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DIRECT DEVELOPMENT IN THE OPHIUROIDEA, AND ITS CAUSES.

by

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With twenty-six figures and one plate.

Part 1

THE EMBRYOLOGY OF THE VIVIPAROUS OPHIUROID *AMPHIPHOLIS SQUAMATA* Delle Chiaje.

Introduction

In a previous paper a general account has been given of the embryology of a New Zealand ophiuroid with direct development, lacking any vestige of a larval form (Fell, 1941). For reasons given there and again made clear in the historical summary below, it became desirable that a reinvestigation of the development of *Amphipholis squamata* should be made. For this purpose the research described in the present paper was carried out at the Department of Zoology in the University of Edinburgh, with the aid of a Shirtcliffe Fellowship grant from the University of New Zealand.

To Professor James Ritchie I have to express my gratitude for assistance and advice during the course of the work, and for all the facilities of the laboratory. I am indebted to Dr. F. Gross for help in the culture *in vitro* of excised embryos, and also to Mr, Richard Elmhirst, director of the Marine Laboratory, Millport, for arranging the forwarding of supplies of fresh, living material to supplement material collected in the Firth of Forth.

Nomenclature

From examination of the literature it appears that *Amphipholis squamata* has received no less than eighteen different names during the last 130 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 Mortenson'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 the 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 existance 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), Cuénot (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 fron what had been found for <u>Ophiothrix fragilis</u>, 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 uot 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 e/ vesicla entirely disappearing. According to him the two posterior vesicles give rise to the perihaemal coelom ("sistema circolotare"). a/

9. Periviceral 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 Cuénot, 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". lc

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

1903 Delage and Hérouard 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 Cuénot 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 Cuénot'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 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 <u>Amphipholis</u> and those of crinoids. f/

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[1] 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 ophiuriod (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 New Zealand ophiuroid *Ophiomyxa brevirima* the perivisceral coelom 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.

The Problem

The present paper is concerned with elucidating three principle questions. These are:-

(1) What is the true nature of the development of *Amphipholis squamata*, and in particular, what is the

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

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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?

Part 1 of 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 Part 2 of the paper, in which are utilised the results given in the first part.

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 here. The embryo of Amphipholis contains yolk material, rendering it both opaque and brittle, but not so markedly 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 <u>Amphipholis</u> 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 a 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 <u>in vitro</u>; 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 embryos is a delicate operation for the disc of the parent measures only from 3-5mm. in diameter. The pregnant adult is anaesthetised by subjection to menthol, as described above. With a fine tenotomy scalpel and forceps the disc is spearated 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 (5cm. diam.) and covered by 2-3mls. 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, uncoordinated 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	0·1gram.
Sodium hydrogen phosphate	0·02 "
Soil extract	50 mls.
Sterile sea water	1,000 mls.

The soil extract is prepared by autoclaving at a pressure of two atmospheres one kilogramme of garden soil in 1,000mls. 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 changes in the density of the sea water as echinoderms are generally supposed to be. Some specimens were alowed 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 was innoculated 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 in so far 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 differences in form or habit from those inhabiting the waters of Britain, twelve thousand miles distant.

Now the existance 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 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 land-bridge, 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 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 that the species has a restricted breeding period at that locality. Specimens from the Clyde area indicate that breeding continues uninterruptedly throughout the year on the West coast of Scotland. l.c. 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 Froth area, tr 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 December, and have little doubt that it breeds there also in the remaining months when I had no occasion to go collect-ing. These observations were made at Island Bay, on the northern shore of Cook Strait.

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 bursa 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

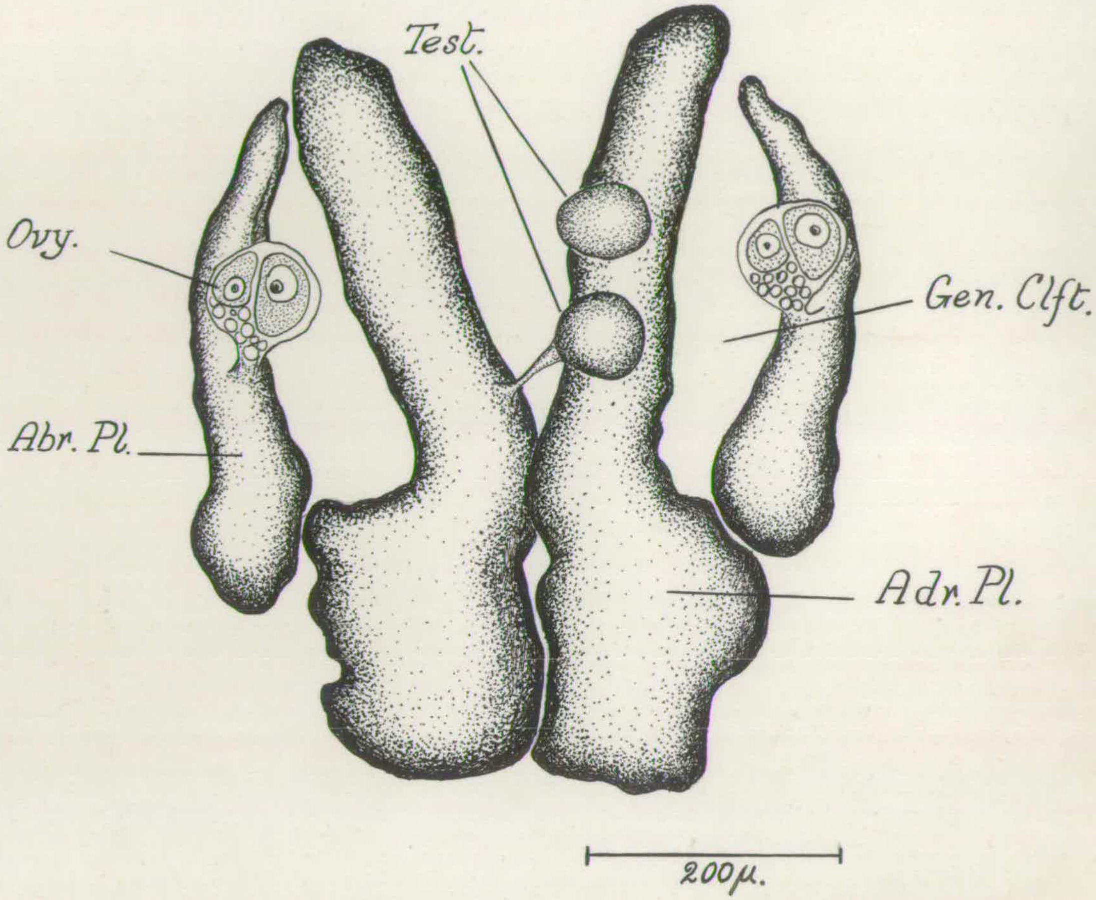


Figure 1. Genital organs of Amphipholis squamata

Ovy., ovary; Test., testis ; Gen.Clft., genital cleft; Adr.Pl., adradial genital plate; Abr.Pl., abradial genital plate .
Viewed from above .

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, three examples are illustrated (Figs. 2 - 4). In Fig. 2 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 the 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 towards 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

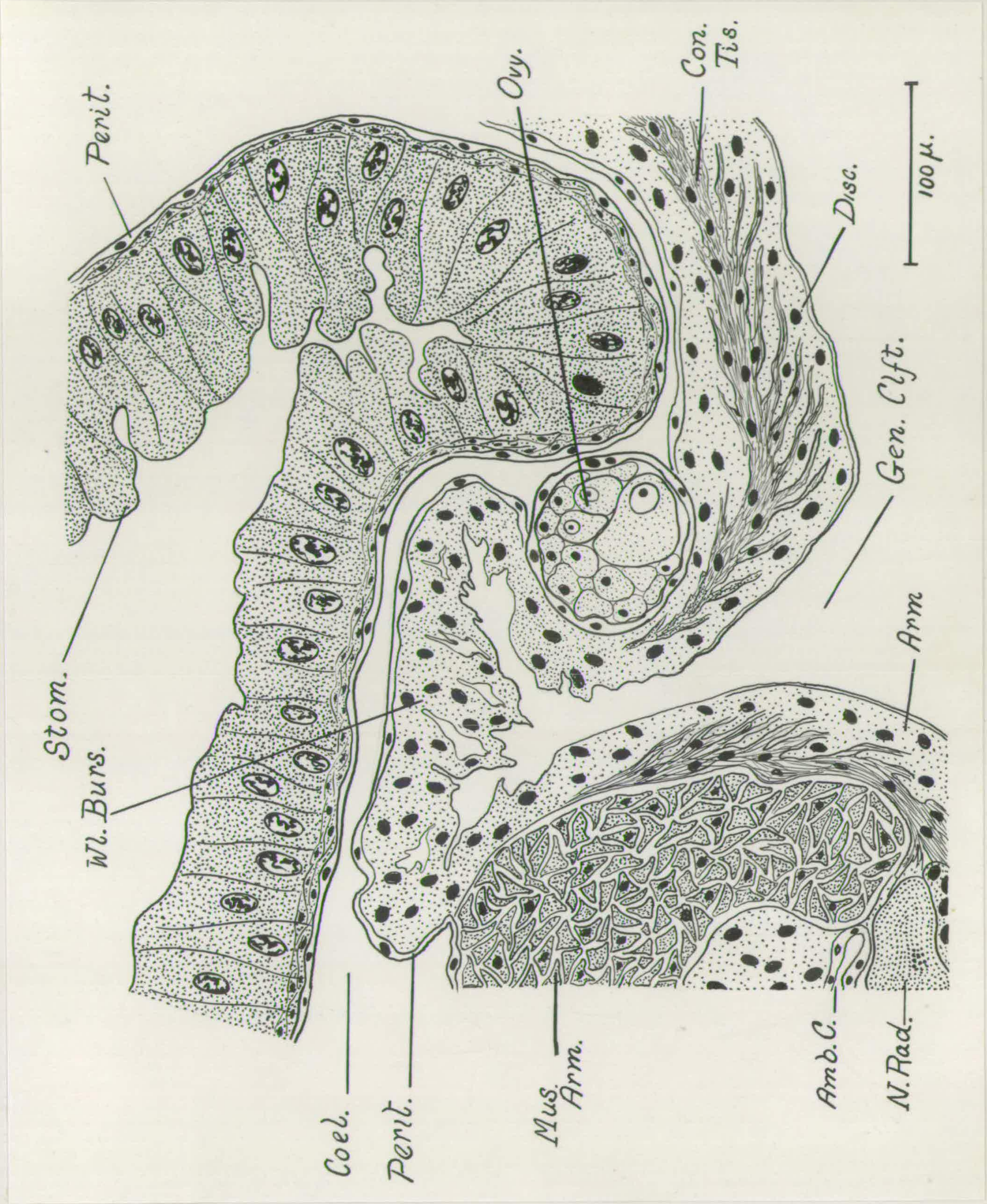


Figure 2. Vertical section shewing relations between the bursa and ovary, and related structures.

Stom., stomach; Perit., peritoneum; Ovy., ovary ; Con.Tis., connective tissue; Disc, lower surface of disc; Gen.Clft., genital cleft; Arm, portion of arm bordering the genital cleft and bursa; N.Rad., radial nerve of arm; Amb.C., ambulacral canal; Mus.Arm., arm muscles; Coel., coelom; Wl.Burs., wall of bursa .

and oocyte.

In Fig. 3 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 inter-radius 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.

Finally, Fig. 4 shows an oblique vertical section so placed as to cut both testis and ovary on either side of a bursa. The latter is in this case non-pregnant, and shows differences from the pregnant organ which will be discussed in the appropriate part of this paper. One of the oocytes in the ovary seen in this section is almost mature.

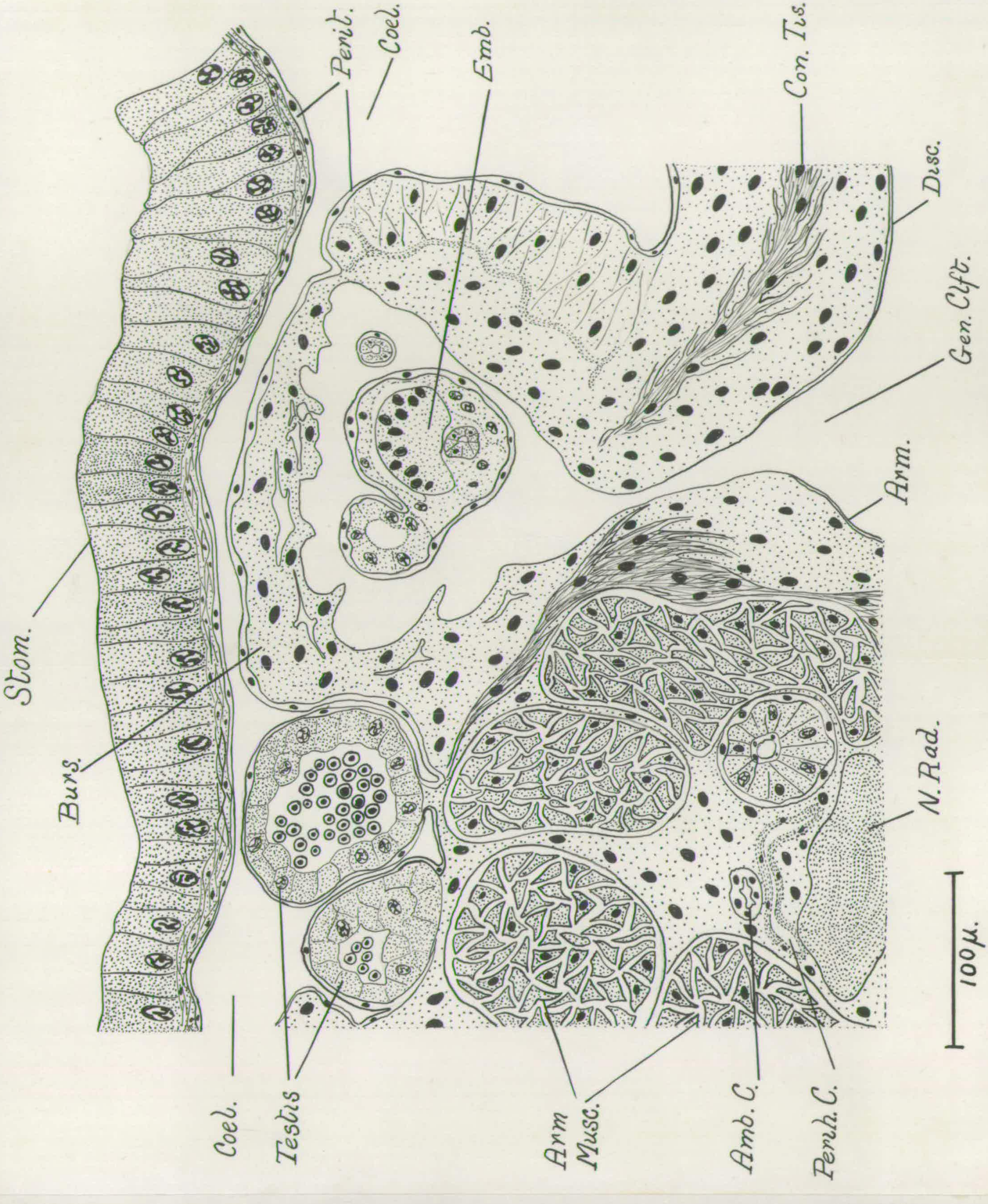


Figure 3. Vertical section showing relation between the bursa, testis and neighbouring structures.

Stom., stomach; Perit., peritoneum; Coel., coelom; Con.Tis., connective tissue; Disc, lower surface of disc; Gen.Clft., genital cleft; Arm, portion of arm bordering bursa and genital cleft; N.Rad., radial nerve; Perih.C., perihaemal coelom; Amb.C., ambulacral canal; Arm.Mus., arm muscles; Burs., bursa .

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 (eg. Just, 1928), that the coelomic fluids of echinoderms exert a strong toxic or inhibitory effect on 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 fluid are carefully excluded. The point arises here of how it is possible for embryos of Amphipholis squamata to develop within the thin-walled bursa 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 fairly certainly be 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 (Cuénot, 1898); Asterina batheri (Ohshima, 1925); Asterias glacialis (Delage, 1902, and Buchner, 1911); Sphaerechinus granularis (Viguier, 1900); Strongylocentrotus droebachiensis (Gadd, 1907); Paracentrotus (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 tendancy 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 animals 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 approached the question from the resulte 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 independant lines of investigation lead to the e/ 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 (Fig. 4) 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 the appearance of bifurcating, when it is viewed in section. As this subdivision of the lumen is of importance during the

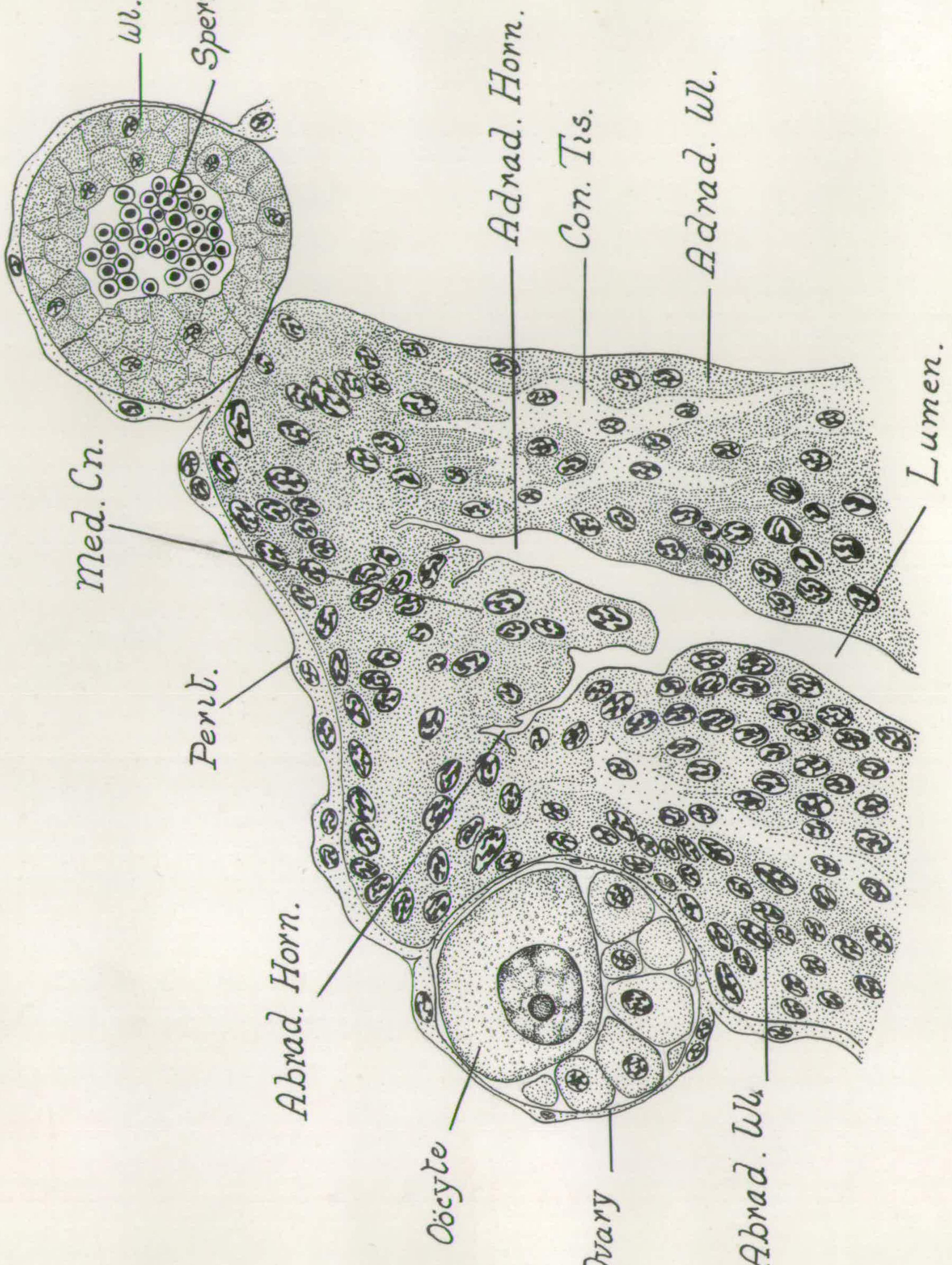


Figure 4. Oblique vertical section of bursa , showing condition when non-pregnant .

Abrad.Wl., abradial wall ; Abrad.Horn., abradial horn of bursa; Perit., peritoneum; Med.Cn., median cone ; Wl. Testis., wall of testis; Spermgon., spermogonia; Adrad. Horn., adradial horn of bursa ; Con.Tis., connective tissue;Adrad.Wl., adradial wall; Lumen, lumen of bursa .

later growth of the embryo, it is of convenience to give distinctive names to the two portions separated by the median cone. The portion of the lumen directed adradially, ie. 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 Oocyte 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 oögenesis, 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 4µ, of which 2µ 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 65µ diameter). Russo probably observed very immature oögonia. Some interesting comparisons, however, can be made with observations made on other echinoderm eggs. A short summary 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 ·1mm. 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 0·3mm. 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 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 0·1mm. in diameter, and is opaque owing to the presence of a brownish yolk. He found no evidence of prelocalisation.

Smith (1940) has described the oögenesis 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 - 100μ 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 500μ, and 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 Strongylocentrotus 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.

In regard to the oöcyte and of egg of Amphipholis squamata, the following facts have been observed :- Within the ovary, which here is very small, -normally

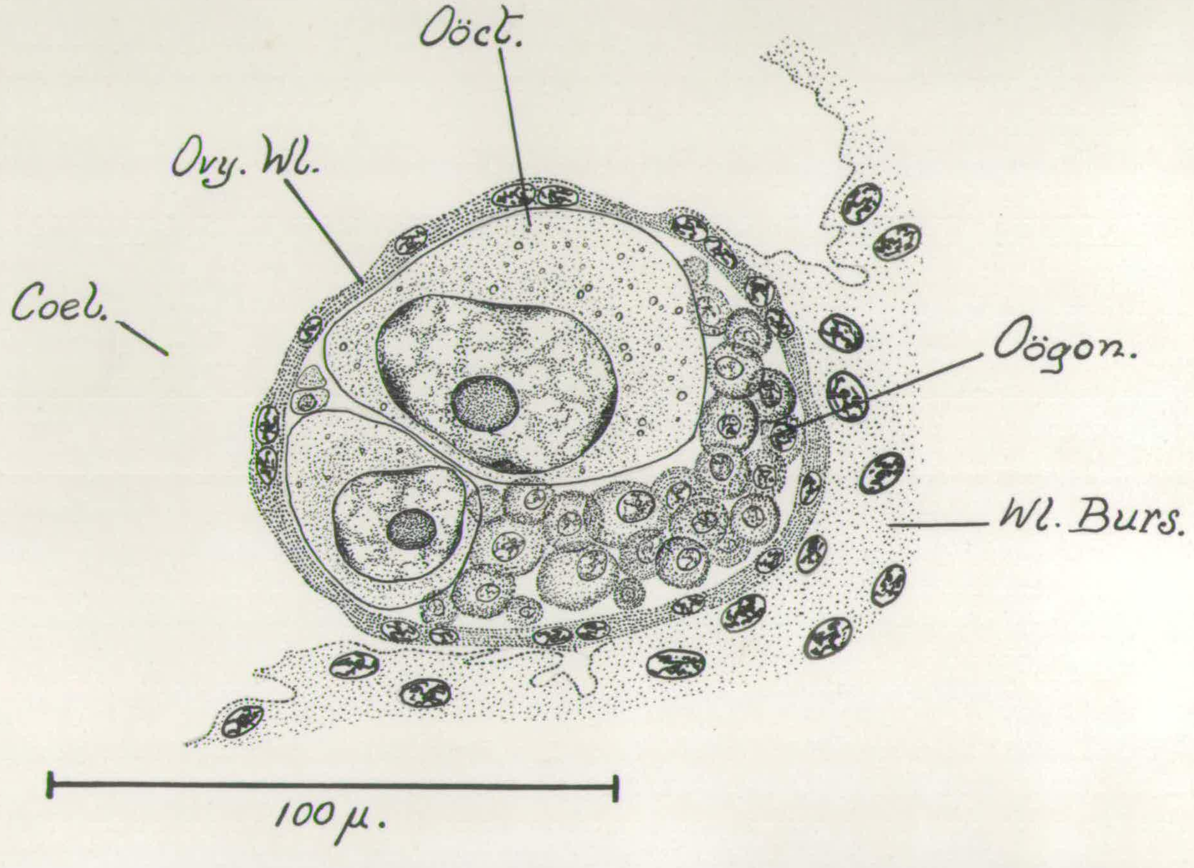


Figure 5 . The Ovary .
Coel., coelom ; Ovy. Wl., ovary wall ; Ooct., oocyte; Oogon., oogonia; Wl.Burs., wall of the bursa .

about 100μ across - there are generally to be seen a number of small, loosely arranged oögonia, each with a prominent nucleus, and usually a small nucleolus to one side of the nucleus. Occasionally a more mature oögonium 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 rrubens*. With increasing maturity the nucleolus tends to stain more deeply, a condition also paralleled in the oögenesis of *Asterias rubens*.

In addition to the oögonia 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.

In Fig. 6 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 100μ, due to 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 50μ 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

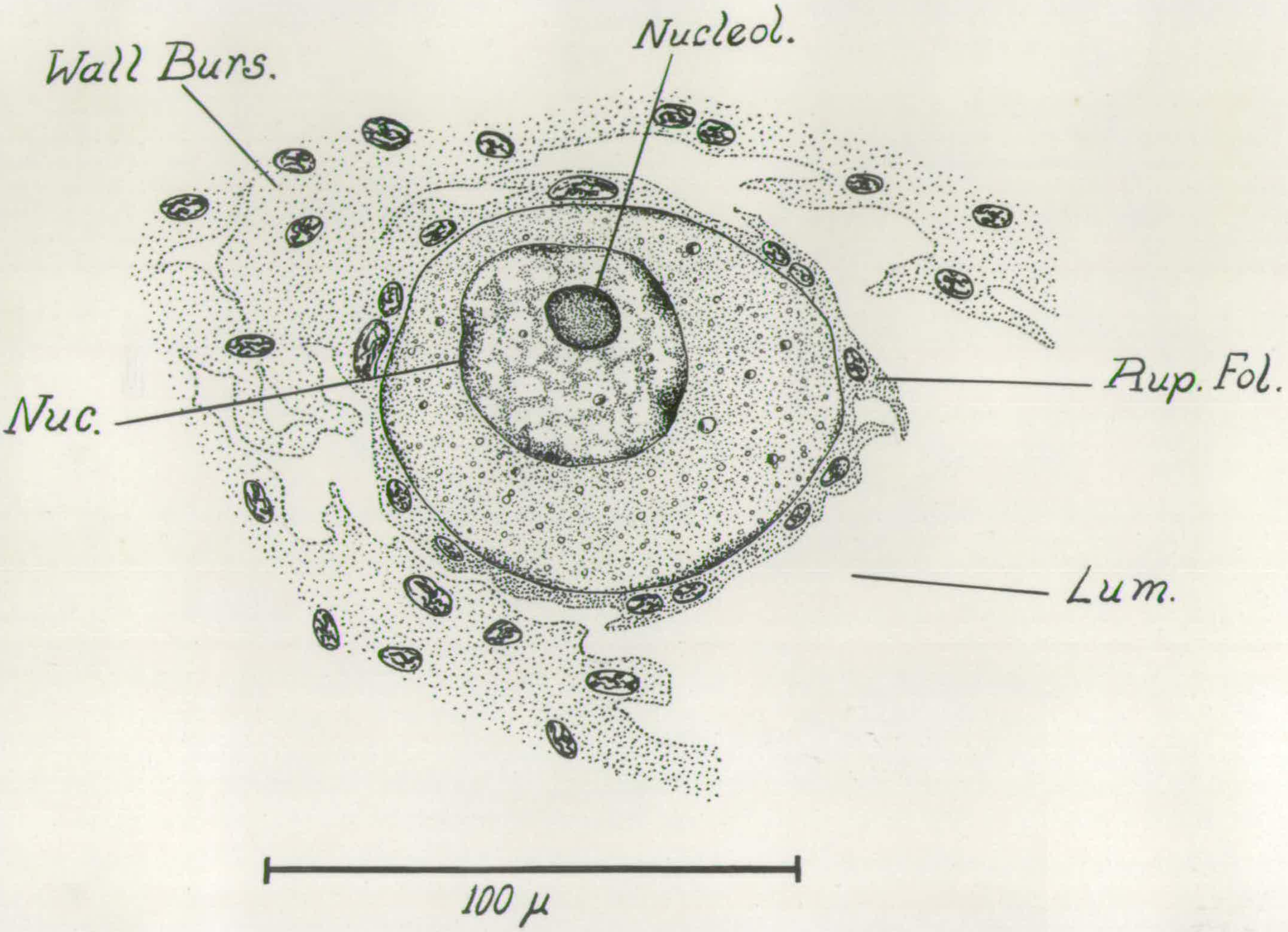


Figure 6. Ovum which has just entered the bursa.
Nuc., nucleus ; Wall Burs., wall of the bursa; Nucleol., nucleolus ; Rup.Fol., ruptured follicle; Lum., lumen of the bursa.

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 on great thickness, it would not require more than a slight cytolytic action of the egg to bring about the perforation of the bursa.

Comparing the egg of <u>Amphipholis</u> <u>squamata</u> 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, is discussed in detail in Part 2 of this paper (p. 88).

<u>Fertilisation</u>

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) sides 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 aggregate in one part of the tank.

Early Development

Subsequent to the liberation on 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 extrenely immature oögonium - 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 radialpattern.

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), though 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 in the second part of this paper, it is only in the most heavily yolked type (Kirk's ophiuroid) 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 preceeding 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.

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. 7) 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 <u>Ophiura</u> <u>brevispina</u>. 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 preceeding blastula,

meso-

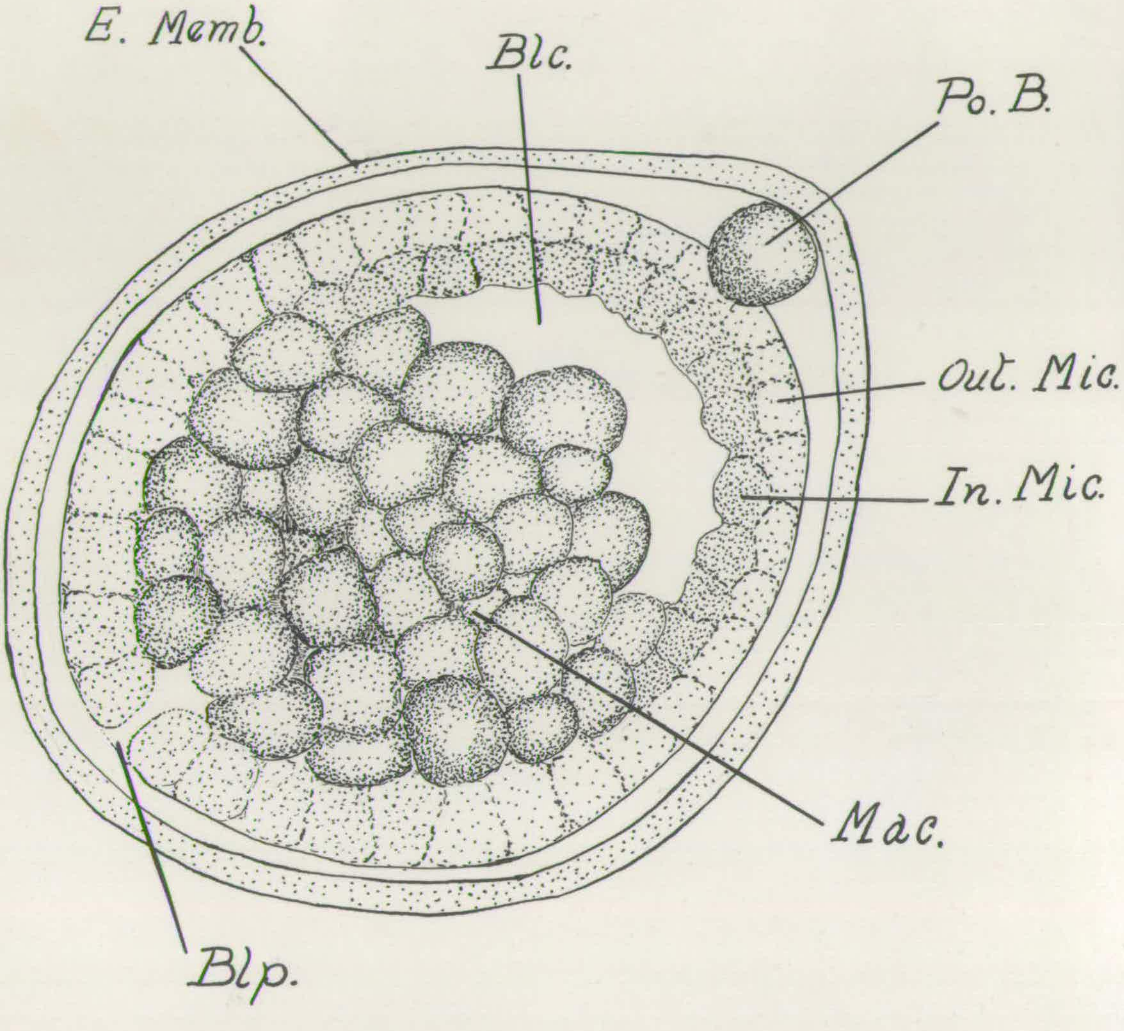


Figure 7 . Early gastrula .

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

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-hypoblsat 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 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 <u>Amphipholis squamata</u>, and the consequent lesser degree of modification which the development has undergone. These facts are further considered in part two of the present paper.

A second difference from Kirk's ophiuroid is provided by the varied extent of the pigmentation of the mes-hypoblsat and epiblast in <u>Amphipholis</u>. 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 <u>Amphipholis</u> shows some degree of similarity to <u>Strongylocentrotus</u>, 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 <u>Amphipholis</u> 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 <u>Strongylocentrotus</u> 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. 8. Here the hypoblast has developed a central cavity, to form the archenteron - evidently through an internal splitting, as in <u>Ophiura brevispina</u> and Kirk's ophiuroid. The archenteron lies 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 <u>Amphipholis</u> 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), and it is further discussed in part two of this paper in relation to direct development in general in the Ophiuroidea.

The series of stages from now onward is fairly

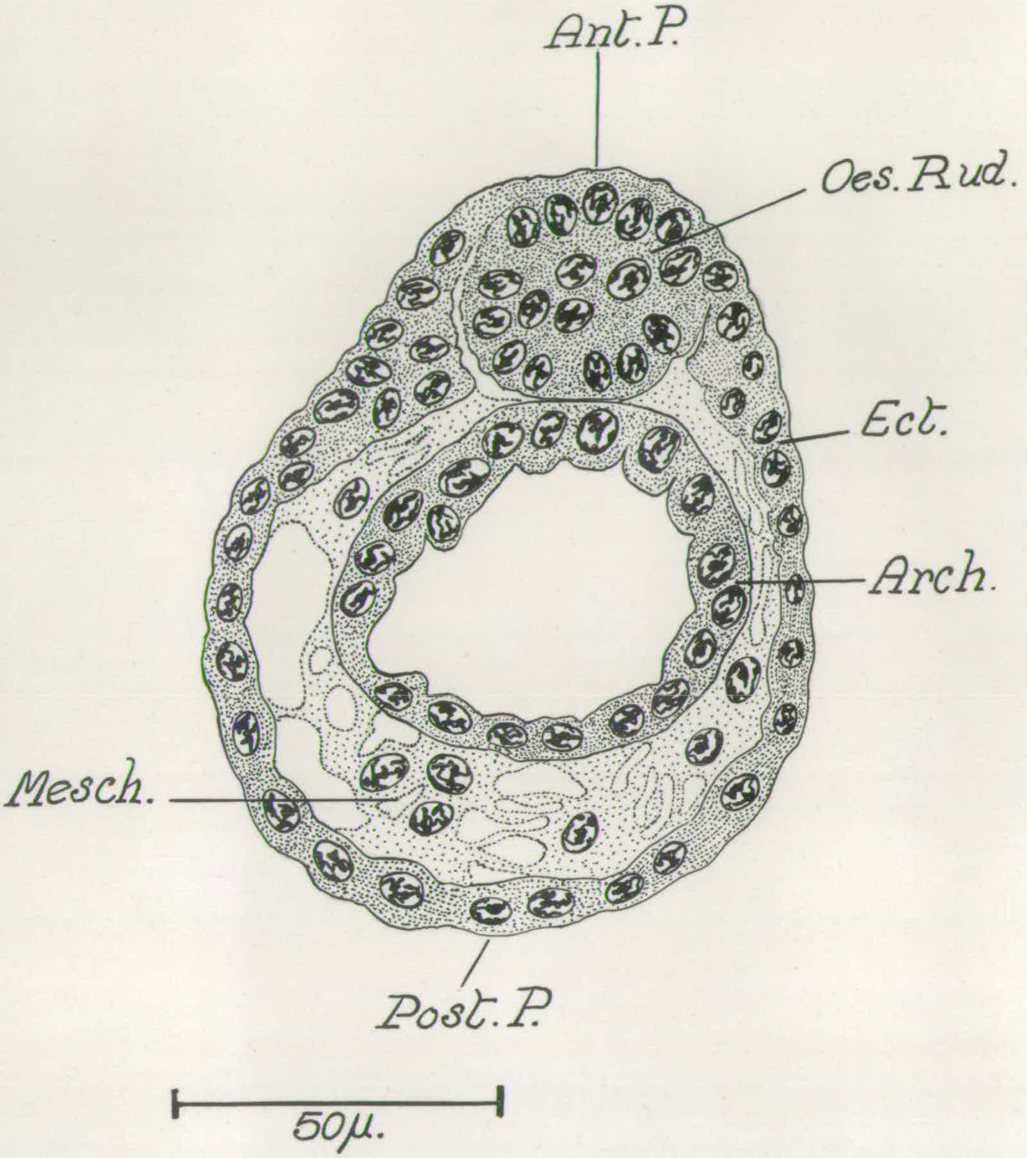


Figure 8. Late gastrula .

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

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 invàgination 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 clàim, 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 substantiated by observation of the development of Kirk's ophiuroid. In regard to his claim that the endoderm arose by delamination in Amphipholis, I was unable 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 been shown, in actual fact the origin of the germ layers and mode of gastrulation differs only in a minor degree from what has 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 is no question whatever of delamination taking place. There can be little doubt that the mistaken interpretation of the gastrula is 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 coelentrate.

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 toward the left side (Fig. 9). Subsequent development shows that the more anterior of these two masses is the rudiment of the hydrocoel (ie. 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 thickwalled bodies with very small cavities. The point is an important one, and it is discussed more fully in part two of this paper.

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

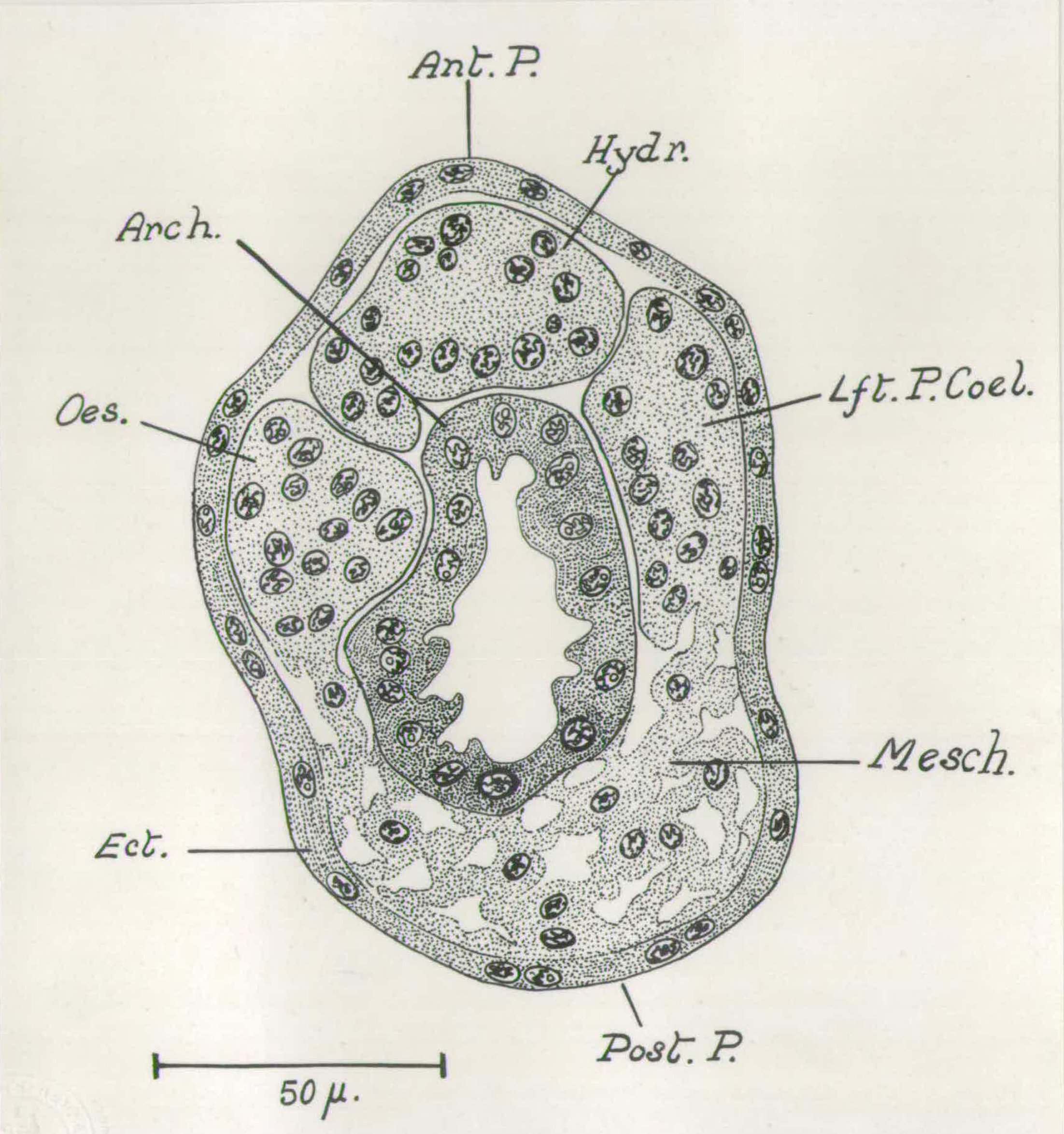


Figure 9 . Ect., ectoderm; Oes., oesophagus; Arch., archenteron; Ant.P., anterior pole ; Hydr., hydrocoel; Lft.P.Coel., left posterior coelomic vesicle; Mesch., mesenchyme.

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

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. 10) 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, 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 tendancy 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. 11 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

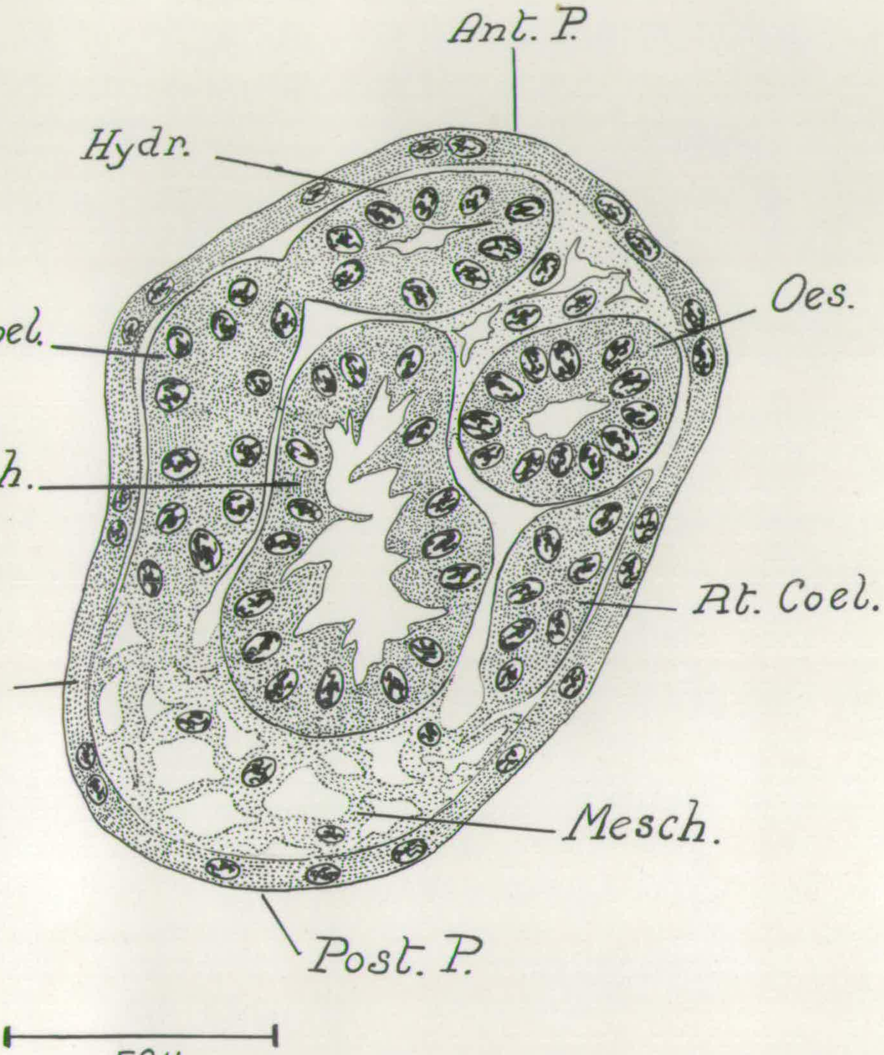


Figure 10.

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

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 100μ in length, and is about fiftyμ broad. The left posterior coelomic vesicle is more rounded, and about fiftyμ in diameter. The right coelomic rudiment is more elongated, about eighty μ in length by twenty 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 250μ with a breadth of 150μ .

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 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 early workers). This structure is more fully described below.

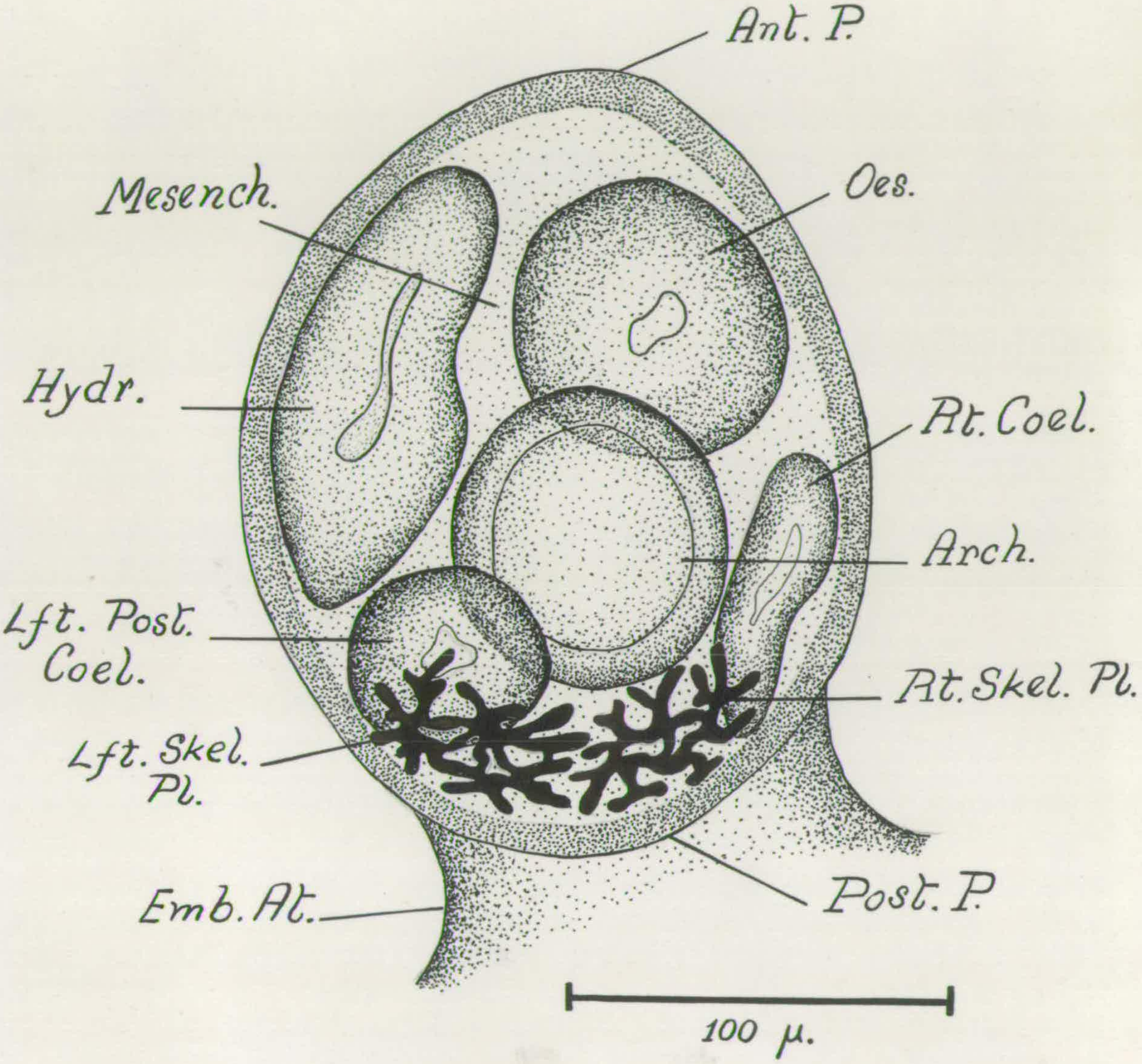


Figure 11 . General view of early larva, when all coelomic vesicles are present. Reconstructed from sections.

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

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. 12. 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 become merged. Thus not all of the organs of the vestigial larva reach 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

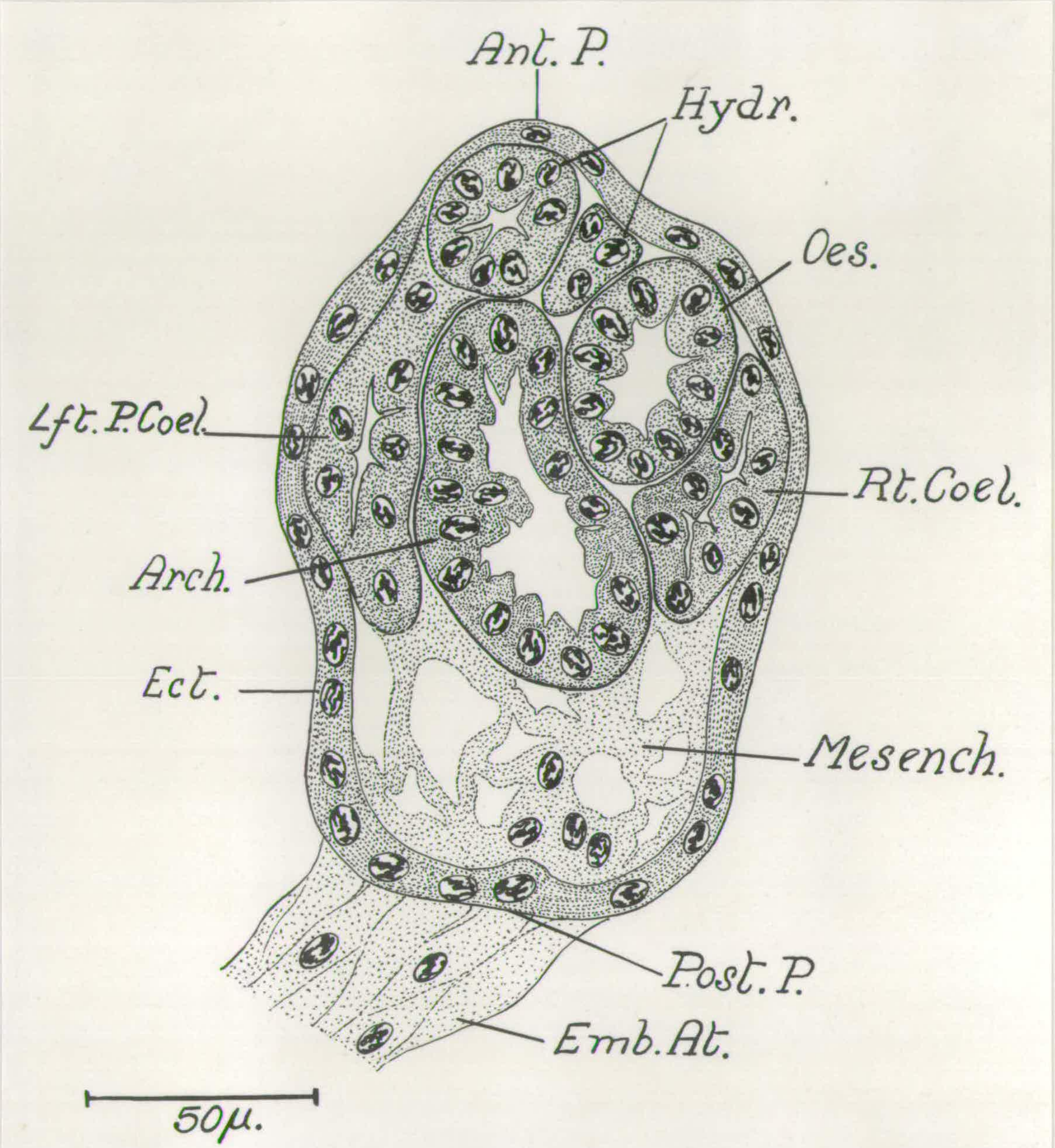


Figure 12. Vertical section of early larva.

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

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. 13 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 until 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. 13 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 has also increased in depth, reaching two or three cells thickness, while the central lumen has assumed a three-lobed shape. The

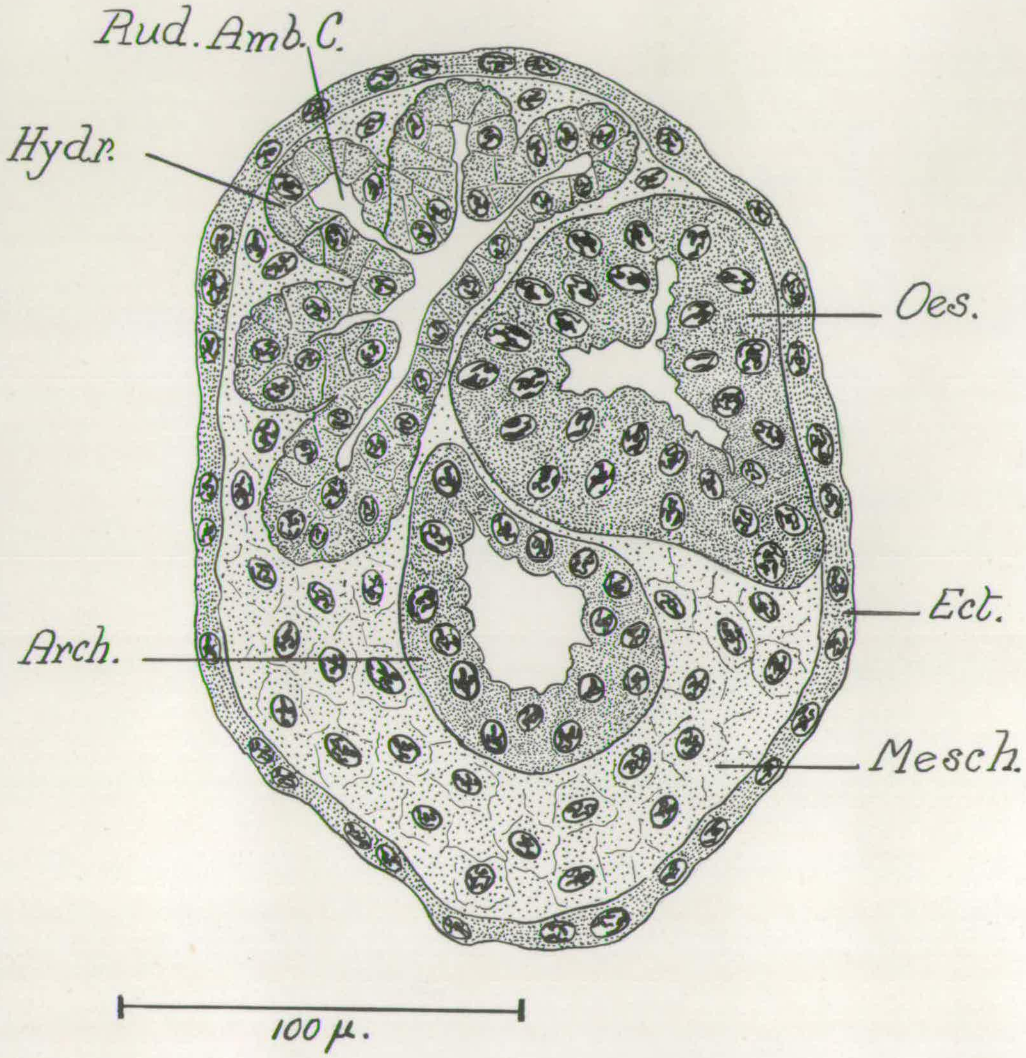


Figure 13. Oblique section of later larva, shewing five-lobed hydrocoel .

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

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. 14, 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 forward 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.

The relationship between this peculiar reduced and vestigial larval stege and a typical pelagic Ophiopluteus is illustrated in diagrammatic form in Fig. 15, where it can readily be seen that the pluteus found in <u>Amphipholis</u> <u>squamata</u> is in reality an armless version

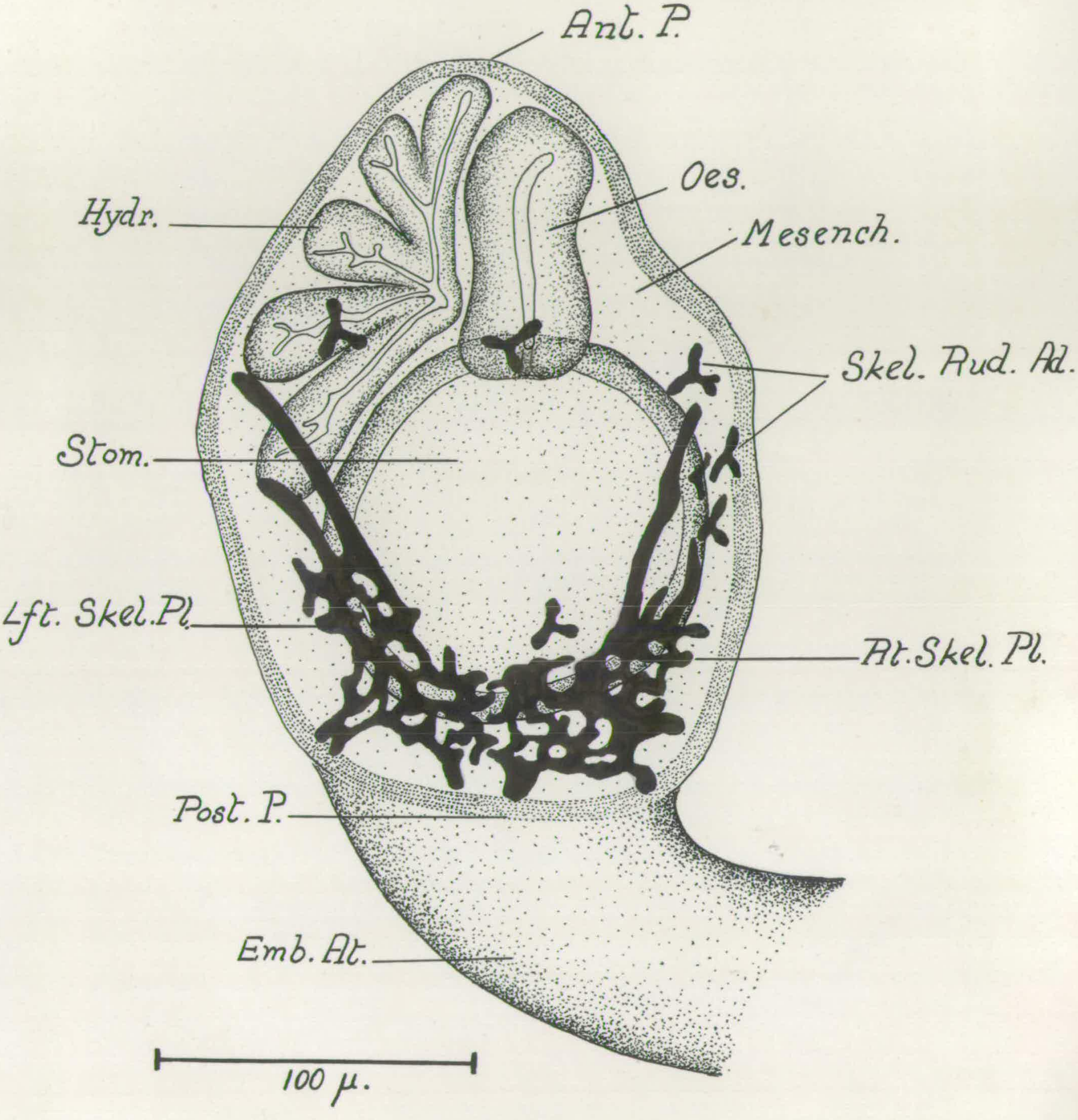


Figure 14. Fully developed larva, with embryonic attachment .

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

of the strongly bilaterally symmetrical larva of those types of ophiuroids which have an indirect, pelagic development. As is shown in the second part of this paper, 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 in the appropriate section of part two of this paper, it will be useful to note here the most salient points in which it differs from the pelagic Ophiopluteus. These are :-

(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 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

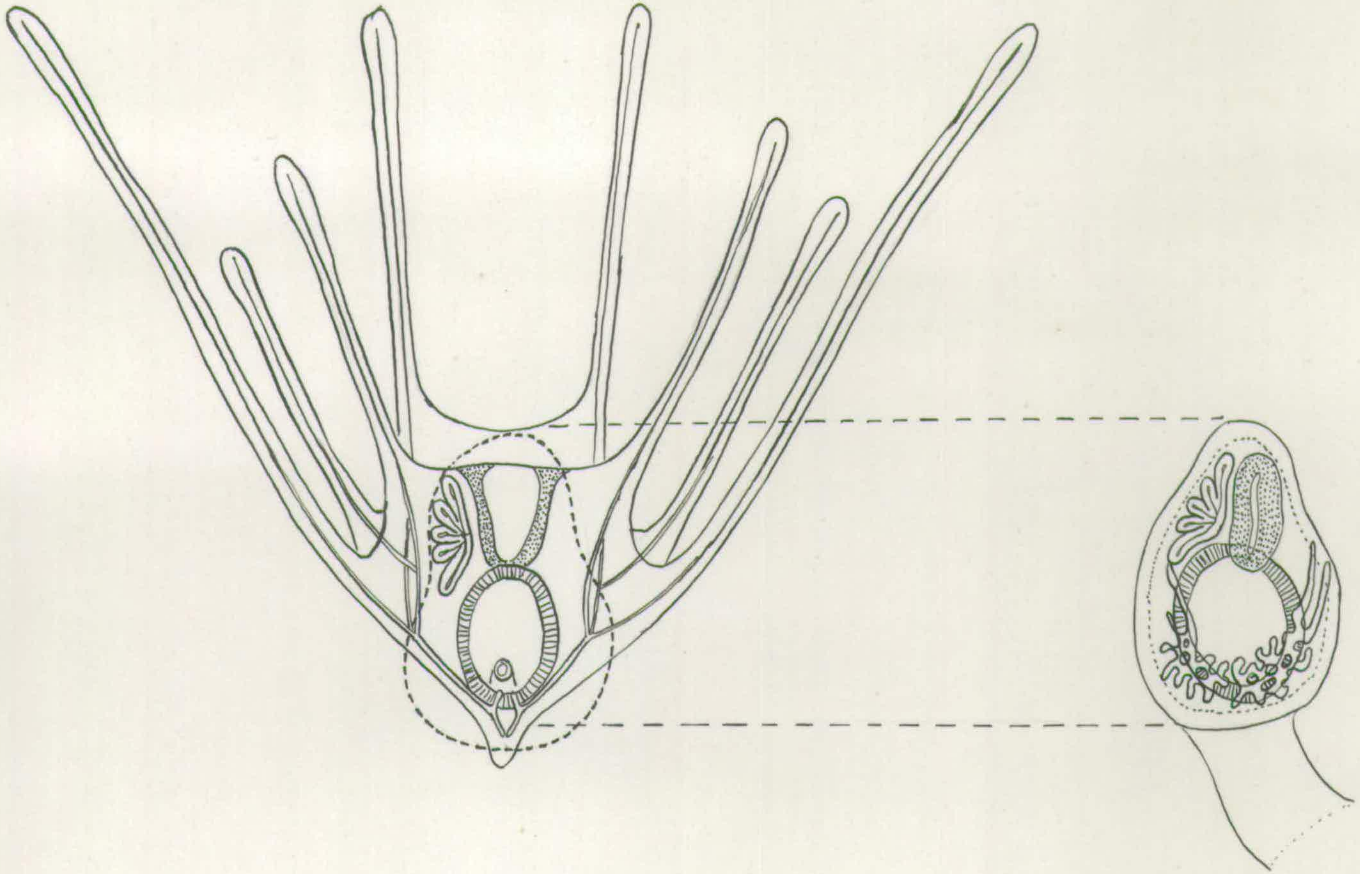


Figure 15. Diagram illustrating the relationship of the vestigial pluteus of Amphipholis squamata (right) to the fully developed Ophipluteus of ophiuroids with indirect development (left)

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 structures 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. 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 analagous "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. 14) this organ is also seen at its greatest development. In section its structure proves to be very simple (see Fig. 12, 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. 24). 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 analagous 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 the 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 matreial - 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 already 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 anatómical 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 name "embryonic attachment" has been used for the organ throughout this paper, and the term "umbilical cord" abandoned.

The appearance of the sinuses in the wall of a pregnant bursa invites comparison with the analagous 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.

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. 16) so that the five lobes originally placed in linnear series on the left side now begin to take up a position such that each lobe begins to point outwards, the lobes being 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

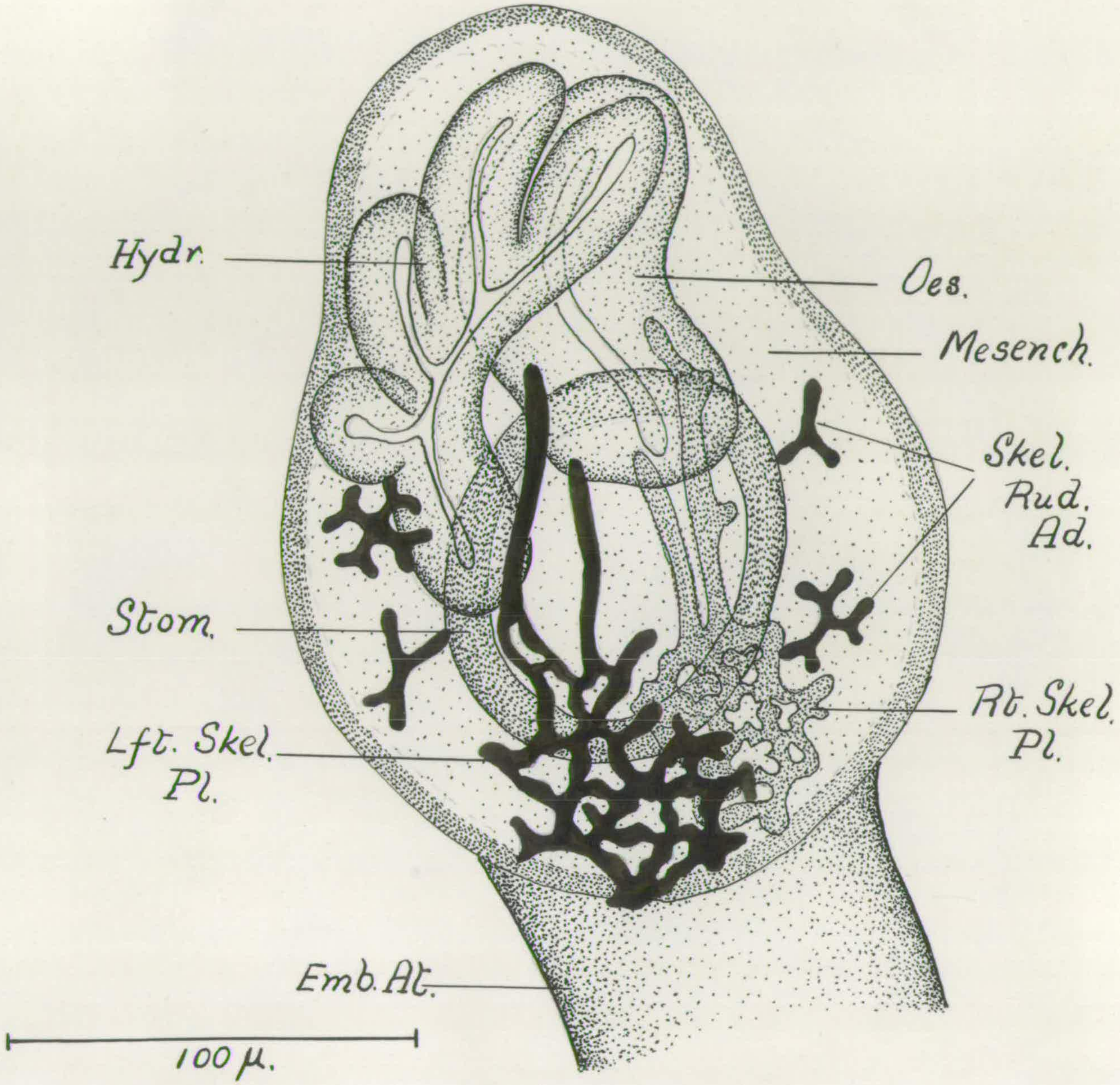


Figure 16. Larva viewed from the left side , at the commencement of metamorphosis.

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

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 previding 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. 24).

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. 17 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

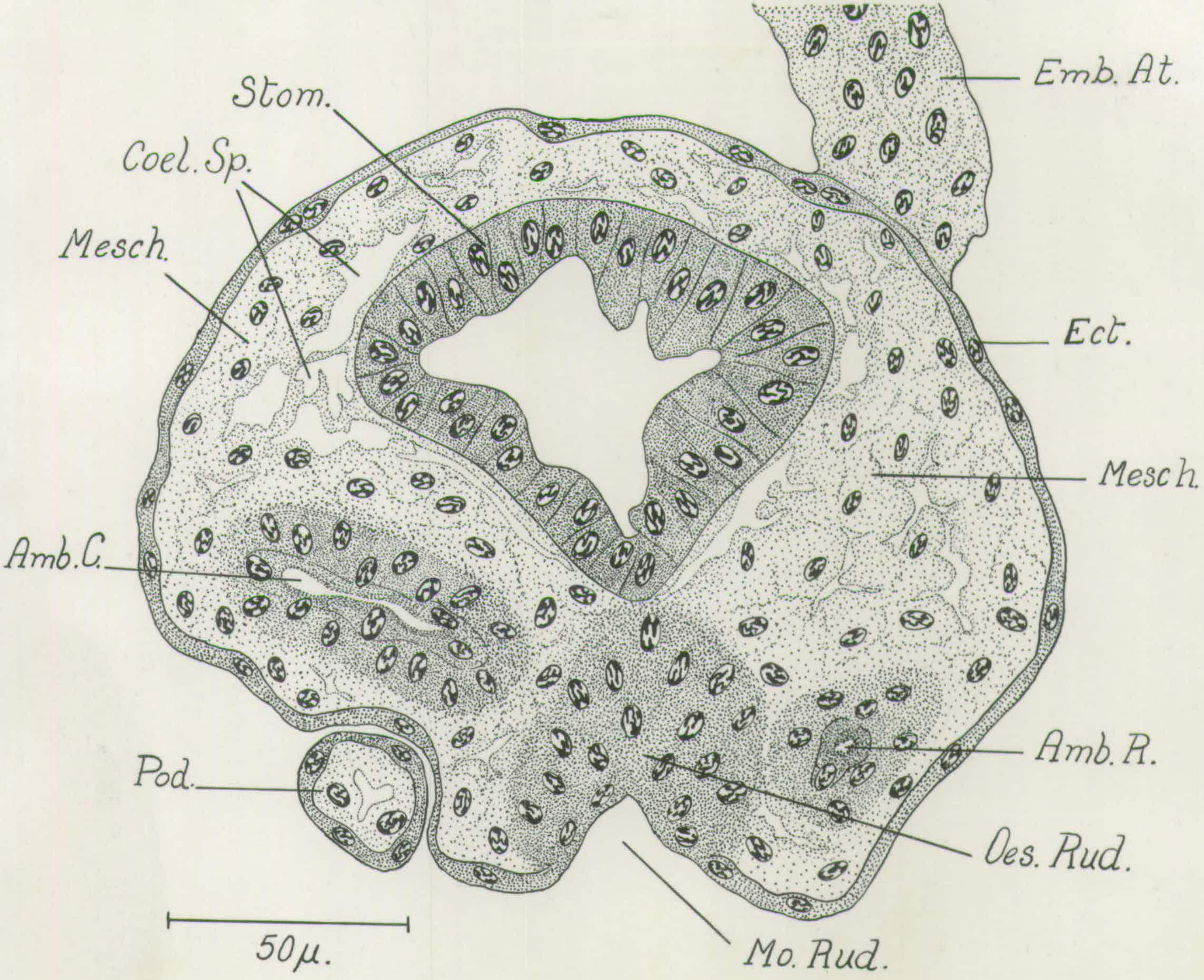


Figure 17. Vertical section through newly metamorphosed star, still attached to parent.
Pod., podium; Amb. C., ambulacral canal; Mesch., mesenchyme; Coel.Sp., intercellular splits forming perivisceral coelom; Stom., stomach; Emb. At., embryonic attachment; Ect., ectoderm; Amb.R., ambulacral ring; Oes.Rud., oesophageal rudiment; Mo.Rud., mouth rudiment .

up with the 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. 18. 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. 19), 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. 20).

At this point it is proper to reiterate that one of the chief purposes of this study was to 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 echinoderms.

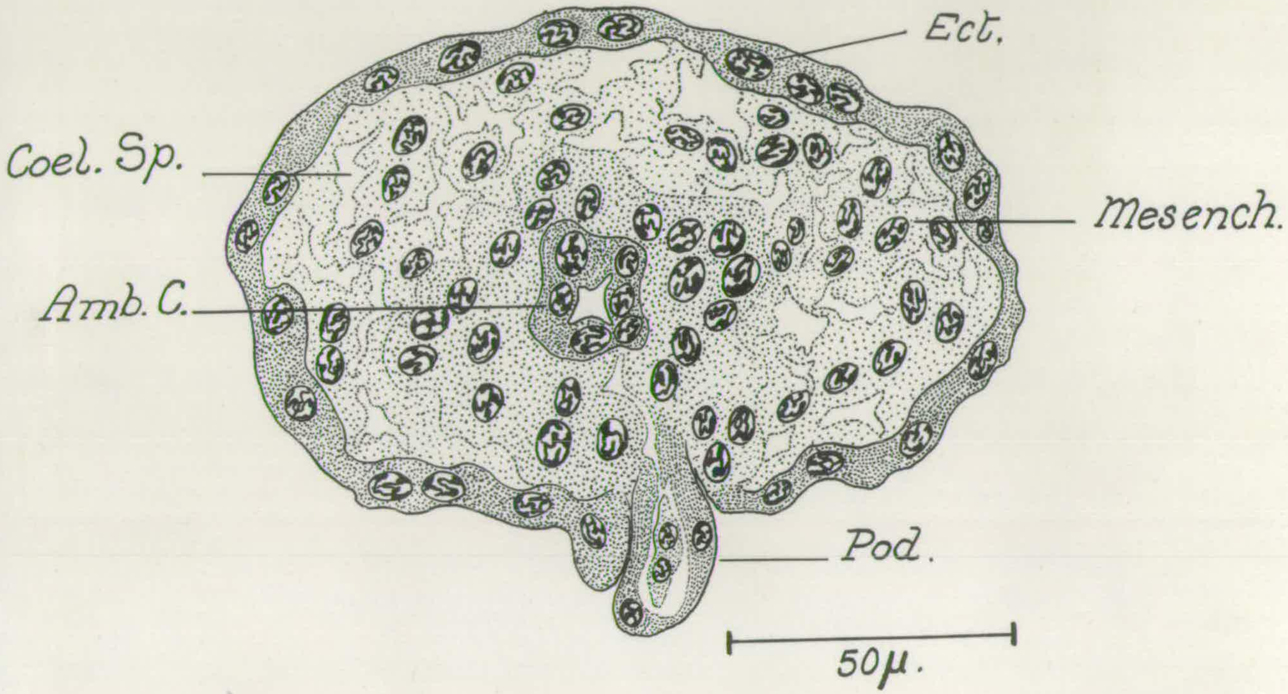


Figure 18. Vertical section through rudiment of arm of young star in which the coelom is appear- as splits between cells of the mesenchyme.

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

Further discussion of the point is reserved for consideration in connection with the problem of direct development among the echinoderms in general in part two of this paper.

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 Hérouard (1903). This statement by Russo is typical of the unfortunate mixture of good and bad observation found in his paper. To

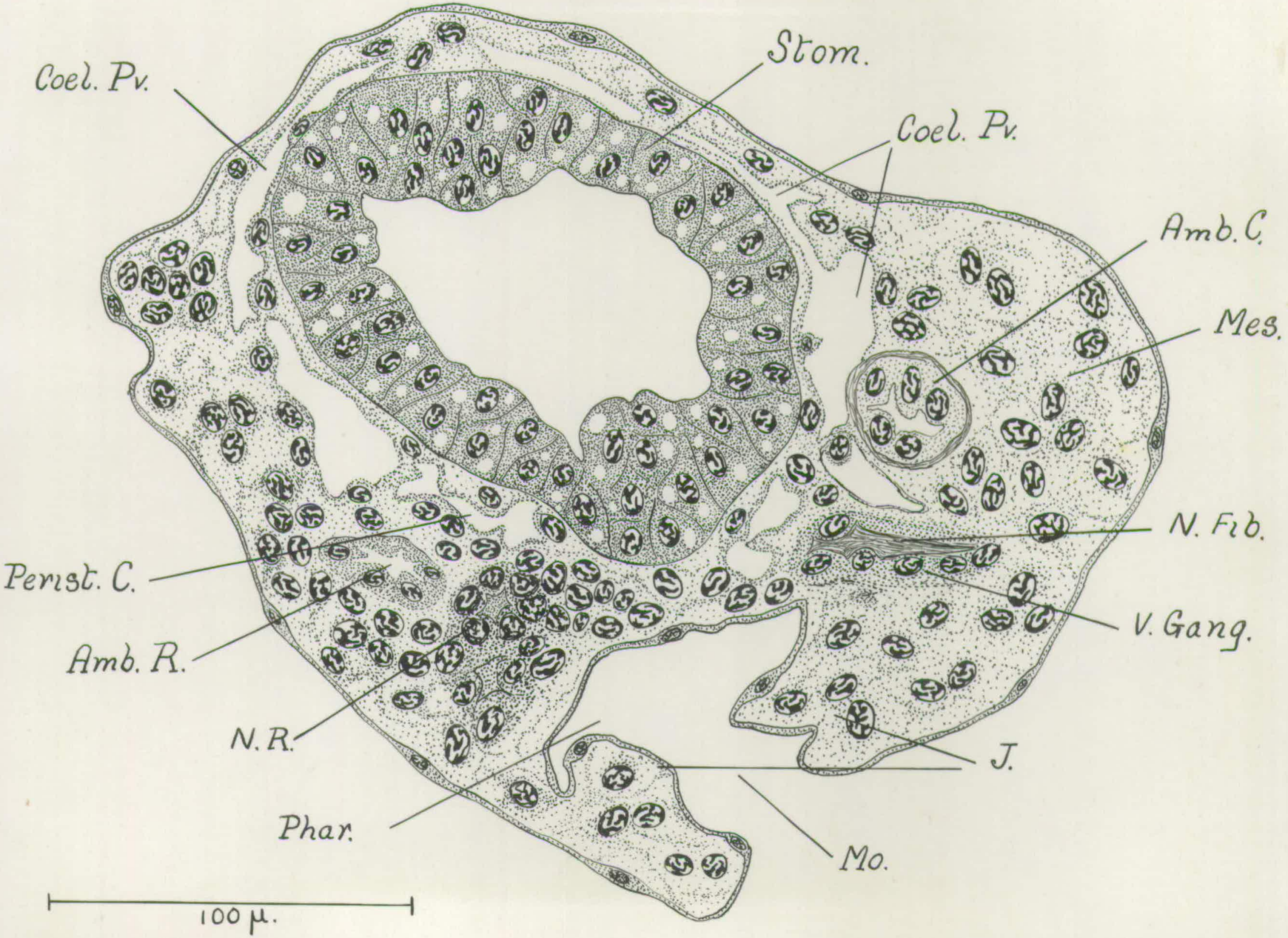


Figure 19. Vertical section shewing later stage in differentiation of the coelom.

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.

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.

Cuénot (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 240μ. He stated that the nervecords 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 Cuénot'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.

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 Cuénot'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 metamorphosis this structure remains as a thickwalled sac, very clearly demarcated from the surrounding mesenchyme, and opening neither to the exterior nor to the stomach, (Fig. 14). After metamorphosis a change occurs in the organ. Vertical sections show that the cells of the periphery of the oesophagus becomes 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

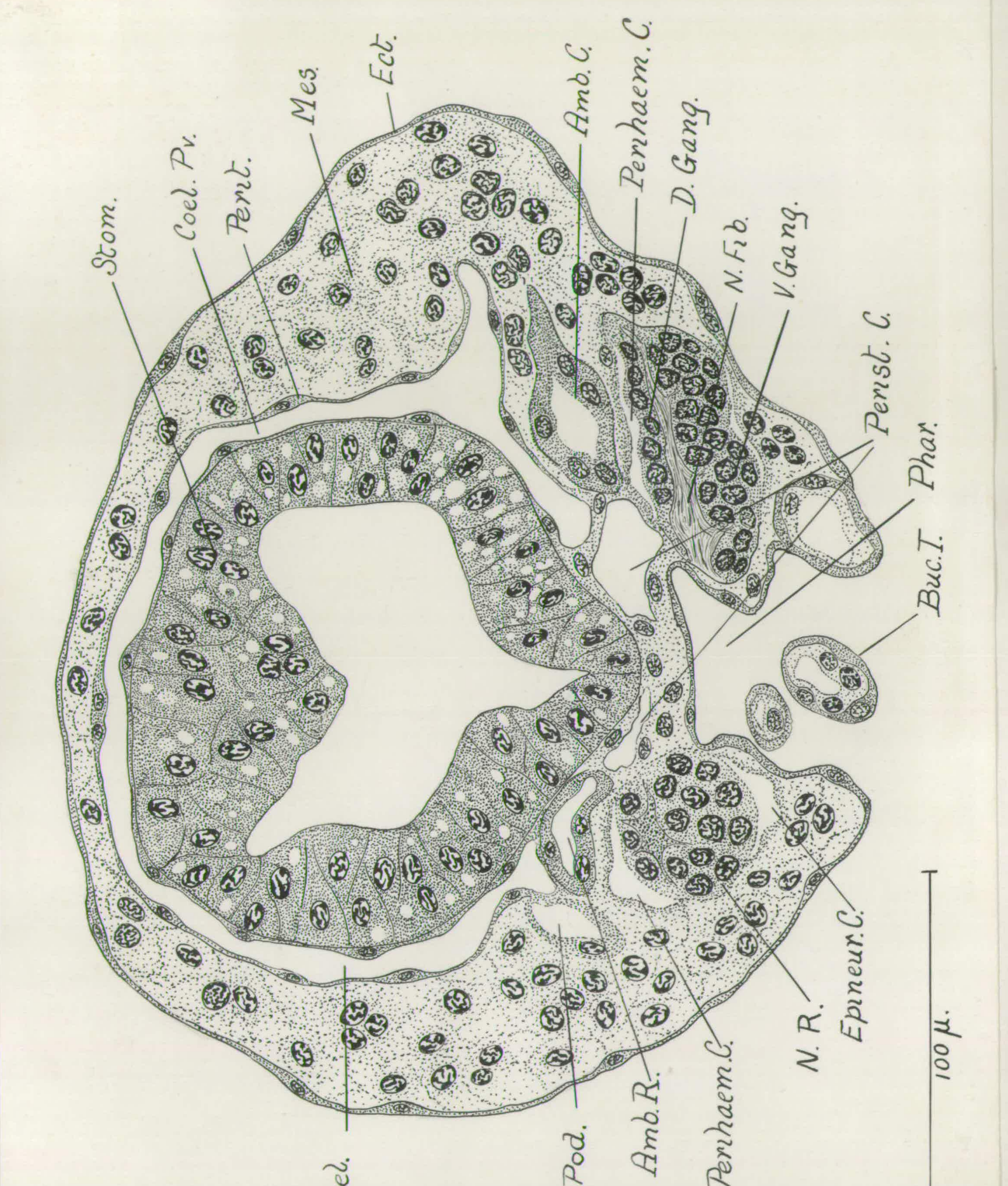


Figure 20. Later stage in differentiation of coelom. Epineur.C., epineural coelom; N.R., nerve ring; N.Fib., nerve fibres; D., V.Gang., dorsal, ventral ganglion; Perihaem.C., perihaemal coelom; Amb.R., ambulacral ring; Amb.C., ambulacral canal; Pod., podium; Coel., perivisceral coelom; Stom., stomach; Perit., peritoneum; Mes., mesoderm; Ect., ectoderm; Perist. C., peristomial coelom; Phar., pharynx; Buc.T., buccal tentacle.

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. 17). In sections of embryos a little more advanced in development, there begin~~s~~ 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. 19). 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. 20 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 Cuénot (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 just 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 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 preivisceral 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. 19 and 20). 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 Hérouard (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 glends 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 this 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 or 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 young 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 orientated with its ventral (oral) surface uppermost. The significance, if there be any, of this orientation

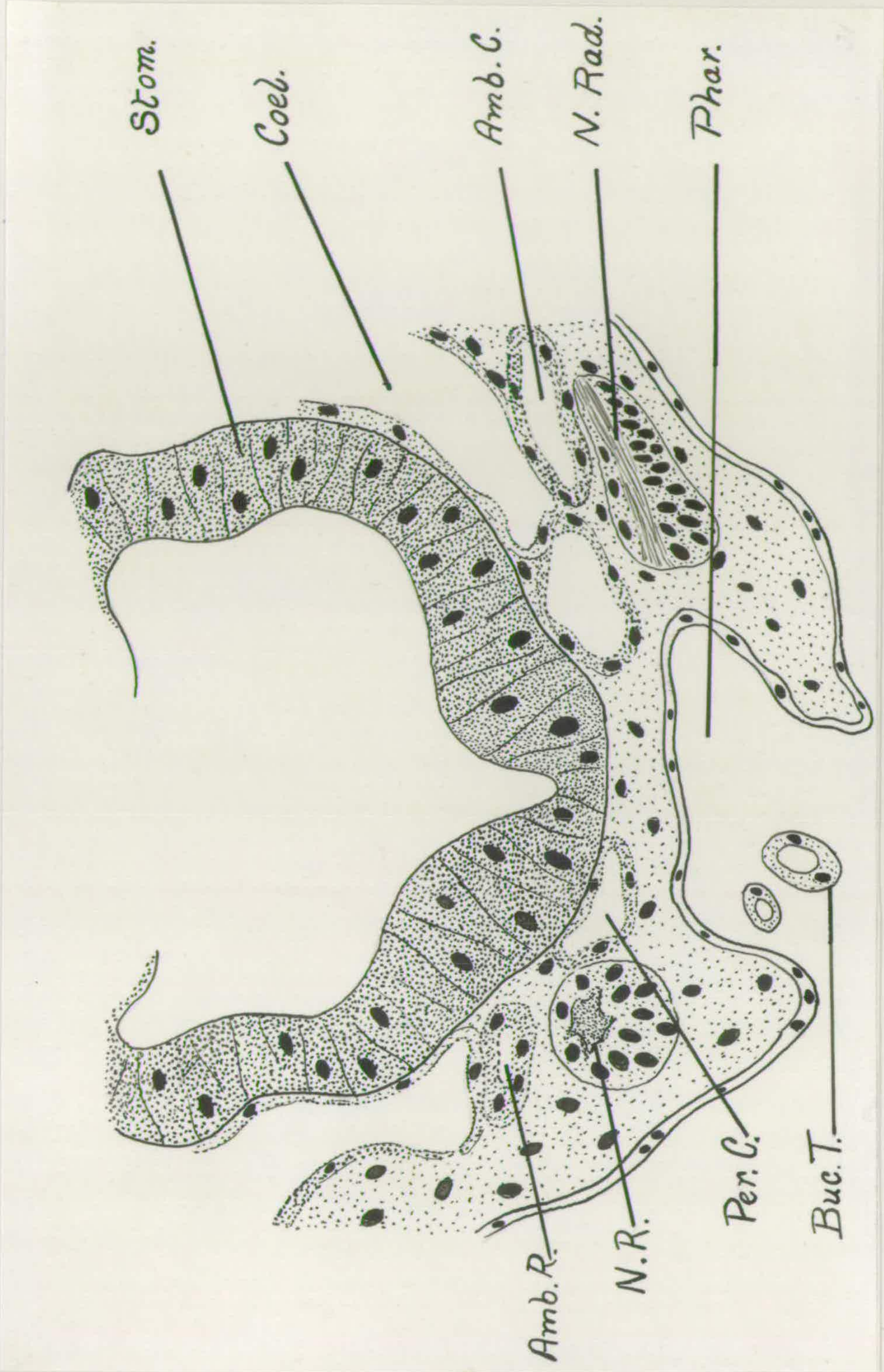
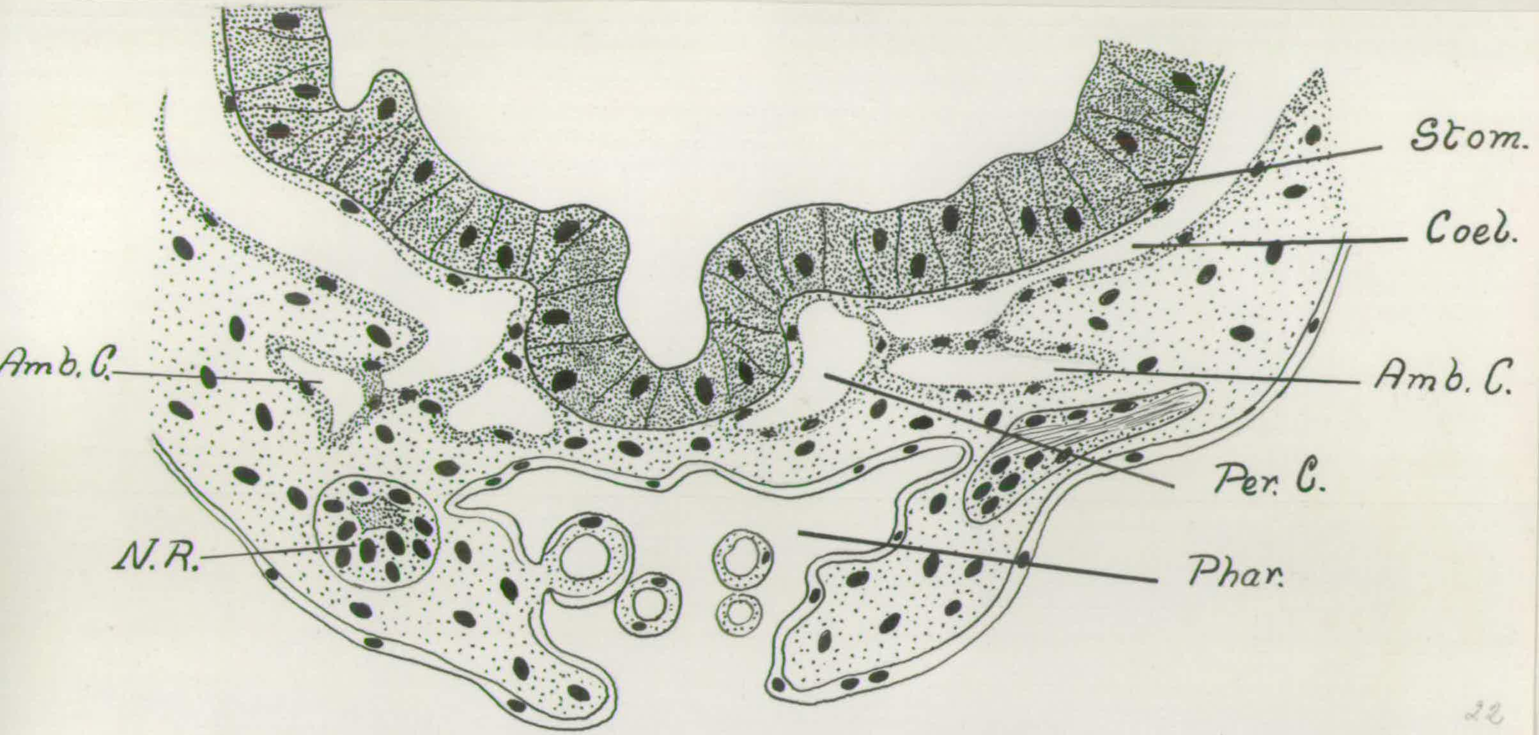
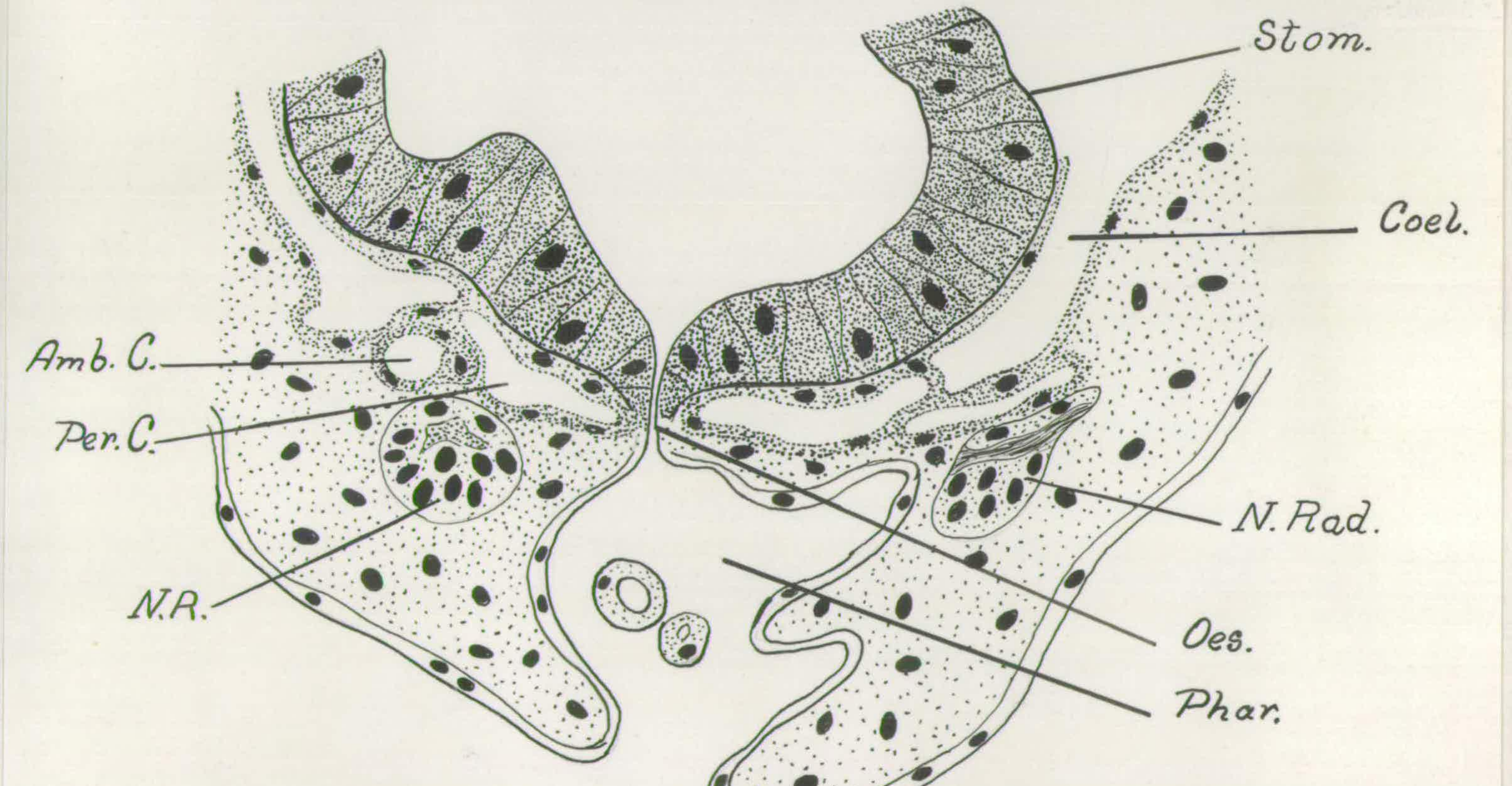


Figure 21. First stage in the piercing of the mouth.
Amb.R., ambulacral ring; Amb.C., ambulacral canal; N.R., nerve ring; N.Rad., radial nerve; Per.C., peristomial coelom; Buc.T., buccal tentacle; Phar., pharynx; Coel., coelom; Stom., stomach.





Figures 22 (above) and 23 (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 .

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 reentrant 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 ectoderm of the mid-ventral surface, As will be seen from the sections shown in Figs. 19 and 20 there has already existed here for some time a hollowing out which is obviously the forerunner of a buccal cavity. Figs. 21, 22 and 23 illustrate the final stages in the process, when the second excavation occurs, downwards from the ventral-most part of the stomach. In this way there forms first a blind pouch from the stomach towards the buccal cavity (Fig. 22), and then finally the oesophageal passage is pierced, and for the first time the alimentary canal is brought into being, (Fig. 23).

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.

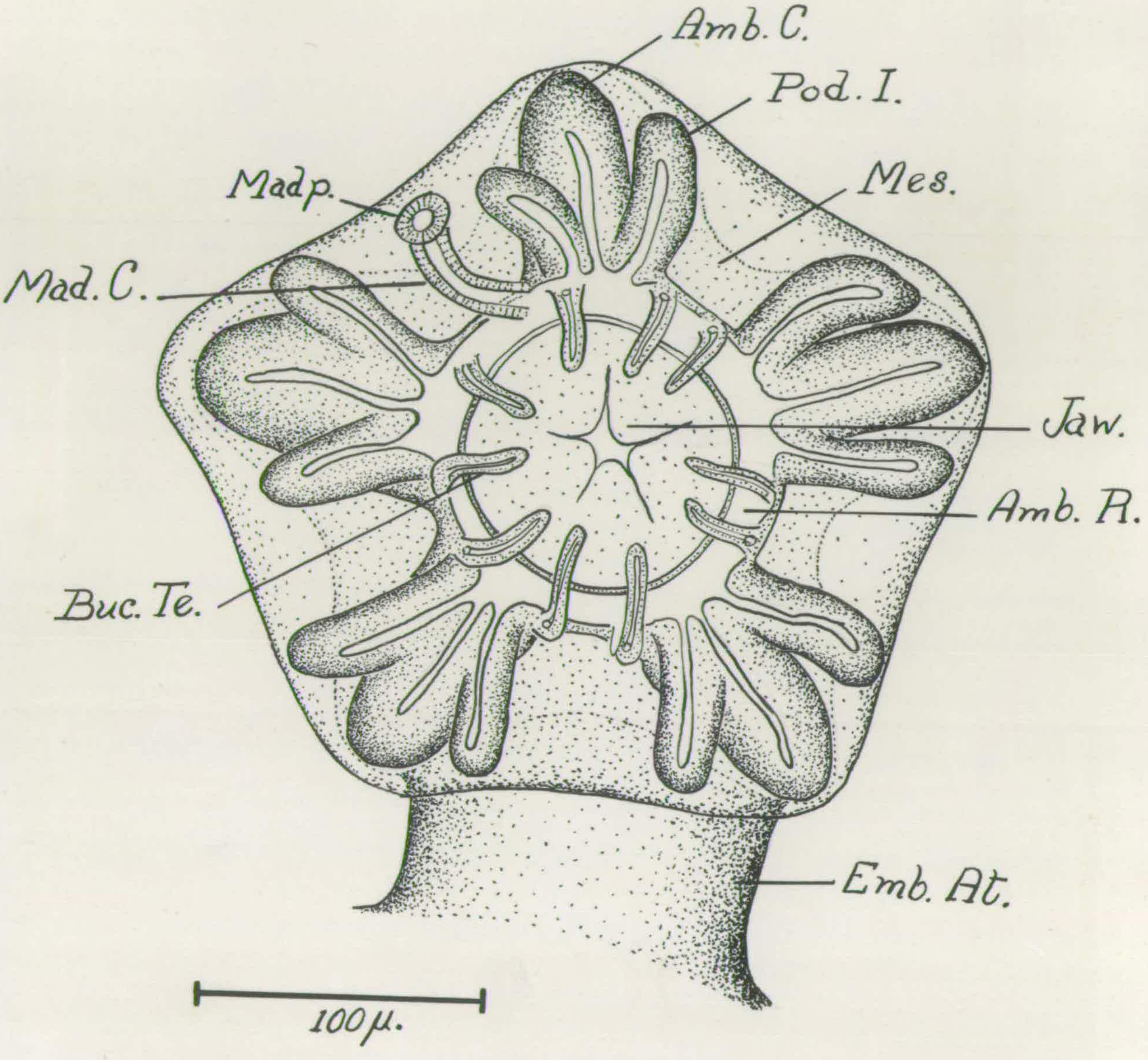


Figure 24. Ventral view of attached 'Pentagon' .
Buc.Te., buccal tentacle; Mad.C., madreporic canal;Madp., madrepore; Amb.C., ambulacral canal; Pod.1., first podium; Mes., mesoderm mass; Jaw, developing jaw; Amb.R., ambulacral ring ; Emb.At., embryonic attachment.

Birth of the Young Ophiuroid

As the arms increase in length they are folded above the disc, twisted in a spiral fashion. Fig. 25 illustrates the orientation of a full-time embryo fixed *in situ* in the bursa, and then dissected out. 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 toward the dorsal aspect of the parent. In Plate 1 is shown a dissection of an interradius of a pregnant adult, with an embryo orientated 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, as in Fig. 26a. 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 existance. The young forms are at first colourless, except for the pinkish disc, but after ten

(This side uppermost)

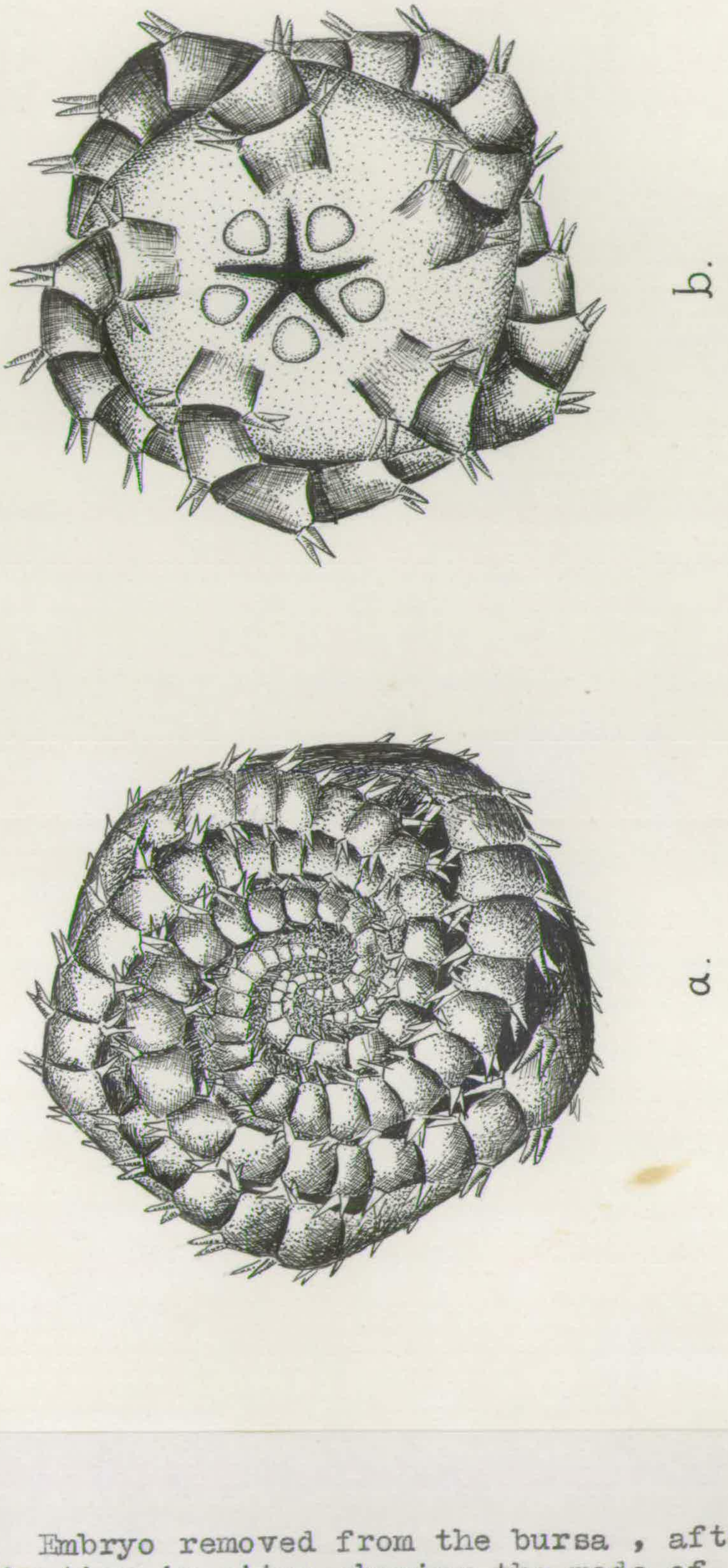


Figure 25. Embryo removed from the bursa , after previous fixation in situ; showing the mode of folding of the arms during the late stages of pregnancy. a, dorsal side ; b, ventral side.

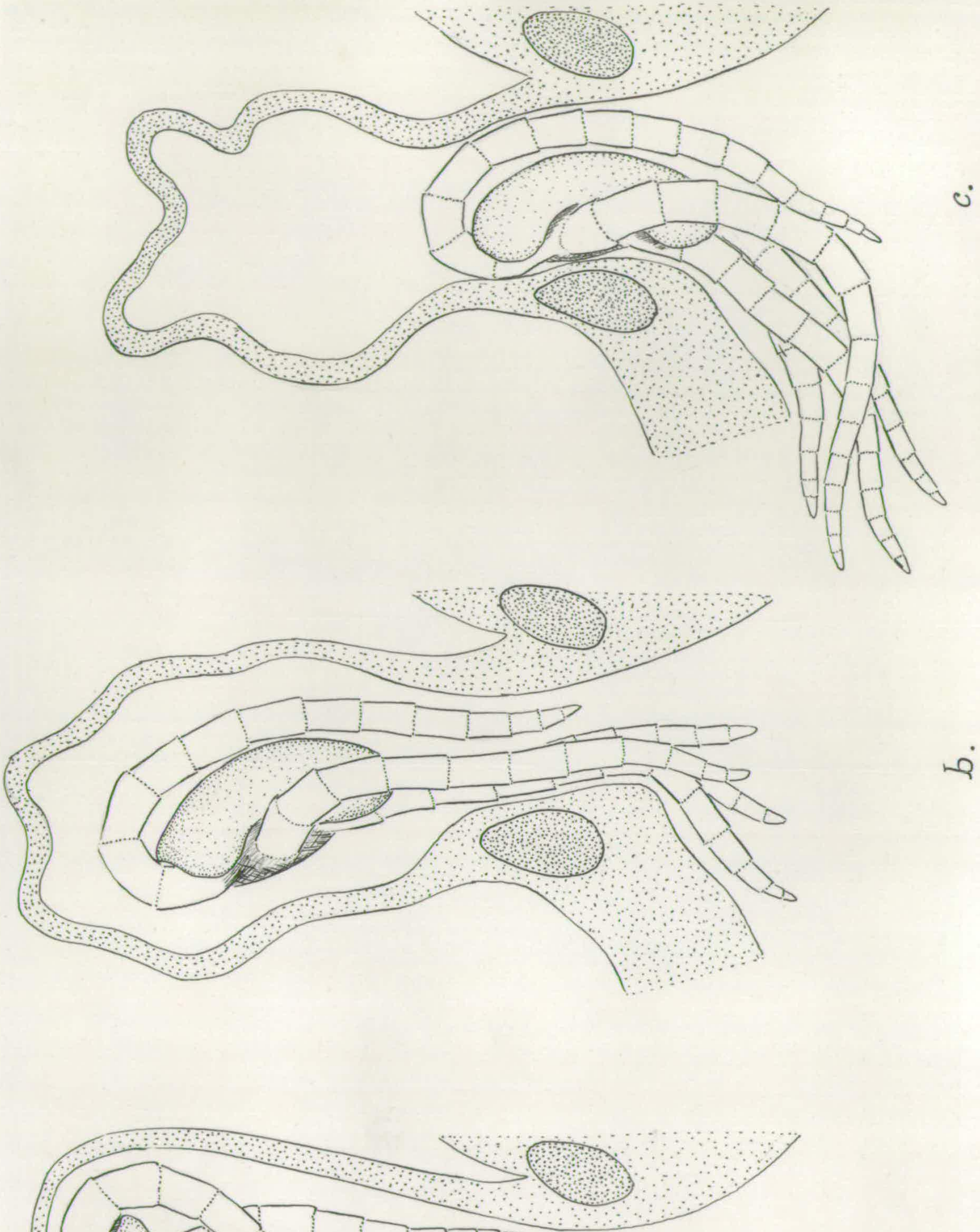


Figure 26, a,b,c. Stages in the birth of the young star. (See text).

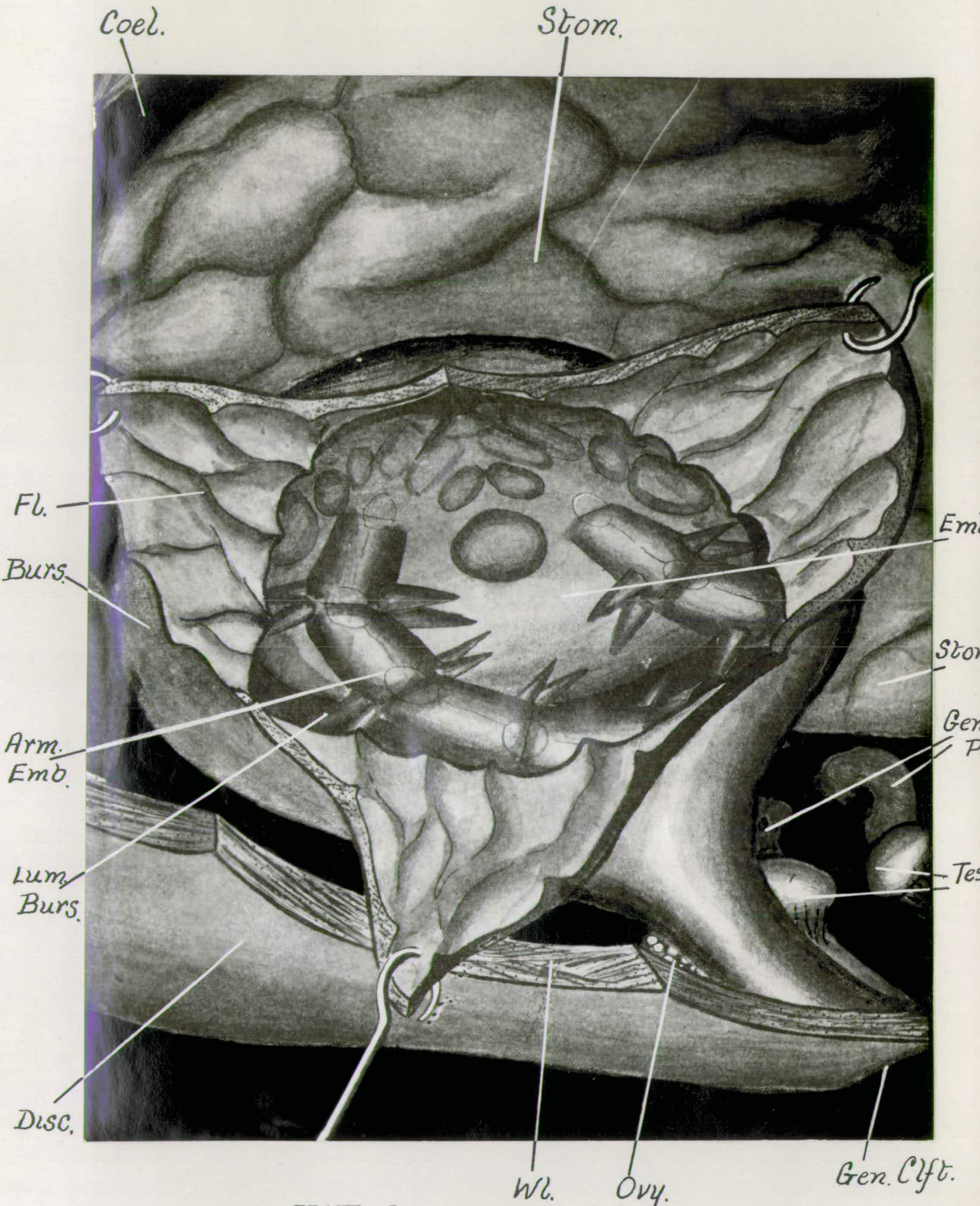
days or so a greyish pigmentation begins to appear. The newly born ophiuroids, and also those which have been free-living for some time after birth, make great use of the podia in progression, these organs having in early life a true tube-foot function. Thus young ophiuroids are enabled to climb vertical 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.

PLATE ONE .

Explanation of Plate 1 .

A dissection of part of one interradius of an adult Amphipholis squamata , with one bursa opened to show the contained embryo . The latter has almost reached the end of its intra-bursal development ; it is oriented in the characteristic inverted position , with the arms directed towards the genital cleft .

Disc., disc of parent; Lum.Burs., lumen of the bursa; Arm.Emb., arm of the embryo; Burs., bursa ; Fl., reflected flap of the bursal wall; Coel., perivisceral coelom of the parent ; Stom., stomach of the parent ; Emb., embryo ; Gen.Pl., adradial genital plates; Test., testis; Gen.Clft., position of the genital cleft ; Ovy., ovary ; Wl., body-wall of the parent cut through to expose the internal organs .


Coel.
Stom.
Fl.
Em
Burs.
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P
Arm.
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Burs.
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Disc.
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Ovy.
Gen. Clft.

PLATE 1 .

PART TWO

DIRECT DEVELOPMENT IN THE ECHINODERMS, WITH SPECIAL REFERENCE TO THE OPHIUROIDEA.

With twenty-nine figures.

Part 2

DIRECT DEVELOPMENT IN THE ECHINODERMS, WITH SPECIAL REFERENCE TO THE OPHIUROIDEA.

Introduction

Nearly one hundred years ago - in October 1846 - Johannes Müller astonished the scientific world of his day by his discovery that a peculiar free-swimming marine animal which he had previously named Pluteus paradoxus was in reality the larval stage of an ophiuroid. This was the first demonstration of the fact that echinoderms undergo an indirect process of development, and the remarkable fact emerging from the observation was that these bottom-dwelling, radially symmetrical animals pass through a stage in their life history in which they are free-swimming and bilaterally symmetrical. Müller's discovery, and his later researches in the development of the echinoderm groups, were destined to exert a great influence on the theories as to the origin and evloution of the echinoderms; but their immediate effect was mainly upon the science of embryology, stimulating research workers to identify and elucidate other free-swimming larval forms.

The subsequent history of echinoderm embryology is too complex to be summarised here, but in its main drift it has resolved itself into the simple statement that all typical echinoderms pass through a free-swimming larval stage. There are various adjuncts to this statement the most notable being the widely accepted statement that the coelom of echinoderms is always an enterocoel. This paper is concerned with a critical examination of the evidence upon which the statement is based.

In 1936 the present writer had the good fortune to be associated with Professor H.B.Kirk of New Zealand in the study of an ophiuroid whose development clearly departed from the preexisting notions of echinoderm embryology. Kirk (1916) had first discovered that the species - as yet unidentified - lacked a free-living larval stage, but was prevented by various circumstances from pursuing his researches further. In the course of the years 1936-38 the writer was able to follow the salient features of development of this ophiuroid and an account of these has since been published (1941). The main points derived from the study were that Kirk's ophiuroid lacked any vestige whatever of a larval stage, and that the coelom arose not as an enterocoel but as a schizocoel excavated in mesenchyme. Examination of the literature revealed the fact that Russo (1891) had made a similar claim in regard to the perivisceral coelom of the ophiuroid <u>Amphipholis squamata</u>, but that his results had not gained acceptance. It was obvious that no worker had actually disproved the statement he made, and therefore in view of the parallel condition found in Kirk's ophiuroid, it was urgently necessary to reinvestigate the conditions in the development of this ophiuroid. This work has occupied my attention during the last two years, and is recorded in Part 1 of this paper. An added reason for research on Amphipholis was the fact of its possessing a vestigial larval form, for it could be expected to throw considerable light upon the process by which the larva has been so completely effaced from ontogeny in Kirk's ophiuroid.

The Problem

The problem under consideration may be briefly enunciated - "To what extent does direct development occur in echinoderms; what are its causes; by what processes does it take place; and what evidence does it provide of the evolutionary history of the echinoderms?"

In view of the more extensive nature of our evidence on the subject in the Ophiuroidea, the present paper is predominantly concerned with that class, but wherever material is available, comparisons are made with conditions holding in the other classes.

Material for Evidence

The evidence upon which this investigation is based is as follows:-

(a) Previous knowledge of the indirect pelagic type of development. This is founded mainly upon the researches of MacBride (1907) on Ophiothrix fragilis and the recent work of Narasimhamurti (1933) on Ophiocomina nigra.

(b) Previous knowledge of the development of a moderately yolky ophiuroid egg, founded solely on Grave's work on Ophiura brevispina (1900 & 1916).

(c) The recently published work of the writer on the development of Kirk's ophiuroid, a form with very yolky eggs, and on Ophiomyxa brevirima, a viviparous form also with very yolky eggs (1941).

(d) The scattered body of data in the literature in regard to isolated stages of development in other ophiuroids - mainly referring to larval stages. Much of this matter is brought together for the first time, for the purposees of comparative study.

(e) The research presented in Part 1 of this paper on the development of Amphipholis squamata, a form with moderately yolky eggs and of viviparous habit.

As will be seen from the above list, there is a fairly representative body of data available, ranging from forms with non-yolky eggs to those with a very high proportion of yolk, and including cases of viviparity. In addition to these data, there is a considerable amount of scattered information on the other echinoderm classes, but in no case presenting so complete a sequence as is now available for the Ophiuroidea.

The method adopted in this investigation is comparative and analytical, i.e. each of the principle phases of development is taken separately, and examined with reference to the forms showing indirect development, those showing intermediate characters, and those showing direct development.

What is the "Typical" Echinoderm Development?

Before commencing the study of the nature of direct development in the echinoderms, it will be useful to examine the position in regard to development in general in this group. As pointed out in the introduct~~a~~ry section of this paper, it has come to be generally assumed that all "typical" echinoderms have an indirect development; consequently, whenever an echinoderm has been found which departs from this criterion, it has been glossed over in the text-books with a remark to the effect that it is "not typical" - and hence presumably need not be considered in an evolutionary sequence.

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Examples of this attitude are abundant. Thus, commenting on the development of Antedon, MacBride (1914) states "We may discount the characteristic features of the development of Antedon, which are obviously due to the yolky egg ..." etc. Antedon, it should be noted is the only crinoid of which we have any embryological knowledge, yet, because it lacks a larva comparable to the Ophiopluteus or Bipinnaria it is automatically rejected as "atypical", - for, it is stated, a "typical" crinoid must undoubtedly possess a bilaterally symmetrical larva.

If echinoderms with yolky eggs - and consequently having direct development to a greater or less degree - are held to be atypical, then obviously a census of the echinoderms whose development we have any evidence should at once reveal that directly developing forms constitute a marked minority. It should be found that most echinoderms have an indirect development, coupled with a pronounced larval stage. Is this the true position? In order to obtain some informationon this question, I have selected three specimen faunas for special examination. These are:- the echinoderm faunas of Great Britain, of New Zealand, and of the Antarctic and Sub-Antarctic Oceans. In these we may expect to find reasonably typical samples of the world's echinoderms.

British Echinoderms

A survey of the literature shows that there are some 61 British echinoderms about which we have sufficient information to be able to deduce with a fair degree of certainty the type of development which they follow. This is a larger proportion of the entire fauna than holds in other regions, and is attributable to the fact that most British species are found over a large part of northern European seas, where most research work on the subject has been done. Taking first those species whose development is either known to be shortened, or which may be regarded as fairly certain to be so, we have the following list:-

Holothuroidea (Seven species)

Mesothuria intestinalis
Bathyplotes natans
Cucumaria frondosa
Cucumaria planci
Psolus phantapus
Leptosynapta minuta
Labidoplax buski

- *Crinoidea* (Two species)
    - *Antedon bifida*
    - *Antedon petasus*
- *Asteroidea* (Twelve species)
    - *Ctenodiscus crispatus*
    - *Psilaster andromeda*
    - *Pontaster tenuispinus*
    - *Ceramaster granulatus*
    - *Hippasterias phrygiana*
    - *Asterina gibbosa*
    - *Solaster papposus*
    - *Solaster endeca*
    - *Henricia sanguinolenta*
    - *Brisingella coronata*
    - *Leptasterias mulleri*
    - *Pedicellaster typicus*
- *Ophiuroidea* (Four species)
    - *Asteronyx loveni*
    - *Ophiomitrella clavigera*
    - *Ophiopus arcticus*
    - *Amphipholis squamata*
- *Echinoidea* (Two species)
    - *Poriocidaris purpurata*
    - *Neolampas rostellata*

In addition to the four ophiuroids named above the larvae of *Amphiura filiformis* and of *Ophiura affinis* although pelagic, lack the full development, and may be regarded as representing stages in the adoption of a shortened development.

Taking now those species whose development is either known or believed to be of the indirect or pelagic type, we have the following:-

Holothuroidea (Three species)

- Stichopus tremulus
- Holothuria forskali
- Labidoplax digitata

Note: Only one Auricularian larva has been taken in British waters - that of L. digitata

Crinoidea

None.

Asteroidea (Seven species)

- Asteropecten irregularis
- Luidia Sarsi
- Porania pulvillus
- Stichastrella rosea
- Asterias rubens
- Marthasterias glacialis
- Pedicellaster sex-radiatus

Ophiuroidea (Twelve species)

- Ophiothrix fragilis
- Ophiocomina nigra
- Ophiactis balli
- Ophiopholis aculeata
- Amphiura Chiajei
- Amphiura filiformis
- Ophiura texturata
- Ophiura Sarsi
- Ophiura albida
- Ophiura carnea

Ophiura affinis

Ophiocten sericeum

Echinoidea (Twelve species)

Cidaris cidaris

Psammechinus miliaris

Echinus esculentus

Echinus acutus

Paracentrotus lividus

Sphaerechinus granularis

Strongylocentrotus droebachiensis

Echinocyamus pusillus

Brisaster fragilis

Spatangus purpureus

Echinocardium cordatum

Brissopsis lyrifera

Analysis

Of ten British holothuriams about whose development we have any knowledge, only three form Auriculariae. The other seven species all have large yolky eggs and a more or less direct development. Two of these are known to have the special simplified yolk-larva which I have termed "Vitellaria" (see below) and the remainder may have an absolutely direct development.

Of the two crinoids whose development is known both have large yolky eggs, and both form the same type of yolk-larva or Vitellaria.

Among the asteroids, only seven out of nineteen species have a pelagic development. The remaining twelve species all have large, yolky eggs, and probably a development more or less direct. In this class there appears to be as yet no evidence of the occurrence of the Vitellaria larva.

In the ophiuroids, of the sixteen species about which we have any knowledge, four have direct development as a result either of viviparity or of large, yolky eggs. Of the remaining twelve species, not all have typical plutei, and stages in the loss of the arms are to be observed. In a species of *Ophiura* a Vitellaria larva has been recorded.

In the echinoids there is far less tendency to have direct development. However, of fourteen species about which we have any embryological information, two at least have large yolky eggs and probably a direct development. Their development is likely to be of the type seen in *Toxocidaris erythrogrammus* of New South Wales. The remaining twelve species have pelagic plutei and indirect development. The following table summarises this information regarding those British echinoderms about which there is any embryological evidence .

Group	% with direct development	% with pelagic indirect development
Holothuroidea	70%	30%
Crinoidea	100%	-
Asteroidea	63%	37%
Ophiuroidea	25%	75%
Echinoidea	14%	86%

Table 1 .

From this it is clear that the notion that echinoderms are typified by having an indirect development with pelagic larvae is without foundation. The idea probably originated from the fact that most laboratory work has been done upon the eggs and larvae of echinoids which, as shown above, are alone notable for the tendency to have pelagic larvae.

New Zealand Echinoderms

Turning now to the New Zealand Echinoderm fauna the ophiuroids may be selected here for particular consideration, as I have had opportunities for studying these in recent years in that country. Mortensen showed in 1924 that at least six New Zealand ophiuroids are viviparous. All of these lack a free-swimming larva and have a more or less direct development. In the case of Ophiomyxa brevirima some further information has been given on its development (Fell, 1940a), and it is hoped to publish a paper on the species at a later date. It has also been shown that the oviparous species at present known as "Kirk's ophiuroid" has an absolutely direct development . The large yolky eggs of Pectinura maculata which have been examined and briefly described (Fell, 1941) almost certainly indicate a direct development . In addition, some species provide an indication as to the nature of their development from the size of their eggs (see later section of this paper). New Zealand ophiuroids which are known, or believed, to have a direct development are as follow:-

Ophiomyxa brevirima
Amphiura magellanica
Amphiura annulifera
Amphiura praefecta
Amphipholis squamata
Pectinura cylindrica
Pectinura gracilis
Pectinura maculata
Amphiocnida pilosa
Amphioplus basilicus
Kirk's ophiuroid
Ophiozonoida picta

In addition, Mortensen (1924) has given reasons for believing that Amphiura hinemoae may have either a direct or a shortened development.

New Zealand ophiuroids whose development is known or believed to be indirect and pelagic are as follow:-

Ophiocoma Bollonsi
Ophiactis resiliens
Amphiura spinipes
Amphiura rosea
Amphiura amokurae
Amphiura alba
Ophionereis fasciata

The development of the remaining twenty-three species is unknown. The above lists show that out of nineteen New Zealand ophiuroids of whose development we have any knowledge at all, twelve i.e. 63% are believed to have direct development. Thus the proportion of ophiuroids with direct development seems to be much higher in New Zealand waters than in thosesof Britain.

Antarctic and Sub-antarctic Ophiuroids

Owing to the detailed work of Mortensen (1936) in his description of the ophiuroids collected by the "Discovery" Expedition, we now have some knowledge of the breeding habits of a considerable number of southern species, together with information in regard to the nature of their eggs. His researches have revealed that out of 56 ophiuroids from this region whose sexual character is known, no less than 31 are viviparous. This high proportion seems to be characteristic of southern seas as it is not paralleled in the Arctic ocean.

These 31 ophiuroids must all have a development which is shortened to a greater or less degree, without free larval forms. There is no need to quote further lists of species here, as details may be obtained from Mortensen's paper (1936). Besides the 31 viviparous ophiuroids, however, it appears that another eight species at least have large, yolky eggs, and therefore a probable direct development. Thus, out of the 56 Antarctic and Sub-antarctic species of whose breeding habits we have any information, it is highly probable that 39 species i.e. about 70% have direct development. Our knowledge of these southern forms must of necessity remain incomplete for a long time, but sufficient is known to show that the majority of them probably have direct development.

Conclusions

No doubt similar evidence of this kind could be provided for the echinoderm faunas of the rest of the world, with the possible exception of the tropics, where larval forms of all types tend to become exaggerated; but sufficient facts have been given to show that the supposition that a pelagic larval development is "typical" of echinoderms is founded on no real evidence. The facts show that no particular kind of development is typical for echinoderms in general, but that the kind of development followed depends on the particular condition obtaining in each species. That these controlling conditions can be analysed and classified it is the aim of the next part of this paper to show.

The Phases of Development Analysed

The Ovum

In the physical characteristics of the ovum I believe that we can find the clue to the factors controlling development in both the directly and indirectly developing forms . These factors , I believe, can be further traced to the cytoplasm of the ovum. But before proceeding to examine the evidence provided by the internal structure of the egg-cell, it will be useful to consider the egg-cell as a whole.

If we take a series of ophiuroids including species with various types of development, and place their eggs in order of increasing volume, a striking fact at once becomes obvious (see Table 2). It is that the eggs of small volume fall into a group possessing as a common character a long period of indirect development , involving a pelagic Ophiopluteus stage and a metamorphosis. The largest eggs form another group whose common character is the absence of a larval stage, the shortening of development, and the absence of any marked metamorphosis. Eggs of intermediate sizes form a third category exhibiting to various degrees , according to the species, intermediate stages in the suppression of larval development. This we can express as a simple and fundamental principle - "<u>The degree of larval development of an embryo varies indirectly as the volume of the egg from which the embryo is derived</u>".

Grave (1916) anticipated this "law", but as no case of absolutely direct development in an ophiuroid had been described at that time, and as only two ophiuroid developments had been fully described then, he was unable to express the fact in a full or convincing manner. His work is further considered below. The same relationship between egg-size and development appears to hold good in the other classes of echinoderms, but as yet there is a complete series

Ophiothrix fragilis	100 μ.	Small eggs , long indirect development, with pelagic Ophioplutei. Group 1.
Ophiocomina nigra	100 μ.	
Amphipholis squamata	100 μ.	Eggs of intermediate size , having a shortened development , and with reduced larvae. Group 2.
Ophiura brevispina	300 μ.	
Ophiopus arcticus	400 μ.	Eggs comparatively very large, with development so shortened as to be direct, and lacking all traces of a larval stage. Group 3.
Kirk's ophiuroid	500 μ.	
Ophiomyxa brevirima	650 μ.	

Table 2. Interrelationship between egg-size and development in ophiuroids.

available only from the Ophiuroidea.

In my previous paper on the development of Kirk's ophiuroid (1941) it was suggested that the immediate ontogenetic factor causing the direct development of that species might be the presence of yolk-material in the tissues of the developing embryo. The yolk, it was thought, might act as a retarding agent, being inert and lifeless. This interpretation accords well with the facts revealed by the sequence of egg types shown in Table 2, for in all cases it is the larger eggs - and consequently those with the larger amount of yolk material - which have direct development.

In the literature hitherto it has been customary to speak of eggs as being "yolky" or "very yolky" or "not yolky" , a method of description which is necessarily inexact. It seemed that a more precise definition of these arbitrary terms would be of use in determining more accurately the influence exerted by the yolk. Accordingly the following method of quantitative estimation of yolk-content in minute eggs was devised, and proved susceptible to mathematical description. Sections of known thickness were cut through the egg the yolk content of which it was desired to estimate. Haematoxylin was used to stain the granules a deep black, utilising their strong basiphilic reaction. Then, by using a squared eyepiece, the average number of granules in a selected number of squares of known area was counted. Knowing the thickness of the section it was then possible to calculate the number of granules in a given volume of egg. Next the average diameter of the yolk granules was estimated, and, combining this result with the former, it was possible to estimate approximately y the actual volume of yolk material in a given volume of egg. In practice the yolk was estimated as a percentage of 100 cubic microns of egg material. Knowing the average diameter of the egg, it was then possible to to determine the absolute volume of the yolk material present in the entire egg.

Example

Species - Kirk's Ophiuroid.

Average diameter of yolk-granules = $1{\cdot}8\mu$

Average no. yolk-granules per unit area = $72{\cdot}5$

Size of one unit area = $18 \times 18\mu^2$

Thickness of section = $(500 \div 60)\mu$

8.3μ

∴ Volume of egg containing $72{\cdot}5$ granules = $(18 \times 18 \times 8{\cdot}3)\ \mu^3$

∴ Number of granules per 100 cubic μ = $\dfrac{72{\cdot}5 \times 100}{18 \times 18 \times 8{\cdot}3} = 2{\cdot}7$

∴ Yolk volume per 100 cubic μ (% yolk content of egg) = $2{\cdot}7 \times {\cdot}5236 \times (1{\cdot}8)^3\ \mu^3$

$= 8{\cdot}24\ \mu^3$

Volume of entire egg = $(500^3 \times {\cdot}5236)$ cubic μ

$= 65{,}450{,}000\ \mu^3$

Total yolk content = $\dfrac{8{\cdot}24 \times 65{,}450{,}000}{100}\ \mu^3$

$= 5{,}300{,}000$ cub. μ approx.

i.e. ·0053 cub. mm.

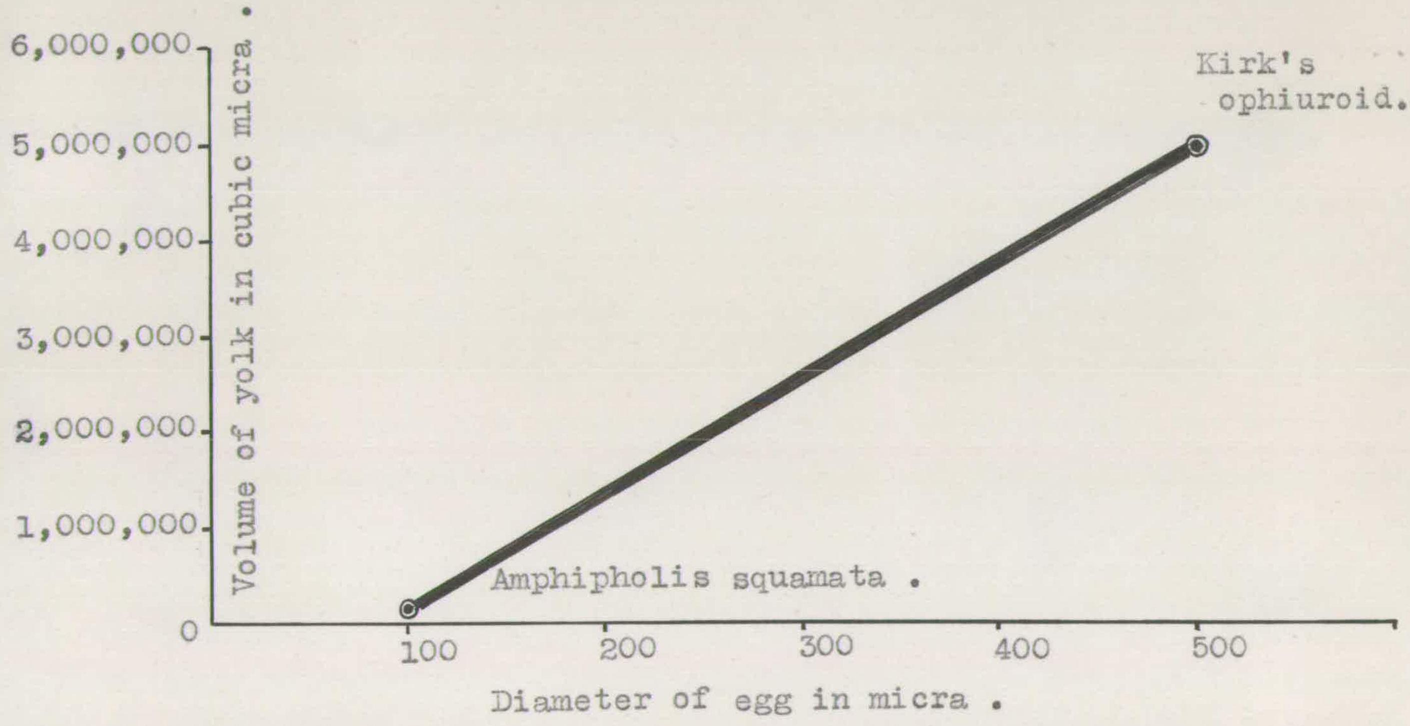
6,000,000
5,000,000
4,000,000
3,000,000
2,000,000
1,000,000
0
Volume of yolk in cubic micra .
Kirk's ophiuroid.
Amphipholis squamata .
100
200
300
400
500
Diameter of egg in micra .

Figure 1 .

A similar estimation for *Amphipholis squamata*, a form with only "moderately yolky " eggs yields a figure of circ. 88,500 cub.μ . Thus we have a much more clear cut picture of the relative "yolkiness" of these two types of egg, and can now proceed to compare the yolk value with the other variables.

Plotting the yolk content against the diameter of the egg we obtain a graph (Fig. 1) which provides a mathematical proof that yolk content increases with size of egg. This of course is merely what has usually been assumed hitherto, though without actual demonstration.

Having noted that the yolk increases proportionately with increasing diameter of the egg, we can now consider the case in regard to the cytoplasm. In this matter the literature provides no information. The tendency, however, has been to suppose that in large yolky eggs the cytoplasm becomes proportionately reduced, remaining more or less constant in amount while the yolk increases. This attitude is maintained by Grave (1916) who also states that it is impossible to measure the ratio of yolk to cytoplasm. In his latter statement he is of course incorrect, since it is possible to make such measurements by the method described above. In the calculation illustrated, at the stage when the yolk-volume per 100 cubic microns was estimated, the resultant is also an expression of the percentage content of yolk of the whole egg. By a simple subtraction, therefore, we obtain an expression of the percentage content of cytoplasm - ignoring the

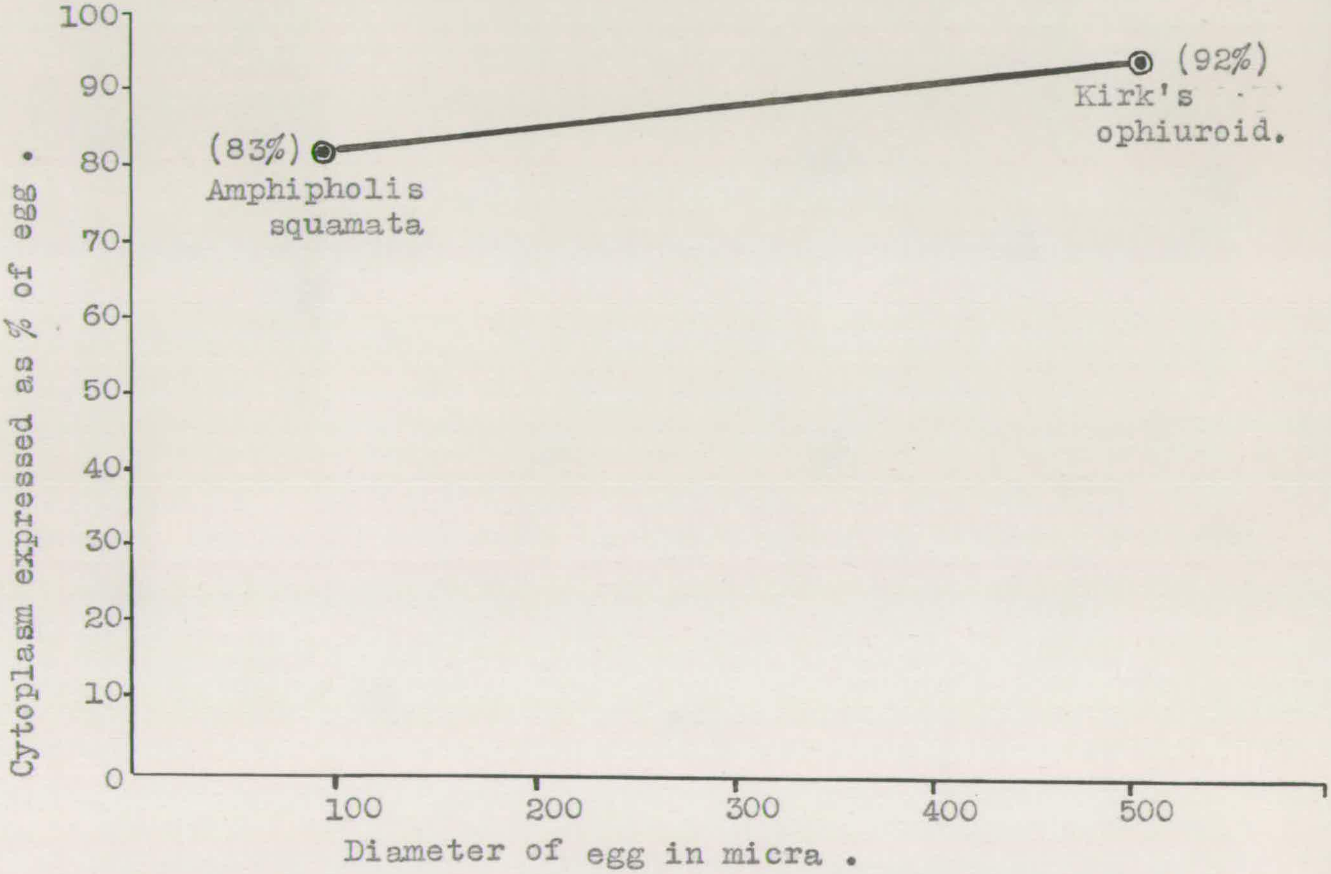
100
90
80
70
60
50
40
30
20
10
0
Cytoplasm expressed as % of egg .
(83%)
Amphipholis squamata
(92%)
Kirk's ophiuroid.
100
200
300
400
500
Diameter of egg in micra .

Figure 2 .

nucleus in both measurements. Plotting now the cytoplasm value as a percentage of egg-volume against the diameter of the egg (Fig. 2) we obtain a graph which shows that comparatively little change in the ratio of cytoplasm to yolk has taken place in the transition from smaller egg-size to larger. This means that as the amount of yolk has increased, so also has the amount of cytoplasm, keeping pace each with the other. The older vague methods of describing "yolky" and "less yolky" eggs failed to bring out this fact, and hence the mistaken notion that the yolk increased and not the cytoplasm. It is therefore necessary to modify the conclusion expressed in my earlier paper on Kirk's ophiuroid (1941) in regard to the influence of increasing yolkmass, by adding that the effect is associated with a parallel increase in the cytoplasm.

Finally if we combine into one diagrammatic graph the three associated conditions we have been considering in this section of the paper - namely, increasing egg, increasing yolk content, and shortening of the development - we obtain a convincing demonstration of how the different types of development are interrelated (see Fig. 3). This graph shows that at one end of the scale we have such types as Ophiothrix fragilis and Ophiocomina nigra, with small eggs and small amount of yolk material . Next comes the group with an increase in the amount of yolk, together with a shortening in the development. This group includes Amphipholis squamata and Ophiura brevispina. In the case of the latter species, the increase in yolk is

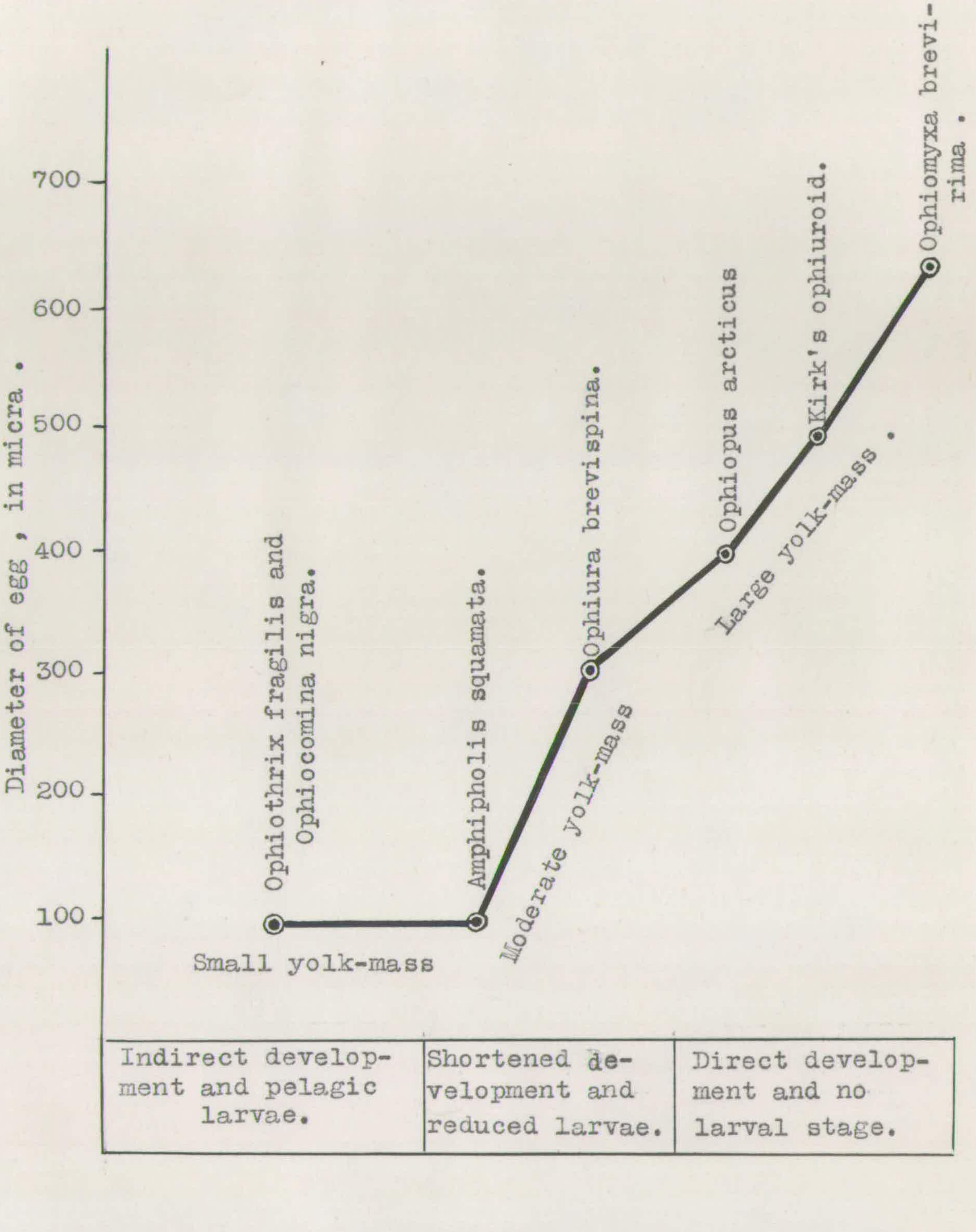

Figure 3. Graph showing interrelationship between size of egg, amount of yolk, and type of development in the Ophiuroidea.

associated aith increase in egg size, together with more marked shortening of the development. At the other end of the scale come the group in which a very much greater increase in yolk material has occurred together with cytoplasm increase, the eggs have become progressively larger, and the development so shortened as to be direct. In this group are such species as *Ophiopus arcticus*, Kirk's ophiuroid, *Ophiomyxa brevirima* and (probably) *Pectinura maculata*.

Conclusions

From these facts it is reasonable to conclude that the factor most important in causing direct development is intimately related with an increase in the size of the egg and an associated increase in the amounts of cytoplasm, and its product the yolk.

Whether other factors may have operated in bringing about direct development will be considered later in the paper, as also will the mechanism through which the factors may have acted.

2. The Early Cleavages

In ophiuroids with small, comparatively non-yolky eggs the plan of cleavage followed is of the usual echinoid type i.e. the first cleavage is equal, as also is the second, and both are vertical. The third cleavage is horizontal and the upper and lower quartets are equal. The fourth cleavage is again vertical and more or less equal, and after that the cleavages occur fairly evenly over the whole embryo to produce a morula. The latter rapidly gives place to a regular blastula. These stages are represented in Figures 4 to 8.

In the case of the moderately yolky eggs of ophiuroids, Grave (1916) has described the cleavages of the egg of Ophiura brevispina. His account shows that no difference is to be observed from the plan followed in the non-yolky group. He concludes therefore that the early stages of development in this species have been disturbed little if any by the increase in the yolk mass that has occurred.

Coming now to the yolky group having very large eggs we have such forms as Ophiomyxa brevirima and Kirk's ophiuroid. The type of cleavage followed in the latter species has been described by the writer (1941). Cleavage in this case follows a somewhat different pattern to that seen in the two preceeding groups, but the alteration nevertheless is not very profound. The first cleavage (Fig. 9) is unaltered[1]. The same applies to the second cleavage (see Fig. 10). When, however, the third cleavage is reached two quartets are formed which show a marked differentiation into micromeres and macromeres (Fig. 11). This fact indicates that a prelocalisation must have occurred during the four-cell stage, or even earlier. As shown in my paper (1940), the macromeres are destined to form mesendoderm, and are ventral (aboral) in position.

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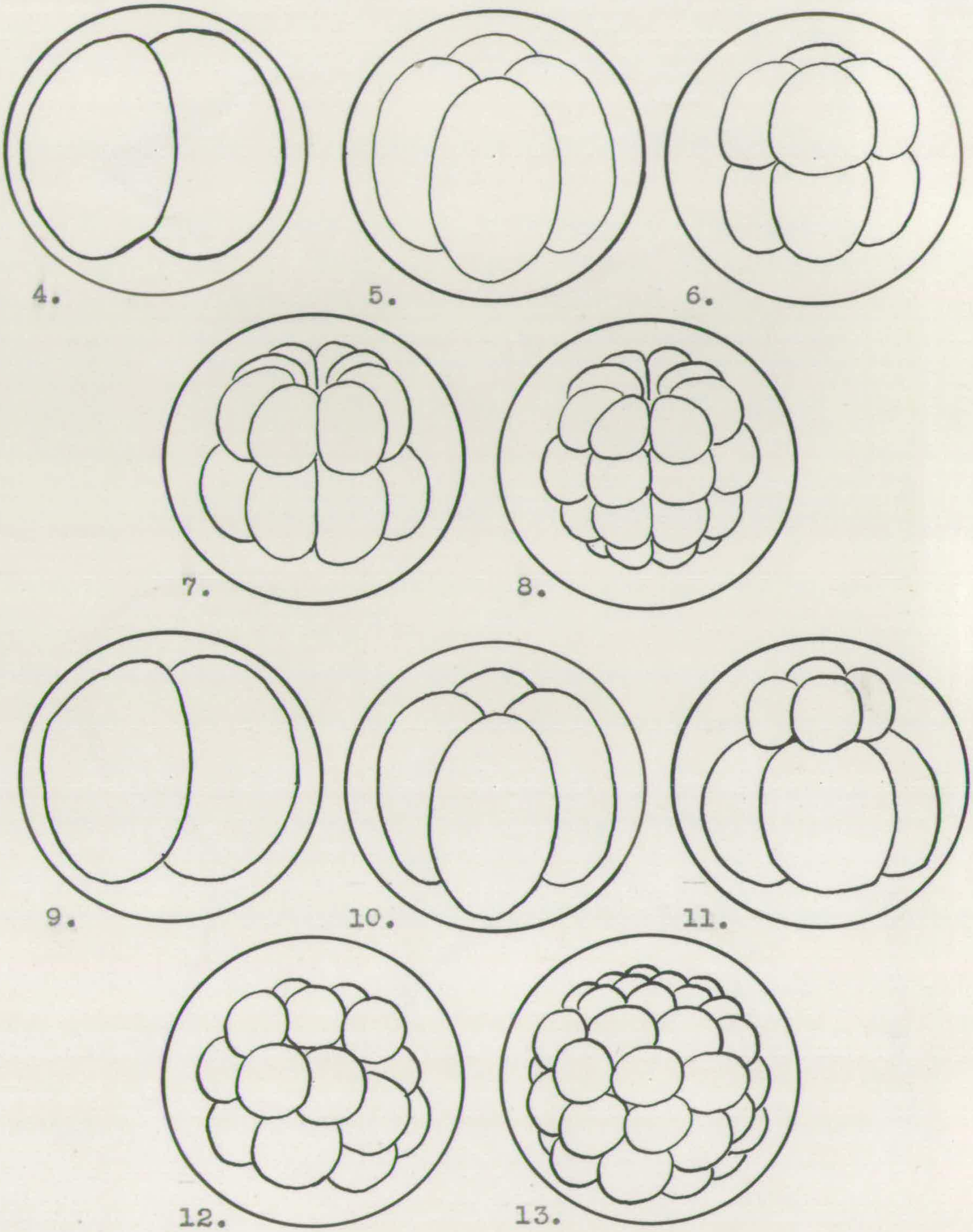
[1] In about 30% of cases, however, an abnormal cleavage occurred in which the first two blastomeres were unequal, the larger undergoing two divisions to form a normal four-cell stage.



Figures 4 - 13 . Diagrams illustrating the effect of increasing yolk mass on the cleavage of the egg.

Figs. 4 - 8, cleavage in ophiuroids with small or moderate yolk mass (eg., Ophiothrix , Ophiocomina , Ophiura). Figs. 9 - 13 , corresponding stages in cleavage of Kirk's ophiuroid , which has heavily yolked eggs .

The micromeres form the ectoderm and its derivatives, and in addition contribute to the mes-endoderm. This will be further discussed in the section of this paper dealing with gastrulation. In the cleavage immediately following the third, the divisions of the blastomeres becomes irregular and "out of step". Finally a morula with very turgid blastomeres is formed (see Fig. 13).

The changes to be observed in the plan of cleavage of this very yolky form are undoubtedly to be correlated with the great increase in the yolk material. It is interesting to note that, despite the comparatively enormous amount of yolk present in Kirk's ophiuroid as compared with <u>Ophiothrix</u> or <u>Ophiocomina</u>, the plan of segmentation is really but slightly altered. One might, for instance, have expected to find some tendancy to adopt partial cleavage, or to form a blastoderm, as has occurred in other groups where increase in yolk-mass has occurred. Indeed such a supposition was put forward by Dr.Th. Mortensen in a private communication to the writer; but so far no evidence of such alteration in development has been found.

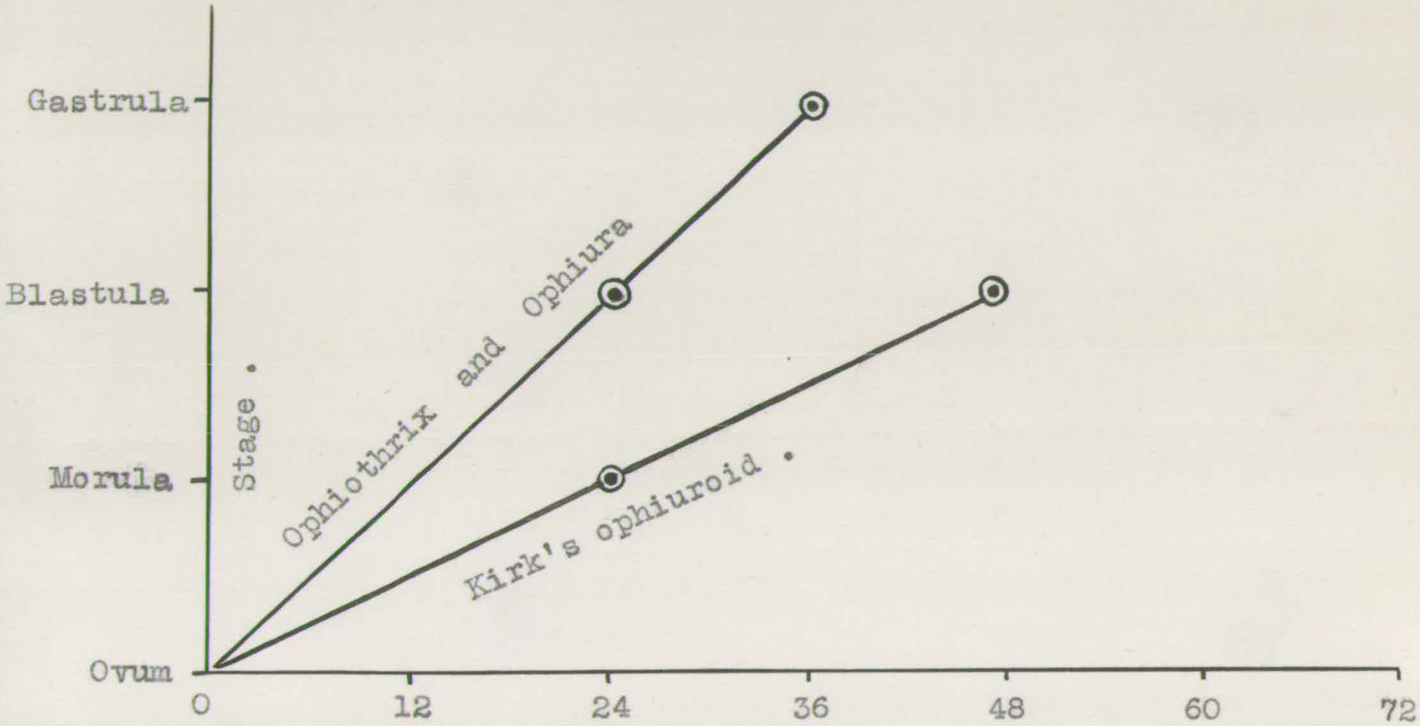
It would appear that the reason for this retention of the primitive type of division may be correlated with the fact already shown in this paper that the cytoplasm mass has increased in step with the increase in the yolk mass. Again, we may consider that the earliest stages of development are always the most rigid, and the last to be affected by factors producing ontogenetic change. This seems to be indisputable, for, as will be shown below, the later stages come to be tremendously altered in the heavily yolked forms.

One further effect of the increased yolk-mass in Kirk's ophiuroid remains to be noted. This concerns the alteration in the rate of cleavage. In my account of the development of this species, a table was given showing the process of cleavage during the first twenty-three hours following the first cleavage. From this it was clear that a pronounced retardation had occurred, for whereas other ophiuroids with non-yolky or moderately yolky eggs reach the blastula stage at the end of the first twenty-four hours, Kirk's form is at that time only at the morula stage. Not till the end of the second day is the blastula completely formed, while the gastrula stage cannot be regarded as complete till the fifth day. On the other hand, other ophiuroids (Ophiothrix and Ophiura) have formed the gastrula at the thirty-sixth hour. Ophiocomina, as described by Narasimhamuti (1933) is slightly slower, forming the gastrula at from the fortieth to the forty-eighth hour.

This retardation is illustrated in graphical form in Figure 14. As the alteration in developmental rate is associated with increase in the yolk-mass, we can justifiably correlate the two conditions. That the presence of a large quantity of inert,nutritive material should have such a delaying action on the ontogenetic processes is not surprising.

3. The Blastula

Passing now to the blastula stage, we begin to



Time after fertilisation, in hours .

Figure 14 .

find that changes in the developmental sequence become more pronounced, and furthermore, these changes not only affect the heavily yolked types, but become extended to the moderately yolked eggs.

In the non-yolky type, exemplified by Ophiothrix, MacBride (1907) states that there is a free-swimming blastula with ciliated cells. These form a layer one cell deep and surround the large central blastocoel. From the vegetal pole mesenchyme cells are budded off, (see Fig. 15);

In the moderately yolky type, Ophiura, as described by Grave (1916), shows a thick-walled blastula with somewhat reduced blastocoel. The disposition of the nuclei shows that there is a tendency to form a blastula wall more than one cell deep - and in the later gastrula stage this tendency receives its full expression according to the earlier paper of Grave (1900) where he refers to the wall of the gastrula being more than one cell deep. From the vegetal pole the mesenchyme in a reduced form bulges into the blastocoel reducing its form in section to a major segment of a circle. (Fig. 16)

Finally, in the heavily-yolked type we see in Kirk's ophiuroid the tendencies already weakly expressed in Ophiura reaching their fullest extent. The wall of the blastula is several cells thick. The blastocoel is reduced to a small cavity crescentic in vertical section owing to the bulging upwards of the macromeres - obviously homologous with the mesenchyme-producing cells of the other two forms.

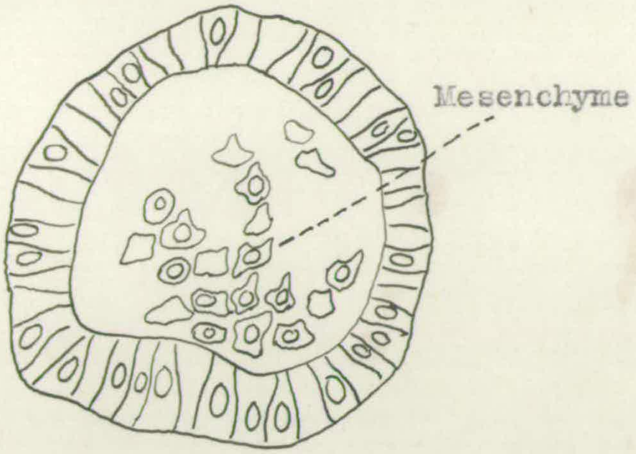


Fig. I5 .

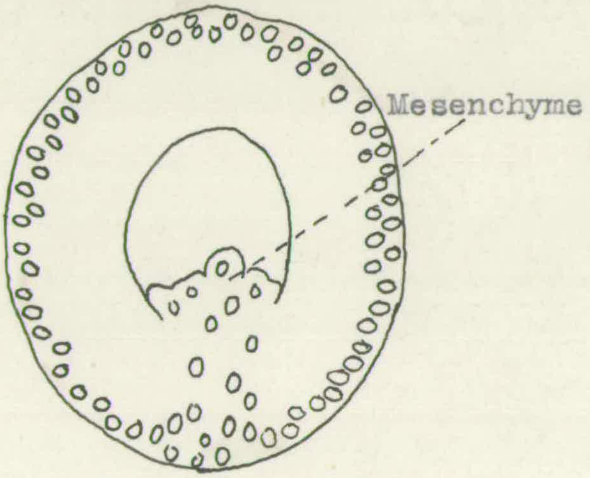


Fig. I6 .

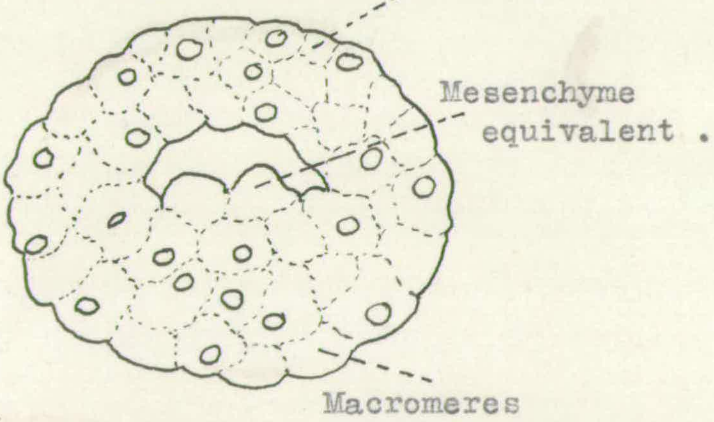


Fig. I7 .

Figures 15 - 17 . Diagrams illustrating changes produced in the blastula by increasing yolk mass.

Fig. 15, Ophiothrix , a non-yolky type (after MacBride, 1907); Fig. 16, Ophiura , a moderately yolked type (after Grave, 1916) ; Fig. 17 , Kirk's ophiuroid , a heavily yolked type (Fell, 1941) .

The macromeres and micromeres occupy respectively the vegetal and animal poles as in the morula (Fig.17).

From this sequence we observe that with increasing yolk-mass the walls of the blastula become successively thicker, steadily reducing the blastocoel to a mere vestige in the animal hemisphere. The mesenchyme fails to separate as such but remains as a great bulging mass projecting upward into the blastocoel. As we see in the development that follows immediately upon this stage, the reduction of the blastocoel has a profound effect upon the process of gastrulation.

4. Formation of the Gastrula

As the onotgenetic process advances the modifications produced in the larger and more yolky forms become ever greater. We thus find that the classical concept of invagination in the echinoderms becomes inadequate; for the invagination doctrine presupposes a static organogeny, whereas in actual fact organogeny is as dynamic and susceptible to moulding influences as any other biological process. Evolution, in other words, can act upon early stages of development as upon later stages. Embryological processes can be altered just as the adult products of these processes. This theory of the ability of evolution to act upon embryonic forms - so convincingly set out in de Beer's essay on "Embryology and Evolution" (1930) - receives strong support from the whole of the evidence provided by the yolky-egged ophiuroids.

Thus it is that in considering together the various types of gastrulation process met with in the

Ophiuroidea we are able to detect the operation of a unidirectional evolutionary force, the strength of the force operating in direct proportion to the size of the yolk-mass. Whereas the doctrine of unchangeable recapitulation during development becomes meaningless in the light of such a process as is observable here, the view which envisages embryonic forms as essentially plastic and subject to a greater or less degree of modification - according to circumstance - provides, in the view of the writer, the only possible explanation of the facts observed.

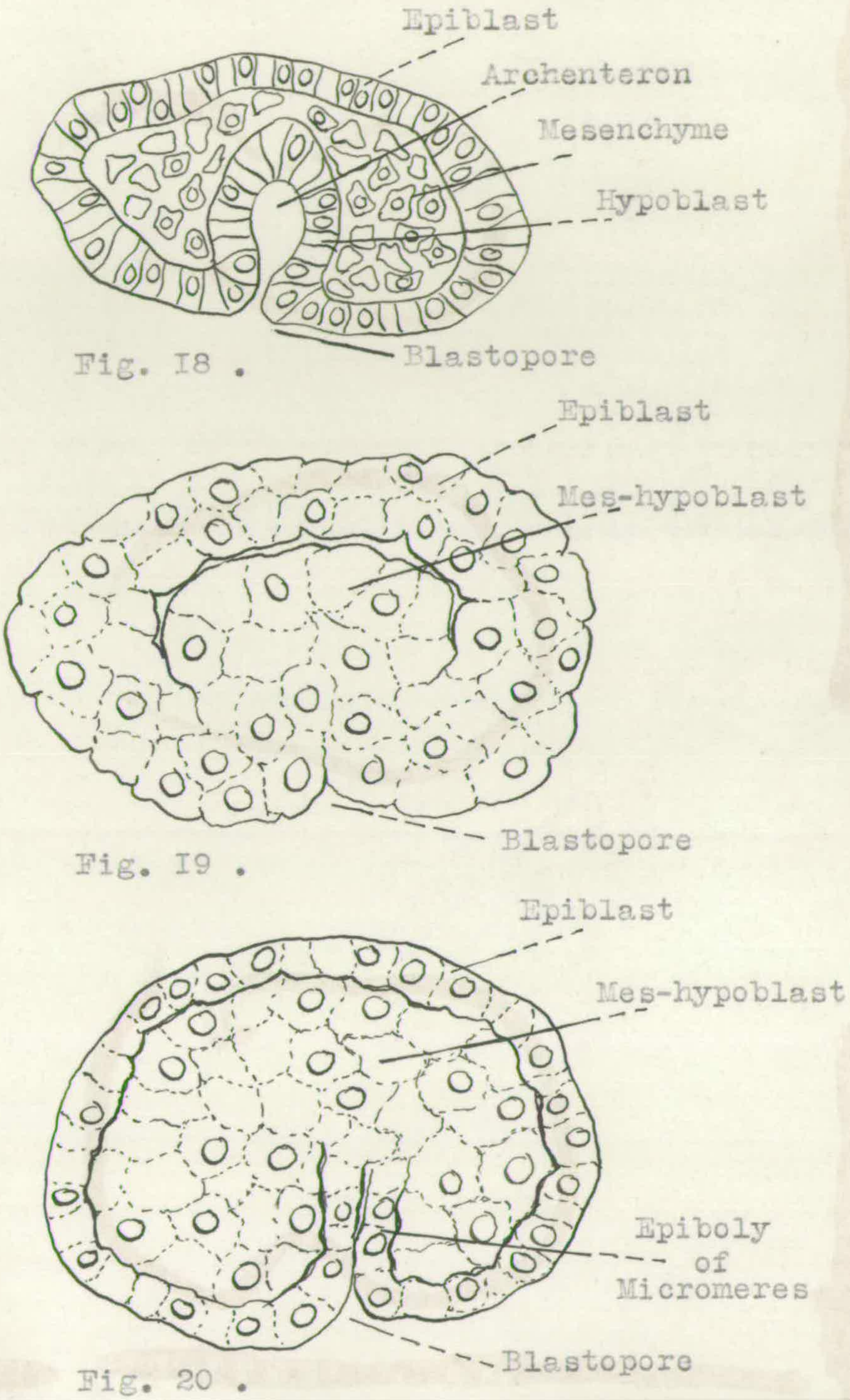
The traditional concept of gastrulation in the echinodermata supposed that it took place by invagination from one pole - the vegetal one. This is indeed true in the dwarf-egged group, such as Ophiothrix and Echinus. But, without any logical reason, it was then immediately assumed that such a process must also occur in all echinoderms.

The first voices raised against this rigid hypothesis were those of Apostolides (1882) and Russo (1891) who both claimed that the endoderm was formed by delamination in the embryo of Amphipholis (Amiphura) squamata. As it happens their claim was based on a misinterpretation - as shown in the accompanying account of the development of that species - but the importance of their observation is that it demonstrated that gastrulation in Amphipholis certainly did not occur by means of simple invagination, for such a process when it occurs is unmistakable.

However, their claim received no recognition although it was never actually disproved. MacBride in fact dismisses the whole of Russo's account as "improbable in the highest degree" and omits it from his account of the echinoderms in his "Text-book of Embryology" (1914). The very fact of such an attitude is itself a demonstration of the inadequacy of the Recapitulation Theory - which of course provided no explanation of the peculiar development of Amphipholis.

Grave (1900) gave reasons for believing that the endoderm in Ophiura arose not by the invagination of a hollow archenteron, but as a solid inpushing which later became hollowed out to form an archenteron. Certain stages were missing from Grave's material, however, and advantage was taken of this fact to throw doubts on the accuracy of his work (Bather 1901, and MacBride 1907). In his later work, however, MacBride (1914) quotes Grave's account without comment. As is seen from the facts quoted below, Grave's account receives strong support from independent evidence derived from the study of Kirk's ophiuroid.

In the latter species gastrulation takes place by means of two processes. Firstly as shown in Figure 19, there is a slight inpushing of the macromeres from the vegetal pole. This results in the complete obliteration of the small blastocoel. The macromeres which were pushed in remain a solid mass of cells without any cavity. So far the process is similar to that described by Grave in Ophiura. A second process commences now and involves extensive epiboly of the micromeres which



Figures 18 - 20 . Effect on gastrulation of increasing yolk mass .

Fig. 18, gastrulation in Ophiothrix (after MacBride); Fig. 19 , first stage in gastrulation of Kirk's ophiuroid; Fig. 20, second (epibolic) stage of gastrulation in Kirk's ophiuroid . Figs 19 and 20 simplified from Fell,(1941) .

migrate towards a central point on the vegetal hemisphere and then turn inwards (see Fig. 20). The point at which the inwandering of these micromeres takes place obviously is homologous with the blastopore, h/ and a temporary small depression at that region is all that represents the archenteron. At a very much later stage a second cavity appears and extends as an excavation up through the solid endoderm mass to form the definitive enteron.

To sum up, the effect of increasing yolk-mass upon the process of gastrulation has been firstly to modify invagination till it takes the form of a solid inpushing of cells. At a later stage an excavation in this mass produces the definitive enteron. This modification applies both to the moderately yolky type (*Ophiura*) and to the heavily yolked type (Kirk's ophiuroid). In the case of the latter however, the modification proceeds to a further stage, for the small blastocoel makes it mechanically impossible to invaginate all the endoderm, and hence a secondary process of epibolic inwandering of the micromeres takes place, surrounding and enclosing the whole of the vegetal hemisphere.

5. The Critical Point in Development

We have now reached the critical point in the development at which the forms with direct development diverge from the forms with a larval stage or with a vestige of a larva. Whereas the former proceed to adopt radial symmetry immediately after the conclusion of gastrulation, the latter begin to assume bilateral symmetry, and retain it for a greater or less period till it is finally obliterated by radial symmetry. At first sight it would seem that there is a complete hiatus separating the two types of development - but in the view of the writer, this is illusory. An important question that this paper seeks to elucidate is how it is that direct development has been brought about, and what the cause of it may be. The first part of the question will be discussed now.

In the larval type of development, there comes a stage at which what is termed "metamorphosis" takes place. Huxley (1928) has shown that metamorphosis is initiated in echinoderm larvae by the liberation of a substance which produces dedifferentiation of the larval organs and development of the adult form. Now if we suppose that in the course of evolution in various species this substance came to be liberated earlier and earlier during development, then metamorphosis would tend to occur in larvae which would be progressively undeveloped. The assumption of radial symmetry, in other words, would tend to be brought nearer and nearer to the gastrula stage. If finally the substance were to be liberated immediately after the completion of gastrulation, the larval stage would be lost altogether. What evidence is there in support of such a theory? This point will be discussed in the next section.

6. The Disappearance of the Larva from Ontogeny; Recession of Metamorphosis

One of the evidences of the Evolution Theory cites the existance of "chains" or "series" of species showing, by small gradations, a progressive directional change. If the theory of receding metamorphosis outlined above be true, then we can reasonably expect to find some evidence of the existence of such a series of forms illustrating stages of the process. A survey of ophiuroid larval forms shows that such a chain does indeed exist.

We may take as the starting point of the bioseries a fully developed larval form such as Ophiura albida. Here there are four pairs of larval arms each supported by slender, calcite, skeletal rods, (see Fig. 21,a). The four pairs of arms are - the anterolateral, the postoral, the posterodorsal and the posterolateral. There is a mouth communicating by the stomodael oesophagus to the stomach, from which a short intestine leads to the anus. To the left lies the five-lobed hydrocoel.

A second term in the bioseries is illustrated by the larva of Amphiura filiformis, (Figure 21,b), in which the posterodorsal arms have disappeared, and at the same time the postoral pair have become reduced in size. The other features remain unaltered.

Next we may select the larva believed to belong to Ophiura affinis (Figure 21,c), in which the postoral arms have disappeared altogether, and the anterolateral pair also. Thus the last pair to disappear will be the posterodorsal.

In the larva known as Ophiopluteus Claparèdei (the parent species being unknown) we have the stage in

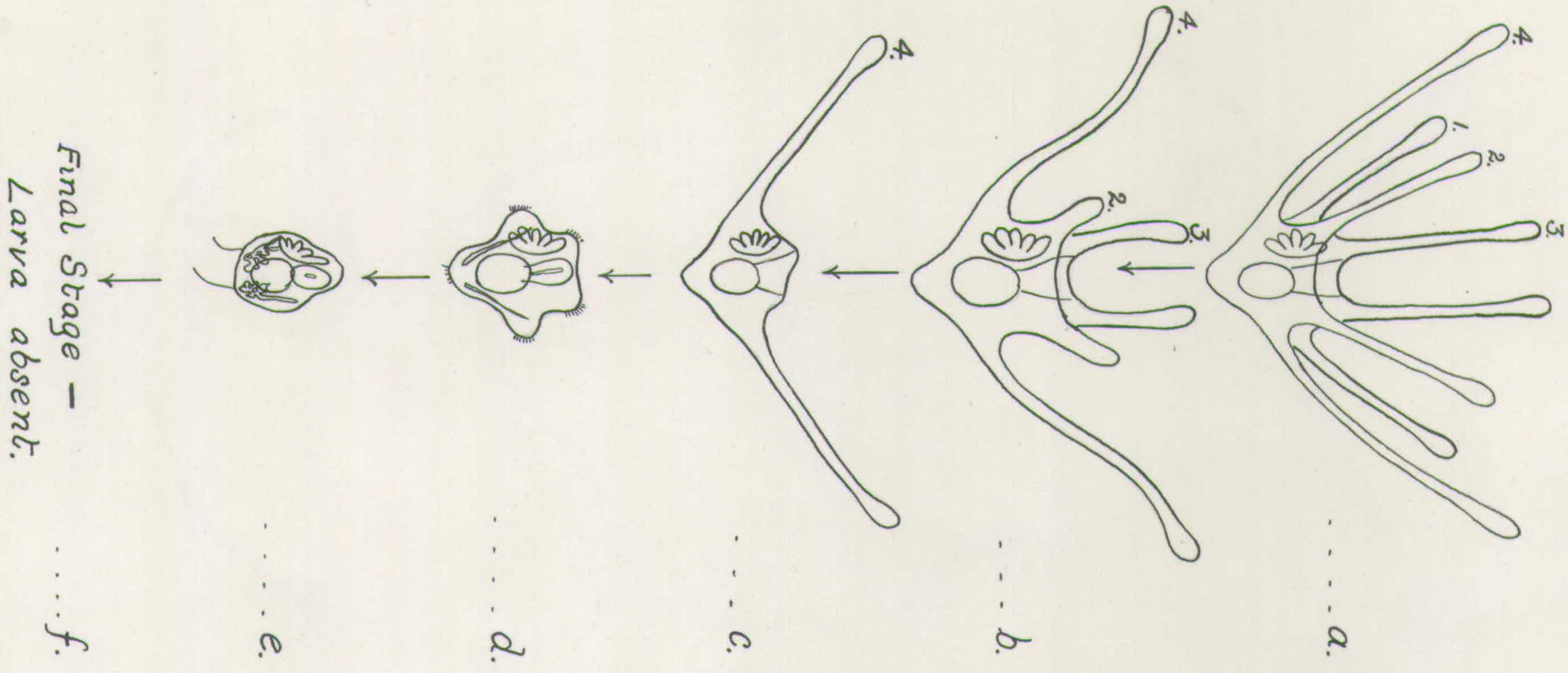


Figure 21. Sequence of Ophioplutei showing successive stages in the reduction and loss of the larva .

a, first stage , eg. *Ophiura albida* ; b, second stage, eg. *Amphiura filiformis* ; c, third stage, eg. *Ophiura affinis* ; d, fourth stage, eg. *Ophiopluteus Claparedei* ; e, fifth stage, *Amphipholis squamata* ; f, final stage , larva absent, and development direct ,eg. Kirk's ophiuroid.

The numbers above the arms indicate the order in which these organs disappear from the larva.

which the posterolaterals have indeed disappeared. That the posterolaterals should be the last to go is of particular interest, because in the metamorphosis of Ophiothrix fragilis it is this pair which is the last to be lost. This peculiar armless larva was taken by Clàparède (1863) swimming on the surface of the sea off the coast of Normandy. In his description of the animal he confuses the hydrocoel with a developing young ophiuroid, owing to its five lobes, but his accurate rendering of the organ in the figure makes clear its true nature. He records that there is a mouth opening, but apparently the anus has disappeared for he does not figure or mention it. At the aboral end are at either side two spicules clearly vestiges of the skeletal rods of the arms. At the aboral apex, and on each of the two pairs of projecting "shoulders" are tufts of cilia. There can be no doubt that these shoulders represent the two main regions whence the arms arise in the fully developed species. But the most significant fact recorded in his account is the fact that the larva was so opaque ("undurchsichtig") that the internal organs were somewhat obscured. Now this opacity undoubtedly indicates the presence of yolk in the tissues, and here we have the first indication of the point in the series at which increasing yolk began to be of importance. The reduction in the alimentary canal is complementary to the presence of yolk (Figure 21,d).

A further step in this sequence brings us to such forms as Amphipholis squamata where the reduction has proceeded so far as to obliterate all traces of arm-roots and cilia, leaving only a vestigial pair of skeletal meshes, recognisable as vestiges of the arm rods by their position and inclination, but having lost the slender spicular form. The mouth opening has now disappeared, the alimentary canal being thus vestigial. The yolk has increased so as to make the larva quite opaque until artificially cleared (Fig. 21,e).

The endpoint in the series is represented by such forms as Kirk's ophiuroid in which the larval stage has disappeared entirely from development (Fig. 21,f).

For this process of shifting backwards of the time at which radial form is assumed I suggest the term "Recession of Metamorphosis".

A further indication that this has actually occurred is provided by the hydrocoel. As is well known, the time at which metamorphosis first commences is indicated by the behaviour of the hydrocoel. For this organ moves from its position on the left side of the gut and begins to encircle the oesophagus, its five lobes becoming the five radial canals of the adult. As recession of metamorphosis proceeds, the encirclement takes place relatively earlier in the life cycle, till in the directly developing form it appears right from the beginning as a canal encircling the future oesophagus, (Fell, 1941).

This process of earlier and earlier metamorphosis is in reality a species of neoteny - the larva becoming "adult", as it were, at successively earlier, and consequently undeveloped, stages. A general diagram

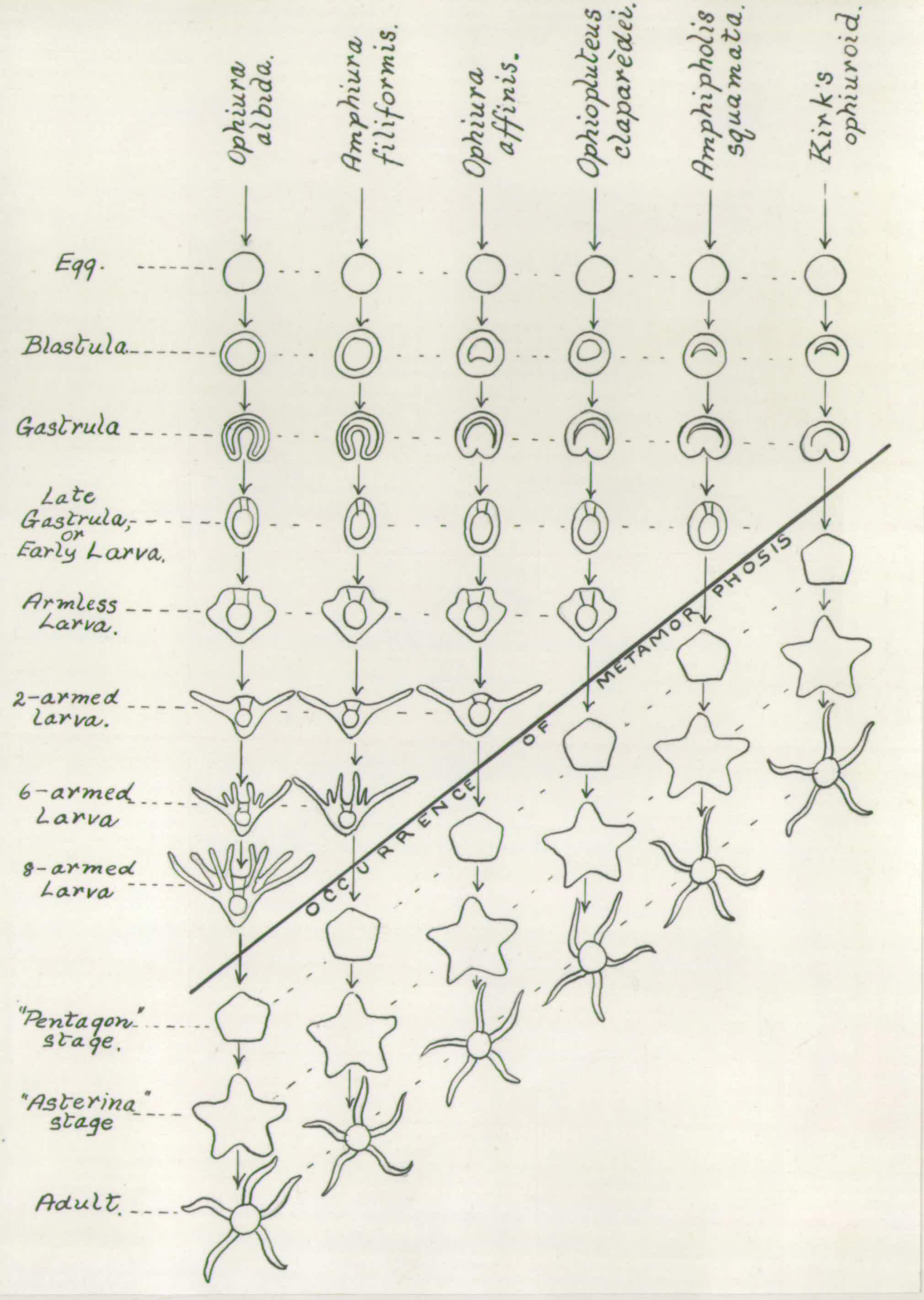


Figure 22 . Diagram illustrating recession of metamorphosis into successively earlier stages , with resultant shortening of development .

(Fig. 22) illustrates graphically the effect of recession of metamorphosis on ophiuroids.

It might be argued that the sequence of larval forms described above represents not a regression but a progression. This veiw would regard the fully developed Ophiopluteus as being a later evolutionary product derived from the simpler forms of larva, such as is found in *Amphipholis squamata*. The following reasons seem to make such a view untenable :-

(1) The presence of what is obviously the representative of the larval arm-skeleton in the armless larvae of *A. squamata* and *Ophiopluteus Clapardei* points strongly to a loss of the arms in these two species. It is very improbable that the skeletal organs of the arms would arise in evolution earlier than the arms themselves. Therefore the larval skeleton in these two species is to be regarded as vestigial, not rudimentary. lc/ e/

(2) The presence of a closed and non-functional alimentary canal in the larva of *Amphipholis squamata* is unlikely to be a primitive condition. An alimentary canal is required for digestion of food by non-yolky larvae, such as those of *Ophiothrix fragilis* where it becomes necessary to swim and obtain food at an early stage. The closed alimentary canal of *A. squamata* is more likely to be a vestige of a formerly functional organ which has become physiologically unnecessary as a result of the presence of yolk.

(3) The simplified larvae, and the species which lack larvae, develop from yolky eggs of large size. It is unlikely that large yolky eggs are more primitive

than small non-yolky ones.

7. The Vitellaria or "Yolk-Larva" Series

The bioseries we have just considered covers most of the ophiuroids. There remains, however, a peculiar divergent series which can be correlated with no larval series at all, but which nevertheless involves free-swimming larval forms with unmistakable characters in common. The amazing fact which emerges from a consideration of the literature is that this peculiar larval form is shared in common by no less than three of the echinoderm classes. Furthermore it is invariably associated with a yolk-mass in the egg. For this distinctive larval form I propose the term "Vitellaria" - or "yolk-larva"; it can only be considered as an independant sequence.

The general characters which distinguish this larval type are as follows ;- The body is simply organised, having no pairs of larval arms, or other projecting organs. It is cylindrical or barrel-shaped, and opaque owing to the presence of yolk-material in the tissues. It is free-swimming, and provided with rings of cilia. These bands are variable in number, but their general disposition is the same. There may be a larger or smaller tuft of cilia at the anterior end. They are commonly deeply pigmented. In figures 23 -26 four larvae of this type are shown together; their common pattern is obvious, though they are drawn from three different classes of echinoderms. Indeed, these larvae, though belonging to Holothurians, Ophiuroids and Crinoids, have far more in common than have many of

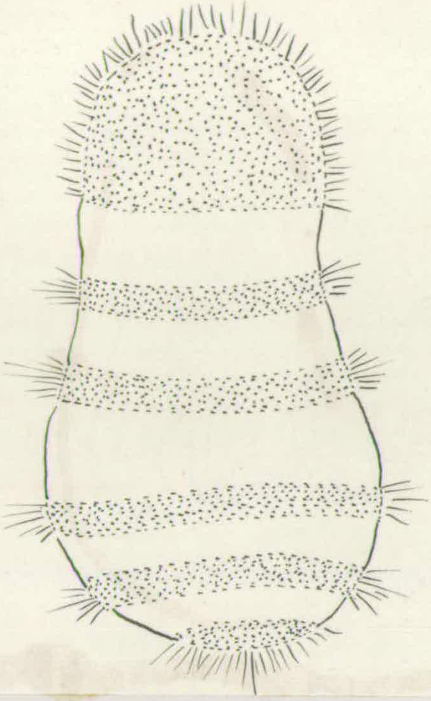


Fig. 23 .

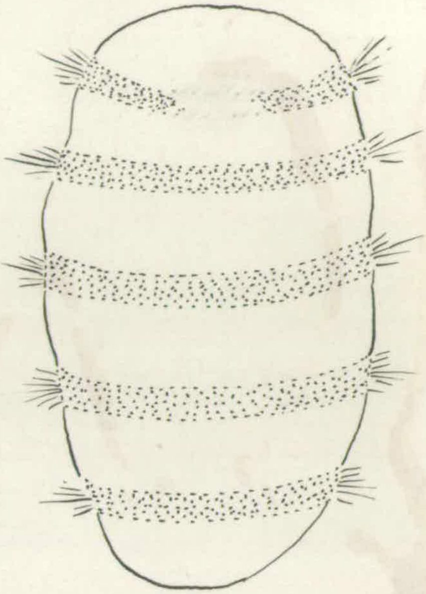


Fig. 24 .

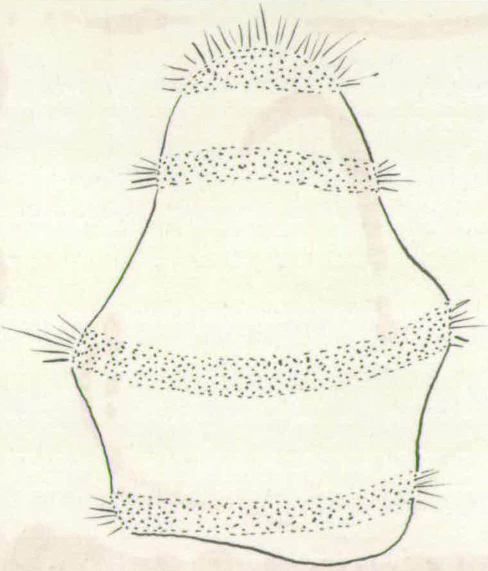


Fig. 25 .

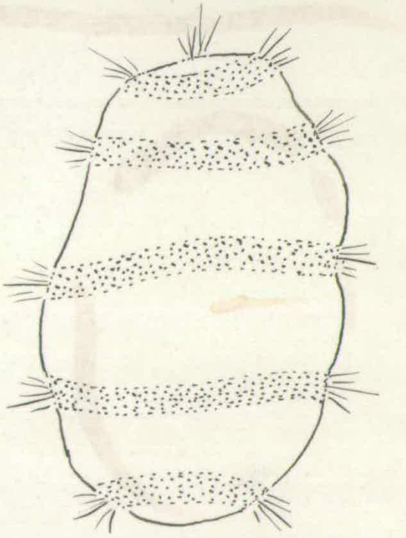


Fig. 26 .

Figures 23 - 26 . Examples of echinoderm 'Yolk-larvae'.
Figs. 23 and 24 , of Holothuroidea (Cucumaria and Labidoplax); Fig. 25, of Ophiuroidea (Ophiura brevispina); Fig. 26, of Crinoidea (Antedon bifida).
Fig. 23, after Selenka ; Fig. 24, after Dawydoff ; Fig. 25, after Grave; Fig. 26, after Seeliger .

the Plutei or Bipinnariae. The Bipinnaria of Asterias, for example, is widely different from the great Bipinnaria of Luidia.

Hitherto in the literature these various forms have been considered separately. The Vitellaria is found among the Crinoids, and from that group no other larva is as yet known. Of Ophiuroidea, species of Ophiura are known to posses the larva in both America and Europe. It is the same as the "Worm-like Larva" of Müller, a fact first pointed out by Grave. Among the Holothuroidea, some species posses only the Vitellaria ("Barrel-shaped larva" or "pupa"), while others possess an Auricularia which later becomes a Vitellaria.

In view of the diverse internal organogeny it is impossible to regard the Vitellaria as representing any ancestral type. It seems to be a generalised yolk form developed independently by the various classes under some special circumstances of which we have no information. The circumstances, whatever they may be, are undoubtedly connected with the yolk mass common to them all but why the larva should assume such a typical annulated form in each case is a problem. It is evidently an example of convergent evolution affecting the larval forms without changing the adult - that is, without leaving its impress upon phylogeny. The process by which the Vitellaria developed is therefore yet another example from the echinoderms of the mechanism termed by de Beer "Clandestine Evolution".

(8) Ontogeny of the Coelom

In parallel with the doctrine that gastrulation always takes place by invagination, there has grown a second doctrine equally dogmatic that the coelom of echinoderms is always formed enterocoelously from pouches nipped off from the archenteron. Russo (1891) again was the first to state that the perivisceral coelom arose in _Amphipholis squamata_ by a process of splitting in mesenchyme. It is instructive to note the way in which his results came to be ignored and forgotten. MacBride (1892) in his own paper on the later stages of development of the same species makes no mention of the earlier development. He does however, mention that "... the coelom in _Amphiura squamata_ is represented at first by a mass of mesenchyme; and as this condition of things is certainly not primitive, I do not think that even if reliable results as to the cavities originating before the coelom clears were obtainable, they would be of much phylogenetic importance". The reasoning here is very confused. Firstly, there is no proof that the mode of origin of the coelom in _Amphipholis_ is not primitive - for, as shown in my analysis at the beginning of this paper, there are as many directly developing echinoderms known to science as ones with indirect development. Secondly, MacBride expresses his opinion that any reliable results as to the development of the cavities originating _before the coelom clears_ (italics mine) they would not be of much phylogenetic importance. Here is a complete reversal in his attitude, for the only cavities which originate in the embryo before the coelom "clears" are the vestigial enterocoelous pouches! If then MacBride considers these vestigial enterocoels of little phylogenetic importance, why does he also regard the schizocoelous coelom as "certainly not primitive"? However, it is clear from MacBride's next paper (1907) that he once more reversed his position, for he now quotes Metschnikoff's account of the enterocoels in Amphipholis and uses this account to disprove Russo's later one. Still no actual reinvestigation had taken place of the development of Amphipholis to test the truth of Russo's account. Finally MacBride in his "Text-book of Embryology" (1914) rejects Russo's results as "improbable in the highest degree" - though still no reinvestigation of Russo's work had been done . Russo's conclusions in regard to the mode of origin of the coelom in Amphipholis squamata remained forgotten till a similar development was found for the organ in Kirk's ophiuroid (Fell, 1941). Such a mode of development was , of course, foreign to embryological theory as taught in the current text-books. On obtaining photostats of Russo's paper from the British Museum the full similarity between his account and the conditions found to occur in Kirk's ophiuroid became evident. As already described in the first part of this paper, Russo's account of the schizocoelous formation of the coelom in Amphipholis has now been confirmed as essentially correct. Having thus briefly referred to the belated recognition of the schizocoel in ophiuroid development, it is now convenient to compare the modes of development of the coelomic cavities in then various types of development (Figs.

27 - 29).

In Ophiothrix fragilis, representing the non-yolky type , MacBride's description records that both right and left enterocoels form. The left divides into anterior and posterior portions, and soon afterwards the right one does so also. From the posterior end of the left anterior coelom arises the hydrocoel. The wall of the left posterior coelom forms the arms, and its lumen the general coelom. The right coeloms become vestigial. MacBride also stated that the right hydrocoel rudiment sometimes developed the five-lobed pattern, like the left normally does, but Narasimhamurti has shown that this was based on a misinterpretation.

In Amphipholis squamata , representing the moderately yolky type with vestigial larva, right and left enterocoels form, but not from the enteron, arising instead from aggregations of mesenchyme tissue. The right enterocoel disappears, and the left gives rise to two small pouches, one of which disappears, and the other (the anterior one) becomes the hydrocoel. The definitive general coelom arises by a process of splitting in mesenchyme, similar to what occurs in vertebrate embryos.

In Kirk's ophiuroid, representing the heavily yolked type where there is no trace of a larva, both the hydrocoel, and the general coelom and its derivatives, arise by splitting in mesenchyme. The hydrocoel is the first to form, and the embryo is free-living for some days before the general (or perivisceral) coelom forms.

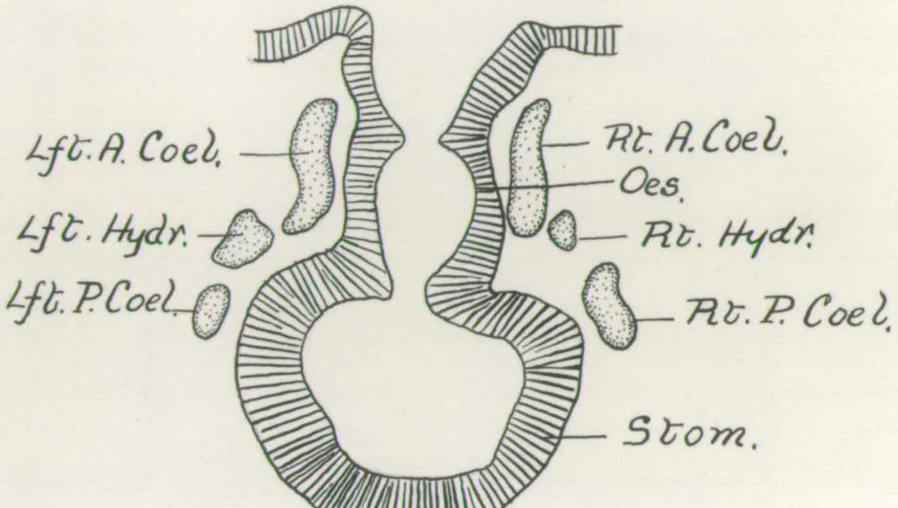


Fig. 27.

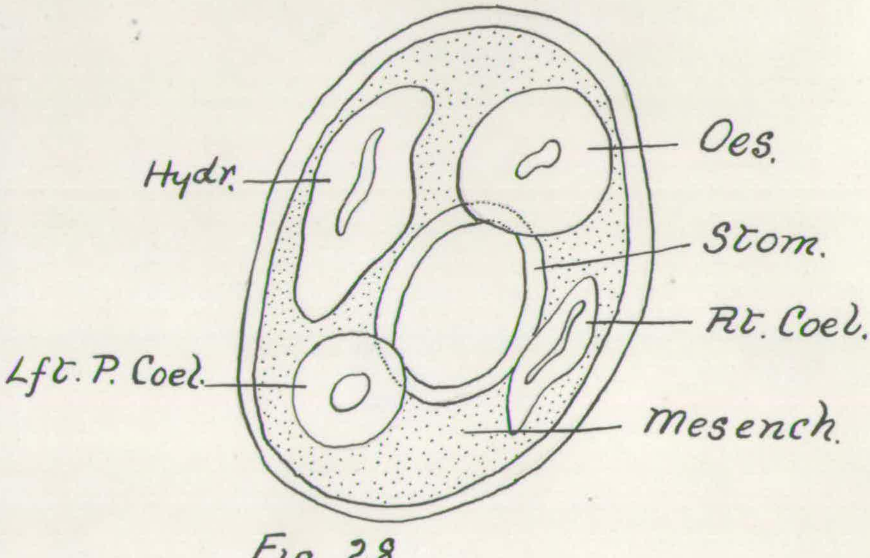


Fig. 28.

Figures 27 and 28 . The enterocoels in *Ophiothrix* and vesicles o *Amphipholis* . In *Ophiothrix* (27) all parts of the coelom arise as enterocoelous vesicles . In *Amphipholis* (28) the number of vesicles is reduced, and none of them is an enterocoel . Only the hydrocoel survives , the other pouches degenerating and contributing to the general mesenchyme, in which the remainder of the coelomic structures is later formed schizocoelously .

Oes., oesophagus; Stom., stomach; Mesench., mesenchyme; Lft., Rt., Hydr., left, and right hydrocoel; Lft., Rt., A.Coel., left, and right anterior coelomic vesicles; Lft., Rt., P.Coel., left, and right posterior coelomic vesicles .

In Ophiomyxa brevirima a similar condition to Kirk's ophiuroid is found, the excavation of the coelom taking place in an extensive mass of mesenchyme , after the formation of the hydrocoel (see Fell 1940a)

To sum up;- as yolk mass increases so does the tendency to form an enterocoel decrease, it being replaced by a schizocoel. The hydrocoel is the last part of the coelom to become a schizocoel . As noted in the previous part of this paper dealing with Amphipholis, the structures termed for convenience "enterocoels" - for they are clearly homologous with the enterocoels of Ophiothrix and Ophiocomina - are in fact formed by splitting within small masses of solid tissue formed from the mesenchyme derived from either side of the archenteron. Thus we have a series leading from enteric pouches with preformed internal cavity, through tissue masses which later acquire an internal cavity, to the forms where the whole definitive coelom arises by an extensive process of splitting in mesenchyme. In a previous paper (Fell, 1940a) reasons were given for believing that the enterocoelous method of development is itself secondary, being a shortened form of development ; in my view the development followed by these yolky forms is to be regarded as a reversion to the more primitive schizocoelous type of development, produced as a result of the retarding effect of the yolk and cytoplasm mass.

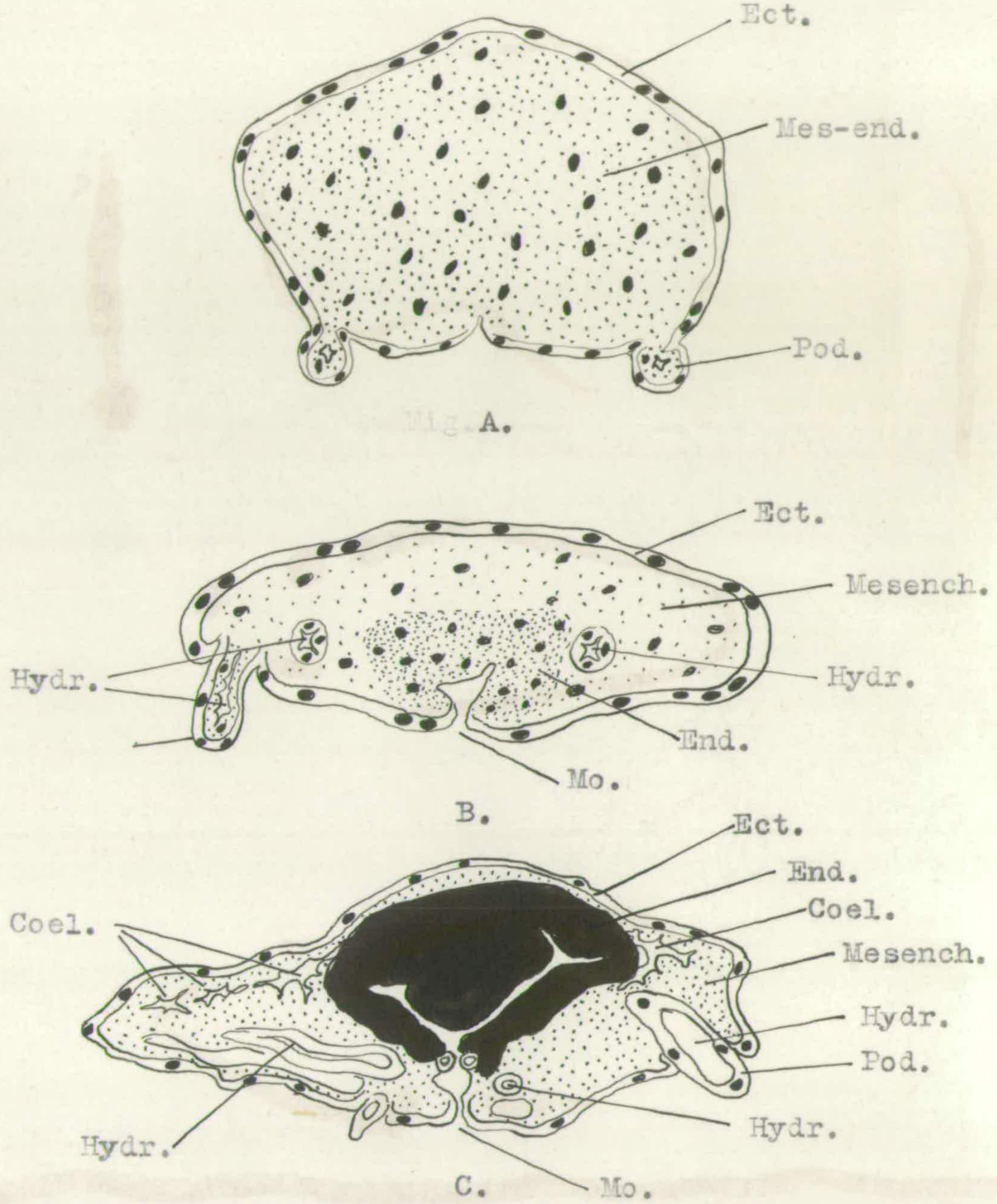


Figure 29. Schizocoelous origin of the coelom in a directly developing ophiuroid (Kirk's ophiuroid).

A, the "rosette" stage, a solid gastrula assuming radial symmetry, and with budding podia. B, later stage with the hydrocoel developing as a system of splits in the mesenchyme. C, general coelom developing by splitting in mesenchyme at a later stage than B. All represented in vertical section. Diagrammatised from Fell (1941).

Ect., ectoderm; Mes-end., mesendoderm; Pod., podium; End., endoderm; Mo., mouth; Hydr., hydrocoel; Coel., general coelom; Mesench., mesenchyme.

9. Later Stages in Development

With the formation of the coelom and its derivatives the principle phases of the early development are completed. The later stages in development are fundamentally similar in all species. In the yolky forms the yolk spherules gradually disappear from the peripheral tissues to become localised in the mesendoderm region, where they are finally absorbed .

Viviparity as a Factor Producing Direct Development

It is clear that viviparity must act as a factor producing direct development for the reason that it automatically effaces the pelagic larval stage from the ontogeny. It had been my hope that a study of the development of the viviparous species A. squamata would have provided some new evidence for the understanding of the effects of viviparity; unfortunately, however, the egg in this species is yolky, and most of the alterations in development are attributable to this factor. The only other viviparous ophiuroid of the development of which we possess much information is Ophiomyxa brevirima of New Zealand, on which species I hope to publish a paper at a later date. Here also the egg is extremely yolky. Indeed, from such information as is at present available, it would seem that a yolky egg is a frequently associated condition in viviparous ophiuroids.

One result of viviparity, however, is certain. This is, that the young embryo is enabled to pursue its development in viviparous forms to a much later stage before emerging to fend for itself. As shown in the first part of this paper, this condition is most probably attributable to the secretion nutritive substances from the wall of the bursa. The effect of viviparity in prolonging embryonic life to a later stage is well illustrated by the following data:- *Ophiocomina nigra* , a non-yolky form with a pelagic Ophiopluteus, has a functional alimentary canal by the third day, and continues to take in food for the remaining 35 days of larval life, before metamorphosis. The conditions are similar in *Ophiothrix fragilis* , where also the alimentary canal is completed by the third day, and a food gathering stage ensues, lasting about twenty-six days. Neither of these species is viviparous. In the case of Kirk's ophiuroid, also not viviparous, where the embryo is well provided with yolk, no food is taken in till the stage at which the arms have about six segments (i.e. when the young ophiuroid is about six weeks old). In *Amphipholis squamata* , on the other hand, which is a viviparous species, although the yolk content of the egg is much less than in Kirk's ophiuroid, no active food gathering stage commences till the stage at which the arms have about 12 segments (i.e. , when the young ophiuroid must be about six months old, if it grows at a similar rate to other ophiuroids) .

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Beyond this one conclusion, there is insufficient evidence available as yet to generalise on the effects of viviparity on the development of echinoderms. Whatever its influence, it is increasing yolk and cytoplasm mass which has brought about the major part of the modifications in development.

The Mechanism Which Has Brought About Direct Development

In the foregoing analysis of the phases of development of ophiuroids it has shown that the principle agent tending to produce direct development has been closely connected with, or actually is, an increased cytoplasm and yolk mass. One striking fact is the uniformity of the changes which have been produced in diverse groups of the Ophiuroidea, ranging from one of the most primitive species, such as <u>Ophiomyxa</u> <u>brevirima</u>, to the more specialised types. The modification of the gastrulation process, the suppression of the larva, and the increase in the amount of the mesenchyme in which the schizocoel comes to form, are some of the salient features of the more yolky-egged forms.

The truly remarkable parallels which are observable in the embryonic stages of these various unrelated ophiuroids which have severally been influenced by increasing yolk content of their eggs, suggests very strongly that there exists some common principle which has operated in a general way upon these diverse forms. Before suggesting a possible answer to the problem of what this principle may be, it will be convenient to summarise the main stages in the sequence of forms showing reduction and loss of the larval stage.

There is to be seen in this sequence a progressive reduction in the size and number of the larval srms, followed by reduction and loss of the arm skeleton. Then occurs reduction and loss of the coelomic pouches on either side with a corresponding increase in the mesenchyme mass in which the coelom comes alternatively to develop by splitting. Finally there is complete loss of bilateral symmetry, loss of the larval stage altogether, and both hydrocoel and general coelom develop schizocoelously in the mesenchyme, the latter tissue increasing in amount and becoming more important.

Now, in this progressive retreat inward of the bilaterally disposed elements of the larval body, with simultaneous recession of the radial symmetry into the earlier stages, we have a process suggesting alterations in the axial gradients of the embryo, these alterations being progressive along the series. Consideraing the modifications of development in this light, the following explanation may be put forward of the nature of the mechanism involved.

If we suppose that in the course of the evolutionary history of these larval forms changes began to take place in the metabolism along the axial gradients on either side of the body of the larva, the changes being in the nature of inhibitions, then a reduction and final loss of the organs on either side would occur - the larval arms, skeletal rods, coelomic pouches; in other words, the bilateral symmetry would become gradually submerged, and the point at which radial symmetry was assumed would undergo a recession towards the gastrula stage. This is just such a sequence of events as has been already suggested from comparative study of the phases of development. Thus, an inhibitory influence acting upon the earlier stages of development is sufficient to bring about entire loss of the larval stage.

Here, as in most scientific theories, a useful test is the experimental one. Is there any experimental evidence that such a process can actually take place? The answer, I believe, is already supplied by the work of Child (1916). In the course of experimental work upon the effect of chemicals upon plutei and earlier embryos of echinoids this worker was able to show that a wide range of chemical substances of a simple , inorganic type could cause an inhibitory effect very similar to what has been postulated above . He was able to produce sea-urchin plutei showing successive degrees of reduction and obliteration of the antero-posterior, medio-lateral, and apico-posterior differences through inhibitions of the axial metabolic gradients. In extreme cases his larvae resembled the peculiar reduced ophiuroid larvae already referred to in this paper. Child drew from his results the deduction that the larval forms of echinoderms may have been evolved by increases in the metabolism in the metabolic gradients, producing the outgrowth of the arms etc. Combining his results with the sequence of forms described in this paper we can similarly add that the larval forms of echinoderms may have been secondarily lost again through inhibitions in the same axial gradients. The inhibitory substance may have been developed in close association with the increase in the total mass of cytoplasmic yolk material, or it may be that the larger mass of yolk material itself provided the inhibitory influence.

A further parallel remains. Child found that the inhibitory effects were differential, acting to a variable extent on different tissues. Mesenchyme, he found, was comparatively unaffected, and consequently underwent a great increase in his reduced larvae, at the expense of the bilateral organs which became reduced. Now this also is closely similar to the effect noted in the transition from indirect to direct development. With the reduction and disappearance of the larva there has been a corresponding increase in the amount and importance of the mesenchyme.

Therefore, in the view of the writer, there is good reason to believe that direct development in the echinoderms has been produced by an influence upon the axial metabolic gradients of the larva. The inhibitory influence, which is associated with an increase in the amount of the cytoplasm, and of its contained yolk, has manifested itself through a recession of the metamorphosis towards the gastrula stage.

SUMMARY

Part One: The Embryology of Amphipholis squamata

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.

3. Only a single egg is brought to maturity normally, at any one time in each of the ten ovaries. The egg measures about 100μ 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 contained 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 <u>Ophiura</u> <u>brevispina</u> 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 in 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 of the embryo, 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 the 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 the other evidence from morphology is taken to indicate that the wall of the bursa secretes a nutritive substance.

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 unfavourably bright conditions. The newly-born embryo sinks to the bottom and begins an independent existence.

Part Two: Direct Development in Echinoderms

1. Direct development in the echinoderms is no less typical than development of the indirect, larval type. Of the five living classes of echinoderms, only the Echinoidea are characterised by being predominantly of the indirectly developing type.

2. Increase in volume of the ovum is directly related to increase of the cytoplasm and of its product, the yolk-mass. With increasing egg-size and amount of yolk, there has arisen a steadily increasing tendency to have direct development.

3. The increase of yolk has not greatly modified the process of cleavage , as segmentation in all forms is total. There is, however, a tendency to form micromeres and macromeres with increasing yolk-mass.

4. With increasing yolk-mass the wall of the blastula becomes successively thicker and the blastocoel becomes reduced to a vestige in the animal hemisphere. The mesenchyme fails to separate as such but projects in a solid mass into the blastocoel.

5. The effect on gastrulation of increasing yolk-mass has been firstly to reduce invagination to a solid inpushing of cells, and secondly to bring about a subsequent epibolic inwandering of micromeres to contribute to the mes-endoderm. The archenteron becomes vestigial and the definitive enteron is later excavated in the solid endoderm by a process of splitting.

6. A succession of stages in the reduction of the Ophiopluteus makes it probable that by a recession of the metamorphosis towards the gastrula stage, the larval period has been shortened , and finally lost altogether.

7. The recession of metamorphosis has resulted in the extreme case in radial symmetry being adopted immediately after the completion of gastrulation. This

occurs in Kirk's ophiuroid.

8. A collateral and independent evolution has been followed by certain echinoderms with yolky eggs, resulting in the formation by convergent evolution of a special yolk larva, termed in this paper the "Vitellaria". This has taken place in the Holothuroidea, Crinoidea and Ophiuroidea, independently in each case. The Vitellaria is characterised by its cylindrical form, opacity due to yolk material present in the tissues, the complete absence of larval appendages, and the development of transverse rings of cilia.

9. With increasing yolk-mass there has occurred a reduction and loss of the enterocoels, with a corresponding increase in the amount of the mesenchyme; in this latter tissue the coelomic cavities arise by schizocoelous, intercellular splitting . The hydrocoel is the last enterocoel to remain as such.

10. Viviparity appears to have been only a secondary factor in producing direct development. It has chiefly acted through prolonging embryonic development by enabling the embryo to obtain nourishment from the parent.

11. The suggestion is made that the mechanism of direct development has been an inhibitory influence upon the axial metabolic gradients of the larva. The inhibitory influence must be closely related to the presence of a yolk-mass, and it has manifested itself through the increase in the mesenchyme, and the recession of the metamorphosis towards the gastrula stage.

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