Transactions of the Royal Society of New Zealand
Vol. 75, Part 4, pp. 419–464, 20 Figs.

The Embryology of the Viviparous Ophiuroid *Amphipholis squamata* Delle Chiaje.*

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[*Read before Wellington Branch, May 2, 1945; received by the Editor, May 3, 1945; issued separately, March, 1946.*]

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Introduction.

In a previous paper a general account was given of the embryology of a New Zealand ophiuroid having absolutely direct development, lacking any vestige of a larval form, and in which the coelom arose as a result of splitting in mesenchyme (Fell, 1941). For reasons stated there, it became desirable that a reinvestigation of the embryology of *Amphipholis squamata* should be made. The research described in the present paper was carried out from 1939 to 1941 at the Department of Zoology in the University of Edinburgh, with the aid of a Shirtcliffe Fellowship grant from the University of New Zealand. Publication has been delayed owing to the absence on war service of the writer.

The work was carried out under the direction of Professor James Ritchie, of the University of Edinburgh, to whom I am much indebted for advice and for the fine facilities of his department. To Professor H. B. Kirk, University of New Zealand, I owe the original suggestion

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* Assistance in the cost of printing this paper was given from the Publications Fund of Victoria University College.

that the problem of direct development in ophiuroids should be investigated. I have also to thank Dr. Fabius Gross, of Edinburgh, for advice on the use of Erdschreiber culture medium for excised embryos, and also the Directors of Marine Laboratories at Millport and Plymouth for supplies of living and preserved material, to supplement that collected in the Firth of Forth and at Island Bay, New Zealand. The terminology employed for the germ layers is that suggested by Professor E. S. Goodrich, of Oxford, and employed by the writer in previous papers. Points of interest are discussed as they arise during the course of the paper, instead of leaving a general dicussion to the end.

NOMENCLATURE.

From examination of the literature, it appears that *Amphipholis squamata* has received no less than eighteen different names during the last one hundred and thirty years. Leach (1815) first described the species under the name *Ophiura elegans.* In 1828 it received the specific name which it now bears, being named *Asterias squamata* by Delle Chiaje. In succeeding years it became *Ophiura neglecta*—under which name it is recorded by Forbes (1841) as occurring at Newhaven " in the Frith of Forth." In 1842 it received the name *Ophiolepis squamata,* and under this designation the first embryological papers on the species appeared, by Milne-Edwards, Krohn, and Schultze (see below). In 1861 Sars gave the ophiuroid the name *Amphiura squamata,* which it was destined to bear for the remainder of the century. More recently it has been separated from the genus *Amphiura,* and it therefore reverts to the generic name *Amphipholis* used by Ljungman in 1872, still retaining the specific name *squamata* of Delle Chiaje. Under this name it appears in Mortensen's " Echinoderms of the British Isles " (1927). It was during the period when it was known as *Amphiura squamata* that most previous work was done on its development. The other names the species has borne are not of importance for the present purpose, for they have not been used in any embryological papers.

HISTORICAL SUMMARY.

The accounts dealing with developmental stages of *Amphipholis squamata* present what must be one of the most confusing series of incompatible statements in the whole of echinoderm embryological literature. From the first it became apparent that its development was quite unlike that of other ophiuroids. Research on its embryology was initiated in 1842 when Quatrefages discovered that the species is viviparous. He communicated his result to Milne-Edwards, who recorded it in a paper. In 1851 Krohn discovered the existence during part of the development of an embryonic attachment to the parent (or " Nabelschnur "), and in the same year Schultze discovered the transitory larval skeleton. Thereafter a series of papers appeared on the embryology of the species. The following authors gave accounts dealing with phases of its development: Metschnikoff (1869), Balfour (1881), Ludwig (1881), Apostolides (1882), Fewkes (1887), Carpenter (1887), Hamann (1889), Cuenot (1891), Russo (1891), MacBride (1892), and Dawydoff (1901).

With the close of the nineteenth century research on the subject came to an end, leaving a series of accounts so conflicting not only with what was then known of other ophiuroids, but also with one another, that MacBride in 1914 made only a passing reference to the species in his "Text-book of Embryology." He quotes, however, Ludwig's work on the development of the skeleton, and his own work on the late development of the ovoid gland and related structures; but as the findings of the previous workers, such as Apostolides (1882) and Russo (1891) differed widely from what had been found for *Ophiothrix fragilis,* he regarded their statements as "improbable in the highest degree."

In order to bring out more clearly the questions most in dispute, it is preferable to give a comparative table of previous results under subject-headings, rather than a purely chronological list of workers and their findings. This is set out as follows:—

1. VIVIPARITY.

1842. Quatrefages discovered the viviparity of the species.
1881. Balfour attributed the reduction of the larva to the influence of the viviparous habit.

2. HERMAPHRODITISM.

1869. Metschnikoff demonstrated that the species is hermaphrodite.

3. EMBRYONIC ATTACHMENT.

1851. Krohn discovered the temporary embryonic attachment of the larva to the parent. He named it the "umbilical cord" (Nabelschnur).
1869. Metschnikoff confirmed Krohn's observation.
1881. Balfour observed the attachment on an excised larva, but failed to realise its true nature, and supposed it to be some larval structure connected with the vestigial skeleton.
1887. Fewkes observed and figured the embryonic attachment, but did not examine its structure.
1891. Russo denied that any such organ existed. In one part of his paper he stated that the embryo is held in place only by a "pocketing" (insaccatura) of the wall of the bursa; elsewhere in the same paper he stated that the embryo is fixed to the parent by a "kind of cement."
1892. MacBride, studying only certain very late phases of development, after metamorphosis, does not mention having observed any attachment to the parent.

4. GASTRULATION.

1869. Metschnikoff did not observe the process, but he assumed that gastrulation would take place by means of invagination.
1882. Apostolides stated that gastrulation occurred by delamination, but gave neither description nor figures of the supposed process.
1887. Fewkes did not observe gastrulation, but the obscure nature of the gastrula led him to express his opinion that delamination might possibly occur "in view of the possibly abbreviated nature of the development."
1891. Russo definitely stated that delamination occurs, and gave a series of figures supposedly illustrating the process. The latter are highly diagrammatic, and very unconvincing.
1914. MacBride, commenting on Russo's claim, stated "such statements are improbable in the highest degree"—but gave no personal observations.

5. LARVAL OESOPHAGUS.

1869. Metschnikoff observed the oesophageal sac, and (erroneously as shown in this paper) figured it as if opening to the exterior.
1891. Russo repeated Metschnikoff's error in stating that the oesophagus opens to the exterior, and, furthermore, stated that the young larva feeds upon the tissues of the maternal bursa by contractions of the oesophagus.

6. LARVAL ANUS.

1869. Metschnikoff discovered that the larva has no anal opening.

1881. Balfour attributed the loss of the anal opening to the effect of viviparity.

1891. Russo stated that there is an anus, and gave figures in which an anal opening is represented.

7. RUDIMENTARY LARVAL SKELETON.

1851. Schultze discovered the existence of a reduced larval skeleton.

1869. Metschnikoff gave a figure of it (a very inaccurate one).

1887. Fewkes gave a more satisfactory figure of the larval skeleton, and described it.

1901. Ludwig, ignoring Fewkes' work, reproduced Metschnikoff's inaccurate figure in Bronn's "Thier-Reichs."

1928. Metschnikoff's figure was again reproduced in Dawydoff's "Traité d'Embryologie Comparée."

8. THE COELOMIC VESICLES.

1869. Metschnikoff described two thick-walled bodies seen by him in a young larva, on either side of the archenteron, but he did not trace their origin. He then described an older larva in which each thick-walled vesicle had divided into two, an anterior and a posterior vesicle on each side thus being formed. He further stated that the left anterior body always, and the right anterior body sometimes, give rise to a five-lobed hydrocoel, on their respective sides.

1891. Russo observed the two vesicles seen by Metschnikoff on either side of the archenteron in the young larva; but he stated that only the left anterior vesicle in the later larva gave rise to a hydrocoel, the right anterior vesicle entirely disappearing. According to him, the two posterior vesicles give rise to the perihaemal coelom ("sistema circolotare").

9. PERIVISCERAL COELOM.

1891. Russo stated that it arose late in development as a series of splits in a mesenchyme mass.

1914. MacBride discounted Russo's claim as "improbable in the highest degree"—but gave no additional information, nor did he disprove Russo's account.

10. EPINEURAL AND PERIHAEMAL COELOMS.

1889. Hamann discovered the schizocoelous origin during development of the perihaemal and epineural sinuses (coeloms).

1891. Cuenot, on the other hand, stated that the epineural sinuses arise by the inturning of "gutters" of ectoderm from the ventral surface of the arm, as had been found previously in some echinids.

1891. Russo gave yet another origin for the perihaemal coelom, stating that it arises from the original left posterior coelomic vesicle.

1901. Hamann's original account of the schizocoelous origin of the structures was quoted by Ludwig and Hamann in Bronn's "Thier-Reichs."

1901. Dawydoff, studying the process of regeneration of ophiuroid arms, found that the epineural sinuses arise schizocoelously.

1903. Delage and Herouard rejected the account of a schizocoelous origin of the structures given by Hamann and Ludwig, and considered Dawydoff's results of little significance. This surprising attitude was supported by no proofs whatever.

1914. MacBride, similarly, without offering any proof or personal observation, rejected as highly improbable the notion that coelomic structures could arise schizocoelously in any echinoderm.

11. NERVOUS SYSTEM.

1891. Cuenot stated that the nervous system arises through the inturning of gutters of ectoderm from the ventral surface of the arms.

1891. Russo, on the other hand, stated that the nervous system takes its origin from "four yellow cells" seen by him in an unstained living embryo on either side of the stomodaeum.

1892. MacBride pointed out the improbability of Cuenot's account, in view of the fact that the nervous system is already deep-seated before any arms are developed. He himself, however, did not offer any alternative account.

12. Nutrition of the Embryo.

1851. Krohn supposed that the embryonic attachment (called by him the "umbilical cord"), had a nutritive function.

1869. Metschnikoff appears not to have contested this belief.

1891. Russo denied that any embryonic attachment to the parent exists. He stated that the embryo actually obtained its nutrition by feeding upon the cells of the lining of the bursa of the parent. He stated that this food was seen by him to be drawn into the stomach of the larva by means of contractions of the oesophagus. (The latter organ, as mentioned above, was supposed by Russo and his predecessors to open to the exterior.)

13. Skeletal Rudiments of the Radial Form.

1881. Ludwig gave a general account of the origin of the skeletal platelets, homologising them with those of Asteroids. His work remains a classic of embryology.

1887. Fewkes confirmed and added to Ludwig's account.

1887. Carpenter attempted to draw a homology between the skeletal plates of the young *Amphipholis* and those of crinoids.

1914. MacBride accepted without question Ludwig's account of the origin of the skeleton, and quoted it in his "Text-book of Embryology."

From the above historical review, it will readily be seen that with the single exception of the skeletal system, very serious conflict exists between the various accounts. It is to be noted that the results which brought upon their originators the greatest criticism were particularly those in which a schizocoelous development of parts of the coelom was described. These latter results, indeed, came to be so completely forgotten that modern text-books almost without exception* omit all reference to any but enterocoelous origins during development of the coelomic spaces of echinoderms.

As a result of this attitude of current text-books, it was with very considerable surprise that I found a schizocoelous coelom in a New Zealand ophiuroid* in 1936. The results of a study of the New Zealand species led to an examination of the older literature, and brought to light the claims of Hamann and Russo, summarised above. In my recent paper on the development of Kirk's ophiuroid (Fell, 1941) I stressed the need for reinvestigating the development of *Amphipholis squamata,* and thus the work described in the present paper came to be done. I should also note here that since the publication of the account of Kirk's ophiuroid, I have found that in the Zealand ophiuroid† in 1936. The results of a study of the New also arises as a mass of mesenchyme in which small clefts form. The fact has been recorded briefly (Fell, 1940a), and a fuller account will be published at a later date.

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* See footnote to page 458.

† The species remains unidentified as yet, for reasons explained in a previous paper (Fell, 1941); in the meantime, it may be conveniently referred to as "Kirk's ophiuroid," after its discoverer, Professor H. B. Kirk.

THE PROBLEM.

My recent research has been concerned with elucidating three principal questions. These are:—

(1) What is the true nature of the development of *Amphipholis squamata,* and, in particular, what is the mode of origin of the coelom in the species?

(2) To what extent has viviparity affected development, and in what ways has it done so?

(3) What are the causes of direct development in ophiuroids, and how have they acted?

This paper is concerned only with the first of these problems, namely, elucidating the real mode of development of *Amphipholis squamata.* The second and third questions are considered in a separate paper (Fell, 1945) in which are utilised some of the results given here.

TECHNIQUE.

The methods used in this work are for the greater part the same as described in an earlier paper (Fell, 1941) and need not be repeated. The embryo of *Amphipholis* contains yolk material, rendering it both opaque and brittle, but not so markedly so as in Kirk's ophiuroid. It is essential not to leave the egg more than a few minutes in each of the xylol and paraffin baths (two minutes in each bath was generally found enough, using strengths of wax to xylol of 25%, 50%, 75%, and 100% wax). The opacity of the earlier stages makes whole mounts impracticable, while unfixed and living material is practically useless. The inaccurate results of previous workers are undoubtedly largely attributable to their use of living material or of imperfectly fixed preparations. It was found that the yolk granules of the egg and embryo of *Amphipholis* are far less basiphilic than those of Kirk's ophiuroid, and were very much smaller. Consequently ordinary staining methods for nuclei and cytoplasm could be employed, and it was unnecessary to adopt the special modified staining technique described in my previous paper.

The viviparous habit of the species, however, presented a problem not encountered in my previous work, and a number of special methods had to be devised. To obtain the embryos for study it was necessary to discover an anaesthetic for the parent of a nature such as to leave the delicate embryo unharmed by any convulsive contractions of the maternal tissues. Chloral hydrate, as used in 5% concentration in sea water in my previous work proved quite unsuitable. Chloroform and ether also produced violent shock, and nicotin was found to bring on an intense muscular rigor. Finally it was found that a solution of 2½–5% of menthol in sterile sea water produced a gentle anaesthesia, followed afterwards by complete recovery if not prolonged.

Owing to the viviparity it is not possible to observe the developmental process taking place within the bursa. Previous workers relied upon isolated stages excised from the parent and examined individually, a method which is only satisfactory if a large number of intermediate stages is available.

After some experimentation, a method was developed by which the embryo may be excised alive from the bursa of the parent and cultured *in vitro*; this proved very useful. A preliminary account of this method has already been given (Fell, 1940b), but owing to its important bearing on the problem of the nutrition of the embryo, it is repeated here in greater detail.

To extract the embryo is a delicate operation, for the disc of the parent measures only 3–5 mm. across. The pregnant adult is anaesthetised by subjection to menthol, as above described. With a fine tenotomy scalpel and forceps the disc is separated from the arms and mouth skeleton, and turned so that its lower (oral) side is uppermost. The bursae will have come away with the disc, and in them the older embryos are usually to be seen moving about. The latter can be disentangled from the membranous walls of the bursa by directing a gentle stream of water from a hypodermic syringe into the bursae. When the disc is removed, the genital plates, gonads, and the younger attached embryos usually remain attached to the bases of the arms; these early embryos can be removed by cutting the attachment with fine scissors. The embryos are now pipetted through several washings of sterilised sea water. Each embryo is then placed in a small watch-glass (5 cm. in diameter) and covered by 2–3 mls. of " Erdschreiber " medium. The watch-glass is set in a larger Petri dish, together with a sterile swab of wet cotton wool to keep the contained air humid and thus minimise changes in the density of the culture medium through evaporation. The whole " set-up " is surrounded by a bath of flowing tap water to keep the temperature moderately constant. In practice the medium was renewed every fourth day, but a longer interval can be allowed. Aseptic technique must be used throughout, as the embryos are very susceptible to bacterial toxins. Embryos treated in this way have been successfully cultured for periods of three weeks; they continued to differentiate as if still within the bursa, but with the advantage that the development can be observed.

As described in my previous account, the embryos if cultured in sterile sea water instead of in " Erdschreiber " medium, even though the pH value be kept at its normal value for unsterilised sea water, underwent a retrograde development, unco-ordinated cell-division took place, and finally they died. The significance of this result is discussed in the section of this paper dealing with the nutrition of the embryo.

The composition of " Erdschreiber " medium, as given by Gross (1937), is as follows:—

Sodium nitrate	0.1 gram.
Sodium hydrogen phosphate	0.02 „
Soil extract	50 mls.
Sterile sea water	1,000 „

The soil extract is prepared by autoclaving at a pressure of two atmospheres one kilogramme of garden soil in 1,000 mls. water for one hour. It is then decanted, filtered, and repeatedly sterilised till it becomes a clear, reddish-brown fluid. Further details are given in Gross's paper (1937). One modification of Gross's technique was made in view of the supposed intolerance by echinoderms of fresh water; to the final filtrate of soil extract was added the equivalent amount of dissolved sea salts from an equal volume of sea water. However, little advantage seemed to result from this addition, and other experiments have since convinced me that *Amphipholis* is by no means so sensitive to change in the density of the sea water as echinoderms are generally supposed to be. Some specimens were allowed to remain in a culture jar from which the sea water very slowly evaporated over a period of ten weeks. At the end of that period the sea water was highly hypertonic, having a salinity of 58 per mil. when titrated, and yet several specimens, both adults and newly-born embryos, remained alive till the end of the period.

Adults kept alive in aquarium tanks and Petri dishes in the laboratory were fed upon a diet of diatoms (*Skeletonema*) which were inoculated into the sea water at intervals.

As before, polarised light was used for the examination of the developing skeleton. For the purpose of decalcifying the embryos before imbedding, the new method of Wilks (1938) was used and gave very satisfactory results. By the use of sodium hexa-metaphosphate calcareous structures may be removed from delicate tissues without any evolution of carbon dioxide or other gases; thus the method is of the greatest advantage in embryology, insofar as it removes all possibility of artificial cavities being produced.

DISTRIBUTION.

Amphipholis squamata is believed to be the only ophiuroid with a world-wide distribution. It appears to vary but little, and in examples taken from the coastal rock-pools of Cook Strait, New Zealand, I have found no obvious difference in form or habit from those inhabiting the waters of Britain, twelve thousand miles distant.

Now, the existence during development of a free-living larval stage in many marine animals has been used as a means of explaining the wide distribution of some species. It is argued by this theory that while in the free-living planktonic stage a species may be carried over distances impossible for it to cover when in the adult, bottom-dwelling stage. In a communication to me, my friend, Mr. J. E. G. Raymont, of Edinburgh University, has pointed out the discrepancy between this theory and the distribution and life-history of *Amphipholis*; for although *Amphipholis squamata* has the most widespread distribution of any ophiuroid, yet it lacks a free-living larval stage, while other ophiuroids with a more restricted distribution possess well-developed larvae. The adult *Amphipholis squamata* is a typically littoral form, never extending below 125 fathoms (Mortensen, 1927).

In view of its strictly littoral distribution, the presence of the species in New Zealand is of the greatest interest, seeing that as it lacks a pelagic stage it can only have reached that country by way of shallow seas which no longer exist in the area. It occurs in South America, and Mortensen (1924) records it from the Sub-antarctic Auckland Islands. This distribution argues strongly in favour of the theory held by many biologists of the former existence of a landbridge, or island chain, between New Zealand and South America, via the Antarctic continent. In the same way certain earthworms have been shown by Benham to be shared in common by New Zealand, the Sub-antarctic islands, and South America; also the floras of New Zealand and South America have remarkable points of similarity.

Further discussion would be irrelevant to the present paper, but it seemed advisable to mention the problem owing to the bearing on it of the mode of development of *Amphipholis.*

Breeding Habits.

There is good reason to believe that *Amphipholis squamata,* unlike most other ophiuroids, breeds throughout the year, a constant stream of eggs being produced one by one and fertilised. Thus at any time of year embryos may be obtained at various stages of development, though the earliest stages are of course very rare, owing to the comparative rapidity of the first cleavages, and the fact that only one egg is formed at a time. The difficulties in obtaining information on these early cleavages are thus closely comparable to those encountered in the mammalia.

Garstang recorded that *A. squamata* breeds at Plymouth, England, from May to September. However, specimens forwarded to me from Plymouth in February, 1940, during an exceptionally cold winter, proved to be carrying embryos at various stages, so that there is no reason to believe the species has a restricted breeding period at that locality. Specimens from the Firth of Clyde area indicate that breeding continues uninterruptedly throughout the year on the West coast of Scotland. In the Forth area, on the other hand, somewhat different results were obtained, probably as a result of the more severe winter conditions of the North Sea. Thus, from January to March, 1940, no specimens at all could be obtained, and it was not till June of that year that the species was again common. However, whenever specimens have been obtainable in the Forth area, they have always been pregnant, so that it would seem that so long as the weather conditions are suitable for the life of the adults, these will breed.

In Forth specimens it is unusual to find more than five of the ten bursae pregnant at any one time, and each bursa seldom contains more than one embryo. In specimens from the Clyde, on the other hand, not only are the adults themselves much larger than examples from the Forth, but they also carry a greater number of embryos, and the latter are often much more advanced. Thus it seems that on the milder Atlantic coast the embryos are carried for a longer period before being born. This is a peculiar fact, as it is more usual for brood protection to be more highly developed in unfavourable conditions. In one particularly prolific specimen, forwarded from Millport, the following embryos were extracted: in one bursa were an embryo having 8–9 arm-segments, an embryo with 3–4 arm-segments, and an embryo at the "pentagon" stage; in another bursa were two embryos, one with 10–12 arm-segments, and one with 3–4 arm-segments; in a third bursa were two embryos with 3–4 arm-segments; one bursa was empty; and the remaining six bursae each contained one embryo, ranging in development from 3–10 arm-segments.

In regard to its habits in other parts of the world there is less information. Fewkes (1887) has recorded it as breeding in August and September at Newport, U.S.A., but he does not make it clear if these are the only months. Bernasconi (1928) records it as breeding at Neochea, Argentina. In New Zealand I have taken breeding specimens from July to March, and have little doubt that it breeds there also in the remaining months when I had no occasion to go collecting. These observations were made at Island Bay, on the northern shore of Cook Strait.

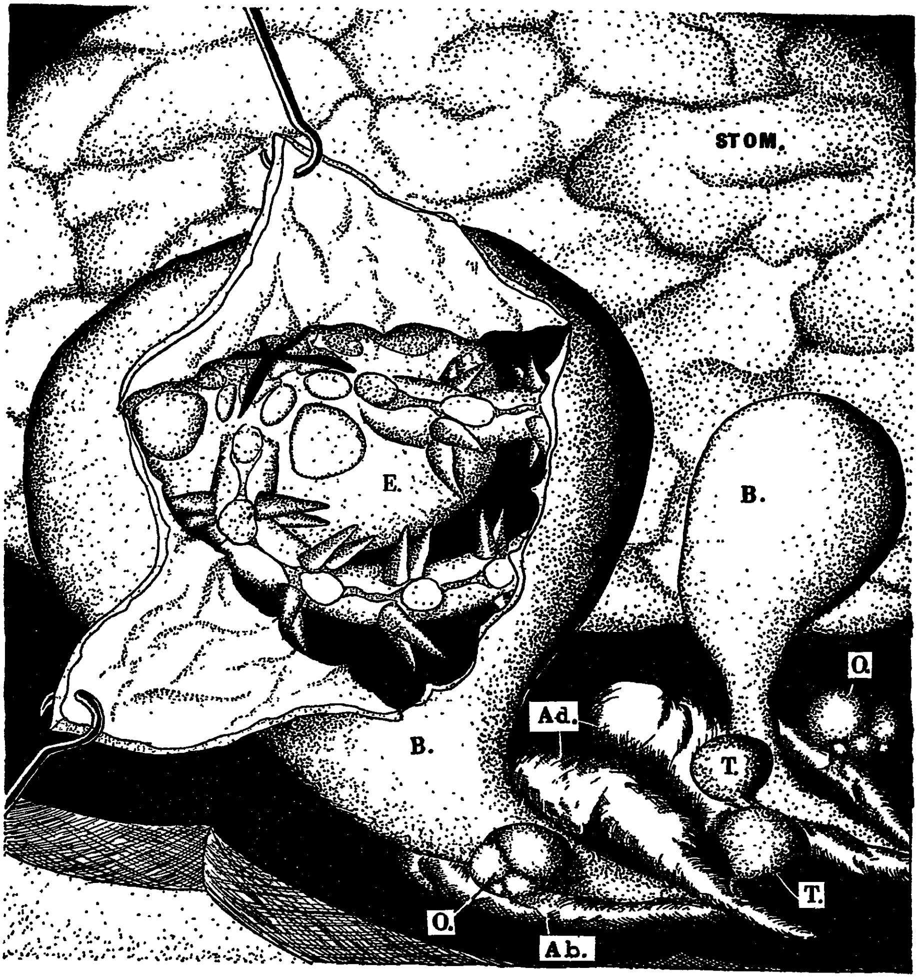

FIG. 1.—The genital organs of *Amphipholis squamata*.

A dissection of an interradius, with the left bursa opened to show the contained embryo. The latter has almost reached the end of its intrabursal development, and is oriented in the characteristic inverted position with the arms directed toward the genital cleft.

Ab., abradial genital plate; Ad., adradial genital plate; B., bursa; E., embryo; O., ovary; T., testis; Stom., stomach.

THE REPRODUCTIVE ORGANS.

The morphology of the reproductive organs of *Amphipholis squamata* follows the general pattern of the whole genus, and the arrangement of the gonads can scarcely be distinguished from the condition described by Mortensen for *A. tenuispina, A. japonica,* and *A. sabrina* (Mortensen, 1920). As, however, the relationship of the gonads to the bursae is of great importance to understanding the subsequent history of the embryo, it is necessary to give here a somewhat more complete account.

There are ten bursae, so distributed that there are two in each of the five interradii. Each opens to the exterior through the narrow genital clefts, which lie on the under surface of the disc, one on either side of the base of each arm. Each cleft is bordered by two skeletal plates, the abradial and adradial genital plates respectively (Fig. 1). Attached to the dorsal (aboral) surface of each adradial genital plate is a single testis, thus making ten testes in all; the ten ovaries are each similarly related to an abradial genital plate. Occasionally a two-lobed testis is seen, or, less frequently, two testes are found attached to a single adradial plate. Occasionally, also, the ovaries are found to be secondarily increased in number in this way. The ovaries occupy a somewhat more peripheral position than do the testes, and thus when a series of vertical sections is being examined, the testes of any particular sector are seen cut across in sections nearer the centre than those in which the ovaries are cut.

As the relationship of the gonads to the bursa and neighbouring organs can best be understood by means of vertical sections, two examples are illustrated (Figs. 2A and 2B). In Fig. 2A is seen part of a vertical section through an interradius, including a bursa. The ovary of this bursa is seen to the right, on the abradial border of the genital cleft. To the left of the bursa is the basal part of the neighbouring arm, cut in vertical transverse section. The part of the bursa which is cut across does not show any portion of a contained embryo, but the presence of the sinuses in its wall indicate that there is an embryo present in another part of the organ (see section dealing with the bursa, and nutrition of the embryo, below). The ovary, which contains a small number of oogonia in various stages of maturity, is ensheathed by a thin wall one cell in thickness. That side of the ovary toward the bursa is closely adpressed against the latter, so that it would not be difficult for a ripe egg to be liberated through the bursa directly into the lumen. Further details in regard to the ovary are given in the section dealing with the ovum and oocyte.

In Fig. 2B is seen a similar vertical section from the same series, but differing in that it has been cut somewhat further in toward the centre of the disc, and thus the ovary is absent from the abradial side, while the testis is visible on the adradial border. There is also seen the testis belonging to the interradius on the other side of the arm. As the bursa is cut more centrally, an arm of a contained embryo is seen cut in transverse section. The testis, like the ovary, is very simple, comprising only a spherical capsule one or two cells thick containing loosely-arranged spermatogonia. Each of the latter is a spherical cell with a very large and deeply-staining nucleus. It is notable that the condition of the gonads in this species is so extremely primitive, and it is to be contrasted with the complex reproductive glands with gonoducts which have recently been described by Smith (1940) for *Ophiothrix fragilis.*

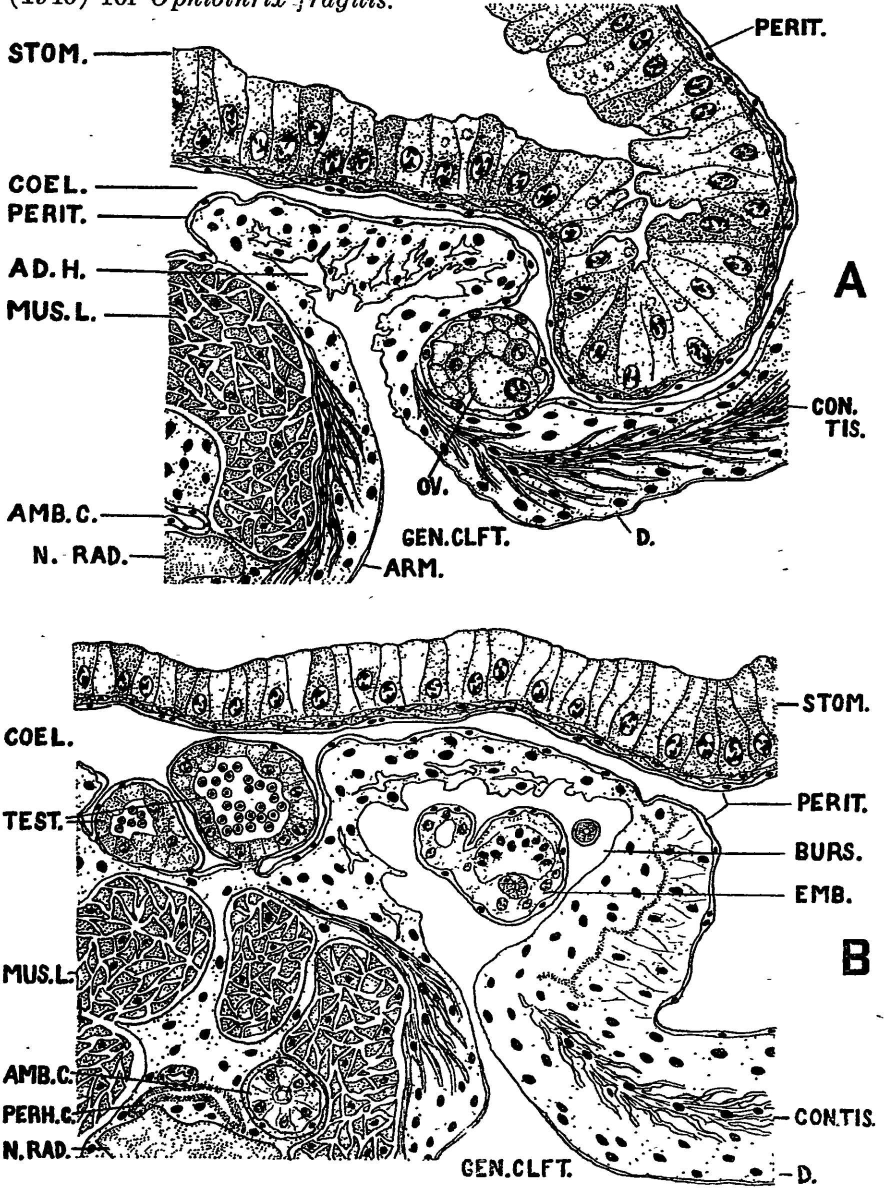

FIG. 2.—Vertical sections showing the relation between the bursa, ovary, testis and related organs.

Stom., stomach; Perit., peritoneum; Coel., general coelom; Con.Tis., connective tissue; D., lower surface of disc; Gen.Clft., genital cleft; Arm, portion of arm bordering the genital cleft; N.Rad., radial nerve; Perh.C., perihaemal coelom; Amb.C., ambulacral canal; Mus.L., longitudinal muscle fibres of arm; Burs., bursa; Emb., embryo; Ad.H., adradial horn of bursa; Ov., ovary; Test., testis.

VIVIPARITY OF AMPHIPHOLIS.

The fact that *Amphipholis squamata,* together with some other echinoderms, is viviparous raises an important question which appears almost to have escaped notice hitherto. It is a belief very common among embryological workers, and one frequently referred to in current papers (e.g., Just, 1928), that the coelomic fluids of echinoderms exert a strong toxic or inhibitory effect upon the ova. This, it is stated, is the mechanism by which embryos are prevented from developing within the body of the parent. In descriptions of methods of artificial fertilisation of echinoderm eggs it is stated with emphasis that eggs will not develop when excised from the gonad and gonoducts unless all traces of coelomic fluids are carefully excluded. The point arises here of how it is possible for embryos of *Amphipholis squamata* to develop within the thin-walled bursae of the parents; for it is improbable that traces of coelomic fluids would not diffuse through the bursal wall when it becomes thin and stretched by the growing embryo; and, in any case, as shown below, there are strong reasons for believing that a fluid is actually secreted by the bursal wall on to the embryo.

Furthermore, a number of cases have been recorded during the last forty years in which viviparity has occurred in species normally oviparous—and in which, according to the theory of "coelomic toxins," internal development is not possible. It is a notable fact that in these cases of viviparous habit in oviparous forms, the condition is associated with, and can be fairly certainly regarded as caused by, a hermaphrodite state of the reproductive organs. The following records are known to me of hermaphroditism occurring abnormally in echinoderms: *Asterina gibbosa* (Cuenot, 1898); *Asterina batheri* (Ohshima, 1929); *Asterias glacialis* (Delage, 1902, and Buchner, 1911); *Sphaerechinus granularis* (Viguier, 1900); *Strongylocentrotus droebachiensis* (Gadd, 1907); *Paracentrotus* (or *Strongylocentrotus*) *lividus* (Herlant, 1918; Gray, 1921; Drzewina and Bohn, 1924; Paspaleff, 1927); *Arbacia pustulosa* (Gray, 1921); *Arbacia* sp. (Heilbrunn, 1929); *Echinocardium cordatum* (Paspaleff, 1927; Moore, 1935); and *Echinus esculentus* (Moore, 1932). The tendency to develop hermaphroditism, as shown by the above list, is thus not confined to one or two examples. Of the instances given above, the hermaphroditism is described as functional in six cases. Thus, in the specimen of *Echinocardium cordatum* examined by Moore (1935) there were larvae and eggs in the gonoducts, eggs having been fertilised by the animal's own spermatozoa.

It is thus quite clear that the supposed inability of echinoderm eggs to develop in proximity to the body fluids does not agree with the facts as observed in nature, and the belief rests entirely upon some data obtained from artificial fertilisations made in the laboratory. All the facts brought together above are in support of a claim made by Bogucki (1930), who also challenged the truth of the theory. He, however, approached the question from the results of experimental cultures of excised echinoderm eggs, artificially fertilised. Bogucki claimed that inhibition of development was not caused by the body fluids of the parent, but happened only if the gut was ruptured. He concluded therefore that this organ is the source of the property incorrectly attributed to the coelomic fluids. As two independent lines of investigation lead to the same result, it seems that there can no longer be any reason to believe in the toxicity of echinoderm body fluids, and the case of *Amphipholis squamata* ceases to appear anomalous.

THE BURSA.

A vertical section through the non-pregnant bursa shows that the lumen of the organ is very small, while the bursal wall is many cells in thickness, and contains few if any blood-sinuses of any importance. At the aboral (dorsal) apex of the organ there is visible a downwardly projecting cone of tissue appearing in section triangular. This may be termed the " median cone." This gives the lumen an appearance of bifurcating, when it is viewed in section. As this subdivision of the lumen is of importance during the later growth of the embryo, it is convenient to give distinctive names to the two portions separated by the median cone. The portion of lumen directed adradially, i.e., towards the testis, may be termed the " adradial horn," and the part directed towards the ovary the " abradial horn."

In the earliest stage of pregnancy the embryo, as might be expected, is found occupying the abradial horn of the bursa, not far below the ovary. After the germ layers have been differentiated the embryo becomes closely invested by the wall of the abradial horn, and at one point a fusion of the parental and embryonic tissues occurs (see later section of this paper). The region of the embryo to be affected in this way is aboral in relation to its own axes of symmetry. The adoral pole of the embryo becomes directed toward the centre of the lumen of the bursa. At the same time numerous blood sinuses begin to become visible in the wall of the bursa, especially in the median cone. At the point where the embryo has become attached to the bursal wall, the latter grows out into a stalk, and thus the embryo now comes to lie suspended in the bursal cavity. As the embryo increases in size the wall of the bursa stretches, till the median cone completely disappears as such, its tissues having contributed to the expanding wall. The embryo thus occupies the entire lumen, and this condition holds during the rest of the development.

THE OÖCYTE AND OVUM.

As the sexual products of *Amphipholis* are never at any time very abundant, it is not possible to give any detailed account of oogenesis, but some general observations may be included here. None of the earlier workers on the embryology of the ophiuroid studied these stages with the single exception of Russo (1891). His account, however, is very unsatisfactory, and cannot be seriously considered. He states, for example, that the ovum has a diameter of 4 μ, of which 2 μ are occupied by the germinal vesicle. Any ophiuroid having so minute an egg would indeed be remarkable, as it would be some 4,000 times smaller than the smallest known echinoderm egg (that of *Toxopneustes variagatus,* of 65 μ diameter). Russo probably observed very immature oogonia. Some interesting comparisons, however, can be made with observations made on other echinoderm eggs. A short summary is given here before noting my own observations for *Amphipholis.*

Boveri (1901) made the important discovery of the existence of prelocalisation. He observed that in the unfertilised ovum of *Paracentrotus* (*Strongylocentrotus*) *lividus* there was an evenly-distributed pigmentation. When fertilisation occurred, and maturation was completed, the pigment became restricted to a transverse band in the lower hemisphere of the egg. Subsequent development showed that it is this pigmented zone alone which normally is able to give rise to the archenteron; eggs which had been deprived of the pigmented zone were unable to gastrulate. Therefore it was evident that a prelocalisation of archenteron-forming elements had taken place in the egg, and that the transverse band of pigment indicated a vegetal hemisphere.

MacBride (1907), in the course of his careful study of the development of *Ophiothrix fragilis* was more concerned with later stages than the egg, and he merely notes in passing that the eggs of that species measure about ·1 mm. in diameter, and are opaque owing to the presence of a yellow yolk. He does not refer to his having noted any evidence of prelocalisation. His earlier work on *Amphipholis* (1892), as already stated, concerned only post-larval stages. In a paper on the development of *Echinocardium cordatum* (1918) MacBride similarly makes little reference to the egg, but he mentions that it is an ellipsoid, not a sphere. Here again he does not refer to any prelocalisation.

Grave (1900) had to omit a description of the egg and early cleavages from his paper on the development of *Ophiura brevispina,* owing to lack of material. But in 1916 he was able to complete his previous work in a second paper. In this he gives a careful description of the egg, as follows: " The mature egg of *Ophiura* has a diameter of approximately 0·3 mm. and is therefore about seventy-eight times the volume of the egg of *Ophiocoma echinata,* the latter being not far from the minimum size of eggs produced by ophiuroids in general. The great difference in the size of the eggs of these two species seems to be chiefly, if not exclusively, due to a difference in yolk content, and not to a difference in volume of ground substance. The yolk material of the egg of *Ophiura* . . . is distributed uniformly throughout the cytoplasm in the form of minute spherules and does not become stratified or localised either before or after fertilisation." Grave further states that the yolk spherules give the egg its characteristic colour—varying from green to lemon-yellow in different broods. His subsequent account of the early development does not refer to any prelocalisation of archenteron-forming material, nor did he find any correlation between pigment distribution and the fate of various regions of the egg.

Narasimhamurti (1933), working on the development of *Ophiocomina nigra,* states that the egg is small, measuring 0·1 mm. in diameter, and is opaque owing to the presence of a brownish yolk. He found no evidence of prelocalisation.

Smith (1940) has described the oogenesis of *Ophiothrix fragilis.* The ovary of this species is large, and has a special gonoduct to the genital cleft. Within the gonad there are to be seen oöcytes which fall into well-defined size-groups. He believes that the sex-cells originate in the rachis, whence they migrate into the gonads. The youngest oöcytes observed had a vesicular nucleus, and a central or slightly excentric nucleolus. The latter is at first dense, but exhibits vacuolation when the oöcyte attains a diameter of thirty micra, and continues to do so till the ovum is 90–100 μ in diameter.

Fell (1941) described the directly developing ophiuroid of New Zealand known at present as " Kirk's ophiuroid." In this species the egg is very large, having a diameter of 500 μ, and is so densely laden with yolk as to be quite opaque, no internal structure whatever being visible without sectioning. The egg is uniformly coloured with a buff or pinkish-brown pigment which is intimately associated with the yolk material. The course of later development shows very clearly that in this species prelocalisation of endoderm-forming and epiblastic material occurs early in development, not later than the second cleavage, and possibly in the egg itself, as in *Paracentrotus lividus.* Unlike the latter species, however, prelocalisation cannot be correlated with pigment distribution. At first the yolk is evenly distributed through epiblast and mes-hypoblast alike, but later it disappears from the epiblastic cells, and becomes confined to the central mes-hypoblast tissues.

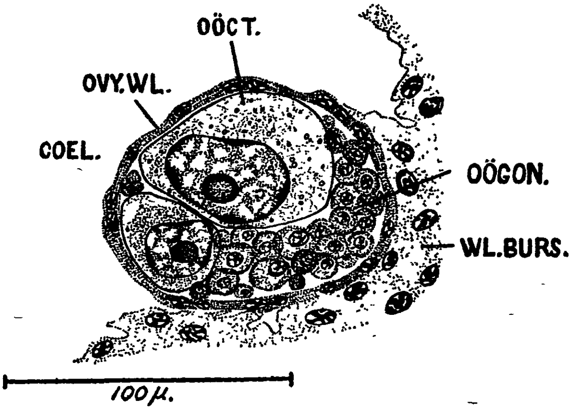

FIG. 3.—The Ovary.
Coel., coelom; Ovy.Wl., ovary wall; Oöct., oöcyte; Oögon, oögonia; Wl.Burs., wall of bursa.

In regard to the oöcyte and egg of *Amphipholis squamata,* the following facts have been observed: Within the ovary, which is here very small—normally about 100 μ across—there are generally to be seen a number of small, loosely-arranged oogonia, each with a prominent nucleus, and usually a small nucleolus to one side of the nucleus. Occasionally a more mature oogonium is to be seen in which the nucleus is somewhat enlarged, and the nucleolus occupies a more central position in the nucleus. This sequence is in agreement with the observations of Harvey (1931) on *Asterias rubens.* With increasing maturity the nucleolus tends to stain more deeply, a condition also paralleled in the oogenesis of *Asterias rubens.*

In addition to the oogonia there are usually to be seen in an ovary two oöcytes of considerable size, one of them twice or thrice as large as the other. In these oöcytes the nucleus has become greatly enlarged, thus taking on the typical form and translucency of the "germinal vesicle." Near the centre of the nucleus a large nucleolus is to be observed. In the cytoplasm around the nucleus yolk-granules are present, and these grow denser and more evenly distributed throughout the egg as growth proceeds. When the egg is mature it is liberated directly into the bursa, the wall of which is thin in the region approximate to the ovary. Only one egg at a time comes to maturity in any one ovary as a general rule, though occasionally two embryos have been observed in one bursa of apparently the same age, indicating perhaps the contemporaneous liberation of two ova.

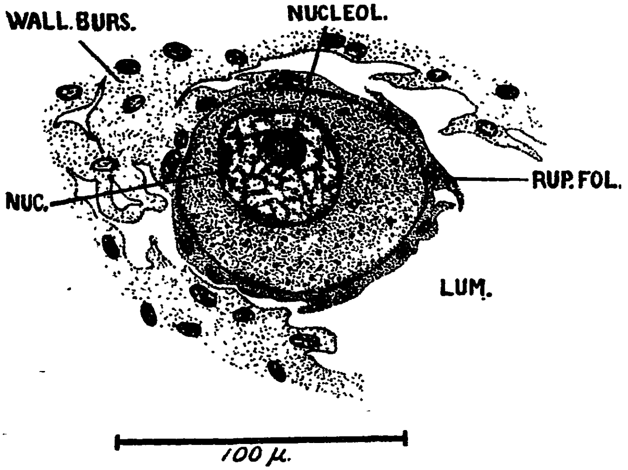

FIG. 4.—Ovum which has just been liberated into the bursa.
Nuc., nucleus; Wall.Burs., wall of the bursa; Nucleol., nucleolus; Rup.Fol., ruptured follicle; Lum., lumen of the bursa.

In Fig. 4 is shown a mature unfertilised egg which has just passed into the bursa and still has part of the follicle adhering to it. It has a diameter of approximately 100 μ, due allowance being made for some shrinkage during fixation. In life the egg is deeply tinted with a reddish opaque pigment which is associated with the yolk in the cytoplasm. The nucleus is large and clear, about 50 μ in diameter, and there is a large and deeply staining nucleolus. Beneath the fragmentary portions of ruptured follicle and lying in close contact with the cytoplasm of the egg is a thin but distinct protoplasmic membrane, but in the unfertilised specimen shown in the figure there is as yet no egg (or "fertilisation") membrane. The yolk material is in the form of numerous fine droplets, much smaller than those of the egg of Kirk's ophiuroid. As in the latter species, the yolk-granules are intensely basiphilic, but, although this property prevents the use of nuclear stains for whole mounts, it is not strong enough to interfere with their use for sections. Thus, it was not found necessary to use the special staining methods which had to be employed for Kirk's ophiuroid, where the basiphilic granules tend to mask the nuclei. As the ovary possesses no gonoduct, it can only be supposed that the ovum enters the bursa through a rupture temporarily formed in the bursal wall. Unlike other ophiuroids, which have large gonads, it cannot be supposed here that pressure of the growing sex-cells ruptures the bursal wall, thus liberating the genital products; but as the portion of wall neighbouring the ovary is of no great thickness, it would not require more than a slight cytolytic action of the egg to bring about the perforation of the bursa.

If the egg of *Amphipholis squamata* is compared with those of other ophiuroids, it is seen to occupy an intermediate place in a series which ranges from small eggs with comparatively little yolk and having a long, indirect and pelagic development to such very heavily yolked types of egg, of large size, and with a shortened, direct and non-pelagic development. The importance of yolk content and egg-size, and their relationship to the type of development followed, has been discussed in detail in a separate paper (Fell, 1945).

FERTILISATION.

Fertilisation has not been observed. Attempts to fertilise artificially such few ova as were obtained did not succeed. It is not known for certain whether the animal is self-fertilised, but it is a significant fact that so many viviparous ophiuroids are also hermaphrodite, a condition which suggests strongly that self-fertilisation accompanies viviparity in ophiuroids. This deduction, however, does not necessarily follow, for in the New Zealand ophiuroid *Ophiomyxa brevirima* the sexes are distinct, though the animal is viviparous. This would indicate that copulation of some kind occurs in that species. The nature of the copulation required by these forms, the eggs of which cannot come in contact with the spermatozoa after extrusion from the body of the parent, may be deduced perhaps from the observations of Mortensen (1933) on the East African form *Amphicyclus androphorus*. Mortensen observed that in this species the female carries the male, which is very much smaller, on her oral side, so disposed that the ventral (oral) side of each animal is adpressed to the other. This type of copulation has also been seen to occur in *Ophiosphaera insignis* and *Ophiodaphne materna.* With the exception of the asteroid *Archaster,* in which a male-female superposition occurs (Ohshima and Ikeda, 1934), copulation is quite unknown elsewhere in the echinoderms. Now, none of the three ophiuroids quoted above is viviparous, so the conditions are not strictly comparable to those obtaining in *Amphipholis squamata,* but till such time as self-fertilisation has actually been observed, copulation must still be regarded as a possibility. The fact that hermaphroditism in the oligochaetes does not dispense with the need for cross-fertilisation gives added reason for caution. It would be necessary to rear young *Amphipholis* in isolated tanks from an early ontogenetic stage, before the ripening of the sex-organs, to establish whether reproduction can occur by self-fertilisation. It should be noted here, however, that during the last two years when adults have been kept under observation in aquaria, no case of copulation has been observed, though it was seen that the animals tended to congregate in one part of the tank.

EARLY DEVELOPMENT.

Subsequent to the liberation of the ovum there is a gap in the series of stages which I have as yet obtained. As is well-known, the early cleavages of eggs of echinoderms are passed through rapidly. The eggs of *Ophiothrix fragilis* reach the gastrula stage within thirty-six hours according to MacBride (1907), as also do those of *Ophiura brevispina* (Grave, 1916). Even in the heavily yolked egg of Kirk's ophiuroid the blastula stage is reached within forty-eight hours (Fell, 1941). As the egg of *Amphipholis squamata* occupies a position morphologically intermediate between these extremes it is most probable that the time occupied in passing through the early stages up till the formation of the blastula is between thirty-six and forty-eight hours. It is obvious that embryos at such stages must be rare, partly because of their short duration, and partly because only one egg is formed at a time, instead of hundreds or thousands, as is more usual in echinoderms.

Russo (1891) and also Apostolides (1882) have given accounts which claim to describe the process of cleavage of the egg of *A. squamata.* These two accounts, however, are conflicting. Since Russo could never have actually observed an ovum of *Amphipholis squamata* —for, as shown above, his supposed description of it can only be applied to an extremely immature oogonium—no particular importance need be attached to his statements in regard to its segmentation. Apostolides' older account, on the other hand, is of some interest, because the peculiar type of cleavage he believed to occur has since been recorded as a variation affecting about thirty per cent. of eggs of Kirk's ophiuroid. He believed that the first cleavage is markedly unequal, resulting in a two-celled embryo in which one blastomere is about thrice the size of the other. In two successive divisions the larger blastomere gave rise to three cells, thus producing a four-celled embryo with equal blastomeres. Thereafter the cleavages are stated to be normal. A very similar plan of cleavage has been recorded and figured for Kirk's ophiuroid (Fell, 1941), and a comparison made with what Apostolides described. However, it is important to note that in Kirk's ophiuroid it affected only a minority of the cases observed, and in more than sixty per cent. of the eggs the first two cleavages did not differ from the usual radial pattern.

Fortunately, there is already available a very satisfactory series of descriptions of the cleavages of eggs of other ophiuroids in which the reproductive cells are produced in far greater abundance. These range from the comparatively non-yolky forms such as *Ophiothrix fragilis* (MacBride, 1907) and *Ophiocomina nigra* (Narasimhamurti, 1933), through the intermediate type, such as *Ophiura brevispina* (Grave, 1916), to the heavily yolked type, illustrated by Kirk's ophiuroid (Fell, 1941). From the facts known about the above species it should be possible to deduce with a reasonable expectation of success the nature of the corresponding cleavages of *Amphipholis squamata;* for all the evidence provided by its development, the greater part of which is now known and described in this paper, indicate that this species is of an intermediate type. As shown elsewhere (Fell, 1945), it is only in the most heavily yolked type that any alteration from radial cleavage occurs in the earliest stages of segmentation. As *Amphipholis squamata* is not of this extreme type, it is therefore highly probable that the early cleavages will in normal specimens differ in no important way from those of *Ophiothrix, Ophiocomina* or *Ophiura.*

For the reasons given in the preceding paragraph I therefore believe that the type of unequal cleavage described by Apostolides, if it occurs at all, is a variation comparable to what has been described for Kirk's ophiuroid.

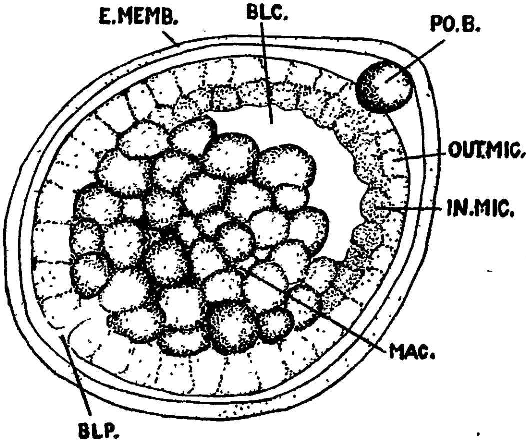

FIG. 5.—Early Gastrula.

E.Memb., egg membrane; Blc., blastocoel; Po.B., polar body; Out. Mic., outer micromeres; In.Mic., inner micromeres; Mac., macromeres; Blp., blastopore.

In regard to the form of the blastula, though again an actual example has not been obtained, it can be reconstructed by examination of an early gastrula (Fig. 5), which is the earliest stage subsequent to the ovum that I have been able to obtain as yet. This early gastrula can be recognised at once as showing affinities with the early gastrula described for Kirk's ophiuroid, and is also very similar to the gastrula which Grave (1900) described for *Ophiura brevispina.* As the form of the gastrula is determined by that of the blastula, it is easy to deduce the latter from the former, particularly when there exist two other species of similar type in which both stages are known. In the specimen figured, a solid mass of macromeres has been invaginated from the vegetal pole, to constitute a mes-hypoblast which is obliterating the blastocoel. The latter cavity is, in the stage shown, reduced to a narrow lumen, crescentic in vertical section, towards the animal pole. Reference to my previous paper (Fell, 1941) will bring out more clearly the similarity of this gastrula to the corresponding stage of Kirk's ophiuroid. As in the latter species, only a very reduced blastopore is present, and there is no true archenteron. A single cell lying at the animal pole, between the epiblast and the egg (fertilisation) membrane is probably one of the polar bodies. It is obvious that the preceding blastula, as in

Kirk's ophiuroid, must have comprised a thick-walled sphere of cells surrounding a somewhat reduced blastocoel, and the cells would be differentiated into micromeres at the animal pole, and macromeres at the vegetal pole. The gastrula shows that it is from the latter elements that the mes-hypoblast is derived; no evidence has been found of any process of inturning of micromeres to form part of the hypoblast as has been described for Kirk's ophiuroid. In *Ophiura* and Kirk's ophiuroid the archenteron is formed much later by a secondary hollowing out of the central inturned mass of macromeres. The next stage obtained in this species shows that a central cavity has arisen in the hypoblast, and there can be no doubt that this, as in the two other species, arises by a similar secondary splitting; for there can be no possibility of an archenteron arising in the way in which it does in the non-yolky forms. As pointed out in previous papers (Fell, 1940a, 1941) this reduced mode of gastrulation is attributable to the reduction of the blastocoel in relation to the large, yolk-laden mass of the blastomeres, making typical invagination an impossibility for physical reasons.

Two important differences are to be observed from the conditions seen in Kirk's ophiuroid, firstly, the blastocoel, though reduced, is not so much so as in Kirk's ophiuroid—resembling more that described for *Ophiura* (Grave, 1916). Thus, in gastrulation all the mes-hypoblast can be inturned in one mass, rendering unnecessary the long process of epibolic inwandering of micromeres which follows in Kirk's ophiuroid. This distinction is consistent with the smaller amount of yolk present in *Amphipholis squamata,* and the consequent lesser degree of modification which the development has undergone.

A second difference from Kirk's ophiuroid is provided by the varied extent of the pigmentation of the mes-hypoblast and epiblast in *Amphipholis.* The epiblast cells are paler and clearer than the inturned macromeres, the latter having an opaque reddish tint. It can be seen that in this respect *Amphipholis* shows some degree of similarity to *Paracentrotus,* for in both cases the pigmentation becomes restricted to the cells that are to give rise to the endoderm. Why changes in the distribution of pigments should be found in these species and not in others is an interesting problem. It may be that it is to be understood in terms of whether or not the yolk—and consequently the pigment associated with the yolk granules—is sufficient in quantity to be present not only in the hypoblast cells, but also in the epiblast. In Kirk's ophiuroid, where there is such an abundance of yolk, all germ layers contain yolk material in quantity, and all, as would be expected, are pigmented. In *Amphipholis,* on the other hand, there is less yolk, and this is mostly aggregated in the mes-hypoblast cells, the pigmentation at the same time being denser in that region. The conditions in this regard in *Paracentrotus* do not appear to have been described, so that it remains to be seen whether this explanation can be applied to it too.

The next stage which has been observed is shown in section in Fig. 6. Here the hypoblast has developed a central cavtiy, to form the archenteron—evidently through an internal splitting, as in *Ophiura brevispina* and Kirk's ophiuroid. The archenteron lies

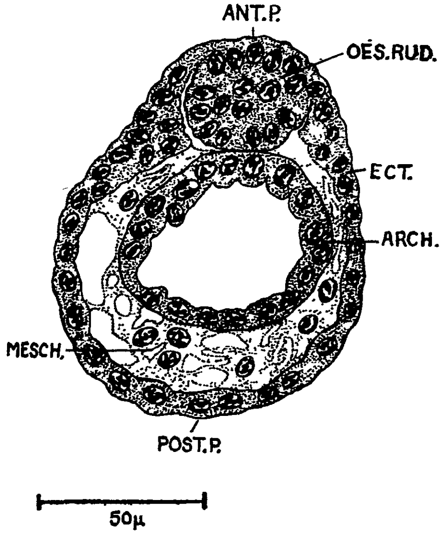

FIG. 6.—Late Gastrula.

Mesch., mesenchyme; Post.P., posterior pole; Arch., archenteron; Ect., ectoderm; Oes.Rud., oesophageal rudiment; Ant.P., anterior pole.

centrally within the outer shell of ectoderm cells, and between the two is a small amount of mesenchyme, probably derived as in Kirk's ophiuroid from the outermost cells of the inturned mes-hypoblast mass. At the future anterior end a solid ingrowth of ectoderm cells as a stomodaeum is commencing, to form the rudiment of the oesophageal sac. Thus, here as in other echinoderms, the oesophageal cells are originally ectodermal. As will be seen below, it is a notable feature of the development of *Amphipholis* that future hollow organs frequently arise first as solid masses of cells, in which cavities later form by splitting. This is a feature to which I have already drawn attention in previous papers (Fell, 1940a, 1941).

The series of stages from now onward is fairly complete, but before proceeding to describe them it is necessary to refer briefly to some statements made by earlier workers on the mode of formation of the endoderm. Metschnikoff (1869) studied only some late stages which he had obtained, and so never had occasion to suspect that there might be anything unusual about gastrulation. Consequently, he assumed that invagination occurs, no echinoderm at that time being known to form its endoderm by any other method. Apostolides (1882), however, stated that the endoderm arises by delamination, though he gave no description of the process, nor did he give any figures, despite the very surprising nature of his claim, seeing that at that period it was believed that invagination is universal in echinoderms. Russo (1891) repeated Apostolides' statement, and gave some highly diagrammatic figures, supposed to illustrate the process—which he compared to that found in *Hydra*. Thus the matter remained till 1914, when MacBride dismissed the statement as highly improbable, and no further attention was paid to the matter till I called attention to Russo's work (1940a), showing that some of his statements, especially those in regard to a schizocoelous origin of the coelom, were in fact supported by observation of the development of Kirk's ophiuroid. But in regard to his claim that the endoderm arose by delamination in *Amphipholis,* I was unable then to comment, because such a process does not occur in Kirk's ophiuroid. It was partly with the intention to examine his claim for delamination that the present research was undertaken. As has now been shown, in actual fact the origin of the germ layers and mode of gastrulation probably differ only in a minor degree from what have been described for Kirk's ophiuroid—and in both species the mode of gastrulation can be seen to be merely a peculiar modified form of invagination, produced by the effect of yolk material. There seems to be no possibility whatever of delamination taking place. There can be little doubt that these mistaken interpretations of the gastrula were in large measure the result of the unsatisfactory histological methods employed by earlier workers; it is not surprising that the opaque appearance of gastrula in unfixed, or improperly fixed, and cleared material led these workers to confuse it with the superficially similar gastrula of a coelenterate.

ORIGIN OF THE HYDROCOEL AND COELOMIC VESICLES.

Sections through somewhat older embryos indicate that the mesenchyme mass occupying the aboral region of the embryo continues to increase, the cells proliferating particularly along that side of the embryo which is destined to be the right side of the larva. At the same time the mesenchyme cells in this right anterior region begin to round up into two well-defined masses, at first solid. These come to bulge forwards, gradually pushing the oesophageal mass over

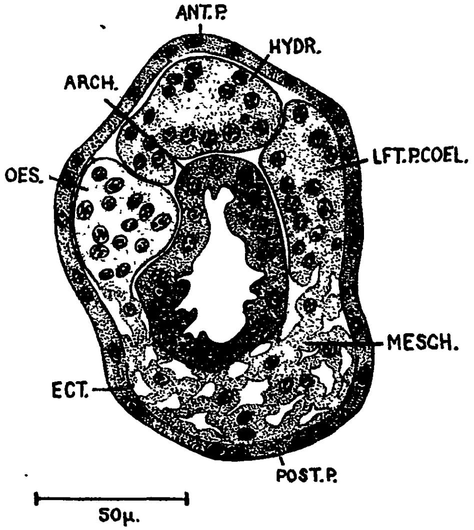

FIGURE 7.

Ect., ectoderm; Oes., oesophageal sac; Arch., archenteron; Ant.P., anterior pole; Hydr., hydrocoel; Lft.P.Coel., left posterior coelom; Mesch., mesenchyme.

Note: as the section is viewed from the (future) ventral aspect, right and left are reversed.

toward the left side (Fig. 7). Subsequent development shows that the more anterior of these two masses is the rudiment of the hydrocoel (i.e., the left anterior coelom), and the other one is the left posterior coelomic rudiment. These coelomic organs are derived therefore from mesodermal mesenchyme in the region lateral to the archenteron, and are in no way pinched off as pouches from the archenteron. It is to be noted that the origin of the coelomic vesicles in this way as bodies at first solid can be regarded as an extension of the tendency to which Narasimhamurti (1933) drew attention in *Ophiocomina nigra,* where the coelomic vesicles arise initially as thick-walled bodies with very small cavities. The point is an important one, and it is discussed more fully elsewhere (Fell, 1945).

At this time the oesophageal rudiment, or stomodaeum, is still solid. The archenteron, meantime, is taking on more of an epithelial arrangement of its component cells, the nuclei in sections showing up in regular series, one deep. Its cells begin to stain more intensely with cytoplasmic dyes than do the surrounding tissues, a fact probably related to some chemical change accompanying the absorption of yolk in this region.

All this time the mesenchyme continues to proliferate actively, and it becomes very prominent in sections of these and succeeding stages. Indeed, mesenchyme plays a most important part in the development of *Amphipholis squamata,* as it does also in Kirk's ophiuroid. A section through a somewhat older stage (Fig. 8) shows that a cavity begins to develop by splitting within the oesophageal mass, and almost at the same time the hydrocoel rudiment begins to form a small internal cavity, also by splitting. The left posterior coelomic rudiment remains for a while without any trace of an internal lumen. Meanwhile, on the right side of the archenteron,

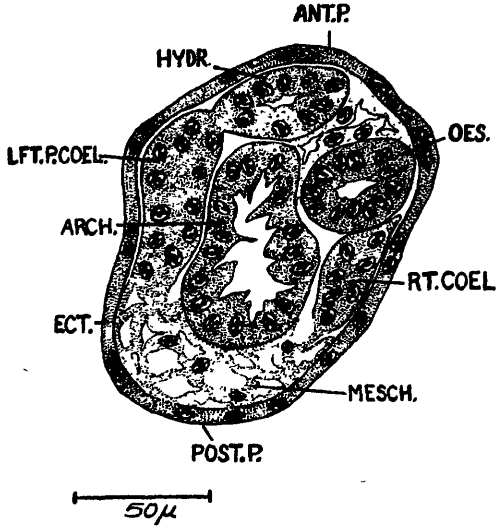

FIGURE 8.

Ect., ectoderm; Arch., archenteron; Lft.P.Coel., left posterior coelomic vesicle; Hydr., hydrocoel; Ant.P., anterior pole; Oes., oesophagus; Rt.Coel., right coelomic vesicle; Mesch., mesenchyme.

some more of the mesenchyme cells have rounded up to form a small body which from its position undoubtedly represents the rudiment of the right coelomic vesicle. There is no trace in any of my specimens of any tendency of this body to separate into anterior and posterior portions, as Metschnikoff (1869) had claimed it does.

The embryo is still very opaque at this stage of development, even after treatment with clearing reagents, and the only satisfactory way of gaining a comprehensive picture of its structure is by means of sections and reconstructions made from them by Pusey's projection method. In Fig. 9 is drawn an embryo—or young larva as it may now be called—of slightly more advanced development. It is represented as a transparent object, having been reconstructed from sections by means of projection drawings, as described by Pusey (1939). The left posterior coelomic vesicle, and the right coelomic rudiment have each by this time developed a small internal cavity, by splitting as in the case of the other vesicles. With the exception of the hydrocoel, the coelomic vesicles have now reached the greatest degree of development that they ever attain, being, like the larva as a whole, quite vestigial. The hydrocoel now measures over 100 μ in length, and is about 50 μ broad. The left posterior coelomic vesicle is more rounded, and about 50 μ in diameter. The right coelomic rudiment is more elongated, about 80 μ in length by 20 μ across. The entire embryo has a somewhat oval outline, the major axis of length corresponding with the antero-posterior axis of the larva, and measuring about 250 μ, with a breadth of 150 μ.

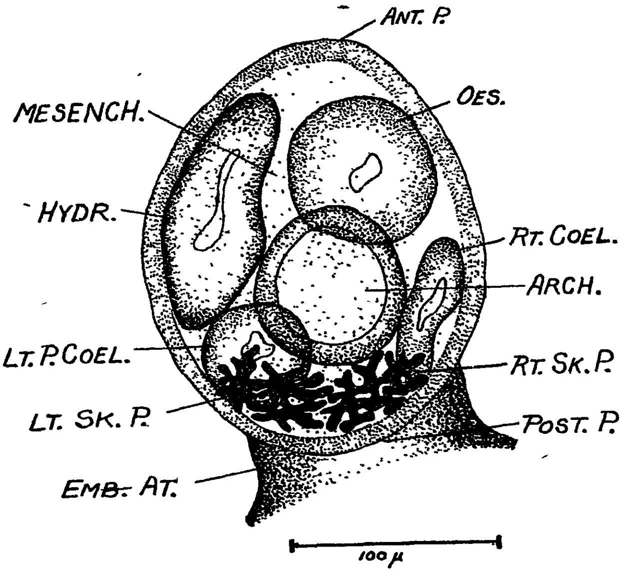

FIG. 9.—The early larva when all the coelomic vesicles are present. Reconstructed from sections.

Emb.At., embryonic attachment; Lt.Sk.P., left skeletal plate; Rt.Sk.P., right skeletal plate; Lt.P.Coel., left posterior coelom; Hydr., hydrocoel; Mesench., mesenchyme; Ant.P., anterior pole; Oes., oesophageal sac; Rt.Coel., right coelomic vesicle; Arch., archenteron; Post.P., posterior pole.

At the posterior pole of the larva some of the mesenchyme cells have taken on the character of spiculoblasts, and have secreted two meshes of calcareous matter which are the vestiges of the larval skeletal plates. These will be described more fully below. While this differentiation of the coelomic vesicles from the mesenchyme has been occurring, the embryo has achieved an organic attachment to the wall of the bursa of the parent, and the cells of the wall of the abradial horn of the bursa begin to grow out to form the embryonic attachment (called " umbilical cord " by the early workers). This structure is more fully described below.

Certain changes now begin to take place at the anterior end of the larva. The cavity of the oesophageal rudiment has been steadily extending, and its cells begin to take on the appearance of an epithelium, as in the section shown in Fig. 10. The hydrocoel has also begun to differentiate, and in the same section it can be seen that a division into two lobes has occurred, these being cut across. In the right coelomic vesicle, and the left posterior coelomic vesicle, no changes or growth in size have occurred, and soon these structures are seen to undergo a process of degeneration, for they are vestigial, like so many structures of the peculiar reduced larva which forms. The vestigial larval skeleton, however, is still increasing in size and degree of differentiation. Soon the right and left posterior coelomic rudiments become disorganised and no longer distinguishable from the surrounding mesenchyme, into which their component cells come to be merged. Thus not all of the organs of the vestigial larva reach

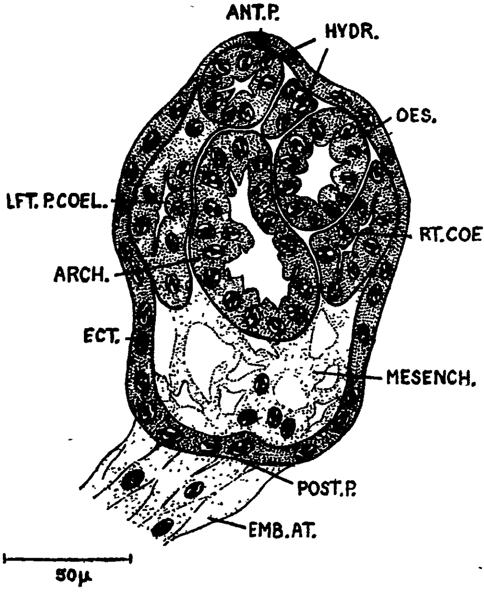

FIG. 10.—Vertical section of early Larva.

Ect., ectoderm; Arch., archenteron; Lft.P.Coel., left posterior coelomic vesicle; Ant.P., anterior pole; Hydr., hydrocoel; Oes., oesophageal sac; Rt.Coel., right coelomic vesicle; Mesench., mesenchyme; Post.P., posterior pole; Emb.At., embryonic attachment.

their greatest development simultaneously, for while the coelomic vesicles are disappearing the vestigial larval skeleton and the hydrocoel are growing.

Contrary to certain inaccurate figures and statements made in the papers of Metschnikoff (1869), Russo (1891) and others, and unfortunately reproduced in subsequent literature, there is no opening to the exterior either via an oesophagus or anus. Both the archenteron and the oesophageal sac are still closed vesicles, neither communicating with the other.

THE VESTIGIAL PLUTEUS.

The embryo is gradually growing larger, there being a notable increase in the amount of the mesenchyme, especially at the aboral (posterior) pole. This tissue comes to surround completely both the oesophagus and hydrocoel as well as the archenteron, filling up the space between these organs and the ectoderm. At the same time the mesenchyme cells become more compacted together, so as to eliminate the intercellular spaces originally present. Fig. 11 is an oblique section through the larva, so directed as to pass through all lobes of the hydrocoel—these having now reached the maximum number of five; for no further differentiation of the organ occurs till the pluteus stage has been superseded. Each lobe is a rudiment of a future radial ambulacral canal, and at present they are placed in linear series in an antero-posteral direction on the left side of the oesophagus. The cells of the archenteron—or stomach as it may now be termed—have multiplied to form a wall two cells deep. In Fig. 11 the section, being oblique so as to show all lobes of the hydrocoel, does not pass through the centre of the stomach, and so only a small part of it is seen in the section. The wall tissue of the oesophagus

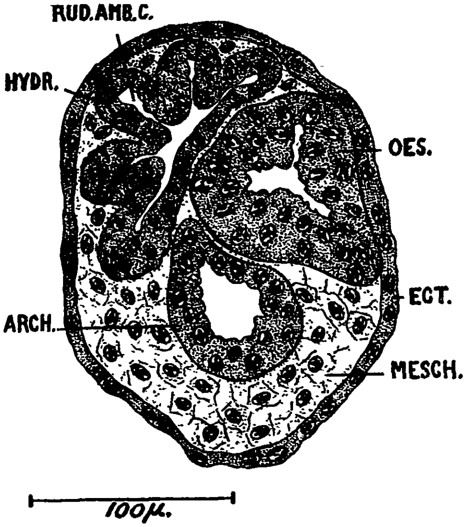

FIG. 11.—Oblique section of later larva, showing five-lobed hydrocoel.

Arch., archenteron; Hydr., hydrocoel; Rud.Amb.C., rudiment of ambulacral canal; Oes., oesophageal sac; Mesch., mesenchyme; Ect., ectoderm.

has also increased in depth, reaching two or three cells thickness, while the central lumen has assumed a three-lobed shape. The embryo is now becoming more transparent, so that it is possible to utilise whole mounts if well cleared. A slightly older stage, when the larva has reached its fullest development as a pluteus, is drawn in Fig. 12, from a cleared whole object. The pluteus has a somewhat pyriform shape, rather pointed at the anterior (oral) pole, and rounded in the posterior part to which is joined the embryonic attachment. The oesophagus in the specimen figured is somewhat elongate in form, but this character is variable, some specimens having the organ rounded. The wall of the oesophagus is thickened and its lumen consequently very narrow. To the left is seen the five-lobed hydrocoel with the rudiments of the ambulacral canals visible within the five lobes. The large, spherical stomach occupies most of the aboral part of the larval body. The ectoderm is in the form of a thin sheet of investing cells of regular arrangement. On either side of the stomach extend forwards the two vestigial skeletal rods of the pluteus, whilst triradiate rudiments of some of the plates of the future adult are to be seen scattered in the more superficial regions of the mesenchyme just below the ectoderm.

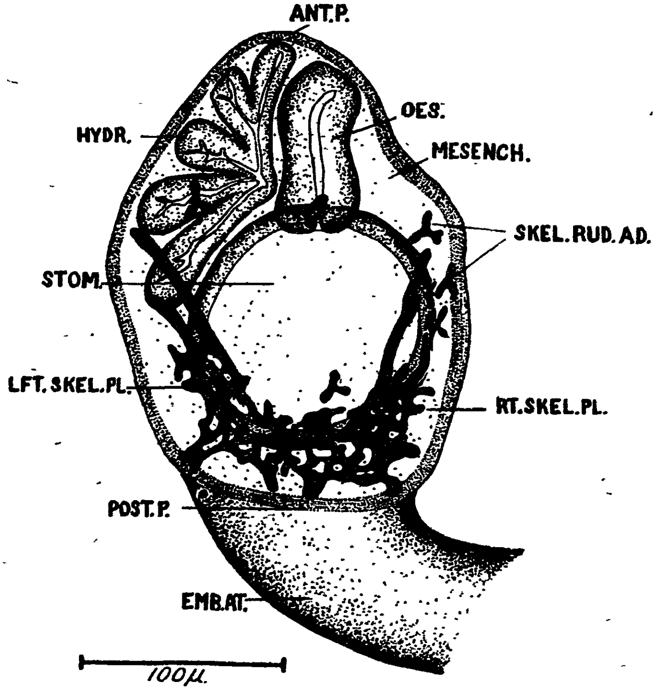

FIG. 12.—Fully-developed larva, with embryonic attachment.

Post.P., posterior pole; Lft., Rt., Skel.Pl., left, and right skeletal plate of larva; Stom., stomach; Hydr., hydrocoel; Ant.P., anterior pole; Oes., oesophageal sac; Mesench., mesenchyme; Skel.Rud.Ad., skeletal rudiment of the adult; Emb.At., embryonic attachment.

The relationship between this peculiar reduced and vestigial larval stage and a typical pelagic Ophiopluteus is illustrated in diagrammatic form in Fig. 13, where it can readily be seen that the pluteus found in *Amphipholis squamata* is in reality an armless version of the strongly bilaterally symmetrical larva of those types of ophiuroids which have an indirect, pelagic development. As is shown elsewhere (Fell, 1945), this vestigial larva of *Amphipholis* can be regarded as but one of a series of forms which illustrate various stages in the gradual reduction and disappearance of the larva in the ontogeny of ophiuroids. It has been one of the purposes of this study to obtain information on such a reduced larva as this in order to achieve some further degree of understanding of the manner in which the larva has been so completely lost by Kirk's ophiuroid. While the general relationships of the reduced pluteus of *Amphipholis squamata* are more fully discussed elsewhere, it will be useful to note here the more salient points in which it differs from the pelagic Ophiopluteus. These are:—

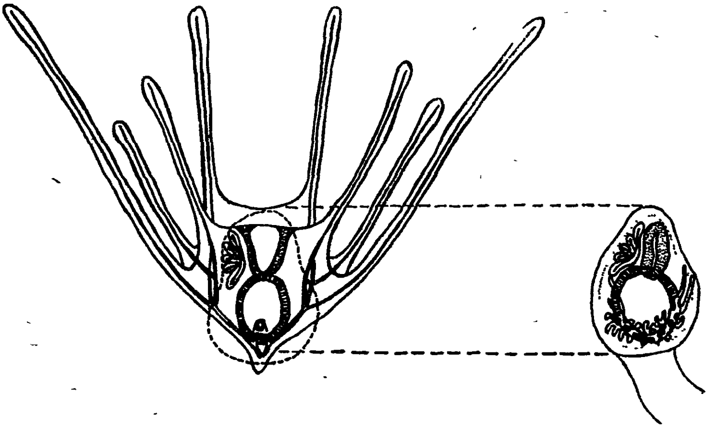

Fig. 13.—Diagram illustrating the relationship of the vestigial pluteus of *Amphipholis squamata* (right) to the fully-developed ophiopluteus of ophiuroids with indirect development (left).

(1) All four pairs of larval arms have disappeared, leaving only traces of their skeletal rods in the two calcareous plates on either side of the stomach.

(2) The pluteus here is not free-living, being formed within the bursa of the parent, and attached to it by an outgrowth of cells from the wall of the bursa. This organ is termed here the "embryonic attachment."

(3) The tissues are more heavily charged with yolk material. Nutrition is obtained partly from this substance, and partly from secretions of the bursal wall (see below), and thus a pelagic food-gathering stage is rendered physiologically unnecessary.

(4) As a condition correlated to the previous one, the alimentary system is not yet functional. The oesophageal sac is still closed off from the exterior, and there is no anus developed.

(5) The only part of the original coelomic sacs to survive is the well-developed hydrocoel. This is interesting in view of the conditions found in Kirk's ophiuroid, where the hydrocoel is the part of the definitive coelom which is first brought into being. Here also the hydrocoel is the first part of the definitive coelom to form, there being no general coelom present.

(6) The mesenchyme is very greatly developed, a condition also paralleled in Kirk's ophiuroid. In the present instance, as in Kirk's ophiuroid, the mesenchyme is destined to play a most important part in the subsequent differentiation of the coelom of the adult.

Before passing on to describe the metamorphosis, it is convenient at this point to consider in greater detail the larval skeleton, the embryonic attachment, and the mode of nutrition.

THE LARVAL SKELETON.

As previously noted, the larval skeleton originates as two small meshes of calcareous material situated towards the aboral pole of the larva. Although I have not been able to observe a specimen showing the structure at the moment of their earliest appearance, there can be little doubt that here, as in the case of nearly all other skeletal plates of ophiuroids, the rudiment is at first a triradiate spicule in either case (see Fewkes, 1887; Woodland, 1907; and Fell, 1941). In my paper on Kirk's ophiuroid (1941) a description was given of a new method of observing the development of the echinoderm skeleton by means of polarised light. The same method has been employed in the present research, giving similar results. Here also each skeletal plate consists of a single crystal of calcite, shining with a bright golden light under crossed nicols, the remaining tissues being darkened. Extinction takes place along two axes which are at right angles. It was found here also that the longer and shorter morphological axes of the skeletal plates correspond approximately with the crystalline axes of the plates, as indicated by extinction. Thus in *Amphipholis,* as in Kirk's ophiuroid, there is a correspondence between molecular orientation and the anatomical orientation of skeletal plates. It is an extraordinary fact that protoplasm should have the power of causing calcite crystals to be precipitated with their molecular chains aligned along predetermined axes. It is perhaps to be compared with the property of mica of causing crystals of other substances to be formed in parallel series on its surface, a suggestion for which I am indebted to Dr. C. A. Beevers, of Edinburgh. This property of mica depends on the molecular orientation of its surfaces; possibly therefore the surface layers of the protoplasm of the skeletogenous cells may act in some similar way.

An average of eight readings gave the angle of inclination of the axes of the skeletal plates of the pluteus as approximately 41°. Comparative study shows that as a general rule each of the two plates has one long, slender branch extending laterally on either side of the stomach, and sometimes two of these branches may occur, one longer than the other. These clearly are vestiges of the slender supporting rods which in the typical Ophiopluteus extend beyond the central body-mass and out into the arms.

THE EMBRYONIC ATTACHMENT ("NABELSCHNUR").

This structure, as already indicated, is an outgrowth of the wall of the bursa, from the position at which the embryo at first becomes adpressed to it, after liberation of the egg from the ovary—that is, from the abradial wall. With the growth of the embryo the portion of the bursal wall immediately related to it begins to grow out, to form an elongate stalk-like organ with the embryo at its distal extremity. One is reminded of the analogous "placenta" by which the gametophyte of an angiosperm plant is attached to the wall of the seed-capsule formed by the parent sporophyte. In the figure of the fully-developed larva (Fig. 12) this organ is also seen at its greatest development. In section its structure proves to be very simple (see Fig. 10, Emb.At.), comprising only a tissue of undifferentiated cells. These are somewhat spindle-shaped, elongate and staining only lightly with cytoplasm dyes. There are no sinuses or other vascular structures present in the organ, and there is little but superficial resemblance to an umbilical cord. After the assumption by the embryo of radial symmetry (see below), the attachment survives for a while, but becomes shorter again. It is then attached to one of the interradii of the young star, towards the dorsal side (Fig. 20). After the completion of the "pentagon" stage of the young ophiuroid, the attachment atrophies, the embryo breaking off and lying freely in the lumen of the bursa. A young free embryo is sometimes found in the bursa with the stump of the embryonic attachment still projecting from one interradius.

NUTRITION OF THE EMBRYO.

The earliest workers on *Amphipholis squamata* (Krohn, Schultze, Metschnikoff) had noted the existence of the embryonic attachment, which was named "Nabelschnur" in reference to a supposed nutritive function analogous to that of the umbilical cord of a mammalian embryo. Russo (1891), as already mentioned, denied the existence of the organ, saying that the embryo merely adhered to the parent by a "pocketing" (insaccatura) of the bursal wall. Elsewhere in his paper he says that it is held in place by a "kind of cement" (una specie de cemento). This substance is said to be derived from the degeneration of the epithelium of the bursa. Needless to say, these statements are absurd and untrue; it is difficult to understand how such an error of observation could have been made. As will now be seen, these errors were but the forerunners of far worse confusion.

When Russo denied the existence of the umbilical cord described by earlier workers, he thereby deprived the embryo (as he described it) of what had hitherto been regarded as its organ of nutrition. This led him to develop what can only be described as a fantastic hypothesis, though Russo himself actually set it down as an observed fact. He states that certain cells degenerate from the bursal epithelium, and the embryo devours these, drawing them into the stomach by means of contractions of the oesophagus. It can only be observed that (a) Russo did not explain the methods by which he was enabled to observe the embryo feeding within the bursa of the parent; (b) in any case, there is no mouth opening, nor oesophageal passage (save in the inaccurate diagrams of Metschnikoff and Russo himself); (c) at this early stage there are no muscle fibres developed about the oesophagus to perform such contractions; and (d) no other observer has seen the phenomena. It is therefore necessary to reject this bizarre notion of a cannibal embryo.

In regard to the actual mode of nutrition of the embryo, it can readily be seen that the embryonic attachment cannot be essential, for two chief reasons. Firstly, it disappears soon after the metamorphosis of the embryo, whereas the greater part of the growth in size takes place after the atrophy of the attachment. Secondly, there never occur any traces of vascular organs or sinuses in the structure. The embryo lies closely invested by the wall of the bursa. Now, it will be remembered that during the course of pregnancy numerous sinuses appear in the wall of the bursa. These are absent from it at other times. This significant fact suggests that the wall of the bursa may itself be a nutritive organ, by supplying a secretion which is poured into the lumen and there absorbed directly by the tissues of the developing embryo. However, there still remains the possibility that the embryo, like that of Kirk's ophiuroid, may develop solely upon the food provided by its own yolk material—though the yolk in *Amphipholis* is much smaller in amount than that of Kirk's ophiuroid.

In order to test whether or not the yolk is sufficient for development to continue, experimental culture of excised embryos was carried out. As recorded (Fell, 1940b) it was proved that the embryo is unable to develop for more than five days *in vitro* unless certain substances (contained in Erdschreiber culture medium) be added to the culture fluid. In the light of this evidence it is reasonable to assume that secretions are in fact poured from the sinuses in the wall of a pregnant bursa, since experimental investigation confirmed the results of previous anatomical study.

It follows that the function of the embryonic attachment can be no more than that of an anchoring organ while the embryo is still minute. With increase in size the embryo no longer requires such an attachment, for it is safely held within the bursa by contact with its sides. The attachment then atrophies. It is for this reason that the term "umbilical cord" has been abandoned, and the name "embryonic attachment" used for the organ throughout this paper.

The appearance of the sinuses in the wall of a pregnant bursa invites comparison with the analogous changes in the vascular supply of the uterus of viviparous vertebrates. In vertebrates the changes in the uterus or oviduct are known to be brought about by the liberation of a hormone. The question arises whether the same holds good for *Amphipholis squamata.* It can be answered quite safely, I think, that hormones cannot be responsible—at least, not in the way that they are in vertebrates. For, whereas in vertebrates if one uterus be pregnant and not the other, both uteri respond to the hormone, in *Amphipholis,* on the other hand, it is only the pregnant bursae which become enlarged and develop sinuses, the others remaining unchanged. Therefore the anatomical and physiological changes brought about in the bursa must be attributed to the direct contact stimulation provided by the embryo itself.

THE METAMORPHOSIS.

The description of the development of the embryo has been brought up to the stage of the fully-formed larva. The transformation of this simplified, but nevertheless easily recognisable pluteus with bilateral symmetry into the radially symmetrical ophiuroid can now be described.

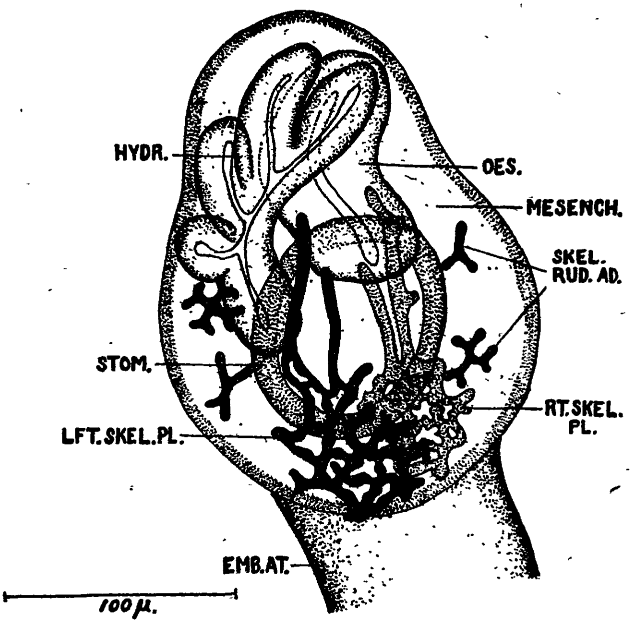

FIG. 14.—Larva viewed from the left side, at the commencement of metamorphosis.

Emb.At., embryonic attachment; Lft.Skel.Pl., left skeletal plate of the larva; Stom., stomach; Hydr., hydrocoel; Oes., oesophageal sac; Mesench., mesenchyme; Skel.Rud.Ad., triradiate skeletal rudiment of the adult; Rt.Skel.Pl., right skeletal plate of the larva.

As usual in the ophiuroids, metamorphosis seems to be initiated by a change in the position and shape of the hydrocoel. The organ begins to curve round the oesophagus (Fig. 14) so that the five lobes originally placed in linear series on the left side now begin to take up a position such that each lobe begins to point outwards, the lobes being at equally spaced intervals about the oesophageal sac. Not all stages of this process have been obtained, but there is no reason to believe that there are any important differences from what has already been described for *Ophiothrix fragilis* by MacBride (1907) and *Ophiocomina nigra* by Narasimhamurti (1933). No doubt the change in the shape and relations of the hydrocoel is brought about by the liberation of a growth substance, as first recorded in echinoplutei by Huxley (1928). The ring canal becomes established about the oesophagus when the two extremities of the hydrocoel meet and fuse on the right side of the oesophagus, whilst the five lobes already present form the five radial ambulacral canals. Nothing was found comparable with the peculiar process of rotation through 360° of the hydrocoel of *Ophiura brevispina*, as described by Grave (1900). While the hydrocoel is encircling the oesophagus, there occurs a flattening of the embryo, shortening the antero-posteral axis and causing the embryo to become more spherical in form. The anterior surface of the embryo becomes at the same time rather flattened.

It thus happens that the anterior (or apparent anterior) hemisphere of the larva becomes directly transformed into the ventral half of the young ophiuroid, and the posterior hemisphere becomes dorsal. The correspondence of the regions, however, is not an exact one, for the actual posterior pole of the larva is found after metamorphosis to have become dorso-lateral, being midway between the central point of the dorsal surface of the star and the periphery of one interradius. The only landmark of value which remains unaltered during the metamorphosis is the embryonic attachment—which, it will be recalled, is united to the posterior pole, thereby providing a useful index to the position of that region. This organ therefore comes to be dorsal and interradial in the young attached ophiuroid. The interradius to which the embryonic attachment is joined is always either of the two interradii which are furthest from the sector subsequently occupied by the madreporic canal (see Fig. 20).

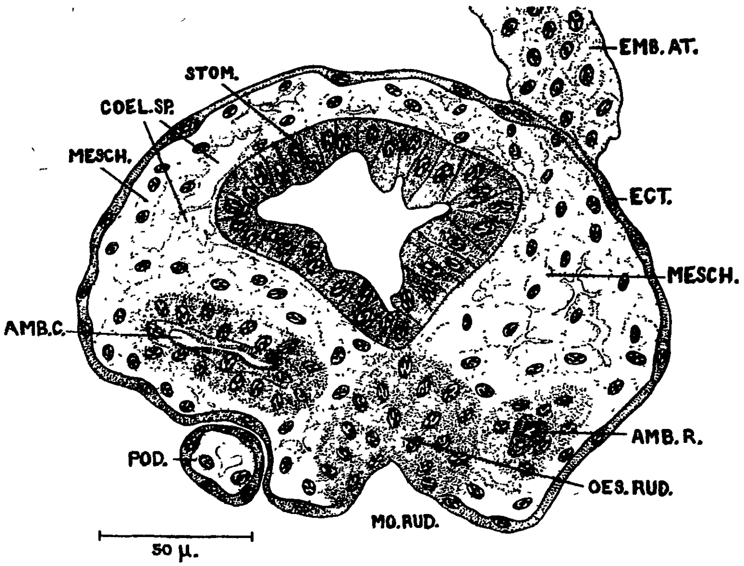

FIG. 15.—Vertical section through a newly-metamorphosed star, still attached to the parent.

Pod., podium; Amb.C., ambulacral canal; Mesch., mesenchyme; Coel.Sp., intercellular schizocoelous splits forming the perivisceral coelom; Stom., stomach; Emb.At., embryonic attachment; Ect., ectoderm; Amb.R., ambulacral ring; Oes.Rud., oesophageal rudiment; Mo.Rud., mouth rudiment.

ORIGIN OF THE PERIVISCERAL COELOM.

Soon after the embryo has assumed radial symmetry a change is observed in the texture of the mesenchyme, closely similar to that which has already been described for Kirk's ophiuroid (Fell, 1941). Between some of the cells of this tissue a process of splitting begins to take place, producing a number of intercellular cavities which are at first small. Serial sections reveal that there is another similarity to what occurs in Kirk's ophiuroid, in that this process of splitting begins in the outermost zone of mesenchyme, extending only subsequently to the more dorsal and central region. Thus, the mesenchyme immediately above the stomach is the last to be affected in this way. In Fig. 15 is illustrated a vertical section through the central region of an embryo in which the schizocoelous splitting is still at an early stage. There are a number of isolated cavities in the mesenchyme, and only in the outer and more lateral regions have they extended so as to link up with neighbouring clefts. Continuation of the process results in the fusion of all the spaces to produce the general body cavity, or perivisceral coelom. A vertical cross-section of one of the incipient arms of a young star, showing the earliest stage in the schizocoelous excavation of the perivisceral coelom, is seen in Fig. 16. Further extension of the splitting, with consequent linking up of the spaces produced, causes the coelom to take on what is essentially its adult relationship with the neighbouring organs. Its lining is at first irregular (Fig. 17), with cells and cytoplasmic bridges projecting into the lumen; but soon a coelomic epithelium is differentiated from the bounding mesenchyme cells, so that the perivisceral coelom takes on a more regular form (Fig. 18).

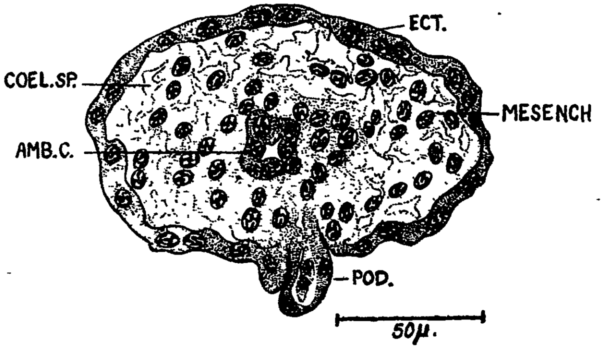

FIG. 16.—Vertical section through the rudiment of the arm of a young star in which the coelom is appearing as splits between the cells of the mesenchyme.

Amb.C., ambulacral canal; Coel.Sp., schizocoelous intercellular splits forming the coelom; Ect., ectoderm; Mesench., mesenchyme; Pod., podium.

At this point it is proper to reiterate that one of the chief purposes of this study was to re-examine the statement made by Russo that the general coelom arises in *Amphipholis squamata* by splitting in mesenchyme (Russo, 1891). It can now be seen that (1) Russo's claim in this regard is completely confirmed, and that he thus is the original discoverer of this mode of origin of the perivisceral coelom in an echinoderm; and (2) the mode of origin of the perivisceral coelom in *A. squamata* differs but little from what has been described for Kirk's ophiuroid. It now remains for future research to show whether the coelom can arise by schizocoelous development in any other groups of the echinoderms. Further discussion of the point is reserved for consideration in connection with the problem of direct development among the echinoderms in general in a separate paper (Fell, 1945).

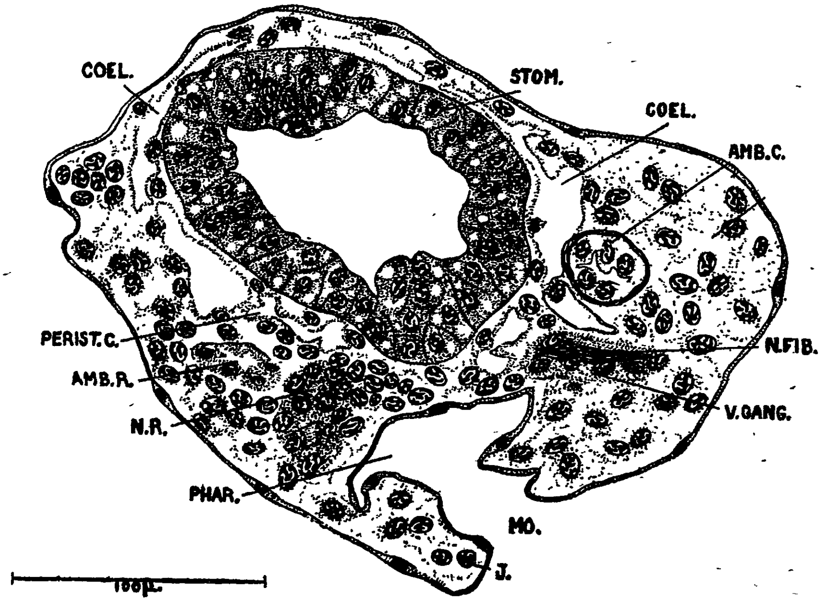

FIG. 17.—Vertical section of an elder embryo showing complete differentiation of the coelom.

Epineur.C., epineural coelom; N.R., nerve-ring; N.Fib., nerve fibres; D.Gang., V.Gang., dorsal and ventral ganglia; Perihaem., perihaemal coelom; Amb.C., ambulacral canal; Amb.R., ambulacral ring; Coel., general coelom; Stom., stomach; Perit., peritoneum; Mes., mesoderm; Ect., ectoderm; Perist.C., peristomial coelom; Phar., pharynx; Buc.T., buccal tentacle; R., radius; I.R., interradius.

One notable difference from Kirk's ophiuroid concerns the relative times of appearance of the mouth opening. Kirk's ophiuroid is already free-living, with a completed mouth opening leading into the stomach before any perivisceral coelom is formed. In *A. squamata,* on the other hand, when the perivisceral coelom has been formed, the alimentary canal is still very rudimentary, the buccal region being as yet unpierced.

NERVOUS SYSTEM.

Russo (1891) claimed to have recognised the nervous system at its earliest appearance as " four yellow cells " said to be attached to the ectoderm and stomodaeum soon after the formation of the latter. The cells were stated by him to be recognisable by their distinctive colour. This observation, according to his description, was made on living embryos without any use of sections. His figures represent the four cells in a highly diagrammatic manner, situated two on either side of the stomodaeum. His account has been quoted by Ludwig in Bronn's *Thier-Reichs* (1901) and by Delage and Herouard (1903). This statement by Russo is typical of the unfortunate mixture of good and bad observation found in his paper. To anyone who is familiar with the extreme opacity and dense pigmentation of early embryos of echinoderms with yolky eggs such as *A. squamata*, the improbability of his claim is obvious. In fact, the nervous system cannot be traced back to any such early stage with the help of such differential staining as is given by Mallory or Heidenhain's haematoxylin in sections. Still less probable is it that four cells of an unfixed embryo could be distinguished from the mass of deeply coloured mesenchyme which surrounds the stomodaeum without any staining at all.

Cuenot (1891), working also on *A. squamata*, and in the same year as Russo, was unable to detect any nervous tissue till the embryo had reached a diameter of 240 μ. He stated that the nerve-cords arise by an invagination of " gutters " of ectoderm from the ventral sides of the arms. However, MacBride, in the following year, pointed out that the nervous elements are already deep-seated before any arms are developed, so that Cuenot's observations must have been faulty. As he was engaged on a study of certain features of the late, post-embryonic development (the genital rudiments, ovoid gland, etc.), MacBride was unable to give any account of the true mode of origin of the nervous system, and so the matter remained thus indeterminate till the present day.

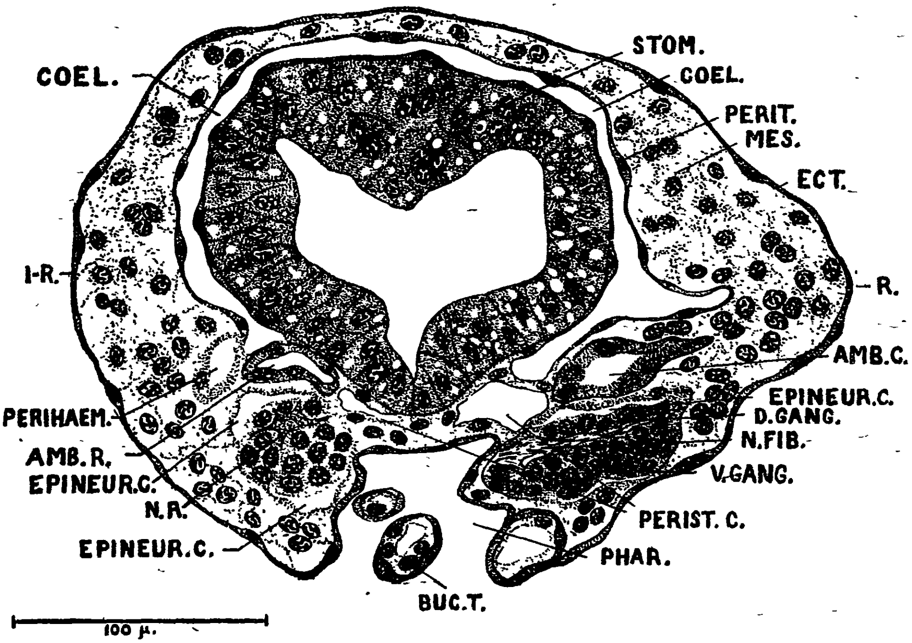

FIG. 18.—Vertical section showing later stage in the differentiation of the coelom.

Phar., pharynx; N.R., nerve-ring; Amb.R., ambulacral ring; Perist.C., peristomial coelom; Coel.Pv., perivisceral coelom; Stom., stomach; Amb.C., ambulacral canal; Mes., mesenchyme; N.Fib., nerve fibres; V.Gang., ventral ganglion; J., jaw; Mo., developing mouth region, In my paper on Kirk's ophiuroid, I did not give any account of the origin of the nervous system, as it seemed desirable to examine a wider range of material than I possessed at that time; but I mentioned that presumably it would originate as an invagination from the ectoderm of the lower arm-surface, as stated in text-books (a belief probably to be traced back to Cuenot's account, after repetition from one text-book to another). I have since obtained more material of Kirk's ophiuroid, and find that the origin of the nervous system is essentially the same both for it and for *A. squamata.* This is now described.

It will be recalled that the larval oesophagus was derived from an inpushing of a solid mass of ectoderm cells, in the centre of which a small cavity later developed by splitting. As the larva grew larger, the cells of the wall of the oesophageal sac proliferated, to make the wall thicker, and at the same time reducing the size of the lumen. Up to the time of the metamorphosis this structure remains as a thick-walled sac, very clearly demarcated from the surrounding mesenchyme, and opening neither to the exterior nor to the stomach (Fig. 11). After metamorphosis a change occurs in the organ. Vertical sections show that the cells of the periphery of the oesophagus become merged with the surrounding tissue (mesoderm), so that the organ is no longer easily distinguishable from the mesoderm. Also the small cavity present in the organ during the larval stage continues to become reduced, and finally it disappears. It is now possible to speak only of an "oesophageal region," for no oesophageal sac remains as such, nor is there any mouth present (see Fig. 15). In sections of embryos a little more advanced in development, there begin to appear the first clear indications of nervous tissue, taking the form of a ring of rudimentary ganglionic masses about the periphery of the oesophageal region (Fig. 17). There can be little doubt that these ganglionic rudiments are derived from the outermost cells of the oesophageal mass, and thus, although deeply imbedded in mesenchyme from the time of their earliest appearance, they have nevertheless an ectodermal origin. As seen in Fig. 18, which is a vertical section through the central region of a young star in which arm rudiments are growing out (one of them being cut through on the right side of the section), the ganglionic rudiments extend abradially into the arm-bud, each coming to lie just below its corresponding ambulacral canal. The nerve-ring about the oesophageal region is completed by lateral extension of the ganglionic rudiments.

It is quite certain that the nervous elements do not arise in the manner described by Cuenot (1891). Although their ultimate origin is from ectoderm, right from the time when they first become differentiated as recognisably nervous structures, they are deep-seated. In this matter my results are in agreement with what MacBride pointed out in 1891.

Within the nervous tissue which has thus appeared a process of differentiation soon commences. The cells occupying the more ventral half of the nerve-mass in each arm-bud and in the nerve-ring develop large nuclei, while the relative amount of their cytoplasm becomes reduced. Thus are formed the rudiments of the ventral ganglion mass of the first segment of the arm in each radius, and the ventral ganglia of the nerve-ring. Immediately dorsal to these ganglion cells there develops from them a zone of fibres which send out slips to the various body tissues. Dorsal again to these fibres there develops a narrow zone of ganglion cells, similar to those of the ventral ganglion, but forming a nervous sheet only one cell deep. Subsequently this dorsal ganglion sheet comes itself to be overlaid by the perihaemal sinuses (see below). Later growth of the arms outward results only in a fuller development, and, with segmentation of the arms, serial reduplication of the regions of the radial nerves already described. In the nerve-ring a similar differentiation into ventral ganglia, intermediate fibre-zone, and dorsal ganglion sheet also occurs.

The staining reactions of the differentiating nerves are very characteristic, especially when stained with Mallory Triple stain. Before differentiation all nervous elements are evenly stained with acid Fuchsin (cytoplasm) and Orange G (nuclei). Once the three main zones are laid down, the nuclei enlarge and become more prominent, taking up the orange dye more deeply. Most stains do not affect the fibre-zone, but Aniline Blue, which is present in Mallory, gives it a pale greyish-blue tinge, thereby making it contrast strongly with the strongly nucleated ganglionic tissue on either side.

The probable ectodermal origin of the nerve rudiments from an outer plastic region of the stomodaeum, even though it is deeply imbedded in mesenchyme, is of interest, the more so now that it is realised that nerve cells do not necessarily arise from ectoderm.

PERISTOMIAL COELOM, PERIHAEMAL SINUSES AND EPINEURAL SINUSES.

The regions of the coelom so far described are the hydrocoel and the perivisceral coelom. The differentiation of the remaining parts can now be dealt with. While the process of splitting which gave rise to the perivisceral coelom was occurring, a portion of the schizocoel thus formed remained distinct from the general coelom. This is the rudiment of the peristomial coelom (or peripharyngeal coelom), which is derived from a series of small splits about the most ventral limb of the stomach, dorsal and lateral to the oesophageal mass (Figs. 17 and 18). As in the case of the perivisceral coelom, it has at first a very irregular form, with its bounding cells projecting into the lumen; but subsequently a lining epithelium is differentiated.

The perihaemal and epineural sinuses are the last coelomic structures to appear, and they arise simultaneously, or almost so. As in the case of Kirk's ophiuroid (Fell, 1941), they form in a very simple manner, as splits above and below the nerve-cords and nerve-ring, never achieving any great degree of differentiation. In much older forms a fairly well-marked lining epithelium is developed in the case of the perihaemal sinuses above the nerve-ring and radial nerve-cords; but so far as I have observed in my sections of adult individuals, the epineural sinuses remain throughout life as little more than intercellular splits without epithelia. Indeed, as Delage and Herouard (1903) have pointed out, the whole epineural system is considerably reduced in *Amphipholis squamata*. The fact is perhaps connected with the small size of the species, and is to be correlated with the simplification of the reproductive glands and other organs.

It is an interesting fact that the perihaemal and epineural sinuses, though comparatively unimportant subdivisions of the coelom, were the structures for which a schizocoelous development was first recorded. The discoverer was Hamann, in 1889, whose results were afterwards quoted in Bronn's *Thier-Reichs* (1901). Dawydoff (1901) also found that the epineural sinuses have a schizocoelous origin in the arm of an ophiuroid which is regenerating lost radii. Yet, despite the evidence produced by these workers, Delage and Hérouard (1903) rejected Hamann's account of the schizocoelous origin of the structures, and considered Dawydoff's results of little importance, though they produced no evidence of their own to the contrary. The eminence which Dawydoff has since attained in the realm of invertebrate embryology is perhaps the most suitable reply to the dogmatic attitude which has unwarrantably been adopted in the instance quoted. MacBride (1914) similarly rejected as "improbable in the highest degree" the notion of a schizocoelous development of the coelom in echinoderms, though, like Delage and Hérouard, he produced no evidence of personal research to confirm or disprove Hamann and Dawydoff. Thus the matter came to be almost forgotten, and no mention is to be found of a schizocoelous origin of the echinoderm coelom in current text-books on embryology.*

ATROPHY OF THE EMBRYONIC ATTACHMENT.

At about this stage in development, when the outline of the embryo is roughly a pentagon, the embryonic attachment becomes completely atrophied, and the embryo thus comes to lie freely in the bursa. From this stage onward the embryo is usually found oriented with its ventral (oral) surface uppermost. The significance, if there be any, of this orientation is not clear, but it is noteworthy that Sladen (1889) quotes Wyville Thomson as having observed a similar condition in the Antarctic asteroid *Leptoptychaster kerguelensis.* Little is known of the embryology of this species, but Thomson records that the young stars develop within the oviducts, with their oral faces turned uppermost. After birth they adhere for a while in the re-entrant angles between the rays of the parent, still with the oral surface upward.

OESOPHAGEAL AND BUCCAL CAVITIES.

When the original oesophageal sac became transformed into a mass of undifferentiated cells, the change not only paved the way to the development of the nervous system, but also initiated a further step in the differentiation of the alimentary canal. Hitherto the stomach has remained a blind sac, having at no stage any anal opening, nor any connection with the exterior via the oesophageal sac. There now occur two convergent cone-shaped excavations into the oesophageal mass. One of these occurs upwards from the outer

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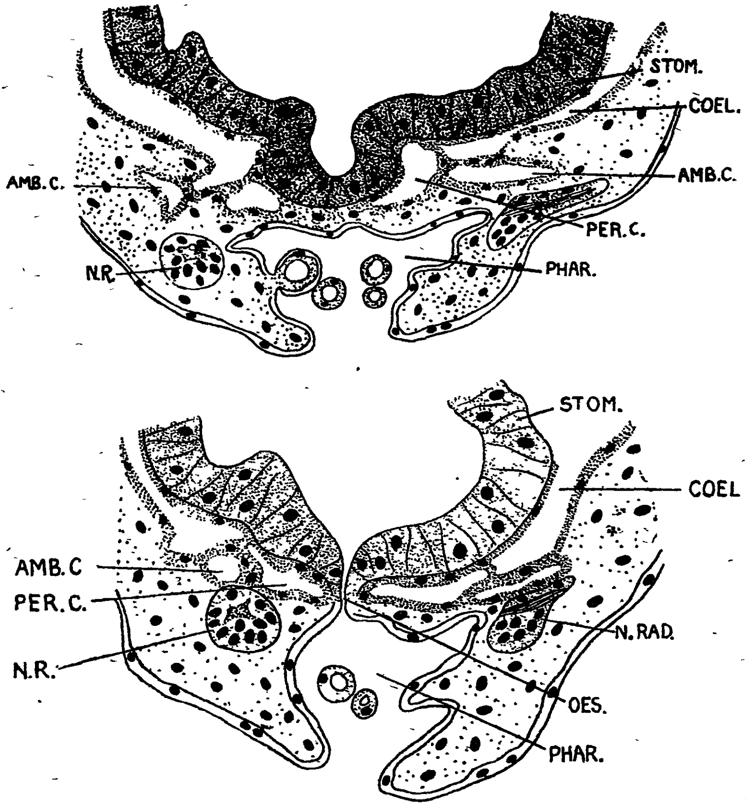
* The above was written in 1941. The ninth edition of Thomson's *Outlines of Zoology* (1944), which was edited by Professor James Ritchie, contains this information.

ectoderm of the mid-ventral surface. As will be seen from the sections shown in Figs. 17 and 18, there has already existed here for some time a hollowing out which is obviously the forerunner of a buccal cavity. Figs. 19A and 19B illustrate the final stages in the process, when the second excavation occurs, downwards from the central-most part of the stomach. In this way there forms first a blind pouch from the stomach towards the buccal cavity (Fig. 19A), and then finally the oesophageal passage is pierced, and for the first time the alimentary canal is brought into being (Fig. 19B).



Figs. 19a (*above*) and 19b (*below*).—Later stages in the piercing of the mouth.

Amb.C., ambulacral canal; N.R., nerve-ring; N.Rad., radial nerve; Per.C., peristomial coelom; Phar., pharynx; Coel., coelom; Stom., stomach.

Later Changes.

From now onward the growth of the embryo is closely similar to what has already been described for Kirk's ophiuroid (1941). Soon after passing the "Asterina" stage, the arms begin to show the first signs of segmentation. The ambulacral system extends by outward growth into the arms. There arise the first set of podia, then the buccal tentacles between the first podia and the mouth, and then the second and third, etc., sets of podia are developed centrifugally.

These later changes have been described for Kirk's ophiuroid, and thus need not be repeated here in detail. The skeletal system develops within the mesenchymatous tissues in the disc and arms in the way already described by Ludwig (1881), Fewkes (1887), and Fell (1941). As in Kirk's ophiuroid the madrepore is situated on an oral shield, at first near the periphery of the disc, but afterwards carried downwards on to the ventral surface when the oral shields take their place among the mouth ossicles. There remains thus only to describe the birth of the young ophiuroid.

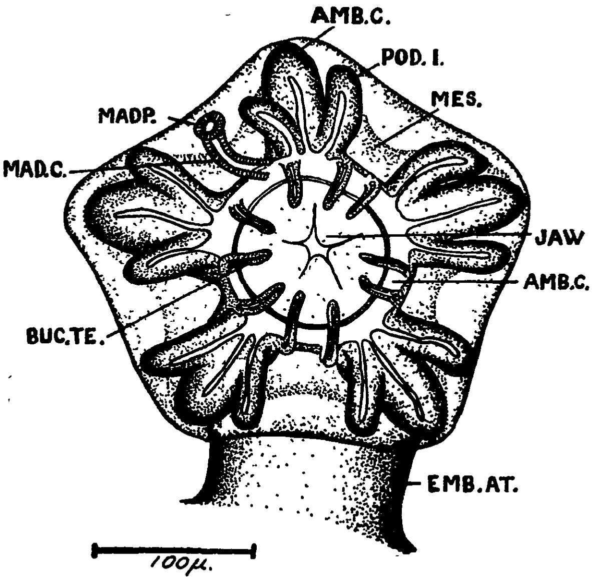


FIG. 20.—Ventral view of an attached "Pentagon."
Buc.Te., buccal tentacle; Mad.C., madreporic canal; Madp., madrepore; Amb.C., ambulacral canal; Pod.1, first podium; Mes., mesoderm mass; Jaw, developing jaw; Amb.R., ambulacral ring; Emb.At., embryonic attachment.

BIRTH OF THE YOUNG OPHIUROID.

As the arms increase in length they are folded above the disc, twisted in a spiral fashion. The mode of folding of the arms causes the young animal to have a roughly spherical form, thus occupying the smallest possible amount of space within the body of the parent. As mentioned before, the embryo usually lies in an inverted position, with its ventral surface turned towards the dorsal aspect of the parent. In Fig. 1 is shown a dissection of an interradius of a pregnant adult, with an embryo oriented in this manner within the bursa.

The actual process of birth was observed on one occasion. The embryo uncoiled its arms so that they became directed downwards to the genital cleft. Then, solely by its own muscular efforts, the young animal crept out of the bursa in such a manner that it emerged with its arms directed away from the nearby arm of its parent. The process occupied over three hours, but may have been delayed by the unfavourably bright conditions on the microscope stage (strong light, as recorded in a previous paper, having a narcotic effect on young ophiuroids). When free from the parent, the young ophiuroid, which had about fifteen arm segments, fell to the bottom and commenced an independent existence. The young forms are at first colourless, except for the pinkish disc, but after ten days or so a greyish pigmentation begins to appear. The newly-born ophiuroids, and also those which have been free-living for some time after birth, make great use of the podia in progression, these organs having in early life a true tube-foot function. Thus young ophiuroids are enabled to climb vertical glass surfaces, and to a certain limited extent the capacity is retained by the adult. These facts go to confirm the observations of Smith (1937), and also of some earlier workers, on the function of the tube-feet of ophiuroids. It is still commonly stated in text-books that the podia of ophiuroids have only a sensory function—with possible use as respiratory organs; such statements are based on inadequate observation.

SUMMARY.

1. The morphology of the reproductive organs is described. Both the testes and ovaries are very simple, and have no ducts.

2. Reasons are given for believing that *Amphipholis squamata* breeds all the year round in British waters, except in severe winters, when the species temporarily disappears from the east coast of Scotland, and probably breeds all the year in New Zealand.

3. Only a single egg is normally brought to maturity at any one time in each of the ten ovaries. The egg measures about 100 μ in diameter, and is opaque owing to an intense reddish colouration closely associated with the yolk material in the cytoplasm.

4. The structure of the gastrula indicates a close similarity at this stage of *A. squamata* to Kirk's ophiuroid. As in the last-named species, gastrulation in *A. squamata* almost certainly takes place by means of a very reduced form of invagination, in which a solid mass of hypoblast cells is inturned without any inturned archenteron. Gastrulation does not occur by means of delamination as has been supposed by some earlier authors.

5. The archenteron is formed subsequently within the inturned hypoblast, almost certainly by means of splitting, though intermediate stages of the process were not observed. A similar process of excavation of the archenteron in hypoblast has been recorded in *Ophiura brevispina* and Kirk's ophiuroid.

6. The oesophageal sac arises first as a solid inpushing of ectoderm (stomodaeum) from the anterior pole. A cavity is subsequently formed in the mass by means of splitting.

7. An extensive mesenchyme mass arises by proliferation of mesodermal cells derived from the outermost layers of the hypoblast.

8. Although development is partially direct and the coelom (except for the hydrocoel) forms later as a schizocoel, there form nevertheless vestigial coelomic sacs. These vesicles do not arise from pinched off portions of archenteron, but by the aggregation of some of the mesenchyme cells derived from the wall of the archenteron.

These aggregations are at first solid, but a small cavity afterwards forms within each mass by a process of splitting. On the left side two such vestigial coelomic vesicles form, an anterior one which becomes the definitive hydrocoel, and a posterior one which subsequently disappears. On the right side there forms one small vesicle, representing the right hydrocoel, but it subsequently disappears without giving rise to any permanent structure.

9. The hydrocoel is thus the first part of the definitive coelom to form. This also is the case in Kirk's ophiuroid, where, however, it is formed in a purely schizocoelous manner.

10. There is formed a vestigial, bilaterally symmetrical pluteus, which corresponds to the central part of the normal pelagic Ophiopluteus, and possesses a reduced larval skeleton in the form of two calcareous plates on either side of the archenteron. It lacks an anus, as also any mouth-opening, having only a closed archenteron and closed oesophageal sac.

11. Metamorphosis is initiated in the usual way by the encircling of the oesophageal region by the five-lobed hydrocoel.

12. After assumption of the radial form the perivisceral coelom arises by means of the fusion of a number of splits which take place between the component cells of the mesenchyme. The latter tissue is unusually well developed in the embryo of *A. squamata,* which in this respect is comparable to the young stages of Kirk's ophiuroid.

13. The peristomial, perihaemal and epineural coeloms all arise by means of a continuation of the process of splitting in mesenchyme. Thus, except for the hydrocoel the whole coelom is a schizocoel, and in having this mode of origin it is comparable to that of Kirk's ophiuroid.

14. The late piercing of the oesophageal passage is notable, and may perhaps be accounted for in the light of the information which has been obtained in regard to the nutrition of the embryo.

15. The existence of an embryonic attachment during part of the development, uniting the larva and the parent, is confirmed; but evidence is provided showing that it is very improbable that the attaching organ is used in nutrition, as earlier workers had supposed. It is more probably to be interpreted as an anchoring organ while the embryo is yet very small.

16. The changes which occur in the structure of the bursa during the course of development of the contained embryo are described. Sinuses appear in the bursal wall during the later stages of pregnancy. Evidence obtained from culture *in vitro* of excised embryos shows that the embryo is unable to develop for long on its own yolk material alone, requiring the addition of other substances. This, in conjunction with morphological evidence, is taken to indicate that the wall of the bursa secretes nutritive substances.

17. Birth of the young ophiuroid is brought about solely by its own muscular efforts. It occupied about three hours in an actual case observed, but the length of time may have been artificially increased by unfavourable conditions. The newly-born embryo sinks to the bottom and begins an independent existence.

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