stage (Stage 11), the lung rudiment becomes further invaginated into the substance of the mantle, just posteriorly to the

osphradium (Text-fig. 23c). Brooks and McGlone's description of this stage agrees with mine, but I find that in their fig. 13, Pl. 4, and fig. 14, Pl. 5, they wrongly label as lung rudiment the thickened epithelium which surrounds the extreme left end of the mantle-cavity, and from which the gill rudiments and the visceral ganglion have already arisen. The actual lung rudiment lies a little anteriorly (Text-fig. 23c), and occurs in the anterior sections of the same series, the posterior sections (Text-fig. 19c) of which show the condition exactly comparable to the diagrams of Brooks and McGlone in which they have misinterpreted the part of the mantle-cavity as the lung-groove.

Similarly, Brooks and McGlone have missed the lung rudiment in their fig. 11, Pl. 3, and fig. 12, Pl. 4. In these two figures, they have indicated the posterior part of the mantle-cavity as the lung rudiment. It is a mistake which is quite natural if the part of the mantle-cavity labelled as "l.g." in figs. 13 and 14 be accepted as the lung-groove.

Text-fig. 23d is a transverse section of an embryo which is slightly older. It passes through the lung-opening as well as through the lung itself, and shows the latter as a flat, elliptical structure, dorso-lateral in position. From this stage onwards, right up to the hatching stage, the position and structure of the lung in *Pila globosa* completely agrees with that given in figs. 15, 16 and 17 and 18 of Pls. 5, 6 and 7 respectively of Brooks and McGlone in *Ampullaria depressa*. The lung having grown larger, now occupies a considerable space within the substance of the mantle, and only two very thin layers of connective tissue separate it from the inner and the outer epithelia of the mantle.

In later stages the dorsal epithelium of the head-vesicle forming the floor of the mantle-cavity becomes raised into a fold-like valvular structure, known as the epitaenia, which acts like an incomplete septum and divides the mantle-cavity into two incomplete chambers of unequal size. The smaller chamber lies towards the right, and is known as the branchial chamber, as it contains the gill, while the left is the spacious pulmonary chamber and contains the opening of the lung and the osphradium.

According to Brooks and McGlone (1908) "the lung of *Ampullaria* becomes functional before the gill does; for, the newly hatched young die very quickly if they are prevented from leaving the water while the adults survive a long immersion" I also found that the newly hatched young, lying in open glass dishes containing moist earth and a little water, crawled out of the dishes probably in quest of food, but after moving a few feet, shut themselves up within their shells and remained so for a considerable time (6 to 10 hours), and were found to be alive when brought back to water.

*Previous work and discussion.*—Prashad (1925) considers the lung as a new structure or only a part of the second ctenidium which, in response to aërial respiration, has been developed in close association with the gill and the osphradium as a specially modified structure" *Pila* belongs to the family *Pilidae* (=Ampullariidae) which is the only family of Streptoneura in which the gill is present simultaneously with the lung. The Pectinibranchiata (Monotocardia), to which the family *Pilidae* belongs, are characterised by having a single ctenidium, and

no trace of a second gill, even in a rudimentary form, is found throughout the course of development. Therefore, from a study of the development of the lung in *Pila globosa*, I arrive at the conclusion that it is a new structure altogether which has developed by the modification of the mantle, and that it is not at all a modification of a second ctenidium.

Unfortunately little work has been done on the origin and development of this organ in other Gastropoda, and the little that is known concerns only the Pulmonata. While there is some unanimity concerning the origin of the lung in the terrestrial Pulmonates (Stylommatophora), in the case of the aquatic Pulmonates (Basommatophora) opinions differ. The lung arises after the formation of the mantle-folds and the mantle-cavity, and is considered as a derivative of the latter, as in *Lymnaea*, (Wolfson, 1880) and *Planorbis* (Rabl, 1879). Fol (1880), on the other hand, does not agree with this view, and believes not only in the independent or separate origin of the lung-cavity, but even in its earlier formation, for he says: "the mantle-cavity is only a secondary invagination and should not be confused with the pallial-cavity", *i.e.*, the lung-cavity. Though Lankester (1874) believes in an independent origin of the lung-cavity, yet he agrees with Rabl (1879) and Wolfson (1880) in its secondary formation, that is to say, that the lung-cavity arises when the mantle-folds have already been formed. Again, all the four authors do not agree on the manner of its origin. Lankester describes (in *Lymnaea*) the lung as a simple depression arising on the right side, hidden below the overhanging mantle-lobes, when the latter are already covering a considerable part of the body. Rabl (1879), on the other hand, describes in *Planorbis* that as the mantle grows further, its growth takes place more on the right than on the left side. Soon a shallow indentation takes place on the right side, which soon deepens and forms the rudiment of the respiratory cavity.

Wolfson (1880) gives an altogether different origin of the respiratory cavity in *Lymnaea*. It arises "by the fusion of the right margin of the mantle which extends more on the right than on the left side with the body-integument, only a small aperture, the respiratory aperture, being left which leads into a deeply widening part of the mantle-cavity—the lung-cavity" Thus the lung-cavity is shown to be a transformed mantle-cavity.

In the Stylommatophora, on the other hand, we find that the lung-cavity arises quite independently of the formation of the mantle-fold or the mantle-cavity, as in *Helix* (Fol, 1880), *Limax* (Meisenheimer, 1898) and *Arion* (Heyder, 1909). Not only this, but Heyder has also stated that in *Arion* the lung arises as an ectodermal invagination even before the presence of any trace of either a mantle-cavity or a mantle-fold. Heyder has traced the complete development of the lung-cavity in *Arion* and has found that it originates as an altogether separate structure from the mantle-cavity which is a secondary formation. Other authors have described it as an ectodermal invagination arising almost simultaneously with the mantle-cavity, but according to Heyder they have either "overlooked the first invagination of the lung or failed to recognise its significance, and have noticed it together with the mantle-cavity appearing a little later"

From the above review of the origin of the lung amongst the different forms both of the Basommatophores and the Stylommatophores, one is led to the conclusion that the lung has two different kinds of origin amongst the Plumonata, *i.e.*, while it arises separately in the case of Stylommatophores (*e.g.*, *Arion*), in the Basommatophores on the other hand it is a derivative of the mantle-cavity and is thus a secondary formation.

When we compare the lung in *Pila* with that of the Pulmonata, we find that the two can hardly be considered as homologous structures, because in the former case it is a secondary formation and at the same time arises as an indentation of the mantle itself; in the latter case, however, especially in the Stylommatophora (*e.g.*, *Arion*), where it has been thoroughly studied, the lung is a primary formation and develops ontogenetically before the mantle-cavity.

### G. *The Nervous System*.<sup>1</sup>

The earliest rudiments of the nervous system appear at an early stage when the embryo is still bilaterally symmetrical externally, although internally it is asymmetrical, because of the unequal growth of the pericardial rudiments and the opening of the anus on the left ventral side. The rudiment of the mantle-cavity is as yet mid-ventral in position and the shell-gland is situated on the aboral dorsal surface (Stage 3). The head plates become thickened and many-layered near their extreme anterior ends, and some of the cells lying innermost project inwards from the thickened region. These delaminating cells of the ectodermal plates form the first rudiments of the cerebral ganglia (Text-figs. 24*a*, *b*) which are thus the first ganglia to be formed.

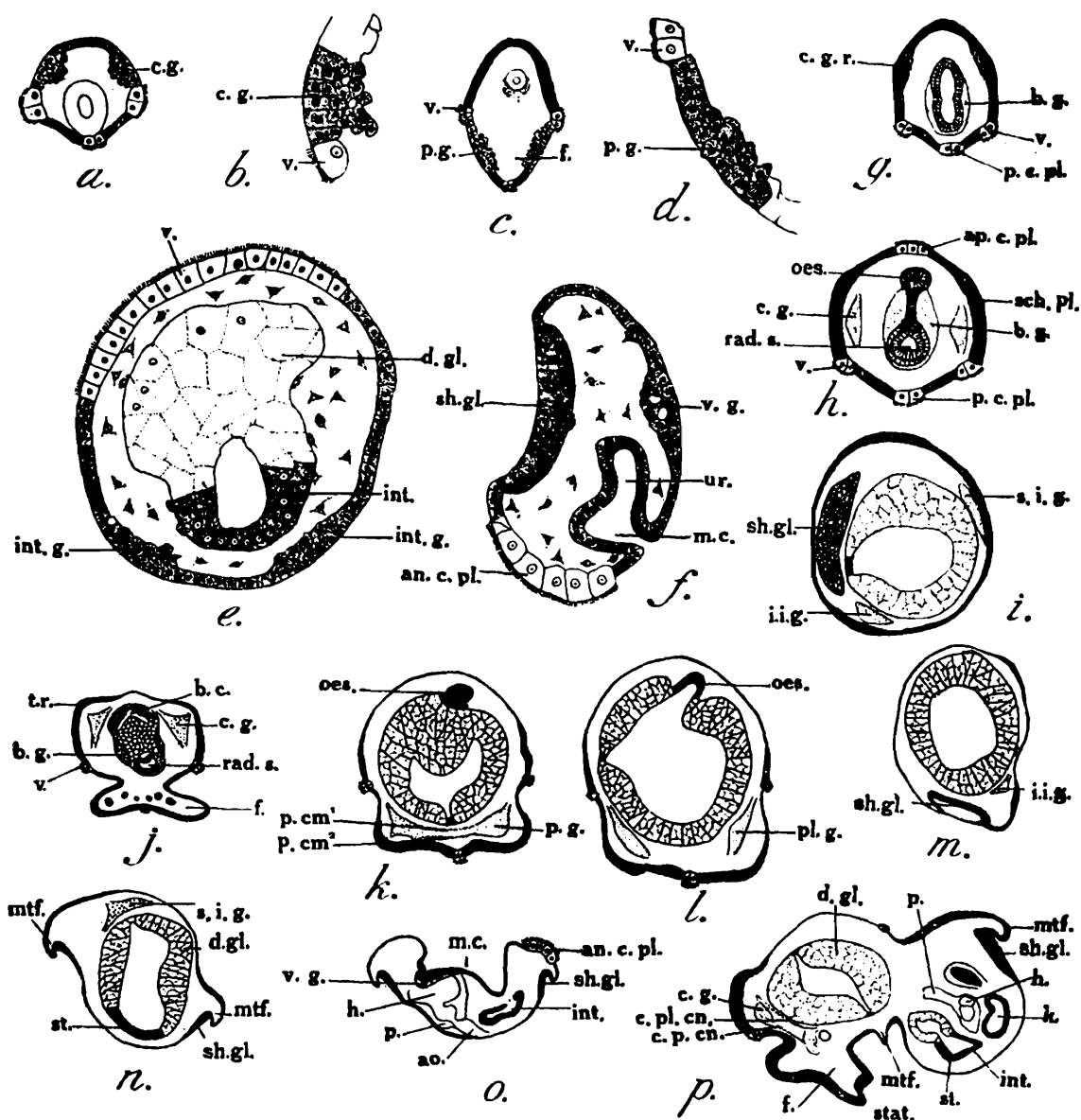
As development proceeds, more cells are delaminated from the head plates and we get thin sheets of closely arranged cells pressed against the inner surfaces of these plates. These cells closely resemble the mesenchyme cells and it is extremely difficult, at this stage, to differentiate the ganglion cells from the adjacent mesenchyme cells.

The rudiments of the pedal ganglia (Text-figs. 24*c*, *d*) are the next to be formed, one on each side, in an embryo slightly older than Stage 3. At this stage the embryo is asymmetrical even externally, the shell-gland having passed over more or less to the left side (Text-fig. 4*d*). The lateral walls of the foot which, to begin with, consist of a single layer of tall columnar cells become thickened and appear many-layered at about their middle regions. From these thickenings, cells are delaminated in the same manner as in the case of the cerebral ganglia, and are laid against the inner surfaces of the walls of the foot on each side. Text-fig. 24*c* is a transverse section of an embryo in Stage 4, and passes through the rudiments of the pedal ganglia which lie more or less posteriorly to the cerebral ganglia.

Almost simultaneously with the appearance of the rudiments of the pedal ganglia, two other similar thickenings appear, one on each side, in the lateral walls of the "Kopffuss" They are situated immediately

---

<sup>1</sup> In the following account the terminology used for the various ganglia and their connections (commissures and connectives) is that proposed by Spengel (1881) and adopted by Prashad (1925).



TEXT-FIG. 24. Stages in the development of the nervous system.

*a.* Transverse section of an embryo in Stage 4, passing through the rudiments of the cerebral ganglia:  $\times 92$ ; *b.* Portion of (*a*), showing the delamination of the ectoderm cells to form the rudiment of the cerebral ganglion:  $\times 280$ ; *c.* Transverse section of an embryo in Stage 5, passing through the rudiments of the pedal ganglia:  $\times 120$ ; *d.* Portion of (*c*), showing the delamination of the pedal ganglion rudiment from the wall of the foot:  $\times 280$ ; *e.* Transverse section of an embryo in Stage 5, passing through the rudiments of the intestinal ganglia:  $\times 232$ ; *f.* Transverse section of an embryo passing through the visceral sac rudiment and showing the differentiation of the visceral ganglion from the body-wall of the right side:  $\times 232$ ; *g.* Transverse section of an embryo, showing the cerebral ganglion and the buccal ganglion:  $\times 80$ ; *h.* Transverse section of an embryo passing through the cerebral and buccal ganglia, and showing the cerebral pair moving inwards and lying parallel to each other:  $\times 80$ ; *i.* Reconstruction of a few transverse sections of an embryo, showing the changed positions, as a result of torsion, of the left (infra-intestinal) and right (supra-intestinal) intestinal ganglia:  $\times 80$ ; *j.* Transverse section of an embryo in Stage 10, passing through the cerebral and buccal ganglia, and showing the cerebral ganglia moving towards each other above the buccal cavity:  $\times 47$ ; *k.* Transverse section of the same embryo as in (*j*), passing through the pedal ganglia and showing the formation of the first and second pedal commissures:  $\times 47$ ; *l.* Transverse section of the same embryo as in (*j*), passing through the pleural ganglia:  $\times 47$ ; *m.* Transverse section of the same embryo as in (*j*), passing through the infra-intestinal ganglion which now lies on the right side of the embryo.  $\times 47$ . *n.* Transverse section of the same embryo as in (*j*), passing through the supra-intestinal ganglion lying on the left dorsal side and above the posterior rudiment of the digestive gland:  $\times 47$ ; *o.* Transverse section of the same embryo as in (*j*), passing through the visceral ganglion lying between the roof of the pericardium and at the extreme left end of the mantle-cavity:  $\times 47$ ; *p.* Sagittal section (left of the median line) of an embryo in Stage 10, showing the formation and the relative position of the cerebro-pedal and cerebro-pleural connectives:  $\times 47$ .

below the velum at a level higher than and posterior to that of the rudiments of the pedal ganglia, and constitute the first rudiments of the pleural ganglia.

In the next stage in which the first rudiment of the radular sac is already evident (Stage 6), a number of cells are laid on either side of the junction of the rudiment of the radular sac with the oesophagus. These cells arise from the columnar cells lining the walls of the fore-gut, and are thus ectodermal in origin. They are the rudiments of the buccal ganglia. Unfortunately, I have not been successful in obtaining sections in which the cells of these rudiments can be observed in a delaminating state; in my sections the cells are already laid against their place of origin. By the time of the appearance of these rudiments of the buccal ganglia, the cerebral, the pedal and the pleural ganglionic rudiments have not yet completely separated from their places of origin.

The first rudiments of the intestinal ganglia are differentiated about the same time as those of the buccal ganglia. Just before the differentiation of these rudiments, the ectodermal cells of the ventro-lateral surfaces of the body-wall, almost at the junction of the "Kopffuss" with the visceral-sac rudiment, become differentiated from those of the rest of the body-wall by becoming tall and columnar. On further development, these cells multiply and form two ectodermal thickenings, one on each side, in the region of the hinder end of the stomach (Text-fig. 24e). These thickenings are almost symmetrical in position and consist of more than one layer of cells. As development proceeds, cells are delaminated from the inner surfaces of these thickenings and form the first rudiments of the intestinal ganglia.

Of all the rudiments, that of the visceral ganglion is the last to appear, and is differentiated immediately after the origin of the intestinal ganglia. In an embryo in Stage 5, a part of the right wall of the visceral-sac rudiment, situated just posteriorly but dorsally to the level of the pericardium, which also lies on the right side, becomes differentiated by its constituent cells becoming tall and columnar. These cells soon proliferate and delaminate inwards a sheet of compact cells stretching lengthwise in an antero-posterior direction: this sheet of cells is the rudiment of the visceral ganglion which, unlike the rudiments of other ganglia, is single and unpaired (Text-fig. 24f).

Thus, by the time of the differentiation of the visceral ganglion, the rudiments of all the ganglia are laid. All these rudiments are ectodermal in origin and the delaminated cells forming these rudiments originate always from tall columnar cells. In the case of the cerebral, the buccal, the pedal, and the pleural ganglia, the cells are already columnar in shape, but in the case of the intestinal ganglia and the visceral ganglion, the cells are at first small and cubical, and become tall and columnar only at the time of the differentiation of these ganglia.

The rudiments of the different ganglia, even after their complete delamination, remain at first thickly pressed against their respective regions of origin, but can be easily distinguished, as the latter again become single-layered, and acquire a distinct outline.

The rudiments of the cerebral ganglia are the first to detach themselves completely from the head plates, while the rudiments of the pedal

and pleural ganglia are still attached to their respective places of origin. After being detached, the thin sheets of cells of the cerebral ganglia begin to thicken at about their middle on each side (Text-fig. 24*h*). When an increase in length takes place in the anterior part of the embryo, it appears as if the rudiments of the cerebral ganglia are being gradually pushed back, but in reality they maintain their position. They are as yet irregular in shape and consist simply of two masses of cells which, on account of their vertical position, lie parallel to each other and to the median axis. Soon, however, they extend along the inner side of the roof of the head-vesicle and become convexly arched and, at the same time, move slightly inwards.

The pedal and pleural ganglia have, meanwhile, become further differentiated, but from the time of their origin, the boundaries of these two ganglia cannot be marked off from each other, as the two form a common diffused mass of ganglion-cells. In transverse sections of the "Kopffuss", these ganglia appear as long thread-like structures placed against the lateral walls of the foot. The pedal ganglia lie at the anterior lower part of the foot, and when the latter flattens at its antero-ventral end, these rudiments come to lie nearer, at the junction of the basal and the lateral walls of the foot. The pleural ganglia grow just behind the pedal ganglia but a little dorsally to them. While in their early stages they lie along the lateral walls of the foot, in the later stages they appear to move away from these walls, and thus their longitudinal axes make acute angles with the median axis of the embryo. The pleural ganglia fusing with the pedal ganglia form the characteristic pleuro-pedal ganglionic masses, one on each side of the median line.

The rudiments of the buccal ganglia have, by this time, become compact masses of cells. They remain, however, at their original position and do not shift in any direction. They lie almost in the same transverse plane as the cerebral ganglia (Text-figs. 24*g*, *h*), while the pedal and pleural ganglia are situated more or less posteriorly.

During the course of development represented in the abovementioned stages, the rudiments of the intestinal ganglia still consist of a small number of cells which no longer occupy their original positions, but have shifted a great deal as a result of torsion. The left ganglion shifts inwards towards the median plane directed more or less towards the anterior end, while the right ganglion shifts upwards on the right side and lies a little posteriorly to the left one. Text-fig. 24*i* is a reconstruction from a few consecutive sections of an embryo and shows the positions of the two intestinal ganglia. The rudiment of the visceral ganglion still lies on the right wall of the visceral sac rudiment towards its dorsal side, and though it has completely separated from its place of origin on the body-wall, it has not yet receded into the interior.

The next stage of development to be described is an embryo (Stage 10) in which complete torsion has just taken place; the rudiments of the eyes and tentacles are already formed and the foot has flattened ventrally along its entire length. Some of the ganglia, *viz.*, the cerebral, pedal, pleural and intestinal, lie towards the interior at some distance away from the body-epithelium. These ganglia are now well differentiated, and fibrous cells (neurofibrils) are already formed within them.

Text-fig. 24j is a transverse section which shows the cerebral ganglia advancing dorsally towards the median line to meet each other at their anterior ends through a string of cells which constitutes the rudiment of the cerebral commissure. This rudiment is not shown in the figure as it lies in the sections immediately preceding it, where it is seen to arise from the inner surface of the anterior end of each ganglion to meet in the mid-dorsal line above the pharyngeal mass. The figure shows that each cerebral ganglion, while it is thickest in the middle especially opposite the rudiment of the tentacle, thins out at its two ends and thus appears, in a transverse section, triangular in shape with the apex of the triangle directed dorso-laterally. At this stage the cerebral ganglia extend posteriorly up to the region of the eye rudiments. The first rudiment of the cerebral commissure, which is well developed by now, is laid down in Stage 9. Some of the peripheral ganglion-cells from each ganglion advance towards the interior and are so arranged as to form rudimentary strings which proliferate and advance inwards, till finally the two rudimentary strings projecting from the opposite ganglia meet each other at about the median line of the embryo and thus give rise to a thin cord of cells, which on further development forms the cerebral commissure. No mesenchyme cells lying scattered within the body-cavity take part in the formation of this commissure. Similarly, a nerve arises from the outer or dorso-lateral part of each cerebral ganglion and advances towards the tentacle rudiment to form the tentacular nerve.

The buccal ganglia are well developed and are connected together through a buccal commissure, which lies behind the radular sac and beneath the oesophagus.

Text-fig. 24k is another transverse section of the same series passing through the pedal ganglia which now lie in the same vertical plane as the eye rudiments, and have moved inwards both from the lateral as well as the ventral walls of the foot. A delicate cord of the ganglion-cells, arising in the same way as the rudiment of the cerebral commissure, connects the inner dorsal ends of these ganglia and thus forms the rudiment of the first pedal commissure. Similarly another cord of cells lying below the first pedal commissure joins the inner ends of the antero-ventral regions of the pedal ganglia, and forms the rudiment of the second pedal commissure. A comparison of these commissures with the cerebral commissure at once convinces us that the latter originates much earlier than the former. Nerves arise both from the antero-ventral and the postero-ventral ends of the pedal ganglion on each side—the former are the anterior pedal nerves and run forward to supply the anterior part of the foot, while the latter represent the rudiments of the main trunks of the pedal nerves which extend right up to the posterior end of the foot. The pedal nerves arise from the antero-lateral sides of these main trunks.

The pleural ganglia (Text-fig. 24l), like the pedal, are well developed and are completely fused with the latter at their postero-dorsal surfaces. The pleural ganglia, however, can only be differentiated from the pedal by their relative positions as well as by the fact that the median axis of each pleural ganglion lies slightly to the outside of that of the pedal ganglion of the same side. Besides, the pleural ganglia are obliquely

placed and form acute angles with the median axis of the embryo. They taper posteriorly and continue as the connectives which join them with the intestinal ganglion and the visceral. The relative positions of the pleural and the pedal ganglia can be easily understood by a comparison of Text-figs. 24*k* and *l*. The statocysts are now closed vesicles, but they still lie dorsally and just posteriorly to the pleural ganglia.

The first rudiments of the cerebro-pedal connectives (Text-fig. 24*p*) are also laid down at this stage by the meeting together of the ganglion-cells which project both from the cerebral as well as from the pedal ganglia of the same side, and now form a delicate string of cells. Almost simultaneously with the formation of the rudiments of the cerebro-pedal connectives, another string of cells becomes differentiated and connects each cerebral ganglion at its postero-ventral end with the antero-dorsal end of each pleural ganglion; this forms the rudiment of the cerebro-pleural connective. Each cerebro-pleural connective lies postero-dorsally but almost parallel to the cerebro-pedal, the two connectives serving to differentiate the pedal and the pleural ganglia from each other.

As a result of complete torsion, the intestinal ganglia undergo a complete change of position; the morphologically right intestinal ganglion (*i.e.*, the ganglion on the right ventro-lateral side at the time of its differentiation) passes over to the left dorsal side and is now topographically the left, and is known as the supra-intestinal ganglion (Text-fig. 24*n*). The morphologically left intestinal ganglion now lies on the right ventral side and is, therefore, known as the infra- or sub-intestinal ganglion (Text-fig. 24*m*).

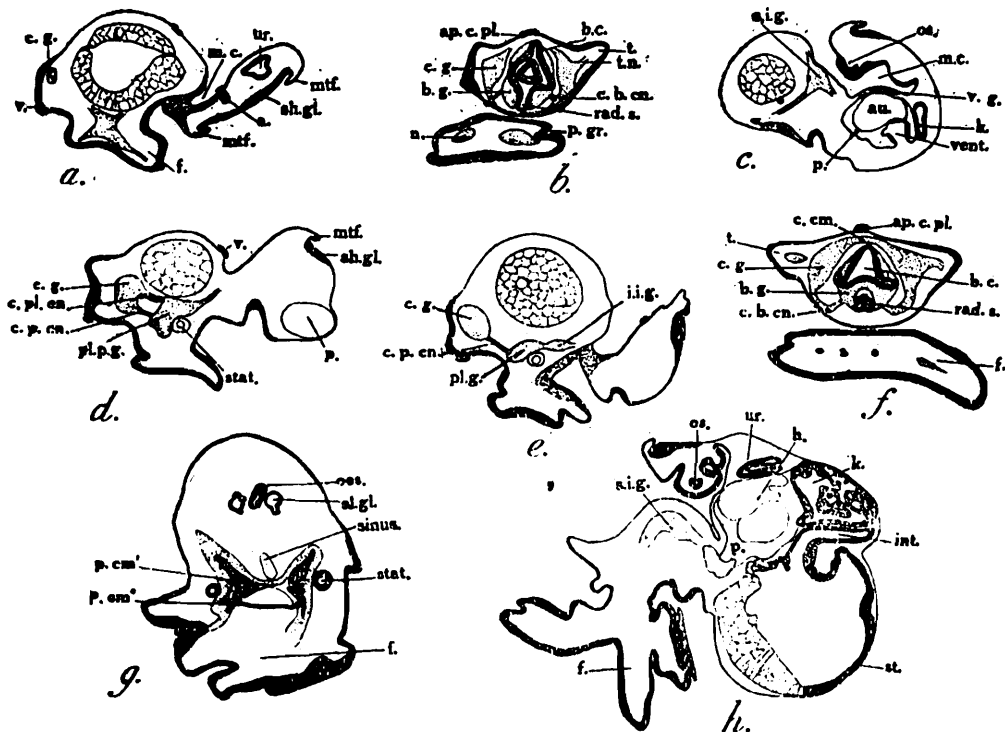
Similarly, as the dorsal part of the visceral sac (=mantle rudiment) comes to lie on the left side, the visceral ganglion, which was formerly on the right side and dorsal to the mantle-cavity, now comes to lie between the floor of the mantle-cavity at its extreme left and the roof of the pericardium (Text-fig. 24*o*). However, unlike the other ganglia, it does not recede into the interior, but remains closely applied to the wall of the mantle-cavity as a ribbon-like structure.

In the next stage of development (Stage 11), in which the rudiments of the eyes as closed vesicles have appeared, the various ganglia have developed further, and all the commissures and connectives have been laid down. The cerebral ganglia extend posteriorly, slightly beyond the eye rudiments, and the two ganglia are connected with each other anteriorly through another commissure running beneath the buccal mass, which is the rudiment of the labial commissure (Text-fig. 25*b*), that is quite slender as compared with the strong and band-shaped cerebral commissure. A nerve arising from the cerebral ganglion runs downwards and inwards and then curves upwards to join the buccal ganglion of its own side and its latero-ventral end. This is the rudiment of the cerebro-buccal connective which lies behind the plane of the cerebral commissure (Text-fig. 25*b*).

The cerebral ganglia have receded to their innermost limit and lie almost pressed against the buccal mass anteriorly. The tentacular nerves arising from the cerebral ganglia are well developed and run through the substance of the tentacles. The eye rudiments lie closely

pressed against the outer margins of the posterior part of the cerebral ganglia, but there is no indication as yet of the optic nerves.

The pedal ganglia develop further and approach each other closer than in the preceding stage. The first pedal commissure develops further and becomes band-shaped, but the second pedal commissure remains slender. Both the commissures are situated immediately behind the plane of the eye rudiments. The statocysts, which have shifted further downwards and inwards, now lie pressed against the outer margins of the pleural ganglia, immediately behind and more or less dorsally to the first pedal commissure. Text-figs. 25*c, d* represent sagittal section of an embryo of this stage (Text-fig. 5*d*) passing through the supra-intestinal ganglion and a part of its connective with the left pleural ganglion, and the cerebro-pedal and the cerebro-pleural connectives.



TEXT-FIG. 25. More stages in the development of the nervous system.

*a.* Sagittal section (right of the median line) of the same embryo as in Text-fig. 24*j* (Stage 10), showing the fusion of the pedal and pleural ganglia to form the pleuro-pedal ganglionic mass:  $\times 39$ ; *b.* Transverse section of the embryo in Stage 11, passing through the plane of the cerebro-buccal connectives, the labial commissure, and the tentacular nerve:  $\times 39$ ; *c.* Sagittal section (right of the median line) of an embryo in Stage 11, showing the fusion of the infra-intestinal ganglion with the pleuro-pedal ganglionic mass:  $\times 39$ ; *d.* Sagittal section (left of the median line) of an embryo in Stage 11, showing the cerebro-pedal and cerebro-pleural connectives, and a part of the supra-intestinal nerve meeting the pleural ganglion:  $\times 39$ ; *e.* Oblique sagittal section (left of the median line) of an embryo in Stage 11, passing through the supra-intestinal ganglion, and a part of the supra-intestinal nerve running downwards:  $\times 39$ ; *f.* Transverse section of an embryo in Stage 12, passing through the cerebral commissure, the cerebro-buccal connectives and the tentacular nerve:  $\times 39$ ; *g.* Transverse section of the same embryo as in (*f*), showing the first pedal commissure, the slender second pedal commissure, and the position of the statocysts:  $\times 39$ ; *h.* Sagittal section (left of the median line) of an embryo in Stage 12, showing the relative position of the supra-intestinal ganglion in relation to other organs. The section passes through the plane of the oesophradium, the heart, the kidney and the opening of the stomach into the intestine:  $\times 39$ .

The infra- or sub-intestinal ganglion lying on the right side has shifted forward and has almost joined the posterior end of the pleural ganglion

of its own side. Text-fig. 25e is a sagittal section passing through an embryo of this stage and shows the union of the infra-intestinal ganglion with the postero-dorsal part of the right pleural ganglion, dorsally and slightly posteriorly to the statocyst.

Text-fig. 25f is a transverse section of an embryo in Stage 12 (Text-figs. 5f, 11, 25h and 26a, b) and passes through the region of the cerebral commissure and the cerebro-buccal connectives. The inner surfaces of the triangular cerebral ganglia are almost flat, while their outer surfaces are steeply arched. The optic nerve arises from the ventro-lateral area of the posterior part of the cerebral ganglion. Text-fig. 25g is still another section of the same series and shows that the statocysts have further sunk into the substance of the foot and now lie closely pressed against the pedal ganglia, at the level of the posterior limit of the band-shaped first pedal commissure and the second pedal commissure, which lies immediately behind and a little ventral to the extreme posterior club-shaped end of the radular sac. The infra-intestinal nerve running transversely, connects the left pleural ganglion with the right.

The supra-intestinal ganglion comes to lie on the left dorsal side, while the infra-intestinal ganglion fuses with the pleuro-pedal ganglionic mass of the right side.

Text-fig. 26c is an obliquely transverse section also of an embryo approximately in Stage 12. It shows the connection of the left pleural ganglion with the supra-intestinal ganglion through the left pleuro-supra-intestinal connective. The osphradio-pallial nerve is seen leaving the side of the pleuro-supra-intestinal connective and running dorso-laterally backwards to innervate the osphradium. The supra-intestinal nerve runs obliquely across the anterior lobe of the digestive gland, and connects the right pleural ganglion with the supra-intestinal ganglion.

As regards the positions of the various ganglia, they can be best understood by examining text-figs. 25h, and 26a, b which represent a series of sagittal sections of an embryo in Stage 12, passing from the left to the right side. Text-fig. 25h passes through the supra-intestinal ganglion lying on the left side of the embryo, below the mantle-cavity but anteriorly to the pericardium; it shows a part of the left supra-intestinal nerve which is connected anteriorly with the supra-intestinal ganglion of its own side (*i.e.*, the left), which lies to the right of the plane of this section. Text-fig. 26a passes through the left cerebral and pleuro-pedal ganglionic mass; it shows the cerebro-pedal and the cerebro-pleural connectives of the same side running postero-ventrally to connect the cerebral ganglion with the pedal and pleural ganglia respectively; the cerebro-pedal connective lies anteriorly and slightly internally to the cerebro-pleural connective which is thicker and stronger than the former. Text-fig. 26b passes through the right pleuro-pedal ganglionic mass; and shows its fusion with the infra-intestinal ganglion. The three ganglia, namely, the pedal, the pleural and the sub-intestinal, are marked off from one another through slight constrictions as shown in text-fig. 14a.

Text-fig. 26d is an obliquely transverse section of an embryo in Stage 12, passing through the entire length of the cerebro-pedal connec-

tives. A study of this series of sections shows that whereas the tentacular nerve arises from the dorso-lateral part of the cerebral ganglion, the optic nerve leaves the ganglion at its ventro-lateral part and ascends obliquely to reach the inner part of the optic vesicle of its own side and lies posteriorly to the tentacular nerve. Another nerve originating from the ventro-lateral surface of the cerebral ganglion, just ventrally to the origin of the optic nerve, runs downwards to reach the dorsal part of the statocyst of its own side (Text-fig. 26*d*). Text-fig. 26*e* passes through the cerebro-pleural connectives which lie behind the cerebro-pedal connectives (Text-fig. 15*b*) and connect the postero-ventral part of each cerebral ganglion with the inner antero-dorsal surface of the pleural ganglion.

Text-fig. 26*f* passes through the supra-intestinal ganglion. Unlike text-fig. 13*c*, in which the supra-intestinal nerve crosses over the anterior lobe of the digestive gland obliquely and joins the infra-intestino-pleuro-pedal ganglionic mass of the right side, this nerve here runs downwards and crosses over the oesophagus to pass over to the other side, since the anterior lobe of the digestive gland has disappeared by this time.

Unfortunately, I have not been able to trace the development of the intestino-visceral connectives, *i.e.*, the supra-intestinal visceral and the infra-intestinal visceral connectives of the adult. Thus I cannot say definitely whether they are formed in very early stages or later. They are so thin and slender as to make it very difficult to distinguish them from the surrounding mesenchyme cells.

*Previous work and discussion.*—All the ganglia arise independently as cell-thickenings and are of an ectodermal origin. With the exception of the unpaired visceral ganglion, they are paired structures. In *Pila*, unlike *Paludina*, *Littorina*, etc., the cerebral and the pleuro-pedal ganglia arise much earlier than the rudiments of the eye and the statocyst respectively. It is only the intestinal ganglia and the visceral ganglion which are affected by torsion. The rudiments of the intestinal ganglia arise ventrolaterally almost at the junction of the “Kopffuss” with the visceral sac rudiment, and are symmetrical, but they become asymmetrical with the progress of torsion, so that the rudiment of the right intestinal ganglion is carried dorsally and comes to lie more or less on the left side of the median plane of the head-vesicle. The rudiment of the left intestinal ganglion is carried ventrally and to the right and then shifts anteriorly, with the result that it comes to lie close to the right pleural ganglion with which it fuses to form the sub- or infra-intestino-pleuro-pedal ganglionic mass. The unpaired visceral ganglion, on the other hand, is delaminated from the epithelial thickening of the right side of the mantle rudiment and, on the completion of torsion, comes to lie against the extreme left end of the mantle-cavity, dorsally to the pericardium.

The various commissures and connectives arise secondarily after all the ganglionic rudiments have become differentiated, and are formed by the peripheral cells of the ganglionic rudiments projecting and advancing towards each other and thus giving rise to strands of cells, which later develop into “commissures” in the case of those ganglia where the strands connect the components of the same pair and

“connectives” in the case of those ganglia where the strands connect different ganglia of the same side.

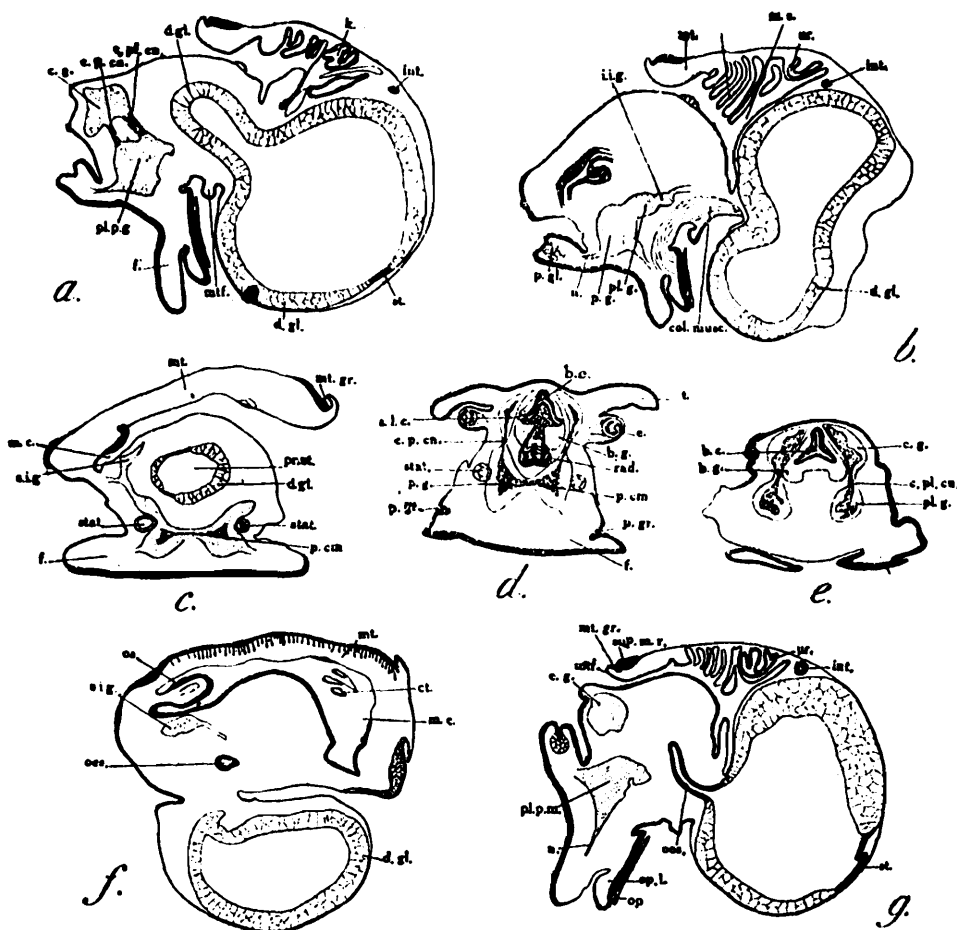
All recent work has shown that the nervous system in all the Gastropoda is of an ectodermal origin (Salensky, Haddon, MacMurrich, Schmidt, Henchman, Ihering, Erlanger, Delsman, Anderson, and others). Bobretzky (1877) in *Fusus* concluded that it was mesodermal in origin. But as MacMurrich (1886) and Rabl (1883) have rightly pointed out, Bobretzky reached wrong conclusions because of the fact that he worked on older embryos and, therefore, could not see the delamination of the nervous mass from the ectoderm. Fol (1876), though believing in the mesodermal origin of the nervous system in Heteropoda, describes the origin of the mesodermal masses (which give rise to the pedal ganglia, etc.), from the ectoderm itself.

With regard to the manner of the origin of the nervous masses, all observers are agreed that, with the only exception of the cerebral ganglia in some forms belonging to different groups of the Gastropoda, all the ganglia arise at first as ectodermal thickenings which are delaminated later to give rise to the rudiments of the various ganglia almost in the same manner as has been described by me in *Pila*. The cerebral ganglia have been observed to arise, wholly or partly, in some Gastropods like the Pteropoda, land-Pulmonates (*viz.*, *Helix*, *Arion*, *Limax*) and *Vermetus*, as ectodermal invaginations in the area corresponding to the head plates. These invaginations, one on either side<sup>1</sup>, become deeper and form tubular structures, the so-called cerebral tubes (Sarasin, 1887) which are, later, detached from the ectodermal wall and develop into cerebral ganglia (Fol, Sarasin, Henchman, Schmidt, Meisenheimer, etc.). It is stated that the cerebral-tubes alone do not form the cerebral ganglia but that they are fused at their inner blind ends with surrounding ectodermal cell-growth which is already delaminated from the head plates to give rise to the cerebral ganglia. Whatever may be the true state of affairs, this much is certain that the cerebral-tubes do take part in the formation of the cerebral ganglia. In no other ganglion has this invagination been observed.

Again, all recent workers are almost unanimous that the various ganglia arise separately and directly from the ectoderm and become connected with one another through commissures and connectives. They have thus denied the existence of a single common median rudiment for the paired ganglia. There are, however, a few exceptions. According to Delsman (1914), the pleural ganglia in *Littorina* do not arise separately and independently but as outgrowths of the cerebral ganglia, and are not sharply marked off from the latter; they are thus the two ganglia differentiated through a constriction “(einschnurung)” of the common rudiment of the cerebro-pleural ganglion. Similarly, Heath (1916) describes that in *Crepidula adunca* “the buccal ganglia are the only ones that do not directly arise from cells migrating from the overlying ectoderm; on the other hand, they give clear evidence of being products of the cerebral ganglia.” Although Erlanger (1891) describes a separate origin of the cerebral and pleural ganglia in *Paludina*,

<sup>1</sup> In *Helix waltoni*, according to P. & F. Sarasin (1887) there are two such invaginations on each side.

Anderson (1924) describes these ganglia as formed from a common rudiment.



TEXT-FIG. 26. Still more stages in the development of the nervous system.

*a.* Reconstruction of sagittal sections of an embryo in Stage 12, passing through the cerebro-pedal and cerebro-pleural connectives:  $\times 29$  *b.* Sagittal section (right of the median line) of an embryo in Stage 12, showing the complete fusion of the infra-intestinal ganglion with the right pleuro-pedal ganglionic mass and the formation of the columellar muscle:  $\times 29$ ; *c.* Oblique transverse section of an embryo in Stage 12, passing through the pleuro-supra-intestinal connective and the origin of the osphradio-pallial nerve:  $\times 29$ ; *d.* Oblique transverse section of an embryo in Stage 12, passing through the cerebro-pedal connective:  $\times 29$ ; *e.* Another obliquely transverse section of the same series as in (*d*), passing through the plane of the cerebro-pleural connective:  $\times 29$ ; *f.* Transverse section of an embryo in Stage 12, passing through the supra-intestinal ganglion, and the osphradium:  $\times 29$ ; *g.* Sagittal section of an embryo in Stage 12, showing the posterior part of the oesophagus opening into the stomach within the visceral sac:  $\times 29$ .

Lastly, with regard to the origin and formation of the commissures and connectives, all the authors, with the exception of Delsman (1914), agree that these arise from the peripheral cells of the ganglionic masses and, as they grow, extend across the spaces between the ganglia to meet similar cell-growths of either the same ganglion of the opposite side (commissure) or a different one of the same side (connective). Thus at first slender cords of irregularly arranged cells are formed which can be hardly distinguished from the surrounding cells. These commissures and connectives become distinct only when the fibrils are laid down within them. Anderson (1924) is, however, not definite as to whether the commissures and connectives arise in the manner described above, or are formed, even though partly, by direct delamination from the ectoderm, like the ganglia. He observed that in the early stages of

*Paludina*, at the time of formation of the rudiments of the cerebral commissure, the ectodermal wall lying between the rudiments of the cerebral ganglia also becomes many cells thick, but after the differentiation of the cerebral commissure, the ectodermal wall becomes single-layered again.

Delsman (1914) holds quite a different view. According to him, the ganglion, as a result of growth, becomes differentiated into a centrally situated fibrous mass, the neurofibrils, surrounded by ganglionic cells in which the nuclei are prominent and are thickly concentrated. In consequence of the strong growth, the two ganglia of each pair touch each other mesially and at the place of their meeting a bundle of nerve-fibres arising from the centrally situated fibrous mass penetrates through the surrounding layer of ganglionic cells to pass outwards to reach the other ganglion. In this way, the commissures are formed. As regards the formation of the connectives, Delsman holds a more or less different view. He says that in the embryo, though the different pairs of ganglia do not touch each other, they are also not far distant from one another. Here also a bundle of nerve-fibres arising from the neurofibrils pierces through the surrounding nuclear layer to go out and grow towards the other ganglion.

#### H. *The Receptor Organs.*

In *Pila* there are five localised receptor organs. Four of these, *viz.*, the statocysts, the eyes, the tentacles and the labial palps are paired, while the fifth, *viz.*, the osphradium (Spengel's organ) is unpaired. Unlike some other Gastropoda, such as *Paludina* (Erlanger 1891), *Littorina* (Delsman 1914), in *Pila* all these organs appear after the formation of the nerve ganglia.

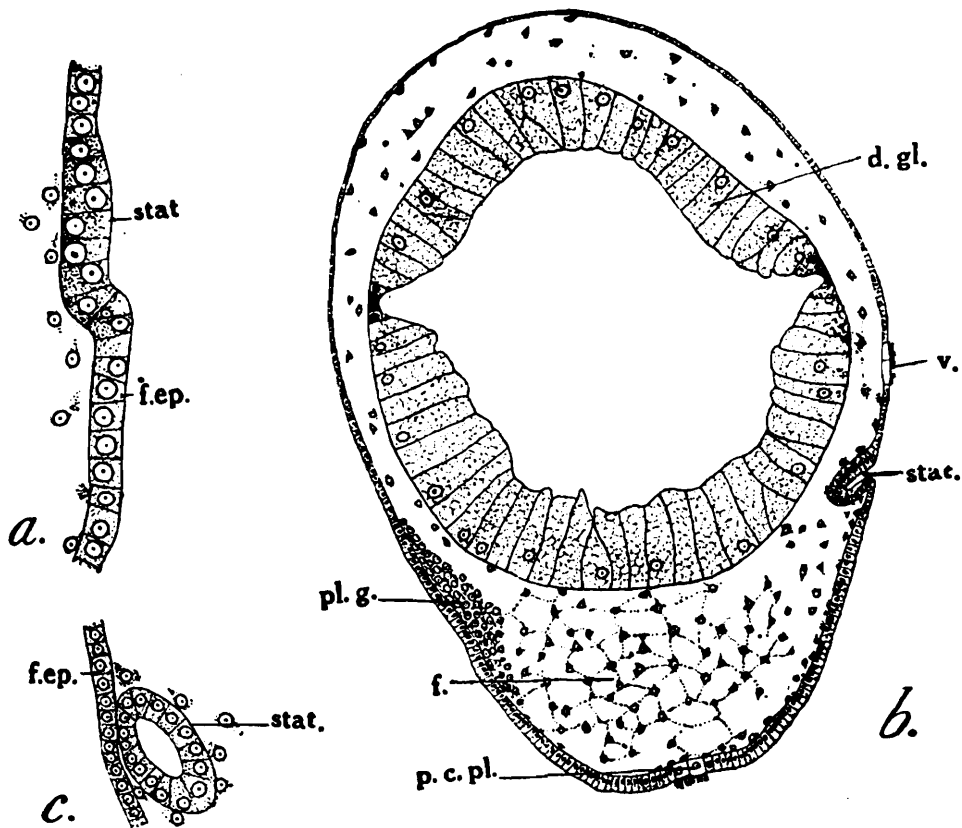
#### *The Statocysts.*

The rudiments of the statocysts are the earliest to appear as two ectodermal thickenings (Text-fig. 27a) which soon develop, first into flat plates and then into depressions (Text-fig. 27b) in the "Kopffuss" region at the base of the foot, a little posteriorly to the velum (Stage 8). The epithelial cells lining each depression increase in size, and multiply rapidly so that the invagination becomes deeper and tubular, although it still opens widely to the outside (Text-fig. 27b). These rudiments of the statocyst are placed obliquely and are directed inwards and downwards. The cells lining the walls of each invagination are not equal in size, those situated at its inner blind end being taller than those towards the outer opening (*cf.* *Paludina*, Erlanger, 1891).

As development proceeds, these invaginations become constricted off from the epithelium, and form closed vesicles lying beneath the continuous epithelium of the foot (Text-fig. 27c). The lumen of the statocyst is still narrow and tubular, and the difference in size of the cells forming its walls still persists.

With further development, the statocysts begin to shift inwards and downwards so that not only does each statocyst lie a short distance away from the epithelial wall, but a change also takes place in its shape. It now assumes an oval appearance due to a widening of its lumen, and

all its cells become more or less alike. It is still situated between the ventro-lateral wall of the digestive gland and the foot-epithelium, but is now surrounded by a few scattered mesenchyme cells.



TEXT-FIG. 27. Development of the statocyst.

a. Section of the wall of the "Kopffuss" of an embryo in Stage 8, showing the depression to form the rudiment of the statocyst:  $\times 390$ ; b. Transverse section of an embryo in Stage 8, showing the position of the statocyst rudiment:  $\times 170$ ; c. Transverse section of an embryo passing through the statocyst which is now a closed vesicle lying against the "Kopffuss" epithelium:  $\times 340$ .

With further growth, the statocysts become large while their cells become cubical and uniform in size. They shift more and more inwards, till they finally come to lie against the outer surfaces of the pleuro-pedal ganglionic masses. The outer surface of each ganglionic mass shows an indentation or depression for the statocyst (Text-fig. 28f), but in spite of this close association between the two structures the statocysts are not innervated by the pleuro-pedal ganglia. On the other hand, a nerve arising from the outer ventro-lateral margin of each cerebral ganglion runs downwards to innervate the statocyst of its own side, a part of this nerve going to the left statocysts being shown in Text-fig. 28f. This marks the final position of the statocysts. Hereafter they grow in size and statoliths are secreted within them. It has been observed that in embryos of Stage 12, a single calcareous or horny particle (statolith) or many particles (statoconia) may be present. As development proceeds, the statocysts become larger so that in a newly hatched animal these organs are large and thin-walled, the cells becoming quite flattened. In an embryo which is ready to hatch the diameter of these statocysts is about  $187\mu$ .

*Previous work and discussion.*—Semper (1862) described the formation of the statocysts in *Ampullaria (Pila) polita*, but did not make any mention of the exact process of its development. Similarly, Scott

(1934) has only described the time and stage in which these organs appear, without making any mention of their mode of origin. Semper described the otoliths (statoconia) appearing as amorphous granules which gradually become larger and crystalline. He was able to count as many as 40 such crystalline otoliths in an advanced stage. Scott counted 14 in a stage where the statocyst is  $85\mu$  in diameter.

In most Gastropoda the statocysts, as in *Pila*, originate as ectodermal invaginations, e.g., in *Patella* (Patten, 1885), *Lymnaea* (Wolfson, 1880), *Paludina* (Bütschli, 1877; Erlanger, 1891), Marine Prosobranchs (Salensky, Bobretzky, Patten, Conklin), *Bythinia* (Erlanger, 1892) and *Physa* (Wierzejski, 1905). In *Planorbis*, Rabl, considered it to be formed by invagination, though he was not quite definite at all about this. On the other hand, *Limax Maximus* (Henchman, 1890; and Meisenheimer, 1898) and *Littorina* (Delsman, 1914) show a different method of its formation. In these cases, each statocyst arises through a process of cell growth ("wucherung"), the cells being constricted off from the ectoderm as a solid mass, in which a lumen develops later.

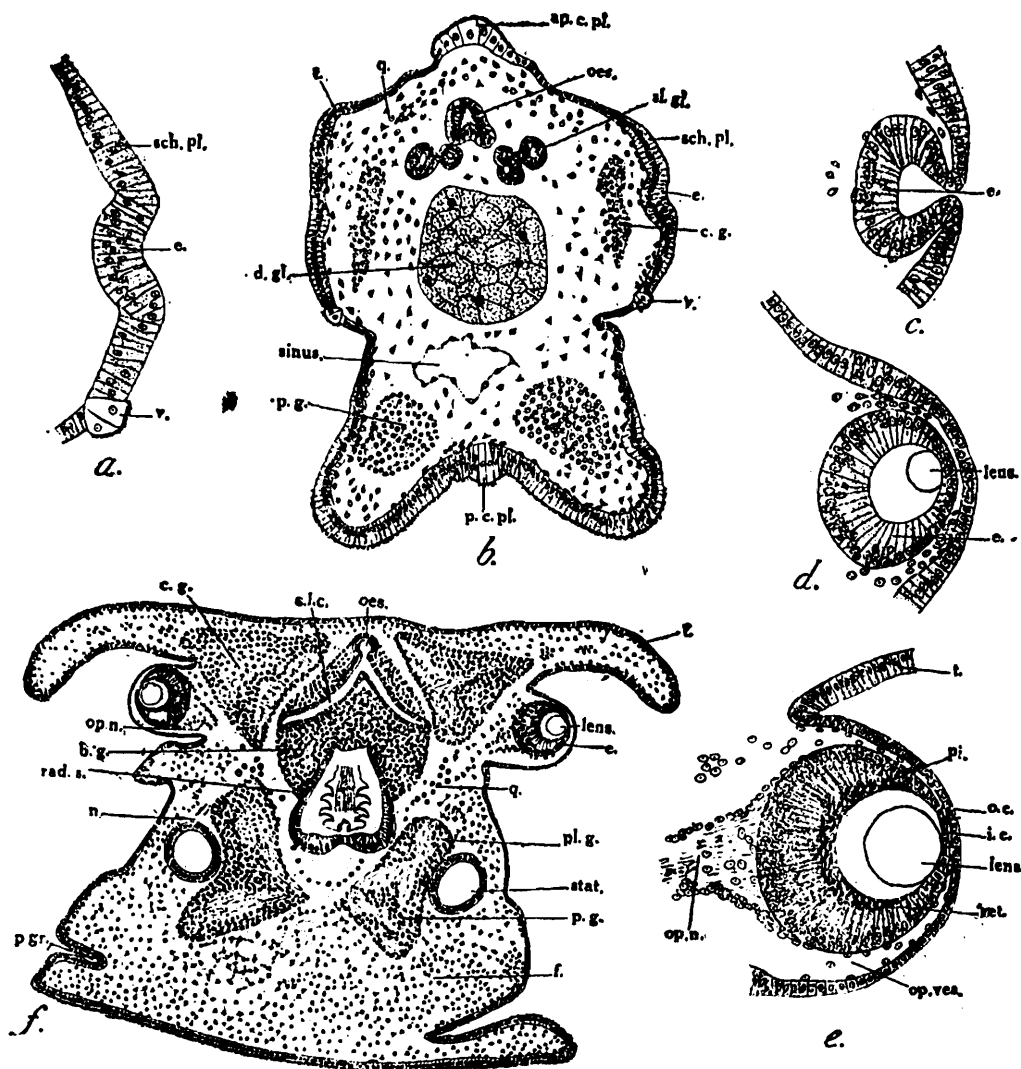
### The Eyes.

The eyes appear later than the statocysts (cf. *Littorina*, Delsman, 1914; *Paludina*, Erlanger, 1891), at a stage in which the torsion is just complete (Stage 10). The head plates become thickened about their middle and form two thick plates of tall, cylindrical cells. On each plate there appears a depression which forms the first rudiment of the eye (Text-fig 28a, b). The eye rudiments appear simultaneously with the tentacular rudiments, the former arising on the outer and posterior sides of the latter. The cerebral ganglia, now connected with each other through a cerebral commissure, lie between the rudiments of the eyes and the digestive gland. The eye rudiments, therefore, arise in very much the same way as the statocyst rudiments.

As development proceeds, the eye depression deepens. However, unlike the statocyst, the cavity of the eye invagination is never narrow, but remains wide from the beginning up to the stage shown in Text-fig. 28c. Here, as in the case of the statocysts, a difference in the size of the cells is noticed (Text-fig. 28c), the cells forming the base of the cavity being tall and cylindrical, while those towards the outer ends and continuous with the cells of the epithelium being smaller in size. In Text-fig. 28c, the invagination shows a deep cavity which is not yet completely separated off from the epithelium of the head plate. The inner wall of the eye rudiment is nearly flat, with the result that the cavity is broader towards the inner wall, but gradually narrows down towards the outer, where it communicates with the outside through a narrow aperture. The cavity thus appears triangular in shape.

In the next stage (Text-fig. 28d), the eye rudiment has been completely constricted off from the surface epithelium, which now forms a continuous layer over the eye vesicle and consists of squarish cells. The eye vesicle is a spherical sac with a rounded cavity. The cells of the inner wall of the vesicle have multiplied and their nuclei have shifted towards the periphery, but the cells of the outer wall remain small and

have centrally situated nuclei. So far, no differentiation of parts has taken place, except for the formation of the lens which has already made its appearance in its rudimentary form. The lens is secreted by the side walls of the eye vesicle, and is a homogeneous crystalline body which later becomes spherical in outline. It lies within the lumen of the vesicle partly attached to its outer wall.



TEXT-FIG. 28. Development of the eye.

*a.* Transverse section of an embryo in Stage 10, showing the invagination of the eye rudiment:  $\times 223$ ; *b.* Transverse section of an embryo in Stage 10, showing the relative position of the eye and tentacle rudiments:  $\times 112$ ; *c.* Transverse section passing through the eye invagination which still communicates with the outside:  $\times 283$ ; *d.* Section of eye, showing the formation of the lens:  $\times 283$ ; *e.* Section passing through a fully differentiated eye of an embryo in Stage 12:  $\times 283$ ; *f.* Oblique transverse section of an embryo in Stage 12, showing the position of the eyes and showing a part of the nerve innervating in the statocyst:  $\times 81$ .

In the next stage (Stage 12), a further differentiation takes place in the vesicle (Text-figs. 28*e, f*). A black pigment is laid down at the inner ends of the tall, cylindrical cells lining the inner and side walls of the eye vesicle. The pigment, sparse in the beginning, increases in quantity as the inner layer forms the perceptive part of the eye, *viz.*, the retina. The oval nuclei become thickly crowded and lie towards the bases of the cells. The lens becomes enlarged and occupies the greater part of the lumen within the eye vesicle. It consists of a crystalline refractory substance. Fine rod-like structures develop along the inner surface of the

retina. The cells of the outer wall of the eye vesicle become flattened and their cytoplasm becomes clearer; this outer wall forms the inner cornea or pellucida of the eye. Similarly, the epithelium overlying this inner cornea forms the *outer* cornea, the cells of which become flattened in contrast to the adjacent cells which remain cubical. The nuclei of these flattened cells appear to be compressed dorso-ventrally and are hyaline in appearance, their cytoplasm also being hyaline. The epithelia of the outer and inner cornea are separated from each other by a narrow space occupied by scattered connective tissue cells. The optic nerve has already appeared at this stage and extends obliquely from the cerebral ganglion of its own side to the eye.

With further development, the eye grows in size, so that in an embryo about 3.4 mm. long, *i.e.*, when it is about to hatch, the diameter of the eye is 190 $\mu$ . It has already been described clearly that each eye is situated at the base of a tentacle at its postero-lateral margin, and is raised on a short peduncle, the ommatophore.

*Previous work and discussion.*—On comparing the development of the eye in *Pila* with that of other Gastropods, it is noticed that in *Pila* (as in *Paludina*, Erlanger, 1891; *Planorbis*, Rabl, 1879; *Limax*, Meisenheimer, 1898; *Physa*, Wierzejski, 1905), the eye arises as an ectodermal invagination which deepens and detaches itself from the ectoderm to form a rounded vesicle in which differentiation takes place later. In *Littorina* (Delsman, 1914), on the other hand, there is no invagination, but a thickening is formed which, later, gets detached from the ectoderm as a solid, rounded growth, and later develops a cavity.

The lens in *Pila*, as in *Paludina*, *Littorina*, and *Limax*, is formed as a cuticular deposit secreted by the cells of the vesicle, which increases in size by the addition of concentric layers around it (*cf.* Hilger, 1885). In *Ampullaria polita*, Semper (1862) found that the cavity of the eye-vesicle was full of a colourless fluid secreted by the wall of the vesicle before the formation of the retina. On the formation of the retina, the fluid turns yellowish, becomes thick and forms the lens. In *Lymnaea*, however, according to Wolfson (1880), the lens is formed in quite a different manner; one cell of the wall of the eye-vesicle becomes metamorphosed, its cytoplasm shrivels up and the nucleus becomes homogeneous and greatly refractory and forms the lens.

### *The Tentacles.*

The tentacles arise simultaneously with the formation of the eye. That part of each plate which lies antero-dorsally to the eye rudiment, takes part in the formation of the tentacle. At the time of its origin, this part of the head plate forms a small bulge at the extreme antero-dorsal region of the velar area (Text-fig. 28*b*). As soon as the tentacle-forming part of the head plate bulges outwards, the mesenchyme cells also enter the cavity of the tentacles and very soon some of the cells are transformed into muscle-fibres, which, with the growth of the tentacles, become thick in texture.

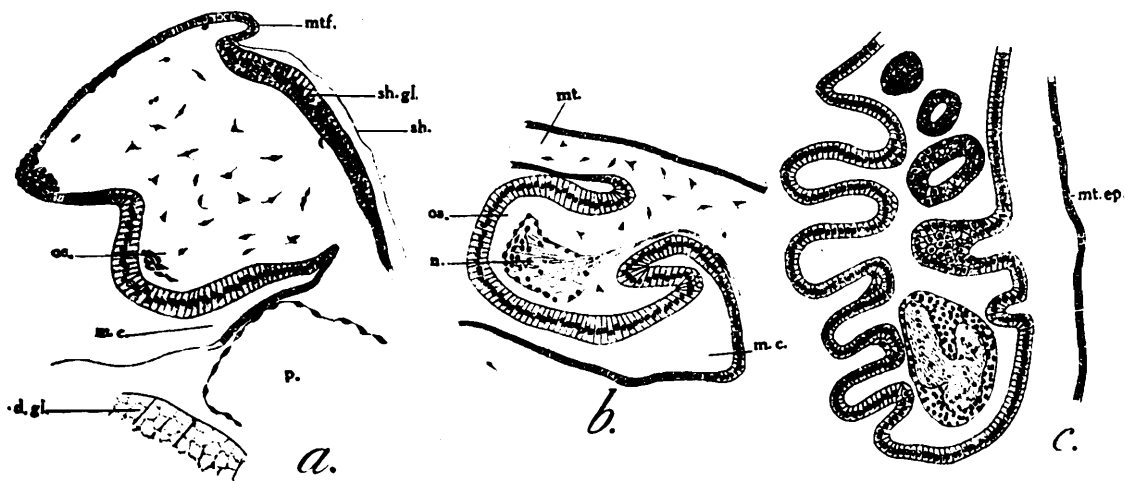
As development proceeds, these tentacles grow considerably in size, become long and tapering, and are directed antero-dorsally as well as

towards the outside. They are lined by columnar ectodermal cells (Text-fig. 28f).

As regards the development of the tentacles, complete agreement prevails amongst workers in nearly all the groups of the Gastropoda. We can thus homologise the position and development of the tentacles in *Pila* with those of *Paludina* (Erlanger, 1891), *Limax* (Meisenheimer, 1898), *Littorina* (Delsman, 1914), and others.

### *The Osphradium.*

The osphradium is an unpaired structure which arises simultaneously with the formation of the gill-rudiments just after the rudiments of the statocysts are laid down. In an embryo in Stage 8 the epithelium forming the right or inner wall of the mantle, situated just above the postero-dorsal part of the pericardium, gets thickened into a ridge-like structure from which the rudiments of the gill, osphradium and the lung arise (Text-fig. 23b). A little later, when the eye rudiments begin to appear (Stage 10), this thickened epithelium is pushed outwards into the mantle-cavity as a lamellar fold, which forms the first rudiment of the osphradium, and lies to the left of another similar formation, *viz.*, the gill-rudiment (Text-fig. 23b).



TEXT-FIG. 29. Development of the osphradium.

*a.* Section of an embryo in Stage 11, passing through the osphradial rudiment:  $\times 157$ ; *b.* Section of embryo, passing through the osphradium which now has a distinct plexus of nerves:  $\times 157$ ; *c.* Oblique section of the osphradium of an embryo about to hatch, showing its pectinate character:  $\times 157$ .

As development proceeds, the osphradium no longer remains lamellar, but changes into a more or less rounded structure (Text-fig. 29*a*).

With the growth of the mantle, the osphradium is also carried forward along with it. As it spreads out to enclose the head-vesicle, the osphradium comes to occupy its final position. It hangs down like a frill at the anterior end of the mantle-cavity on the left side of the embryo, a short distance behind the free edge of the mantle. It is now an oval structure and is broadest in the middle; its nerve has already reached its substance and is seen prominently in Text-fig. 29*b*.

As development proceeds, the ectodermal wall on either side of the osphradial rudiment is pushed inwards, thus giving rise to folds or leaflets, whilst the central part forms the median axis of the osphradium (Text-fig. 25*h*).

Text-fig. 29c is a section of the osphradium of an embryo ready to hatch. The number of folds on the two sides of the central axis has increased, giving the osphradium a bipectinate appearance.

The osphradium is an unpaired organ and retains its position on the left side throughout the course of its development. In spite of careful search, I could not observe any trace of a rudiment of the organ on the right side. According to Prashad (1925), this organ "in spite of its position on the left side is homologous to the osphradium of other Gastropods"

### *The Labial Palps.*

The labial palps or the anterior pair of tentacles arise last of all. They appear first when the embryo has already assumed an adult appearance by the forward growth of the mantle enclosing the head-vesicle (Stages 11 and 12). Like the tentacles, they arise as outpushings of the ectoderm, at the extreme antero-dorsal end of the snout, *i.e.*, the region which bounds the mouth on its dorso-lateral sides. In a newly hatched animal they appear as "short conical prolongations of the snout on the two sides of the mouth".

## X. THE LARVAL ORGANS.

In *Pila globosa*, as in other Mollusca, some organs of a transitory nature appear at one phase or another of embryonic life. These organs perform their function, if any, and then disappear completely, leaving no trace behind. Since the organs are met with only in the earlier stages of development and are not found in later embryonic stages, they have been termed as larval organs. In *Pila*, three such larval organs are present, *viz.*, (1) the velum, (2) the larval heart and (3) the "neck cells" or nuchal cells. These organs do not arise simultaneously, but originate at different periods of embryonic life.

### *The Velum.*

The velum is the first of these organs to appear at the gastrula stage, and transforms the embryo into a trochophore. Various workers who have investigated cell-lineage in the Gastropoda, such as *Crepidula* (Conklin, 1897), *Physa* (Wierzejski, 1905), *Littorina* (Delsman, 1914), have traced back the origin of the velum to the primary and secondary trochoblast cells, and this appears to be the general rule in all molluscs.

The velum consists of two band-like rows of cells which in their fully differentiated condition, are large and transparent, and bear cilia. In transverse sections, the velar cells appear wedge-shaped with the broader ciliated surface to the outside (Text-figs. 15a and 28b). To begin with, the velum is equatorial in direction, lying more towards the animal pole. Later, however, on account of the ventral protrusion of the foot-rudiment and the stomodaeal invagination, it becomes displaced on its ventral side towards the anterior end forming a curve above the mouth-opening, as a result of which two small lobe-like structures, the velar lobes, are formed on the antero-lateral sides, which are characteristic of a veliger.

The velum is a ciliated organ which helps the embryo to rotate within the egg-shell, although I have never seen these rotatory movements myself. The velum persists up to a pretty late stage (Stage 11), but when the embryo is fully developed, it becomes absorbed within the body-wall epithelium and disappears. The disappearance begins on the dorso-lateral sides and is completed before the embryo hatches out.

#### *The Larval Heart.*

The second larval organ is the so called larval or embryonic heart. In the posterior region of an embryo in Stage 6 (Text-fig. 4f), between the posterior part of the foot and the anus, irregular contractile movements are seen in the body-wall on the right side. These movements occur long before the definitive heart starts pulsating. It is the thin body-wall in this region which exhibits the pulsations in the form of strong movements of expansion and contraction. The position and functioning of the larval heart can be best studied in the living condition, since after fixation this region generally evinces a systolic phase, and it is very rarely that a distended condition is met with. The larval heart consists of a bulge of the ectoderm to which a large number of mesenchyme cells are closely attached on the lower side.

Although the first pulsations of this organ are seen in Stage 6, I am inclined to believe that it functions even earlier, and the pulsations have been overlooked on account of the opacity of the embryo and the feebleness of the movements. Only when this "heart" has developed appreciably and the embryo becomes more or less transparent with the complete absorption of the food-yolk, that the pulsations are readily observed.

These pulsations have been connected with embryonic circulation by some authors, *e.g.*, Fol. (1880), who suggest that circulation of the body-fluid is promoted through its contractions. The larval heart continues to pulsate even when the true or definitive heart has begun its rhythmic beats. I could observe the contractile movements of the larval heart at a stage when it is enclosed by the forwardly growing mantle (Stage 11, Text-fig. 5d). At this stage the shell covering the visceral sac is still thin and sufficiently transparent to allow the movements within to be readily seen. The duration of the larval heart could not be definitely ascertained, as the shell, becoming opaque, shuts it out of view, but it is certain that it disappears long before the hatching of the embryo.

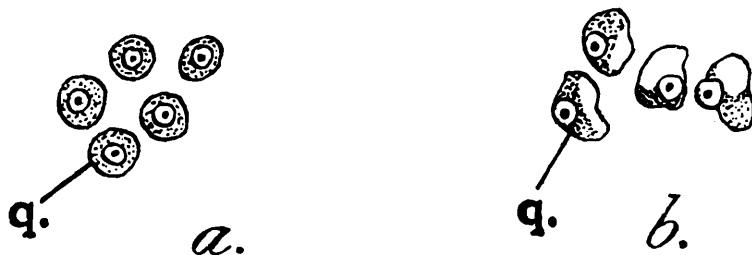
Semper (1862) describes in *Ampullaria polita* such a larval organ as a chamber at a place exactly comparable to that in *Pila*. He, however, describes the presence of a short muscle extending from the skin to the liver mass and dividing this chamber into two (*cf.* his fig. 14 S, Pl. 11). An examination of living embryos and whole mounts, as well as transverse and sagittal sections, failed to show any trace of such a muscle. Scott's observations on *Ampullaria canaliculata* (1934) support my conclusions about the absence of this muscle.

Salensky (1872) has likewise demonstrated the presence of a larval heart in *Calyptraea*, while Robretzky (1877) observed it in *Nassa* and *Fusus*. Fol. (1880) describes the appearance of this larval organ

amongst the *Pulmonata*, such as *Helix*, and similarly, *Delsman* (1914) noticed its appearance in *Littorina* and *Fusus*.

### The Nuchal Cells.

The third larval organ is a group of specialised cells which first appear within the velar area in the region of the head plates. They are definitely noticed for the first time, when the torsion of the embryo has just been completed (Stage 10). These cells are mostly rounded in shape and have centrally situated nuclei containing distinct nucleoli (Text-fig. 30*a*). They differ from the rest of the mesenchyme cells found scattered within the body-cavity not only in their much larger size but also in their capacity to take a deeper stain. After observing their constant presence within the neck region amongst the *Pulmonata*, Fol (1880) designated them as the "neck-cells" ("cellules nucales"). Since nothing definite has been established as regards their morphological or physiological nature, this designation has been accepted by subsequent authors like Erlanger (1891) and Wierzejski (1905).



TEXT-FIG. 30. Nuchal cells.

*a.* Nuchal cells of an embryo in Stage 11. *b.* Nuchal cells of an embryo older than Stage 12, showing the formation of vacuoles. Both figures semi-diagrammatic.

At the time of their appearance, the nuchal cells are few in number and are situated in the dorsal part of the head-vesicle near the part of the head plates lying in the neighbourhood of the posterior part of the cerebral ganglia. They are already arranged in a semi-circle, corresponding to the next stage of development in *Paludina* and *Physa*. But I believe that in *Pila* too they probably originate as two lateral plate-like cell-groups in a stage immediately preceding the one under discussion, but are not sufficiently differentiated to be readily recognisable. These cell-groups extend towards each other, meet dorsally above the oesophagus, and finally produce a semi-circular or horse-shoe-shaped appearance.

As development proceeds, these cells multiply, and spread inwards and downwards on either side of the radular sac, so that they lie scattered in the space between the body-wall and the fore-gut, right up to the lower end of the radular sac. The nuchal cells not only increase in number but also show a marked growth in their size and become more prominent.

Later, vacuoles appear within these cells, as a result of which they become distended, while their shapes get distorted and irregular (Text-fig. 30*b*). The vacuoles are small to begin with, but soon grow large and displace the nuclei from their original central position towards the periphery.

A close study of these cells in various stages of development shows that they continue to lead an independent existence

without participating in the formation of any organ whatsoever, that they persist for some time after the formation of vacuoles and changes in their shape, and that they disappear completely when the embryo is ready to hatch. No trace of their existence has been observed either in embryos ready to hatch or in the newly hatched animals.

*Previous work and discussion.*—Nuchal cells have been described in some other fresh-water Gastropods, viz., *Paludina*, *Bythinia* (Prosobranchs), and *Lymnaea*, *Planorbis*, *Ancylus* and *Physa* (Pulmonates); but they have not been observed in the terrestrial Pulmonates. Wierzejski (1905), on account of their position, considers them to be analogous with the external kidney of the marine Prosobranchs.

As regards their morphological and physiological significance nothing definite is known. Fol. studied them in *Ancylus*, *Planorbis* and *Lymnaea* and describes them as special mesodermal cells which have delaminated from the ectoderm. According to Erlanger (1891), the nuchal cells arise as an unpaired plate-like extension ("verbreitung") in the mid-dorsal line of the posterior border of the velum, and are thus ectodermal in origin. Wierzejski (1905), on the other hand, describes them in *Physa* as mesodermal in origin, having been derived from the secondary mesoderm as a paired rudiment close to the dorsal edges of the head plates which later extend towards each other in the middle line above the pharynx and thus form one semi-circular cell-plate. According to him, there is no genetic relation either with the velum as considered by Erlanger, or with the head plates. In *Pila*, so far as I have been able to ascertain, the origin and development of these cells accords with the findings of Wierzejski.

Similarly, contradictory views are held as regards their physiological significance. Lereboullet (1862) regarded them as nervous elements, while Lankester (1874) mistook them to be rudiments of cerebral ganglia. Fol. (1880) believes them to be remnants of a rudimentary organ. Erlanger (1891), though silent about their actual significance in *Paludina*, mentions a casual connection between the appearance of these cells and the absorption of the velum. Wierzejski (1905) suggests that on account of (1) the presence of these cells close to the primary kidneys, (2) the early appearance of these cells, and (3) their becoming large and vacuolated at the time of the atrophy of the primary kidneys, these cells seem to be concerned with an excretory function. But this view cannot hold good in *Pila*, since the nuchal cells (i) appear in later stages, i.e., in Stage 10; (ii) are not at all related to the primary kidneys, as the latter are absent in *Pila*; and (iii) because in *Pila* these cells attain their maximum size and get vacuolated in stages when the real kidney is already functioning (Stage 12). I, therefore, hold that the nuchal cells constitute an independent larval organ of problematic function.

## XI. THE FOOT.

### *General Development of the Foot.*

The origin of the foot and the structural changes it undergoes have already been described above. The foot is composed exclusively of ectodermal and mesenchymatous cells. As development proceeds, the base of

the cone at its junction with the head-vesicle anteriorly and the entire anterior surface of the cone both increase in size. The posterior surface of the cone does not grow at the same rate. The result of this differential growth is, that the area at the anterior junction of the foot and head-vesicle forms the anterior ascending surface of the foot, the anterior surface of the cone comes to form the flat sole of the foot, while the slow-growing posterior surface forms the postero-dorsal wall of the foot. The pedal cell-plate persists up to Stage 12 but disappears later. When the foot has flattened (Stage 10), cilia develop on its entire ventral surface (Text-fig. 28 *b*.) These cilia are extremely fine in the earlier stages of their development and are evenly distributed over the sole of the foot (*cf.* Barr, 1928 ; Roth, 1929).

In later stages of development when the embryo has assumed a snail-like appearance (Stages 11 and 12 : Text-figs. 5*d*, *e*, *f*), a notch-like invagination appears close to the extreme posterior end of the foot, at the junction of the sole and the posterior surface (Text-fig. 10). This invagination gradually deepens and extends upwards on either side of the foot so as to reach as far as the junction of the foot with the posterior end of the head-vesicle, close to the place where the latter meets the visceral sac. As a result of this extension of the invagination, the postero-dorsal part of the foot becomes raised and is distinguished as a disc-like structure more or less constricted from its ventral part. This may be called the operculigenous disc or lobe of the foot, *i.e.*, that part of the foot which secretes and bears the operculum (Text-figs. 10 ; 11 ; 25*h* ; and 26*a*, *b*, *g*).

Similarly, at about this stage (*i.e.*, Stage 12<sup>1</sup>), an ectodermal invagination takes place in the anterior wall of the foot. This is notch-like to begin with, but soon deepens posteriorly into a small and narrow groove-like structure. This is the rudiment of the pedal gland (Text-fig. 11).

#### *The Epithelium of the Foot.*

In the earliest stages, the cells lining the walls of the foot are all alike in being tall and cylindrical (Text-fig. 15*a*). They are closely pressed against one another, are rich in protoplasm, and are provided with centrally situated oval or ovoid nuclei. Gradually, however, the cells towards the base of the conical foot become smaller than those towards the apex of the cone (Text-fig. 27*b*). This change in size and shape proceeds dorso-ventrally, both along the anterior wall of the foot as well as along its lateral walls, till, finally, in a completely flattened foot only the cells of the sole of the foot remain tall, cylindrical and glandular with oval or rounded nuclei (Text-fig. 28*f*). The cells bounding the postero-dorsal area of the foot, *i.e.*, those forming the operculigenous disc, remain unaffected by these changes and they retain their columnar and glandular character. The horny, membranous rudiment of the operculum is secreted by these cells in the same way as the shell is secreted by the shell-gland, with this difference that there is no invagination in the area of the operculigenous disc, and the surface remains flat. The rudimentary operculum later becomes calcareous<sup>1</sup>.

---

<sup>1</sup> I have, however, not followed the development of the operculum.

The cells lining the sole of the foot undergo a change. Their nuclei move towards their bases, *i.e.*, towards the end opposite the one that bears the cilia. These cilia are not confined to the sole of the foot but also extend upwards along the edges of the foot so as to reach dorsally the lower margin of the lateral grooves, which are formed at this stage as ectodermal invaginations, one on either side, occurring in the anterolateral part of the foot close to the foot-fringe<sup>1</sup>. These lateral grooves correspond to the peripodial grooves of *Arion* as described by Barr (1928), with this difference that in *Pila* they do not extend as far back as the posterior end of the foot, but are confined to its anterior part. They are deeper anteriorly but gradually become shallow till they disappear altogether. I noticed the presence of these grooves for the first time in Stage 11.

Another structure within the foot, is the columellar muscle. In very early stages, when the foot has just flattened at its anterior end (Stage 6), the mesenchyme cells become thickly concentrated in the posterior part of the foot. With further development, the number of these cells increases and they aggregate towards the left posterior side. When the embryo has assumed a snail-like appearance (Stage 11, Text-fig. 10), the cells of the aggregated mass begin to differentiate into elongated spindle-shaped cells with centrally situated nuclei; these are the muscle cells. This differentiation extends from the posterior to the anterior end, till nearly all the cells of the mass become transformed into the muscle cells. These cells form the rudiment of the columellar muscle. A fully differentiated columellar muscle extends over the entire surface of the operculigenous disc of the foot beneath the operculum. In an extended foot, the muscle appears as an arched structure directed anteriorly with the convexity of the arch lying at the junction of the foot with the head-vesicle (Text-fig. 11). The muscle is broad at its anterior end, but posteriorly it is narrow and band-like and lies to the left of the oesophagus. It helps in the retraction of the foot within the shell.

In addition to this muscle, other muscle fibres and strands, which lie interspersed within the foot and cross one another, develop by the transformation of the mesenchyme cells and thus form the musculature of the foot.

#### *The Pedal Gland.*

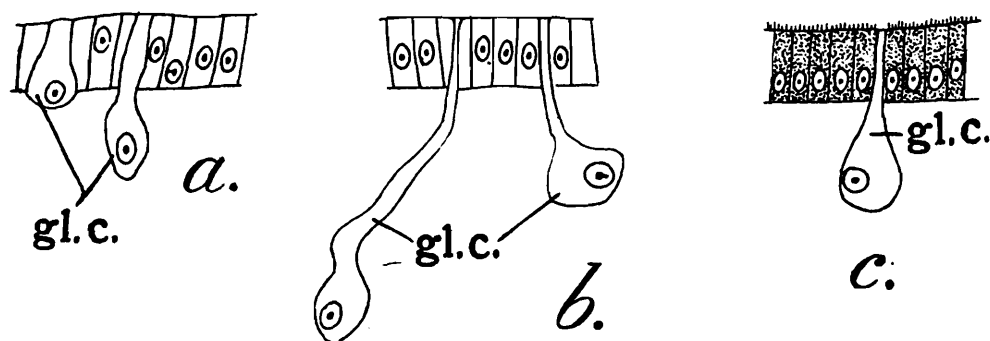
The origin of the pedal gland as an ectodermal invagination at the anterior end of the foot has been already pointed out. With further development the invagination deepens and extends backwards in a horizontal direction within the substance of the foot, almost parallel to its ventral creeping surface. It is tubular in appearance and is confined to the anterior part of the foot (Text-fig. 11).

At first this tubular rudiment is lined throughout with small cubical cells but soon some of the cells at the inner blind end of the tubular invagination increase in size and sink into the subjacent connective tissue mass. Here they swell up into irregular oval bulb-like shapes

---

<sup>1</sup> The lateral margins of the sole are designated as the foot-fringe.

with their nuclei in their swollen bases. These swollen cells vary in size and together form a compact mass around the posterior end of the invaginated tube, and constitute the glandular mucous cells of the pedal gland, while the undifferentiated tubular part functions as the canal for the slime secreted by the glandular cells. These secretory cells do not take up stains easily.



TEXT-FIG. 31. Three successive stages in the development of the gland cells of the foot in the developing embryo. In (c) a gland cell is seen opening on the sole of the foot:  $\times 447$ .

As development proceeds, the secretory cells increase in size and sink further into the subjacent mass of connective tissue, but retain their connection with the tubular canal through their narrower proximal parts. The slime secreted by the mucous cells is thus discharged directly into the secretory canal.

#### Other Glands of the Foot.

Besides the pedal gland, there are a few others found within the foot. I have arranged them, on the basis of their position, into three different groups.

(1) The first group includes glands that are situated in the neighbourhood of the lateral grooves into which they open. These correspond to the "peripodial glands" of *Arion* (Barr, 1928), and resemble them in form and structure and are ectodermal in origin. They arise almost simultaneously with the pedal glands and are always unicellular. They are mucous glands which discharge their slimy secretion into the lateral grooves. From the lateral grooves the secretion is carried anteriorly to the opening of the pedal gland by the cilia lining the outer borders of the grooves<sup>1</sup>.

(2) The second type of glands are confined to the antero-dorsal part of the foot. The covering epithelium of this part of the foot consists

<sup>1</sup> Barr (1928) states that in *Arion* "the cells discharge their mucus into the intercellular spaces, especially into those that are situated just below the canal. From there the mucus passes out into the lumen of the canal. This process is probably assisted by the contraction of the muscles which are found between the cells. Some of the glandular cells (*i.e.*, those in the roof of the canal and round its dorsal half) discharge their contents directly into the canal". I do not see any justification for this view as the whole of the secretion is directly discharged into the lumen of the canal itself. The mucous cells are of epithelial origin and sink into the substance of the connective tissue but they never lose their connection with the epithelium. In sections, one can not always see their connections with the epithelium without reconstruction, and hence can easily be misled to think that they discharge their contents into the intercellular spaces. Barr himself has noticed the mucous cells in the roof opening directly into the canal.

of tall cylindrical cells with oval or elliptical nuclei in the basal ends of these cells.

In an embryo in Stage 11, some of the epithelial cells lining this surface sink inwards (Text-fig. 31a) and take up a deeper stain than that of the adjacent epithelial cells. The nuclei in these cells become large and round and pass into their epithelial portions.

As growth proceeds these glandular cells sink deeper till they come to be imbedded in the deeper parts of the sub-epithelial mass of the foot (Text-fig. 31b). The proximal parts of these cells become very much narrowed into long tubular neck-like structures which serve as the ductules for the secretion to be discharged on the surface of the foot. On their further migration inwards, these ductules elongate and become tortuous in their course, with the result that one must examine a number of sections to trace the entire course of these cells and their ductules, which are continuous right up to the outer epithelium (Text-fig. 31b). Since these cells are continuously being differentiated from the ordinary columnar cells, they are seen at different stages of development and are of different sizes in the same embryo. They vary in shape from the vesicular to rhomboidal.

(3) The third set of glands are confined to the sole of the foot and are, therefore, aptly described as the foot-sole glands. These are also unicellular like the others, and appear much later, when the embryo is ready to hatch. They arise and develop from the epithelium of the sole of the foot and resemble more or less the gland-cells belonging to the second group (Text-fig. 31c).

#### *Some other structures connected with the Foot.*

The lateral grooves, on either side, mark the boundary between the epithelium of the foot-fringe and that of the lateral walls, the cells lining the foot-fringe and the sole being tall and ciliated, while those lining the lateral walls are small and non-ciliated.

The other structures found within the foot are: (1) two statocysts, (2) the two pleuro-pedal ganglia with their commissures and connectives, and (3) the lower distal part of the unpaired radular sac with its associated musculature (Text-fig. 28 f). Of these, the first two are directly related to the foot because of their origin by invagination and delamination, from the ectodermal epithelium of the foot. These two structures have been separately described in the chapter on the nervous system and the receptor organs.

The radular sac, however, arises from the fore-gut and developmentally is entirely unconnected with the foot. It is only due to its increase in length that in later stages it penetrates into the foot, lying between the two pleuro-pedal ganglionic masses. The musculature of the radular sac and the so-called supporting cartilages of the odontophore is partly derived from the mesenchymatous cells of the foot.

## XII. THE SHELL-GLAND AND THE MANTLE.

The origin and development of the shell-gland as a thickened plate of tall glandular cells on the postero-dorsal surface of the embryo (Text-figs. 4 a, b; and 6 a) and the further changes it undergoes, firstly through

invagination, and secondly, through eversion and change in position, have already been fully described already.

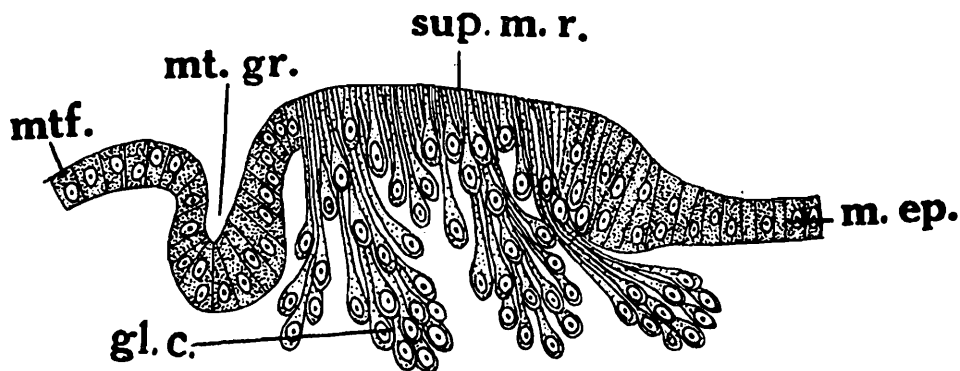
After the invagination of the shell-gland is complete there is an eversion of the gland. The cells lining the central, everted area become smaller in size and are more or less flattened, while the peripheral area, which is bounded on the outside by the mantle fold and the mantle groove, still consists of tall and columnar cells (Text-figs. 9 and 23a). This peripheral area represents the rudiment of the shell-gland of the adult.

At the time the shell gland invaginates, its margin forms a raised fold all round the gland, the fold projecting inwards towards the centre of the invagination. This is the mantle fold which, in earlier stages, is more marked anteriorly than posteriorly (Text-fig. 4 f), with the result that the mantle groove, running all round beneath the mantle fold, is also deeper anteriorly than posteriorly. In later stages, the mantle fold forms the anterior free end of the mantle (Text-figs. 10 and 11).

The rudiment of the shell is secreted first as a thin cuticular membrane from the shell gland when the latter still forms an invagination on the left side of the visceral-sac rudiment (Stage 6, Text-fig. 4 f). It forms a thin cap-like structure covering the shell-gland. It is against the inner surface of this cuticular shell that the calcareous parts of the shell (ostracum and hypostracum) are later deposited.

The rudiment of the mantle is differentiated from that part of the visceral-sac rudiment which is bounded by the mantle fold and the mantle groove at its free end, and is lined on its left side by tall columnar cells of the shell gland (Text-fig. 23a).

On the completion of torsion, the mantle comes to lie dorso-laterally, and with further growth moves forward to enclose the head-vesicle dorso-laterally (Text-fig. 5 d). During this period, the mantle fold becomes thick and the mantle groove well-defined. The central everted area of the shell-gland, which now consists of completely flattened cells, forms the outer epithelium of the visceral sac.



TEXT-FIG. 32. Sagittal section passing through the adult shell gland and the mantle groove, and showing the glandular cells of the supra-marginal ridge:  $\times 447$ .

The mantle epithelium, at the free edge of the mantle, *viz.*, the mantle fold, is lined by a single layer of cubical cells of uniform height, with a rounded nucleus in the middle of each cell. These cells continue posteriorly and line the inner epithelium of the mantle. On the outer side of the mantle, however, the mantle groove (=supra-marginal groove of Prashad, 1932), which is still sufficiently deep in Stage 11, is lined with a single row of columnar cells of varying height, the cells lining

the anterior wall of the groove being shorter in height than those which line its posterior wall. Immediately posterior to the mantle groove lies, as in earlier stages, the rudiment of the shell-gland of the adult. The latter still consists of a single layer of tall columnar and glandular cells with a centrally situated nucleus in each cell. These cells gradually diminish posteriorly in their height and merge imperceptibly into the small and flattened cells of the mantle (Text-fig. 10).

In later stages of development (Stage 12), the region of the shell-gland becomes more marked and its surface becomes raised a little above that of the rest of the mantle (Text-fig. 11). This raised area forms the rudiment of the supra-marginal ridge (Prashad, 1928). The cells constituting the supra-marginal ridge have become further differentiated. They are now taller than in the preceding stages and have sunk deep down into the subjacent connective tissue. The nuclei of these cells moving from their original central position, come to lie at the inner or ventral ends of the cells. The supra-marginal ridge is now restricted to a limited and defined area (Text-fig. 11).

With further development, the tall glandular cells of the supra-marginal ridge become swollen or rounded at their inner ends and thus appear more or less flask-shaped (Text-fig. 32). The ovoidal nuclei lie in the rounded ends of these cells, which are now arranged more or less in bundles, the individual cells being curved in their lower halves (*cf. Paludina*, Prashad, 1928). This curvature is due to lack of space, the cells being pushed sideways into the connective tissue, a view also held by Prashad.

In an embryo about to hatch, some calcareous structures are found lying imbedded in the connective tissue at the base of these flask-shaped cells. They are small refractive bodies which, according to Prashad, gradually pass into the glandular cells of the supra-marginal ridge which secretes the ostracal layers of the shell. At this time pigment is found in the outer epithelial covering of the mantle, which extends posteriorly behind the supra-marginal ridge. The pigment granules<sup>1</sup> are confined to the upper parts of the cells. It is the outer general covering of the mantle which is responsible for the secretion of the innermost or hypostacal layer of the shell.

The mantle groove, during the course of its development, does not remain unchanged. Its lumen becomes reduced on account of the encroachment of the differentiated cells of the supra-marginal ridge (Text-fig. 32).

*Previous work and discussion.*—Semper (1862) did not notice in *Ampullaria polita* the first formation of the shell-gland on the aboral surface of the embryo. The stage which he described as the "erste Anlage der Schale" is a later stage, where the shell gland has already shifted to the left side (Stage 5) and the shell secreting area is lying as an oval disc consisting of small polygonal cells. Fernando (1931) describes it as a depression on the postero-dorsal surface of a trochophore, but he does not describe its structure, though his figs. 1 and 4,

---

<sup>1</sup> I have not traced the origin of the pigment granules, but according to Distaso (in *Helix* and other *Gastropoda*, 1908) and Prashad (in the *Viviparidae*, 1928), and others, they are derived from the nuclear chromatin of the chromatophores.

Pl. I show it as being composed of polygonal cells, a condition which is also noticed in *Pila globosa* when the sections are oblique.

Scott (1934), while making no mention of the structure of the shell-gland, in *Ampullaria canaliculata*, describes it as a depression on the postero-dorsal surface of the embryo. This depression follows the same course in later development as I have described in *Pila globosa*.

In the case of those Molluscs whose cell-lineage has been completely traced, e.g. *Crepidula* (Conklin, 1897), *Planorbis* (Holmes, 1900), *Physa* (Wierzejski, 1905) *Littorina* (Delsman, 1914) and others, it has been definitely established that the shell-gland is formed by the descendants of the first somatoblast, 2nd or X, showing thereby that in all Molluscs it is derived from the same region of the developing egg. This region forms the aboral surface and extends from behind the velum to the posterior end of the embryo.

### XIII. THE MANTLE-CAVITY.

The rudiment of the mantle-cavity, first formed in the mid-ventral region of the visceral-sac rudiment, arises as an ectodermal invagination almost in the same transverse plane as the right and left rudiments of the pericardium (Text-fig. 16e). The invagination sinks deeper and forms a groove-like structure from the inner end of which the rudiments of the right and left ureters take their origin, these latter being hardly distinguishable in their earlier stages, from the rudiment of the mantle cavity.

All the stages of development of the mantle cavity, from its mid-ventral position (Stage 2) to its final definitive dorsal position (Stage 10), have already been described above (pp. 233 and 246). It was stated that by the time the mantle-cavity reaches its definitive dorsal position, it no longer remains tubular but becomes a wide and spacious cavity enclosed dorso-laterally by the mantle (Stages 10 and 11). The anus opens for the first time into the mantle-cavity just when the latter, on account of complete torsion, comes to lie on the dorsal side of the embryo, and the rudiments of the gill and the osphradium are projecting into it from the inner surface of the mantle (Stage 10).

As the embryo assumes a snail-like appearance (cf. Stage 11), an antero-posterior fold, the *epitaenia* running in an obliquely longitudinal direction, arises from the base of the mantle cavity and grows vertically upwards. It forms a septum incompletely dividing the mantle cavity into two chambers of unequal size. The larger right chamber contains the gill and the anal, excretory and genital apertures and is called the branchial chamber. The smaller left chamber is, on account of the presence in it of the osphradium and the lung, known as the pulmonary chamber.

Thus, we find that the mantle cavity in *Pila globosa* is an ectodermal structure, mid-ventral in position at the time of its origin. But as a result of dextral torsion, it is rotated through 180° to occupy its final definitive dorsal position.

*Previous work and discussion.*—According to Fernando (1931), the rudiment of the mantle cavity in *Pila gigas* arises as an ectodermal invagination which later acquires a lumen. He does not, however

specify the exact position of this rudiment. His description of the later stages of its development agrees with mine.

As regards the origin of the mantle-cavity in other Gastropoda, Erlanger (1891) describes it as an unpaired small depression on the ventral surface of the posterior part of the embryo, while Drummond (1903) and Otto and Tönniges (1905) describe a paired rudiment of the mantle-cavity, one on either side of and below the intestine, the right one being larger than the left. Drummond says that "it is only at a later stage that a portion of the body immediately in front of the anus sinks in and unites the two original depressions, thereby including the anus in the mantle-cavity" The two depressions are, according to Drummond, never symmetrical.

Delsman (1914), who studied it in *Littorina* only in the earlier stages of development, describes the rudiment of the mantle-cavity as situated in the beginning on the right side.

Amongst marine Prosobranchs, viz., *Nassa mutabilis* and *Fusus* (Bobretzky, 1877), the rudiment of the mantle-cavity, being asymmetrical, arises as a sickle-shaped ectodermal invagination on the right side of the embryo.

Amongst the Pulmonates also, the rudiment of the mantle-cavity arises either as a simple shallow depression or slit-like invagination which on deepening leads to the formation of the mantle-cavity. It arises independently of the lung, though the two are intimately connected. In *Planorbis*, according to Rabl (1875), the rudiment of the mantle-cavity originates in a different manner: it originates by the raising of the mantle-folds from the body-surface to which they are closely attached in the earlier stages, and thus a slit-like space is produced which later develops into the mantle-cavity. But in *Helix* (1880) he describes the rudiment of the mantle-cavity to be formed as an ectodermal invagination. In *Limax*, according to Meisenheimer (1898) the mantle-cavity arises by the "secondary" rolling up of the shell-folds (=mantle-folds) but Heyder (1909) considers that "the formation of the mantle-cavity is a continuous and progressive growth so that a primary formation and a secondary rolling up (as pointed out by Meisenheimer) can hardly be admitted" On this basis, Heyder regards the primary arching of the mantle-fold as the first rudiment of the mantle-cavity. Heyder (1909) describes the rudiment of the mantle-cavity in *Arion* as a groove on the right side of the embryo which, therefore, is asymmetrical.

#### XIV SUMMARY.

1. Oviposition takes place on land. Each egg is composed of a thick calcareous shell, two thin membranes (the shell-membrane and the albumen-membrane), a solid albuminous sphere, and the albuminous fluid in which the embryo floats.

2. Cleavage is total and of the spiral type, the first two divisions being equal, inequality stepping in at the third division. Only three quartettes of micromeres are segregated to give rise to the ectoderm. The macromeres give rise to the endoderm and the mesoderm.

3. The cleavage-cavity appears very early at the 2-cell stage.

4. The blastula has a large blastocoele.
5. Gastrulation is embolic.
6. The mesoderm is teloblastic in origin.
7. The blastopore persists and is transformed into the anus.
8. All the essential organs are developed during the embryonic stage, and the embryo hatches out when it fully resembles the adult in all important respects.
9. The first rudiment of the alimentary canal is the endodermal archenteron from the anterior blind end of which the stomach and the digestive gland arise, while its posterior part gives rise to the intestine. In early stages, folds arise internally from the wall of the primitive stomach for the assimilation of the ingested albumen ; as development proceeds these folds are absorbed.
10. The stomodaeum or fore-gut is ectodermal and is formed by invagination. Later, the stomodaeum becomes differentiated into an anterior part including (i) the buccal-cavity, and (ii) a posterior part including the oesophagus. The radular sac, the sub-lingual cavities, the oesophageal pouches and the salivary glands all arise from the anterior part.
11. The radular sac arises as a mid-ventral outpushing from the floor of the stomodaeum, the basal membrane being secreted by the basal epithelium of the sac. The radular teeth are secreted by the odontoblasts which are many in number and are differentiated at the blind distal end of the radular sac. There are only seven teeth in each cross row. The lateral pair of teeth are the first to be differentiated. These are followed by the marginal pairs, while the median tooth is the last to be differentiated. The dorsal epithelium of the sac forms cell-complexes projecting in between the apices of the teeth.
12. The salivary glands evaginate from the postero-dorso-lateral walls of the buccal-cavity as simple tubular structures which become branched subsequently.
13. The digestive-gland evaginates from two rudiments. Of these, the anterior one is differentiated from the cells lining the walls of the primitive stomach lying within the head-vesicle, while the posterior is formed later from the posterior end of the primitive stomach and lies within the visceral-sac rudiment and develops into the digestive-gland of the adult. The anterior rudiment is absorbed in the later stages of development.
14. The stomach is differentiated from the right ventro-lateral side of the posterior end of the primitive stomach.
15. A common rudiment for the kidney, the pericardium, the heart, and the gonad is differentiated in the form of two ventrally situated mesodermal cell-masses, one on either side of the intestine in the visceral-sac rudiment. A lumen appears in each cell-mass (representing the coelom) giving rise to the rudiments of the pericardium, etc. The two rudiments, meeting together, fuse to form a single structure divided by a septum which subsequently disappears.
16. The rudiments of the right and left kidneys arise in the form of two thickenings of the postero-ventral walls of the right and left pericardial cavities. The right one evaginates and forms a sac-like

structure which subsequently becomes lamellar and develops into the definitive (left) functional kidney of the adult.

17 The left kidney rudiment persists as a rudimentary structure and takes part in the formation of the genital duct.

18. The ureter (=anterior kidney) evaginates from the inner end of the mantle-cavity as a tubular structure and is thus ectodermal in origin. It develops, later, into the lamellar organ of the adult.

19. The efferent duct of the left rudimentary kidney also evaginates from the inner left end of the mantle-cavity and remains rudimentary, to be transformed later into the genital duct.

20. The heart arises as an unpaired thickening of the pericardial wall which invaginates later and forms a tubular organ. This becomes constricted in the middle to give rise to the auricle and the ventricle.

21. The blood-vessels arise independently of the heart and originate in sinuses in the body-cavity, enclosed by mesenchyme cells.

22. The gonad is differentiated from the roof of the pericardium as an unpaired thickening. It lies very close and dorsally to the left kidney rudiment with which it fuses to communicate with the mantle-cavity through the rudimentary efferent duct of the left kidney.

23. The gill arises as an unpaired rudiment in the form of a series of outpushings from the right or future inner wall of the mantle rudiment just dorsally to the right of the pericardium.

24. The lung originates as an invagination of the mantle epithelium between the rudiments of the gill and the osphradium. It is neither an invaginated gill-filament nor is it homologous with the lung of the Pulmonates.

25. The nervous system is ectodermal in origin. All the ganglia arise separately as ectodermal thickenings which are delaminated and only become connected later through commissures and connectives.

(a) The cerebral ganglia arise from "head plates" (=the "Scheitel plattes") of the German authors, and "Sinnesplatte" of Schmidt.

(b) The pedal and pleural ganglia arise as separate rudiments from the lateral walls of the "Kopffuss," more or less ventrally to the place for the origin of the statocysts.

(c) The buccal ganglia arise from the stomodaeum close to the region of evagination of the radular sac.

(d) The intestinal ganglia are symmetrical in the beginning and arise ventro-laterally at the place where the "Kopffuss" and the visceral-sac rudiment meet.

(e) The visceral ganglion is unpaired and delaminates from the right wall of the visceral-sac rudiment, and is situated dorsally to the level of the pericardium. It originates much earlier than the differentiation of the mantle rudiment.

26. The statocysts arise at first as flat, plate-like thickenings which invaginate deeply and get detached from the overlying epithelium of the foot. They originate much later than the pedal and pleural ganglia.

27 The eyes arise as invaginations in the region of the head plates, which become detached from the over-lying epithelium to form the optic vesicles. The lens in each is secreted by the walls of the vesicle.

28. The tentacles originate as outpushings of the "Scheitel plattes"

29. The osphradium arises as an outpushing of the inner wall of the mantle rudiment, close to and almost in the same plane as the rudiments of the gill and the lung.

30. The mantle-cavity arises as a single tubular ectodermal invagination of the visceral-sac rudiment, immediately below the mesodermal cell-masses. The anus, which at first lies posterior to it, opens into it when torsion is complete.

31. The foot arises as a mid-ventral protrusion from the post-velar area. The pedal gland is formed by an invagination of the outer epithelium at the anterior end of the foot. The other glands found within the foot are also of epithelial origin. The operculigenous lobe is demarcated at the postero-dorsal surface of the foot, the operculum being secreted from its dorsal surface.

32. The shell gland arises as an ectodermal thickening at the aboral pole. It first invaginates and later gets everted. During eversion, while the cells of the central region of the everted shell gland become thin and flattened to form the outer epithelium of the visceral sac, its marginal cells remain tall and columnar and give rise subsequently to the shell-gland (=supra-marginal ridge) of the adult.

#### XV EXPLANATION OF LETTERING IN TEXT-FIGURES.

*a.*, Anus. *an. c. pl.*, Anal cell-plate. *ant.*, Anterior end. *a.p.*, Animal pole. *ao.*, Aorta. *ap. c. pl.*, Apical cell-plate. *arch.*, Archenteron (or primitive stomach). *arch. c.*, archenteric cavity. *arch. f.*, Archenteric folds. *au.*, Auricle. *au. vent. ap.*, Auriculo-ventricular aperture. *bas. m.*, Basal membrane. *b. c.*, Buccal cavity. *b. ep.*, Basal epithelium. *b.g.*, Buccal ganglion. *b. gl.*, Buccal gland. *bl.*, Blastopore. *c. b. cn.*, Cerebro-buccal connective. *c. cm.*, Cerebral commissure. *c. g.*, Cerebral ganglion. *cl. c.*, Cleavage cavity. *coe.*, Coelom. *col. musc.*, Columellar muscle. *c. p. cn.*, Cerebro-pedal connective. *c. pl. cn.*, Cerebro-pleural connective. *ct.*, Gill. *ct. f.*, Gill filament. *d. ep.*, Dorsal epithelium. *d. gl.*, Digestive gland. *dr.*, Dorsal side. *e.*, Eye. *ect.*, Ectoderm. *end.*, Endoderm. *f.*, Foot. *f. ep.*, Foot-epithelium. *g.*, gonad. *gl. c.*, Gland cells. *h.*, Heart. *h. ves.*, Head-vesicle. *i. c.*, Inner cornea. *i. i. cn.*, Infra-intestinal connective. *i. i. g.*, Infra-intestinal ganglion. *i. i. n.*, Infra-intestinal nerve. *int.*, Intestine. *int. g.*, Intestinal ganglion. *k.*, Right kidney. *k.*, Left (rudimentary) kidney. *l.*, Lung. *lens.*, Lens of eye. *l. h.*, Larval heart. *l. o.*, Opening of the lung. *lp.*, Lip. *l. r.*, Lung rudiment. *l. r. o.*, Opening of the lung rudiment. *lt. mes. bd.*, left mesoblastic band. *m.*, Mouth. *m. c.*, Mantle-cavity. *mes.*, Mesoderm. *mes. bd.*, mesoblastic band. *mesch.*, Mesenchyme cells. *mt.*, Mantle. *mtf.*, Mantle fold. *mt. gr.*, Mantle groove. *n.*, Nerve. *o. c.*, Outer cornea. *od.*, Odontoblasts. *oes.*, Oesophagus. *op.*, Operculum. *op. l.* Operculigenous lobe. *op. n.*, Optic nerve. *os.*, Osphradium. *os. n.*, Osphradial nerve. *p.*, Right pericardium. *φ.* Left pericardium. *p. cm.*,<sup>1</sup> First pedal commissure. *p. cm.*,<sup>2</sup> Second pedal commissure. *p. c. pl.*, Pedal cell-plate. *p. g.*, Pedal ganglion. *p. gl.*, Pedal gland. *p. gr.*, Pedal groove. *pl. g.*, Pleural ganglion. *pl. p. g.*, Pleuro-pedal ganglionic mass. *post.*, Posterior end. *p. r.*, Pericardial rudiment. *pr. st.*, Primitive stomach (or archenteron). *pr. tel.*, primary teloblasts or primary mesodermal cells. *p. v.*, Pulmonary vein. *q.*, Nuchal cells. *rad.*, Radula. *rad. s.*, Radular sac. *rad. t.*, Radular teeth. *ret.*, Retina. *r. p. o.*, Reno-pericardial aperture of the right kidney. *r.<sup>1</sup> p. o.*, Reno-pericardial aperture of the left kidney. *rt. mes. bd.*, right mesoblastic band. *Sch. pl.*, Head plate or "Scheitelplate." *seg. c.*, Segmentation cavity. *sept.*, Septum. *sh.*, Shell. *sh. gl.*, Shell gland. *s. i. g.*, Supra-intestinal ganglion. *s. i. n.*, Supra-intestinal nerve. *sinus.*, Blood sinus. *s. l. c.*, Sub-lingual cavity. *sl. gl.*, Salivary gland. *sl. gl. o.*, Opening of the salivary gland. *s. oes. p.*, Sub-oesophageal pouch. *'s. rad. or.*, Sub-radular organ. *st.*, Stomach. *stat.*, Statocyst. *stomod.*, Stomodaeum. *str.*, Streak. *sup. m. r.*, Supra-marginal ridge. *t.*, Tentacle. *t. n.*, Tentacular nerve. *ur.*, Ureter of the Right kidney. *ur.*, Ureter of the left kidney. *v.*, Velum. *vac.*, Vacuoles. *vent.*, Ventricle. *vest.*, Vestibule. *v. g.*, Visceral ganglion. *v. h.*, Visceral hump. *v. m.*, Visceral sac. *v. p.*, Vegetable pole. *v. t.*, Ventral side.

## XVI. REFERENCES.

- Aeberhardt, B., 1905.—*Mitt. nat. Ges. Bern.*  
 Anderson, K., 1924.—*Morph. Jahrb.* LIV  
 Andrews, E. A., 1933.—*Nautilus* XLVI.  
 Annandale, N., 1920.—*Journ. Nat. Hist. Soc. Siam* IV  
 Annandale, N., 1921.—*Rec. Ind. Mus.* XXII.  
 Bahl, K. N., 1928.—*Mem. Ind. Mus.* IX.  
 Balfour, F. M., 1881.—*A Treatise on Comparative Embryology.*  
 London.  
 Barr, R. A., 1928.—*Quart. Journ. Micr. Sci.* LXXI.  
 Behme, Th., 1889.—*Arch. Naturgesch.* LV  
 Biedermann, W., 1902.—*Jena Zeit. Naturwiss.* XXXVI.  
 Bloch, J., 1896.—*Jena. Zeit. Naturwiss.* XXXI.  
 Blochmann, F., 1882.—*Zeit. wiss. Zool.* XXXVI.  
 Blochmann, F., 1883.—*Zeit. wiss. Zool.* XXXVIII.  
 Bobretzky, N., 1877.—*Arch. Mikr. Anat.* XIII.  
 Boutan, A., 1886.—*Arch. Zool. Exp.* (2) III.  
 Bouvier, E. L., 1888.—*Mem. Soc. Philom. Paris, Cent.*  
 Braun, M., 1888.—*Nachrichtsbl. deutsch. Malakozool.* XX.  
 Brock, J., 1886.—*Zeit. wiss. Zool.* XLIV  
 Brooks, W. K., 1878.—*Johns Hopkins Univ. Sci. Results.*  
 Brooks, W. K. and McGlone, B., 1908.—*Carnegie Inst. Pub.*, No. 102.  
 Bronn, H. G., 1928.—*Klassen und Ordnungen des Tier-reiches, Mollusca,*  
*Gastropoda*, III.  
 Buchner, O., 1892.—*Jahr. Ver. vaterl. Naturk. Württemb.* XLVIII.  
 Burkhardt, Fr., 1916.—*Das Körperepithel von Helix pomatia.* Marburg.  
 Burne, R. H., 1898.—*Proc. Malacol. Soc. Lond.* III.  
 Bütschli, A., 1877.—*Zeit. wiss. Zool.* XXIX.  
 Bütschli, A., 1887.—*Morph. Jahrb.* XII.  
 Carazzi, D., 1900.—*Anat. Anz.* XVII.  
 Carazzi, D., 1905.—*Arch. Ital. Anat. Emb. Firenze.* IV  
 Casteel, D. B., 1904.—*Proc. Acad. Nat. Sci. Philadel.* LVI.  
 Conklin, E. G., 1891.—*Johns Hopkins Univ. Circ.* X, No. 88.  
 Conklin, E. G., 1897.—*Journ. Morph.* XIII.  
 Conklin, E. G., 1907.—*Journ. Acad. Nat. Sci. Philadel.*  
 Crampton, H. F., 1894.—*Ann. New York. Acad. Sci.* VIII.  
 Crampton, H. F., 1896.—*Arch. Ent. Mech.* III.  
 Dautert, E., 1908.—*Zool. Jahrb. (Anat.)* L.  
 Delsman, H. C., 1914.—*Tijdschr. Nedrl. Dierk. Ver.* (2) XIII.  
 Deschamps, A., 1910.—*Ann. Soc. Sci., Bruxelles* XXVI.  
 Distaso, J. M., 1908.—*Biol. Centrabl.* XXVIII.  
 Eisig, H., 1869.—*Zeit. wiss. Zool.* XIX.  
 Erlanger, R. V., 1891.—*Morph. Jahrb.* XVII.  
 Erlanger, R. V., 1892.—*Quart. Journ. Micr. Sci.* XXXIII.  
 Erlanger, R. V., 1892.—*Mitt. Zool. Stat. Neapel* X.  
 Erlanger, R. V., 1894.—*Morph. Jahrb.* XXII.  
 Fernando, W., 1931a.—*Proc. Zool. Soc. Lond.*  
 Fernando, W., 1931b.—*Proc. Roy. Soc. Lond.* CVII.  
 Fernando, W., 1931c.—*Proc. Roy. Soc. Lond.* CVII.  
 Fol, H., 1875.—*Arch. Zool. Exp.* IV.

- Fol, H., 1876.—*Arch. Zool. Exp.* V  
 Fol, H., 1879-80.—*Arch. Zool. Exp.* VII.  
 Flossner, W., 1915.—*Zeit. wiss. Zool.* CXIII.  
 Fleischmann, A., 1932.—*Zeit. Morph. Ökol. Tiere.* XXV.  
 Fujita, T., 1895.—*Zool. Mag., Tokyo.* VII, No. 79.  
 Giese, M., 1915.—*Zeit. wiss. Zool.* CXIV  
 Gilchrist, J. D. F., 1895.—*Proc. Roy. Soc. Edin.* XX.  
 Goette, A., 1896.—*Ver. deutsch. Zool. Ges.* VI.  
 Goodrich, E. S., 1895.—*Quart. Journ. Micr. Soc.* XXXVII.  
 Gosse, P. H., 1851.—*A Naturalist's Sojourn in Jamaica.* London.  
 Grobhen, C., 1899.—*Arb. Zool. Inst. Wien.* XII.  
 Haddon, A. C., 1882.—*Quart. Journ. Micr. Sci.* XXII.  
 Haeth, H., 1916.—*Proc. Acad. Nat. Sci. Philadel.* LXVIII.  
 Hägler, K., 1923.—*Acta Zoologica* IV  
 Haldane, J. B. S., 1931.—*Nature* CXXVI.  
 Haller, B., 1895.—*Morph. Jahrb.* XI.  
 Hatschek, B., 1880.—*Arb. Zool. Inst. Wien* III.  
 Henchman, A. P., 1890.—*Bull. Mus. Comp. Zool. Harvard* XX.  
 Herbers, K., 1914.—*Zeit. wiss. Zool.* CVIII.  
 Herfs, A., 1922.—*Zool. Jahrb. (Anat.)* XLIII.  
 Herfs, A., 1922.—*Arch. Mikr. Anat.* XCVI.  
 Heyder, P., 1909.—*Zeit. wiss. Zool.* XCIII.  
 Heymons, R., 1893.—*Zeit. wiss. Zool.* LVI.  
 Hilger, C., 1885.—*Morph. Jahrb.* X.  
 Hoffmann, H., 1922.—*Zeit. wiss. Zool.* CIX.  
 Hoffmann, H., 1932.—*Jena. Zeit. Naturwiss.* LXVII.  
 Holmes, S. J., 1900.—*Biol. Bull.* I.  
 Holmes, S. J., 1900.—*Journ. Morph.* XVI.  
 Ihering, H. V., 1875.—*Jena. Zeit. Naturwiss.* IX.  
 Jourdain, S., 1884.—*Compt. Rend. Acad. Sci. Paris.* XCVIII.  
 Joyeus-Laffuie, J., 1882.—*Arch. Zool. Exp.* X.  
 Kessel, E., 1933.—*Zeit. Morph. Ökol. Tiere.* XXVII.  
 Klotz, J., 1889.—*Jena. Zeit. Naturwiss.* XXIII.  
 Knipowitsch, N., 1891.—*Biol. Centralbl.* XI.  
 Kofoid, C. A., 1895.—*Bull. Mus. Comp. Zool. Harvard* XXVII.  
 Korschelt, E., 1936.—*Lehr. Vergl. Entwickl. Tiere*, I, II.  
 Langerhans, P., 1873.—*Zeit. wiss. Zool.* XXIII.  
 Lankester, E. R., 1874.—*Quart. Journ. Micr. Sci.* XIV  
 Lankester, E. R., 1875a.—*Phil. Trans. Roy. Soc. Lond.* CLXV  
 Lankester, E. R., 1875b.—*Quart. Journ. Micr. Sci.* XV  
 Lankester, E. R., 1876.—*Quart. Journ. Micr. Sci.* XVI.  
 Lankester, E. R., 1881.—*Ann. Mag. Nat. Hist.* (5) VII.  
 Lereboullet, A., 1862.—*Ann. Sci. Nat. (Zool.)* (4) XVIII.  
 Leydig, F., 1850.—*Zeit. wiss. Zool.* II.  
 Lillie, F. R., 1895.—*Journ. Morph.* X.  
 List, H. J., 1887.—*Zeit. wiss. Zool.* XLV  
 Lubosch, W., 1931.—*Morph. Jahrb.* LXVI.  
 MacBride, E. W., 1895.—*Quart. Journ. Micr. Sci.* XXXVII,  
 MacBride, E. W., 1915.—*Text-Book of Embryology* I,  
 London,

- MacBride, E. W., 1931a.—*Nature* CXXV  
 MacBride, E. W., 1931b.—*Nature* CXXVI.  
 Manfredi, L., 1882.—*Atti. R. Accad. Sci. Napoli* IX.  
 Matthes, W., 1914-15.—*Jena. Zeit. Naturwiss.* LIII.  
 Meisenheimer, J., 1897-98.—*Zeit. wiss. Zool.* LXII, LXIII.  
 Meisenheimer, J., 1899.—Entwicklungsgeschichte von *Dreissensia polymorpha* Pall. I. Bis zur Ausbildung der Jungen Trochophoralarve. Habit-Schrift, Marburg.  
 Meisenheimer, J., 1901a.—*Zeit. wiss. Zool.* LXIX.  
 Meisenheimer, J., 1901b.—*Zeit. wiss. Zool.* LXIX.  
 Meisenheimer, J., 1921.—Geschlecht und Geschlechter in Tierreichs. Jena.  
 Meisenheimer, J., 1932.—Ontogenie. In *Hivb. Naturwiss.* 2 Aufl. VII.  
 Meuron, P., de 1884.—*Compt. Rend. Acad. Sci. Paris* XCVIII.  
 Naef, A., 1913.—*Ergeb. u. Forts. Zool.* III.  
 Naef, A., 1926.—*Ergeb. u. Forts. Zool.* IV  
 Neumann, G., 1928.—*Jena. Zeit. Naturwiss.* LXX.  
 Osborn, H. L., 1884.—*Stud. Biol. Lab. Johns. Hopkins Univ.* III.  
 Otto, A. and Tönniges, C., 1905.—*Zeit. wiss. Zool.* LXXX.  
 Pabst, H., 1914.—*Zool. Jahrb. (Anat.)* XXXVIII.  
 Patten, W., 1885.—*Arab. Zool. Inst. Wien* VI.  
 Pelseneer, P., 1896.—*Zool. Anz.* XIX.  
 Pelseneer, P., 1907.—Mollusca. In *Lankester's A Treatise on Zool.* V London.  
 Pelseneer, P., 1911.—*Mem. Acad. Sci. Belg. (2)* III.  
 Perrier, R., 1889.—*Ann. Sci. Nat. (Zool.)* (7) VIII.  
 Plate, L., 1895.—*Zool. Jahrb. (Morph.)* IX.  
 Poetzsch, O., 1904.—*Zool. Jahrb. (Morph.)* XX.  
 Portmann, A., 1925.—*Zeit. Morph. Ökol. Tiere* III.  
 Portmann, A., 1927.—*Naturforscher*, IV  
 Portmann, A., 1932.—*Ver. Schweiz. nat. Ges.* CXIII.  
 Prashad, B., 1925.—*Mem. Ind. Mus.* VIII.  
 Prashad, B., 1928.—*Mem. Ind. Mus.* VIII.  
 Prashad, B., 1932.—*Pila*. In *Ind. Zool. Mem.*, No. 4, Lucknow.  
 Rabl, C., 1875.—*Jena. Zeit. Naturwiss.* IX.  
 Rabl, C., 1879.—*Morph. Jahrb.* V  
 Rabl, C., 1883.—*Sitz. k. Akad. wiss. Wien.* LXXXVI.  
 Ramanan, V V.—[On the habits of some non-marine Molluscs of Madras. Ms in Connemara Library, Madras.]  
 Randles, W B., 1905.—*Quart. Journ. Micr. Sci.* XLVIII.  
 Robert, A., 1903.—*Arch. Zool. Exp.* X.  
 Rossler, R., 1885.—*Zeit. wiss. Zool.* XLI.  
 Rottmann, G., 1901.—*Zeit. wiss. Zool.* LXX.  
 Roth, H., 1929.—*Zeit. wiss. Zool.* CXXXV  
 Ryder, J. A., 1889.—*Amer. Nat.* XXIII.  
 Sachwatkin, V., 1920.—*Acta. Zoologica* I.  
 Sachwatkin, V., 1926.—*Mem. Univ. Etat. Extreme Orient., Vladivostok* I,  
 Salensky, W., 1872.—*Zeit. wiss. Zool.* XXII.  
 Salensky, W., 1883.—*Arb. Zool. Inst., Wurzburg* VI,  
 Salensky, W., 1887.—*Arch. Biol.* VI,

- Saunders, A. M. C. and Poole, M., 1910.—*Quart. Journ. Micr. Sci.* LV
- Sarasin, P. & F., 1887.—*Zool. Anz.* X.
- Schimkewitsch, W., 1888.—*Zool. Anz.* XI.
- Schiemenz, P., 1888.—*Biol. Centralbl.* VII.
- Schmidt, F., 1889.—*Sitz. nat. Ges. Univ. Dorpat* VIII.
- Schmidt, F., 1891.—*Sitz. nat. Ges. Univ. Dorpat* VIII.
- Schmidt, F., 1894.—*Zool. Jahrb. (Anat.)* VII.
- Scott, M. I. H., 1934.—*Rev. Mus. La Plata* XXXIV
- Semper, C., 1862.—*Entwicklungsgeschichte der Ampullaria polita*  
Deshayes. Utrecht.
- Semper, C., 1887.—*Arb. Zool. Inst. Wurzburg.* VIII.
- Simroth, H., 1876.—*Zeit. wiss. Zool.* XXVI.
- Simroth, H., 1896-1907.—Gastropoda, in *Bronn's Kl. u. Ord. Tier.*  
3 Bd., Mollusca, II Abt.
- Simroth, H., 1898.—*Biol. Centralbl.* XVIII.
- Soos, L., 1930.—*Zeit. Morph. Ökol. Tiere* XIX.
- Soos, L., 1930.—*Allatt. Koglem.* XXVII.
- Spek, J., 1921.—*Zeit. wiss. Zool.* CXVIII.
- Spengel, J. W., 1881.—*Zeit. wiss. Zool.* XXXV
- Teschow, G., 1911a.—*Arch. Entw. Mech.* XXXI.
- Teschow, G., 1911b.—*Arch. Entw. Mech.* XXXI.
- Thiele, J., 1901.—*Arch. Naturgesch.* LXVII.
- Thiele, J., 1901.—*Biol. Centralbl.* XXI.
- Thiele, J., 1902.—*Zool. Anz.* XXV
- Tönniges, C., 1896.—*Zeit. wiss. Zool.* LXI.
- Tönniges, C., 1899.—*Sitz. Ges. Naturw. Marburg.*
- Vogt, W., 1926.—*Sitz. Ges. Morph. Phys. München* XXXVII.
- Warneck, A., 1850.—*Bull. Soc. Imp. Nat. Moscou* XXIII.
- Wierzejski, A., 1905.—*Zeit. wiss. Zool.* LXXXIII.
- Wilson, E. B., 1904.—*Journ. Exp. Zool.* I.
- Wolfson, W., 1880.—*Bull. Acad. Imp. Sci. St. Petersbourg* XXVI.
- Woodward, M. F., 1901.—*Quart. Journ. Micr. Sci.* XLIV
- Ziegler, H. E., 1885.—*Zeit. wiss. Zool.* XLI.
- Ziegler, H. E., 1912.—Leibeshöhle in *Handwörterb. d. Naturwiss.* II,  
V Lief. Jena.
- Zill, R., 1924.—*Zeit. Anat. u. Entw.* LXXI.