

On the Occurrence of Embryonic Fission in
Cyclostomatous Polyzoa.

By

Sidney F. Harmer, M.A., B.Sc.,

Fellow of King's College, Cambridge, and Superintendent of the University
Museum of Zoology.

With Plates XXII, XXIII & XXIV.

THE results of the present paper have formed the subject of a preliminary communication made to the Cambridge Philosophical Society (16). The case of embryonic fission which I have now to describe in greater detail appears to me, on the assumption that my explanation of the observed facts is the correct one, to be without parallel in the animal kingdom.

My observations refer entirely to the genus *Crisia*, and in particular to a form common at Plymouth, which I have described as a new species under the name *C. ramosa* (17). The general results may be stated as follows:—

(i) The ovicell, which is morphologically equivalent to a zoecium, develops at the growing-point in the same way as an ordinary zoecium.

(ii) A polypide-bud is found in the young ovicell, consisting of tentacle-sheath and a part which represents the alimentary canal of a polypide.

(iii) Small egg-cells are present in various parts of some of the growing-points. One of these acquires a close relation to the potential alimentary canal of the ovicell-polypide.

(iv) This potential alimentary canal grows round the ovum, losing its previous form, and becoming a compact multi-

nucleated follicle surrounding the egg, which at first lies in an excentric cavity in the follicle.

(v) The ovum segments¹ and the blastomeres may, in early stages, be completely separated from one another. The relations of the segmenting egg to its follicle are similar to those described by Salensky (28) in *Salpa* (cf. Salensky's figs. 12, 13, on pl. x).

(vi) The ovicell is meanwhile maturing, and by the end of the segmentation of the ovum has been shifted to some distance from the growing-point by the superposition of new zoœcia above it. Its non-calcified aperture, which, at an earlier stage, formed the wide end of a large funnel, has become constricted, and has grown out into a long tubular orifice.

(vii) At the end of segmentation, the embryo consists of a small mass of undifferentiated cells, lying near the distal end of the follicle, which has increased largely in size, and now forms a spherical knob projecting freely into the interior of a spacious tentacle-sheath. A complicated arrangement of cells connected with the aperture has meanwhile been formed.

(viii) The follicle becomes vacuolated, and is soon transformed into a nucleated protoplasmic reticulum. The tentacle-sheath loses its distinctness.

(ix) The number of blastomeres increases, cell-limits being indistinguishable at this, as at all other stages, excepting the very earliest.

(x) The embryo, having thus considerably increased in size, although remaining a solid mass, without differentiation of organs, grows out into several finger-shaped processes, which are generally directed towards the distal end of the ovicell.

(xi) The finger-shaped processes are divided up by a series of transverse constrictions into rounded masses of cells, each of which becomes a complete larva.

(xii) This process of embryo-formation continues during the whole functional period of the life of the ovicell, and is still actively proceeding at a stage when many of the embryos are mature, or nearly mature. The number of (secondary) embryos

¹ The occurrence of a process of fertilisation was not made out.

present in an ovicell at any one time may exceed one hundred, and these have all been produced by budding from the above-described "primary embryo."

(xiii) Each of the "secondary embryos" acquires its well-known two-layered condition at the time of its separation from the budding mass of embryonic cells. It develops in a vacuole of the protoplasmic reticulum, which presumably supplies it with nutriment since the embryo rapidly increases in size, becoming ciliated externally, and ultimately escaping through the tubular aperture of the ovicell as a characteristic Cyclostome larva.

Taking the above history into consideration, it is not surprising that, as is actually the case, the Cyclostome larva differs considerably in structure from that of other marine Polyzoa. This history also explains the fact that no observer has ever succeeded in giving an account of any process corresponding to egg-cleavage in Cyclostomata.

The protoplasmic mass surrounding the embryos has been figured by Smitt (34),¹ who has alluded to the yellow colour so characteristic of the contents of the ovicell. This colour is contained principally in the protoplasmic reticulum, although the embryos themselves have a yellowish colour.

The first satisfactory account of the Cyclostome larva was, however, given by Barrois (1), who calls special attention to the fact that no previous observer had been able to discover "genital products" in any Cyclostome, and adds, "Je n'ai pour ma part encore réussi qu'à suivre les morulas jusqu'à des stades composés d'un nombre d'éléments de moins en moins nombreux et plus volumineux, sans réussir encore à constater d'une manière bien certaine la présence de l'œuf;" although supposing that the Cyclostomes do not really differ from other Polyzoa in this respect.² Barrois' failure to understand the early development of the embryos is readily explained if my own account be correct; and it is not surprising, con-

¹ See his pl. iv, fig. 2.

² L. c., pp. 58, 59, note.

sidering the great difficulty of making out anything of the nature of the early stages except by means of sections.

Barrois expressly states that the earliest stage to which he succeeded in tracing his morulas with certainty is that represented in his pl. iii, fig. 3. This stage exactly corresponds with the condition at which I have found the embryos to be constricted off from the budding primary embryo (cf. Pl. XXIII, fig. 11). Barrois was, however, once successful in finding a cell, the egg nature of which he considers uncertain (his pl. iii, fig. 1); and in another case in finding what may have been an egg divided into two blastomeres. It is not easy to say whether the former cell was really an egg, or whether it was merely one of the giant-cells described below.

The rest of Barrois' account contains an erroneous history of the later stages, which he himself was the first to correct (2). I am compelled to doubt altogether Barrois' account here given (not accompanied by any figures) of the supposed occurrence of a process of segmentation of the egg, accompanied by the formation of an epibolic gastrula. In a later paper (3) Barrois figures quite accurately the "morula" at the stage at which it becomes independent (his pl. i, fig. 26), although he wrongly supposes that the inner layer of cells disappears in the later stages (his pl. iii, figs. 29, 30).

Although Metschnikoff (23, pl. xx, figs. 61—64) gives admirable figures of the early embryos of *Discoporella radiata*, the earliest stage observed by that author is the stage at which the "secondary" embryo becomes free from the budding mass of embryonic cells. Ostromnoff (25) is no more fortunate in elucidating the early history of the embryo of Cyclostomata.

I. Development of the Ovicell.

This process, which takes place fundamentally in the same manner in all the species of *Crisia* which I have examined, has been to some extent described by Smitt (34), although most writers have paid little attention to the difference

between the form of the adult ovicell and that of the younger stages of the same organ.

The ovicell is developed at the growing point, and it is here that the early stages in the development of the egg take place. A young internode¹ may be described as an acute-angled isosceles triangle with two sub-equal sides (*AB*, *AC*). Within the triangle a calcareous septum occurs parallel to *AB*, cutting off the oldest zoœcium of the internode from the others. The next septum is parallel to *AC*, and is nearer to the base of the triangle. The formation of septa, alternately parallel to *AB* and *AC*, gives rise to a series of alternate zoœcia, an arrangement characteristic of *Crisia*. The oldest zoœcia are, of course, those nearest to the apex of the triangle, and the central part of the base is the region from which, with continued growth, fresh zoœcia are cut off. It remains to be stated that the growing-point, like the adult internode, is flattened, and that the openings of the zoœcia are lateral, and are directed towards one of the flat surfaces of the branch.

As the internode elongates, its proximal zoœcia acquire their full length, and cease to take part in the formation of the growing-point. Or, explaining this by the former illustration, let the internode grow to twice its former length, the growing point remaining of the same width throughout its growth. By producing the lines of the septa already present it will be seen that if the growing point does not grow wider the older zoœcia will be excluded from it, their growth being completed. It follows that the zoœcia, several of which occur in a young state at the end of the branch, become successively shifted to the edges of the growing-point, preparatory to leaving it altogether.

The growth of the zoœcia and of the ovicells takes place by the apposition of fresh material at the distal end. The proximal end of each unit of the colony is first laid down, and the last-formed portion is the aperture. Thus, by drawing a line transversely at any level across an internode, whether the internode bears an ovicell or not, we obtain an accurate idea of

¹ Compare pl. XXIV, fig. 15.

the condition of the branch when the growing-point was at the level of that line. It follows from the shape of the ovicell, that an ovicell which is half grown will have the form of a wide-mouthed funnel, as shown in the figures of Smitt and others. But although it is easy to recognise a young ovicell at this stage, it is anything but an easy matter to distinguish the ovicell while it is still a sub-median member of the growing-point.

The ovicell is indeed merely a modified zoœcium, as is shown by the method of its development, as well as by its internal structure. Further evidence for this statement is afforded by the occasional occurrence of abnormal units of the colony, intermediate in form between the zoœcia and ovicells (17, pl. xii, fig. 12).

In Pl. XXIV, fig. 19, the proximal portion of the ovicell is already developed. The first, second, and third units of the internode are zoœcia, the fourth being an immature ovicell. The growing-point is formed, on the right side, by the base of a lateral branch, which would have been borne by the fifth member of the internode. There follow, in order from right to left, the fifth unit, the actual growing-point capable of producing fresh zoœcia, the sixth unit, and the ovicell. The last occurs at the left side of the growing-point; but while its proximal end is in the same plane with the zoœcia of the internode, the open end of the funnel is already projecting forwards (i. e. in the direction of that surface of the internode on which the zoœcia open). This condition becomes more prominent at a later stage, so that the ovicell, in its most swollen portion, projects considerably beyond the level of the general surface of the internode.

By referring to pl. xii, fig. 11, of my former paper (17) it will be seen that the zoœcium "5" (in fig. 19) would have formed its aperture at the level of the middle of the ovicell, while "6" would have completed its growth at a very short distance above it.

The young ovicell has, at first sight, the appearance of an open funnel. This is not really its condition, since its end is

closed by a chitinous uncalcified membrane (ectocyst). This is the condition of the ovicell, and of the growing-points generally, at all stages before their growth is completed and the definitive apertures are formed. The funnel, which is, in fig. 19, the most conspicuous part of the ovicell, is consequently merely that part of the ovicell in which calcification has occurred.

In fig. 20 (in which the arrangement of the lateral buds does not correspond with that in fig. 19) the zoœcia have been numbered in such a way as to facilitate comparison with fig. 19. The zoœcium "5" is already complete, while "6" is beginning to free itself from the growing-point. The growth of the ovicell has progressed, the most swollen part is already completed, and the aperture (still closed by a membrane of uncalcified ectocyst) is beginning to constrict.

In fig. 21 the zoœcia "6" and "7" are complete. The aperture of the ovicell is still further constricted, and now consists of a slit-like portion which will soon close completely, and of a wider portion which will become the base of the tubular aperture so characteristic of this species (*C. ramosa*). A comparison of figs. 19—21 with one another will show that the method of the growth of the ovicell has been such as to bring its distal portion on to the front of the branch, while its proximal portion is lateral, and in series with the zoœcia. The base of the tubular aperture thus comes to be situated at about the middle line of the internode.

The valve of the ovicell (17, pl. xii, fig. 10) is formed as a ridge from the back of the ovicell at a stage between figs. 20 and 21. The growth of the ovicell will be completed by the outgrowth of the tubular aperture.

So far as I have been able to make out, the aperture is closed by the uncalcified membrane of ectocyst at all stages of its development, and does not become actually perforated until the escape of the first larva. I am quite unable to say when and how the process of fertilisation is effected.

II. The Male Sexual Elements.

There can, however, be no doubt of the existence of spermatozoa in Cyclostomes, although I am not aware that they have previously been described.

In *Crisia* I have usually found them in colonies without ovicells¹ (17, p. 145), although they occur in ovicell-bearing colonies in *Idmonea serpens*.

The spermatoblasts occur in masses filling up a large portion of the body-cavity of sexual individuals. The sperm mother-cells in both *Idmonea* and *Crisia* seem to occur in groups of four (Pl. XXII, fig. 4); and the four flagella when first developed appear, under insufficient magnification, as if they belonged to one cell. The mature spermatozoon (fig. 4) possesses an elongated head (measuring about .0064 mm.), and a long, active flagellum.

In *C. cornuta* it was noticed that a delicate, hyaline layer of endocyst protruded from the aperture of the zoecium, during the escape of the spermatozoa, in the form of a cone at the apex of which the spermatozoa escaped.

III. The Origin of the Secondary Embryos.

My observations on this part of the subject have been made almost entirely by means of sections. The ovicells were preserved and decalcified, at one operation, by placing in a mixture of corrosive sublimate, nitric and acetic acids. The most successful staining was obtained with Grenacher's hæmatoxylin or with borax-carmine, in the latter case washing with alcohol containing picric acid.

The free larva of *C. eburnea* is well figured by Barrois (1, pl. iii, fig. 22). It is, roughly speaking, cylindrical in shape, being covered externally by a complete coating of cilia. At one end of the cylinder is an aperture leading into the "sucker," by means of which fixation is effected; and, at the opposite end, is another aperture leading into the so-called

¹ In one case, spermatozoa were found in a colony of *C. cornuta*, which bore a single very young ovicell.

"mantle-cavity." I have observed no trace of a "pyriform organ." Barrois (2, p. 142; 3, p. 48, pl. iii, fig. 31) states, however, that he has discovered a rudiment of this structure in the larvae of *Discopora*.

The internal structure of a young larva may be illustrated by means of fig. 23, a median longitudinal section. The sucker is already well developed, having been formed, as in most other Ectoproct larvæ, by a process of ectodermic invagination. Cilia have appeared on the greater part of the external surface, the non-ciliated part of the ectoderm representing the portion which will be later invaginated to form the mantle cavity. The inner layer of cells is still perfectly distinguishable, forming a thin layer, closely applied to the ectoderm, and enclosing a cavity which occupies the whole of the interior of the embryo.

The earlier stages, which alone concern us at present, may be realised by assuming that the volume of the embryo shown in fig. 23 has become largely reduced; and that the sucker has become flattened out. Slightly anterior to the stage of fig. 22, the sucker is much shallower, and opens by a wide aperture in the middle of the "oral" surface. Still earlier, the sucker is a very slight depression of the thickened "oral" ectoderm. The inner layer is at this stage a layer of great tenuity, in which a nucleus is thick enough to form a swelling wherever it occurs. Before this, the embryo is plano-convex, the position of the future sucker being represented by its flat side; and, still earlier, it is rounded in section, the inner layer consisting of a few cells, completely surrounding a central cavity. Between this stage and that shown in fig. 22, the inner layer may be separated, in parts or completely, from the ectoderm; so that it would be impossible to overlook its presence in any well-preserved section.

At the earliest stage at which the embryo is free in the ovi-cell, it consists of a small rounded mass (Pl. XXIII, fig. 11) The outer layer is in the form of a continuous mass of protoplasm, enclosing one layer of nuclei. The inner layer also consists of continuous protoplasm, with a very small number

of nuclei arranged in one row; and it encloses a minute central cavity.

Pl. XXIV, fig. 17, represents a median section, slightly magnified, of an ovicell of *Crisia ramosa*. The ovicell contained in all about 115 embryos, which were embedded in a loose protoplasmic reticulum, filling up most of the cavity of the ovicell. In the older embryos, the conspicuous sucker or "internal sac" is clearly seen; and in some of them, a slit-like space which is the mantle-cavity. The aperture of the latter to the exterior is not shown in any of these embryos. To the left of the ovicell is the structure from which all the embryos have been produced. This structure is labelled "primary embryo," the evidence that this name implies its real nature being given in the sequel. The primary embryo is produced into several processes; and indications are seen, in at least one case, that the end of the process is being constricted off, as a rounded mass of cells, which is equal in size to the smallest of the embryos found free in the protoplasmic reticulum.

Fig. 11 (Pl. XXIII) is a longitudinal section of a young ovicell, at the period when the formation of "secondary" embryos (i. e. embryos which are developed by budding from the "primary" embryo) has just commenced. The protoplasmic reticulum includes one or two free embryos, the structure of which has already been described. The most conspicuous structure in the section is, however, the large primary embryo, which consists of a dense mass of granular protoplasm containing numerous nuclei, and having an extremely embryonic appearance. This structure is in a state of active growth, as is shown by the occurrence of nuclei with karyokinetic figures. The proximal end of the primary embryo is compact and rounded, and contains centrally a group of nuclei which are distinguished by the activity with which they are undergoing division. The opposite end of the primary embryo is produced into several irregular processes, which show constrictions at intervals. From the ends of two of these processes, embryos have just been constricted off, and

are seen disconnected from the primary embryo. The constrictions indicate the limits of as many future embryos.

The "primary embryo" contains, distally, an irregular cavity. It is difficult to be sure of the exact arrangement of the embryogenic processes; but in some cases at least it is evident that the distal end of the primary embryo has the form of an irregular cup, the processes forming the wall of the cup, from which they become free at their ends. The irregular cavity seen in fig. 11 is part of the cavity of the cup.

Towards the ends of the processes an ectodermic layer becomes clearly differentiated; while, in the centre of each of the swellings indicating a future embryo, a small group of inner-layer cells can, in some cases, be clearly distinguished. The ectoderm of the processes is continuous with the outer nucleated layer of the primary embryo, and with the similar layer immediately lining its distally-placed cavity. The inner-layer cells of the secondary embryos are continuous with the inner nuclei of the more solid, proximal half of the primary embryo. But these nuclei and the protoplasm surrounding them are not throughout clearly differentiated from the outer layer of nuclei. I am, however, inclined to suppose that the somewhat triangular, clear mass of protoplasm at the proximal end of the primary embryo, containing actively dividing nuclei, is the region which gives rise to the inner-layer cells. This region can generally be distinguished with ease in ovicells at this stage.

The primary embryo consists of a mass of embryonic cells (or, rather, nuclei embedded in continuous protoplasm) which are obscurely differentiated into outer and inner cells (or nuclei). The whole function of this embryo is to act as an embryogenic organ, or producer of secondary embryos, and it possesses no structures which can be described as its own organs.

At its proximal end, the primary embryo is budding off nuclei which migrate into the protoplasmic reticulum, where they become indistinguishable from the rest of the nuclei of that reticulum. I have been unable to make out the

significance of this phenomenon, which I have frequently observed.

I am in a position to multiply indefinitely figures showing the important fact that the young larvæ are really produced as buds from a "primary embryo." I consider that I have the clearest possible evidence of the following statements:

- i. The larvæ are produced as buds from an embryonic mass of cells found in the young ovicell.
- ii. They are produced in no other way than that mentioned under i.

The embryogenic organ is invariably present in all ovicells in which young embryos are found, and in most of the older embryo-containing ovicells as well. It is still active, even at the stage shown in fig. 17. The youngest embryos, free in the reticulum, are invariably identical in structure with the ends of the processes of the primary embryo, and there is not the slightest trace in any of the ovicells, young or old, of the development of larvæ by the ordinary process of the segmentation of an egg.

It might, indeed, be supposed that the bi-nucleated cell shown in the upper part of the reticulum in fig. 11 had the nature of a dividing egg. This supposition is not confirmed by an examination of the actual facts. While the evidence in favour of the origin of the larvæ by a process of budding is unmistakably clear, there are no transitions between such cells as the large one shown in fig. 11 and the young two-layered larvæ. These large cells, which are normally present in the ovicells, are probably of the nature of "giant-cells," similar to those which are found in developing bone. This subject will be considered later; but it may be pointed out that it is possible that the supposed egg-cell figured by Barrois (1, pl. iii, fig. 1) may have been one of these giant-cells.

IV. The Development of the Primary Embryo.

Fig. 15 (Pl. XXIV) is a decalcified internode of *C. eburnea*, possessing a very young ovicell. The internode consists of one complete zoœcium, which bears the beginning of a lateral

branch; of a second zoœcium, which is very nearly mature; of the ovicell as the third member of the internode; and of the real growing-point, which contains a young polypide-bud; although the corresponding zoœcium is not yet separated off from the growing-point by a septum.

The ovicell contains a structure which is the exact equivalent of an ordinary polypide-bud. This consists of (1) a thick (proximal) mass of cells, which in a zoœcium would give rise to alimentary canal and tentacles; (2) a thin-walled portion, next to the above, and corresponding to the tentacle-sheath; and (3) a distal portion, indicated by two parallel lines in the sketch, and which is really an invagination of the distal body-wall of the ovicell. This is formed in a precisely similar manner in any young zoœcium, where it develops into the aperture.

Fig. 1 (Pl. XXII) is a nearly median longitudinal section of an ovicell at nearly the same stage as fig. 15. The body-cavity is, as in ordinary zoœcia, largely filled up by funicular tissue, but contains an obvious polypide-bud, the distal portion of which can be clearly distinguished as a tentacle-sheath, similar in all respects to the same structure in an ordinary polypide-bud. The one fact, indeed, which enables this member of the colony to be distinguished as an ovicell is the presence of a relatively large cell, which is closely applied to one wall of the polypide-bud. The latter shows some tendency to give off cells which are growing round the large cell. This has a diameter of about .0176 mm., and it has a large clear nucleus with one or two nucleoli. Its structure, in fact, reminds one irresistibly of that of an egg; and I believe this cell to be the source from which all the larvæ produced in the ovicell are developed.

"Eggs" of this kind are found in various positions in some of the growing-points. Thus in the particular individual in question there is a second, smaller egg¹ in the same ovicell; and in the next zoœcium there are two eggs¹, one of which is at the apex of the polypide-bud. The fact that these eggs are commonly found in the growing-points leads me to suppose

¹ Not visible in the particular section figured.

that several are produced in each fertile internode, apparently by a modification of cells of the funicular tissue, and that their further development depends on their entering into definite relation with a polypide-bud. If this association is brought about, it may be assumed that what might at first have developed into a zoœcium becomes an ovicell. In abnormal cases, where several polypide-buds enter into relation with ova, two or more ovicells may be produced in the same internode (17, p. 166; pl. xii, fig. 18). It may further be supposed that the failure to bring about the association between the egg and the polypide-bud results in such abnormalities as that shown in fig. 12 of my former paper, and that this or some other cause, such as the failure to get fertilised, results in the development of the empty ovicells which are so frequently observed.

On decalcifying a number of ovicells, it is soon noticed that many ovicells are either completely empty or are abnormally developed. An ovicell, with complete tubular aperture, may be absolutely devoid of any trace of primary or secondary embryos. In some cases, these empty ovicells are probably the result of degeneration which has set in after the completion of the process of development of free larvæ. After the escape of the last larvæ, the remaining tissues of the ovicells degenerate, and are gradually absorbed. Many of my sections bear out this assertion.

In other cases, however, the degeneration takes place in ovicells which have produced no larvæ. Empty ovicells which are near the growing-points are, probably, generally of this character. It is easy to obtain evidence of the fact that, in such cases, degeneration may set in at various periods—sometimes after the egg has developed to a considerable extent. In some cases, this may be the result of the absence of fertilisation—a process of which I have vainly endeavoured to prove the existence. That fertilisation does actually occur at some period can hardly be doubted, considering the fact that normal spermatozoa are developed in some colonies. In other cases, the degeneration is probably due to the atrophy of the poly-

pides in the zoœcia contiguous to the ovicell. It is well known that the thick calcareous ectocyst of the Cyclostomata is perforated by pores. On decalcifying a colony, and staining what is left, it can be easily shown that all the zoœcia are in organic connection by means of the funicular tissue, which passes through the pores from one zoœcium to another, and from the zoœcia to the ovicell. It can hardly be doubted that the nutriment at the expense of which the larvae develop is provided by means of the protoplasmic network which thus connects all the individuals of a colony. The ovum is extremely minute, although it gives rise to a massive primary embryo; and this to numerous free larvæ, each of which is very many times larger than the original ovum. This rapid growth—to say nothing of the development of an extensive reticulum of funicular tissue in the ovicell itself—can only depend on the existence of pores by which the ovicell is connected with zoœcia which possess functional polypides.

In fig. 2 the ovum is completely surrounded by the polypide-bud, whose tentacle-sheath has considerably increased in size; while in fig. 3 further alterations of importance have taken place. The tentacle-sheath has grown very much larger; but, so far as this structure and the invagination which forms the aperture are concerned, the ovicell still resembles an ordinary zoœcium. The proximal part of the polypide-bud, which in the younger ovicell was practically indistinguishable from the corresponding structure in an ordinary ovicell, has now become much modified. The egg is now completely surrounded by it; and the polypide-bud has in fact transformed itself into a round mass of cells which may be termed the "follicle." The ovum lies partly surrounded by a cavity in this follicle.

The fact that the distal endocyst is not in contact with the ectocyst is probably due to shrinkage brought about during decalcification. The side-walls of the zoœcium are of course calcified (*cf.* fig. 19), while the distal ectocyst forms an uncalcified membrane stretching across the mouth of the funnel formed by the ovicell.

It is presumably at this stage that fertilisation takes place; but I have in vain looked for any evidence of perforation in the terminal membrane of the ovicell, or for traces of spermatozoa inside the tentacle-sheath. This fact is not really surprising when it is remembered that the finer details of the highly calcified ovicell of *Crisia* can hardly be examined except by means of sections; and that the spermatozoa are very minute.

In fig. 5 the whole ovicell has considerably increased in length. Its irregular form is of course due to shrinkage caused by the action of reagents. The ectocyst is not represented in the figure. The ovicell was probably at about the stage represented in fig. 21.

The valve (*cf.* pl. xii, fig. 10, of my former paper) is now developed as a fold of the ectoderm on the back wall of the ovicell. The aperture has no longer any obvious opening to the exterior; and the tentacle-sheath has increased in size, its walls having become very thin, except at its distal end, which is considerably thickened.

The follicle is slightly larger than before, and its nuclei have obviously increased in number. In place of the egg found in the preceding stage, there are now three egg-like cells, which are not in contact with one another; and which I regard as blastomeres. Remains of the follicle-cavity are still present.

Although I have no direct evidence that the "blastomeres" are really derived from the egg, their subsequent history leaves room for little doubt on this point. The details of the formation of the primary embryo in *Crisia* remind one strangely of the early development of *Salpa*, as described by Salensky (28). This is true not merely of the segmentation of the ovum, but also of the later relations of the embryo to its follicle. Salensky states, for instance, that the blastomeres of *Salpa* may at first be entirely disconnected from one another (*l. c.* pl. x, fig. 10; pl. xxii, figs. 3, 4).

In the next stages, of which I have numerous preparations, but which I have not figured, the number of blastomeres gradually increases. I have been unable to make out any regu-

larity in the succession of the blastomeres, which are, in fact, inextricably entangled among the follicle-cells. They are not necessarily in contact with one another, but may be separated from one another by ingrowths of the follicle-cells, so that in most preparations it is almost impossible to count the number of the blastomeres, or to distinguish all of them from the follicle-cells. An excellent idea of the general relation of the blastomeres to the follicle-cells (or nuclei) may be obtained by referring to some of Salensky's figures of *Salpa*, as his pl. x, figs. 12, 13. The only difference that I can point out between *Crisia* and *Salpa*, as regards the relation of the blastomeres to the follicle-cells, is that in *Crisia* the follicle is somewhat larger relatively than in *Salpa*, and that the blastomeres occupy only the central region of the follicle instead of filling up most of that structure, as in *Salpa*. Remains of the follicle-cavity may still be detected in some of these stages.

In fig. 6 (which is connected with fig. 5 by numerous preparations, forming a perfectly continuous series, in my possession) the tentacle-sheath has increased in size so much as to fill up nearly the whole of the ovicell. The follicle has largely increased in size, and now forms a sub-spherical knob, projecting freely into the cavity of the tentacle-sheath. This stage is a perfectly constant and easily recognised one.

The scattered blastomeres have at last come together to form a small but compact embryo, in some of the nuclei of which karyokinetic figures are discernible. There is no trace of the differentiation of germ-layers in the embryo, which consists simply of a small rounded mass of undifferentiated embryonic cells, or rather of a continuous mass of protoplasm, containing nuclei scattered through it without any attempt to arrange themselves in definite layers.

At the distal end of the embryo is a clear part of the follicle which contains small nuclei. This is apparently a constant feature of the stages near this one; but I have not been able to make out its significance.

Fig. 7 is not cut quite medianly, so that it does not show that the attachment of the follicle to the tentacle-sheath is

much the same as in the former figure. The tentacle-sheath is, however, now becoming less definite. To the left of the figure it is hardly distinguishable from the follicle, with which it probably fuses.

The embryo is practically unaltered, except that it has come to the surface of the follicle; but the principal difference between this and the earlier stage concerns the follicle itself. This structure no longer forms a compact mass of granular, nucleated protoplasm, as in fig. 6; but it has become distinctly vacuolated. This vacuolation is the beginning of the process by which the follicle of earlier stages is transformed into the protoplasmic reticulum of later stages.

Fig. 9 (Pl. XXIII) well illustrates the manner in which this transformation is effected. The tentacle-sheath is not so clear as in the former stage, and can, indeed, hardly be distinguished except at its upper end. Whilst in earlier stages it filled up most of the ovicell, it has now collapsed to a large extent.

The proximal portion of the follicle is in this ovicell still solid, and is perfectly similar in structure to the solid follicle of fig. 6. Distally the follicle is almost unrecognisable, having become separated by enormous vacuoles into strands of anastomosing, nucleated protoplasm. These strands are, however, most unmistakably continuous with the proximal, solid portion of the follicle. The embryo is practically unaltered, still forming a small rounded mass of undifferentiated embryonic tissue lying in a part of the reticulum.

The great increase in the size of the follicle and in the number of its nuclei up to the stage shown in fig. 11 is probably connected with the development of a nutritive arrangement for the young larvæ. The minute egg-cell of fig. 1 gives rise, as I believe, to the embryogenic organ of fig. 11, and this to the numerous young larvæ with which the mature ovicell is crowded. These larvæ lie in the meshes of the protoplasmic reticulum, from which they are probably supplied with nutritive material.

In figs. 6 and 9 the base of the ovicell has a very characteristic structure, always noticed in young ovicells at certain stages.

Next to the ectocyst comes a very definite nucleated layer, which encloses a network of cells separated by smallish vacuoles.

In later stages the outer definite layer of nuclei disappears, and the network becomes continuous with the reticulum formed by the modification of the follicle (fig. 11). The basal network of cells is obviously part of the ordinary funicular tissue, which, as has already been pointed out, forms a continuous connection from zoœcium to zoœcium, or from zoœcium to ovicell, through the pores in the calcareous septa between neighbouring individuals. It can hardly be doubted that the rich protoplasmic reticulum in which the young larvæ lie is the means by which nutriment is conveyed to the developing larvæ.

Fig. 10 is a stage of which I obtained only one example, and I cannot be sure that what is there represented is really a normal process. This preparation indicates that a kind of invagination takes place in the embryo at this stage (which is certainly very near that shown in fig. 9). If this is really correct, the inner layer of the primary embryo may possibly be formed by a process of invagination, and the inner layer of the secondary embryos is then probably derived from this invaginated layer; but I make these suggestions with all reserve.

Fig. 8 is interesting partly because it supports the view advanced in my former paper (17) that the species there described as *C. ramosa* is not identical with *C. eburnea*. The figure is a longitudinal section of an ovicell of *C. eburnea*; and so far as the general development of the ovicell goes, the age corresponds with the stage shown, for *C. ramosa*, in fig. 6.

The tentacle-sheath is at its period of maximum development; but the relative sizes of follicle and embryo are widely different from their relative sizes in *C. ramosa*. This appears to be a constant difference between the two species. In *C. eburnea*, the follicle is reduced to a minimum, and the quantity of the protoplasmic reticulum of mature ovicells is,

consequently, appreciably smaller than that in *C. ramosa*, although in old ovicells of the latter species even, the quantity of the protoplasmic reticulum may be considerably reduced, by the development of the larvæ at its expense. The primary embryo, on the contrary, is relatively very large. It has differentiated a distinct external layer of nuclei, which will give rise to the external layer of the secondary embryos. Throughout the development, the budding secondary embryo of *C. eburnea* differs considerably from that of *C. ramosa*; although the fundamental facts are the same in both species.

I have, unfortunately, no satisfactory sections of the ovicells of other species, which I found more difficult to obtain than the two former species. It is necessary to have a large stock of material in order to study the development; as it usually happens that a very small proportion of the colonies found are provided with ovicells.

The stages intermediate between figs. 9 and 11 have not been figured; but it is easy to describe their general development. After the stage shown in fig. 9 (but not until then) the embryo increases in size, and rapidly transforms itself into the characteristic mass of embryonic cells from which the young larvæ are budded off.

The history of the aperture of the ovicell has, so far, not been considered in sufficient detail. Its commencement as an invagination of the endocyst has been seen in fig. 3. When the egg has begun to segment (fig. 5), the opening of the invagination has closed. The distal end of the tentacle-sheath is, however, now thickened; and the valve is commencing to develop.

In fig. 8 (*C. eburnea*), the valve is practically complete; the distal thickening of the tentacle-sheath has increased, but the invagination constituting the primary aperture has not materially altered. The ovicell is completely calcified except in the region of its aperture, which is beginning to grow out into its tubular form. This part is covered merely by uncalcified ectocyst.

Fig. 12 represents a slightly earlier stage in *C. ramosa*.

The primary aperture is still present ; the distal thickening of the tentacle-sheath being already distinct, and showing a differentiation of an external epithelial layer, and a more internally placed mass of nucleated protoplasm.

It is very difficult to make out with certainty the later history of the aperture. In stages previous to that at which the solid follicle has its maximum size, the connection of the primary aperture with the distal wall of the ovicell seems to be completely lost in many cases. It is perhaps the case that the original invagination remains connected with the distal wall of the ovicell by a thin cord of cells which is not easily seen in sections—accounting for the apparent discontinuity between endocyst and apertural invagination which is frequently remarked ; and that, later, this cord shortens, bringing the invagination once more nearer to the distal wall of the ovicell, where its aperture once more opens out widely.

In fig. 13, representing the aperture of an ovicell in which traces of vacuolation are beginning to appear in the follicle, the primary aperture still opens to the exterior ; although, in accordance with what has just been said, its opening would probably not have been discernible at a somewhat earlier stage. The formation of the tubular definitive aperture has progressed, and the valve is complete. The differentiation of the thickened part of the tentacle-sheath into two kinds of cells, alluded to in the description of the last figure, has advanced a stage.

In fig. 9 the tubular aperture is practically complete. At its end is seen an invagination which I regard as the remains of the primary aperture, but which has now become disconnected from the thickened part of the tentacle-sheath.

The history of the aperture is thus, according to what I believe I have made out, as follows :—During the calcification of the distal end of the ovicell, the primary aperture, which at first opened in the middle of the mouth of the funnel, becomes shifted nearer the “ back ” wall of the ovicell, into the position where the tube of the ovicell is to be formed. The aperture is thus not closed by the calcification of the ovicell, but finally

disappears in the region of the permanently uncalcified part of the ovicell; i.e. of the definitive aperture.

Fig. 14 illustrates a condition of the tube of the ovicell which I have observed in one or two cases. The ectocyst is drawn out into a long narrow tube, which was probably uncalcified and which opens to the exterior. This recalls the condition described in the zoœcia of certain Cyclostomata, in which the zoœcium is closed by a (calcareous) lid, perforated by a small central aperture.¹ I am not prepared to state whether or not this is a normal character of ovicells at any particular stage, nor can I suggest any satisfactory explanation of the meaning of the phenomenon.

The central mass of cells differentiated from the thickened part of the tentacle-sheath in fig. 12 is destined to undergo certain very important modifications. In fig. 16 (more highly magnified than the previous figures, and belonging to the same ovicell from which fig. 6 was drawn) some of the nuclei of the central mass are growing larger. This is especially the case in the neighbourhood of the lumen of the tentacle-sheath, where there is a tendency for the nuclei to group themselves in small numbers. In fig. 9 some of these multinucleated masses of protoplasm are breaking off into the lumen of the tentacle-sheath.

From this stage onwards the characteristic multinucleated cells, which have been formed from the thickened distal part of the tentacle-sheath, are a normal feature of the ovicell, being found in the vacuoles of the protoplasmic reticulum in which the young larvæ lie. One of them is seen in fig. 11, and others are shown, more highly magnified, in fig. 18 (Pl. XXIV). In the latter figure the multinucleated cells contain nuclear and other structures which are obviously degenerating; and they are clearly not unlike the "giant-cells" which are known to occur in certain tissues in Vertebrates.

The giant-cells make their appearance at just that stage

¹ Cf. *Mesentipora meandrina* (Busk, No. 8, pl. xvii, fig. 2); *Reticulipora dorsalis* (Waters, No. 36, pl. xvii, fig. 4); and other cases referred to by Waters.

when the vacuolation of the follicle is commencing; and they appear to be closely connected with the carrying out of this process of vacuolation, although it is clear that the first small vacuoles (cf. fig. 7) make their appearance independently of the giant-cells.

At later stages each giant-cell is usually seen to lie in a large, sharply-marked vacuole of the protoplasmic reticulum. The cell may be apposed to one wall of the vacuole; or may, apparently, lie quite freely within it. There is good reason to believe that the vacuoles which are at first occupied by giant-cells are later occupied by the young larva. Each embryo, soon after its formation, comes to lie in a sharply-marked vacuole in the protoplasmic reticulum. Taking into consideration the facts (1) that the giant-cells are formed simultaneously with the appearance of large vacuoles in the follicle, and (2) that they contain fragments of degenerating cells or nuclei, it may be concluded that one of the functions of the giant-cells is to excavate spaces in the follicle in which the larvae can develop. These spaces are probably filled with some albuminoid fluid, at the expense of which the embryos develop—probably by diffusion through their tissues, as they have no recognisable means of absorbing nutriment.

The function of the giant-cells would thus be closely similar to that of the osteoclasts or myeloplaques of bone "which excavate small shallow pits . . . in the part which is undergoing absorption" (27, p. 104). Their structure, too, is in accordance with the descriptions of various observers of the multinucleated giant-cells in Vertebrates.

In the mature ovicell the remains of the distal thickening of the tentacle-sheath are always found as a dense mass of nucleated protoplasm which is attached to the ectocyst, not in the tubular aperture of the ovicell, but invariably at its base, on the side which is further from the back of the ovicell (figs. 9 and 16). The valve constantly projects from the back of the ovicell into the proximal part of this mass of cells in the manner shown in figs. 9 and 16. It appears to me probable that the function of the valve is to offer an obstacle to the

escape of the immature larvæ. When mature, the larvæ force their way one by one through the solid mass of protoplasm into the tubular aperture, and so escape to the exterior.

The tentacle-sheath is no longer easily distinguishable in the mature ovicell. With the commencement of the vacuolation of the follicle its distinctness vanishes, and it becomes confounded with the vacuolated follicle. The relations shown in figs. 9 and 17 probably indicate that the follicle ultimately fills up the whole of the original tentacle-sheath, and that that part of the ovicell which is not occupied by the protoplasmic reticulum and its contents is the original body-cavity of the ovicell.

The ovicells which are at their period of greatest activity can readily be recognised in the living condition by the pronounced yellow colour of their contents. This is sufficiently distinct to show clearly through the calcified wall of the ovicell. Although the embryos and larvæ are pale yellow, the colour of the ovicell depends mainly on the pigment in the reticulum which supports the embryos. In *C. cornuta* this is bright red-orange in colour, while in *C. ramosa* the orange colour is not quite so bright.

The oldest larvæ, which are almost ready to escape, lie each enclosed in a distinct vacuole of the reticulum, in close contact with the thick mass of protoplasm which fills up the aperture of the valve.

The production of embryos continues up to a very late stage, but embryos are always developed only from the budding mass of embryonic cells (primary embryo). The budding organ has, however, a somewhat different appearance in old ovicells from that which it first had, both the secondary embryos and their nuclei being markedly smaller than in the younger ovicells.

So far as my observations go, the whole of the budding organ is ultimately used up in the production of embryos. In ovicells which are nearly exhausted the embryos are few in number, and the budding organ has been reduced to small dimensions. Finally, the ovicell is found to consist merely of

a protoplasmic reticulum, which may be richly developed, and which no longer contains any embryos or larvae.

It must not, however, be assumed that all ovicells in this condition have passed through an embryo-producing stage. I have repeatedly obtained evidence to show that degeneration of the ovicell may start at almost any stage in its development. The external form of the ovicell may develop completely, even if the embryo and its follicle are degenerating. It is common to find an ovicell which, from its proximity to the growing-point, should be a young one, but which appears completely empty in sections. I can only assume in these cases that the protoplasmic structures which the ovicell at first possessed have been absorbed through the pores into the neighbouring zoœcia. In other cases the ovicell may contain remains of a degenerating follicle, the degeneration having clearly commenced before the follicle became vacuolated. One may, therefore, distinguish between a "primary" degeneration of the ovicell occurring before any larvæ have been produced, and a "secondary" degeneration, which has taken place after the escape of the last larva. It is sometimes possible to distinguish between these two conditions by reason of the fact that the base of the young ovicell is limited by a marked epithelial arrangement of its endocyst (cf. figs. 6 and 9).

I have so far avoided the use of the term "endoderm" as an equivalent for the inner layer of cells of the secondary embryos. This layer is excessively distinct in the embryos soon after their liberation from the embryogenic mass of cells. It then forms an epithelium, lying more or less close to the inner surface of the ectoderm-cells, and completely surrounding the whole internal cavity of the embryo (fig. 22).

In later stages the distinctness of the cavity becomes lost, and its lining cells send off processes which grow across the cavity and convert it into an irregular set of spaces. At the sides of the sucker these spaces disappear altogether, while between the sucker and the middle of the aboral pole the cavity remains distinct for a time longer. Its cells become, however, almost indistinguishable from the epithelium of the

sucker on the one hand, and from the epithelium lining the mantle-cavity on the other.

I formerly assumed (15, p. 455), on the authority of Ostroumoff's statements (25, pl. vi, fig. 1), that the cavity lined by the inner cells represented the alimentary canal of the larva. But, taking into account the manner in which the larvæ are developed, it appears to me doubtful whether any representative of the endoderm occurs in them. It appears to me to be satisfactorily established that a young polypide-bud in any Polyzoan is developed at the expense of two layers, viz. the ectoderm and a layer of funicular tissue which may be regarded as mesoderm.¹ The metamorphosis of the larva of Cyclostomata has been described by Barrois (3) and by Ostroumoff (25). The observations of Barrois show that the processes of fixation and of metamorphosis take place essentially as in other Gymnolæmata. The larva fixes by the eversion of its sucker, its mantle being rolled downwards so as to come into contact with the flattened plate formed by the eversion of the sucker, and the greater part of the larval tissues undergo a process of histolysis. The larva thus enters into the condition of a zoœcium containing a "brown body," and the young polypide is produced by an invagination of the body-wall from the centre of the surface opposite to the basal surface. While the inner layer of the bud is formed by an invagination of the ectoderm, Barrois was unfortunately unable to trace the history of its outer layer.

Ostroumoff is but little more definite on this point. The inner layer of the bud is formed, according to this observer, not as an invagination, but as a plate of cells split off from the aboral ectoderm. The edges of this plate curve round, so as to transform the plate into a sac, to the outer side of which "mesenchym-cells" apply themselves, and form the outer layer of the bud. The origin of these "mesenchym-cells" is not traced. It is recognised that the "alimentary canal" of the earlier stage disappears, but there is nothing to show how its cells are related to the "mesenchym-cells" shown in

¹ Cf. especially Seeliger, Nos. 32 and 33.

Ostroumoff's pl. vi, fig. 2, which, by the way, are unlike any cells which I have ever seen in a Cyclostome larva.

Whatever be the origin of the outer layer of the bud which forms the primary polypide, it is quite clear that that polypide is formed in fundamentally the same way as any other polypide in the future colony. There can be no question of the "alimentary canal" of the embryo passing over directly into that of the primary zoœcium.

In default of sufficient evidence on this point I am inclined to regard the inner layer of the Cyclostome embryo as mesodermic rather than endodermic, and this principally on the following grounds:

1. The alimentary canal is an excessively rudimentary structure in the great majority of known Ectoproct larvæ.
2. The peculiar character of the early development of *Crisia* suggests that a representative of this rudimentary structure is likely to be found in the primary embryo only, and that the secondary embryos, formed by budding from the primary one, are no more likely to possess an alimentary canal than is a young zoœcium formed at the growing-point of an old colony.
3. The analogy of other Ectoprocta is in favour of this hypothesis.¹

Prouho (26), for instance, has given an account (which I can confirm in the main from my own observations) of the metamorphosis of *Flustrella*. Even before the end of larval life, a distinct aboral mesodermic layer is present, from which the outer layer of the bud is directly developed.

In the course of the budding of an ordinary Ectoproct colony the polypide buds are formed from two distinct layers. The inner layer of the bud is developed at the expense of the ectoderm; the outer layer, either from an already definite layer of mesoderm (*Phylactolæmata*), or from mesoderm-cells of the funicular tissue which arrange themselves as an epithelium round the outside of the ectodermic portion of the bud

¹ Cf. particularly the larva of the *Phylactolæmata*, as described by Braem (5) and by Davenport (8A).

(most Gymnolæmata; cf. especially Seeliger, No. 33). There is no sufficient reason for supposing that a young zoœcium consists of anything but ectoderm and mesoderm. The Ectoproct larva may be considered morphologically as a young zoœcium containing a potential "brown body" (the remains of the purely larval organs), and it is not unreasonable to suppose that the structures found in the larva of the Cyclostomata, developed as it is by a process of budding, are comparable with those which are found in a zoœcium.

We arrive, therefore, at the provisional conclusion that the inner layer of the Cyclostome embryo is more likely to represent the mesoderm than the endoderm of the larva.

There can be no doubt that, on the assumption that my account of this process is in the main correct, the development of *Crisia* takes place in a manner to which there are few known parallels.

The most frequently quoted case of embryonic fission is that of *Lumbricus trapezoides*, in which, according to the statements of Kleinenberg (20), the embryo normally divides into two complete embryos at the gastrula-stage. In some abnormal cases, however (l. c., p. 217), a single embryo is first formed; and this gives rise to one or more embryos produced as buds on the margin of its mouth. The segmentation of the egg is described as being much less regular than in other species of *Lumbricus*, in which no embryonic fission takes place.

An equally striking case of the same kind had previously been described by Busch (7), in *Chrysaora*. In only a few cases does an egg develop into a single embryo. In the other cases, the embryo gives rise to one or two buds, apparently at the gastrula-stage; the buds becoming free larvæ, and developing fresh buds. Not only does Busch claim to have followed the whole process in an isolated individual, but he states that each time that the water in which the young larvæ were kept was changed, two thirds or so of the embryos were thrown away, and that this loss in number was compensated for, by the next day, by the gemmiparous habit of the larvæ

(l. c., p. 28). This account is confirmed by Haeckel (14), who observed the production, in three weeks, of 60—80 buds, from ten isolated gastrulae of *Chrysaora*.

The process of larval fission or gemmation is known to be even more remarkable in *Aurelia* (Haeckel, l. c.). Not only do the gastrulae multiply, in some cases, by budding or by fission, but the same processes are known to occur in the Scyphostoma stage; while numerous variations are recorded in the character of the strobilation, in the multiplication of the tentacles by incomplete fission or budding, and even in the number of the highly characteristic tæniolæ and in that of the Ephyra-lobes.

The property of giving rise to fresh individuals, whether by budding or by fission, has, in this case, become a normal feature of the species; and the process takes place even at very early periods of the development, just as is the case in *Crisia*. The striking variability in the number of the radii of the immature stages in *Aurelia* may possibly be connected with something in the constitution of the egg which predisposes it to develop in an unusual way.

For since the cells which are destined to give rise to a single individual are not normally separated off until a late stage, which varies in different individuals, the existence of a tendency to vary in the number of individuals produced from an egg might also, in all probability, make itself felt in variations in a different direction. If the gastrula contains in itself the power to develop into several individuals, it is hardly surprising that it should in some cases develop an abnormal number of radii.

Similar cases of larval budding have been recorded in other Scyphomedusæ. Thus Goette (12), confirming an older observation of Sars (1841), shows that the formation of a stolon may take place (presumably in *Cotylorhiza tuberculata*) in the larva which has just fixed, but which is still without tentacles. Ciliated buds are also given off from the Scyphostoma of *Cotylorhiza*, the buds fixing and developing a mouth after fixation.

A method of reproduction similar to the last is recorded by Bigelow (4) in *Cassiopea xamachana*.

In *Oceania armata*, Metschnikoff (24) characterises the process of segmentation as a regular "Blastomerenanarchie" (p. 38). The first two blastomeres almost separate from one another; while, in some cases, when the very slight connection which normally exists between them becomes ruptured, the separated blastomeres atrophy. *Oceania* further distinguishes itself, at the eight-cell stage also, from other Medusæ investigated by Metschnikoff; the blastomeres, instead of being arranged in an orderly manner, lying together "ganz unregelmässig." This extraordinary irregularity (see Metschnikoff's pl. i, figs. 33—35) is equally remarkable at later stages, and ultimately gives rise to irregularly shaped masses of cells; the embryos often assuming a quite "abenteuerliche Gestalt," due to the fact that they multiply by division. Those embryos which do not divide form much larger larvæ than the others.

As a converse to this may be mentioned some most interesting results arrived at by Driesch (9) and by Fiedler (10). Driesch showed that by violent shaking of the water containing *Echinus*-eggs which had divided into two blastomeres, or in other ways, the two cells could be isolated from one another. Each segmented in the same way that it would have followed if it had remained connected with its fellow, i.e. it developed into a half-embryo, right or left as the case might be. The segmentation cavity, at first widely open, closed up in course of time so as to form a blastosphere, consisting (as appeared from measuring the cells) of half the normal number of cells, and being half the normal size. Three of these embryos developed into complete Plutei, which differed from normal ones only in size. In cases where the two original blastomeres had been only partially separated, seventeen cases were recorded in which the embryo distinctly consisted, at the end of the first day, of two halves. In several cases each of these embryos divided into two complete embryos, some of which were shown to develop into small normal Plutei. In

another instance an injured two-cell embryo developed apparently normally up to the end of the blastosphere stage, but finally divided into two; and in another case the injury to the two-cell stage resulted in the formation of a double monster.

In *Crystallodes* (Siphonophora) the remarkable amoeboid character of the superficial blastomeres suggested to Haeckel (13) that the embryo, at the end of the second day, could be compared to a colony of *Amoebae*, in consequence of the great individuality of the separate blastomeres; and that, if this comparison were correct, an isolated portion of the embryo might be expected to have the power of further development. The experiments made to test this hypothesis were completely satisfactory. Embryos artificially divided at this stage developed into normal individuals of a smaller size than usual. The cut surface became concave, the edges ultimately joining, so that the embryo again became spherical, and then proceeded to develop in its normal manner.

It is hardly possible to overlook the fact that, in some at least of the above quoted cases, embryonic fission is specially connected with deviation from the normal type of segmentation of the egg. This is most clearly seen in the case of *Oceania*, where a superficial glance at Metschukoff's figures is sufficient to convince one of the extraordinarily abnormal character of the segmentation. The same fact is, however, to some extent true of *Lumbricus trapezoides* and of *Crystallodes*, where Kleinenberg and Haeckel respectively call attention to remarkable features in the segmentation.

The segmentation of the egg of *Crisia* obviously belongs to an unusual type, and, as has already been pointed out, it finds its closest parallel in *Salpa*, an animal which is remarkable for the great extent to which asexual reproduction is carried out.

Doliolum, whose life-history agrees with that of *Salpa* in including two remarkably different generations, offers a further analogy to *Crisia* in the character of its asexual reproduction. The stolon of the asexual generation segments off, according to

the description of Uljanin (35), a series of buds in which there is a very small amount of differentiation. These "Urknospen" consist of a layer of ectoderm surrounding a mass of embryonic cells which are but slightly differentiated (l. c., pl. x, fig. 3). These buds divide up into numerous similarly-constituted buds, so that the sexual individual of *Doliolum* takes its origin from a group of cells which is very similar to the young "secondary embryos" of *Crisia*.

The same method of reproduction characterises the remarkable *Dolchinia*, recently described by Korotneff (22). This animal is closely allied to *Doliolum*, if, indeed, it should not be placed in that genus. The only phase in its life-history which is so far known is a gelatinous axis, bearing very numerous *Doliolum*-like zooids, and which probably corresponds to the dorsal process of *Doliolum*. The axis bears numerous buds, wandering about on its surface by means of pseudopodia. The buds have probably been derived from the segmentation of the ventral stolon of an asexual form. They increase in number by division. Should one of the daughter-buds fix itself on the base of a young zooid, it becomes a bean-shaped body, which gives rise to a large number (as many as forty) of new buds. The young buds, at the stage at which they become free, consist of a solid mass of cells in which a very small amount of differentiation has taken place.

The formation of the secondary buds, as shown in Korotneff's pl. xiii, figs. 14, 15, has thus a striking resemblance to the mode of development of the secondary embryos in *Crisia*; neglecting the not unimportant difference that in the former case the budding organ is itself a bud, and in the latter case an embryo.

A similar process probably takes place in *Anchinia* (21); and Uljanin (l. c., pp. 106—117) brings forward evidence to show that the same is true of some of the compound Ascidians. The larva of *Distaplia magnilarva*, for instance, gives rise to structures comparable with the "Urknospen" of *Doliolum*. Uljanin comes to the conclusion that the budding of adult Tunicates is derivable from a division of "very

young developmental stages." A similar suggestion with regard to the origin of alternation of generations among the Hydromedusæ has also been elaborated by Brooks (6), who supposes that the hydroid stage has been evolved by the acquirement of the power of budding by the fixed larval stage.

A slight modification of the "primary embryo" of *Crisia* would suffice to make it necessary to consider the life-history of that animal as a case of alternation of generations. But since, as I believe, the budding structure consists of a mass of embryonic cells, which ultimately becomes completely converted into "secondary embryos," leaving nothing behind, I have preferred not to describe it as a separate generation.

Enough has been said to show that in the Tunicata at least, and to a less extent in the Cœlenterata,¹ there are remarkable cases of the formation of buds from slightly differentiated masses of cells. These two groups, with the Polyzoa, are certainly the groups of animals in which budding in the adult condition is a more normal event than in other groups of animals.

It may thus be asserted that in the Polyzoa, the Tunicata, and the Cœlenterata the asexual reproduction of certain forms takes place at a stage before the individual which is reproducing asexually has had time to undergo more than the earliest steps in its development. A similar precocious formation of fresh individuals is well known in the reproduction of Trematoda.²

The investigations of Driesch (9) and of Haeckel (13) have shown that blastomeres which have been artificially separated from the embryo are able, in some cases, to give rise to complete larvæ. The question suggests itself: Has the gemmi-

¹ The case of *Cunina*, as described by Uljanin, Schulze, Metschnikoff, and Brooks, and more recently by O. Maas ("Zoolog. Jahrbücher," "Abth. f. Anat. u. Ontog.," Bd. v, Heft 2, 1892), is another remarkable instance of the same kind.

² Compare particularly the remarkable account given by Heckert (18) of the life-history of *Distoma macrostomum*, and particularly the statements referring to its remarkable branched sporocyst, known as *Leucocloridium paradoxum*.

parous method of reproduction in the adults of the above-mentioned groups been preceded by larval fission, possibly induced by the separation from the embryo of individualised blastomeres or groups of blastomeres, or is the embryonic fission the result of the precocious acquirement of the budding habit which characterises the adult? Now in the Polyzoa, embryonic fission is by no means a common phenomenon, although the adults of all known Polyzoa possess the power of budding; and although it is probable that the method of reproduction above described in *Crisia* will be found to be characteristic of all Cyclostomes. I have no sufficient evidence on this point at present, but it may be pointed out that the ovicells of Cyclostomatous Polyzoa invariably (so far as I know) contain a large number of embryos. My own observations enable me to state further that the general structure of the ovicell in *Idmonea serpens* and in *Diastopora patina* agrees with that in *Crisia*; and I have little doubt that I shall be able to show that embryonic fission is characteristic of Cyclostomes in general. The development of the Phylactolæmata possibly offers some analogies to this process. The structure of the larva is somewhat similar to that of Cyclostomes, and the early development, according to the account given by Jullien (19), is not unlike that of *Crisia*. Braem (5) has also given an incomplete account of the development of *Plumatella*, which suggests further resemblances to the Cyclostomata. The two layers which form the wall of the embryo, and which are considered by Braem to represent ectoderm and body-cavity epithelium respectively,¹ are obviously comparable with the two layers shown in Pl. XXIV, figs. 22 and 23, of *Crisia*. The manner in which (in *Plumatella*) a rudimentary bud encloses the egg, forming the "oœcium," is again strikingly suggestive of *Crisia*.² The first stage in which the

¹ The same conclusion is arrived at by Davenport (8A), whose valuable paper should be consulted for a comparison of the larva of *Phylactolæmata* with that of *Gymnolæmata*.

² Compare in particular the woodcut given by Braem in his explanation to fig. 171.

egg is completely enclosed is shown in Braem's pl. xv, fig. 171, while in fig. 172 the "oœcium" has differentiated off a distal portion which may be the exact equivalent of the "tentacle-sheath" shown in my own fig. 3 for *Crisia*. The tendency to precocious fission shows itself in *Phylactolemata*, however, in the precocious formation of a considerable number of polypides, particularly in *Cristatella*;—a process which is of course very different from the embryonic fission of *Crisia*.

The ovicells of the *Cheilostomata* are probably not homologous with those of *Cyclostomata*. They are probably not to be regarded as modified zoœcia, since the ovicell is an appendage of a fertile zoœcium, and ordinarily contains a single embryo.

Similarly in the other groups which have been mentioned precocious fission is not characteristic of the whole group, but occurs sporadically;—in *Cœlenterata*, in *Oceania*, *Cunina*, &c.; in *Trematoda*, in the *Distomeæ*; and in *Tunicata* in the *Thaliacea*, and in some *Synascidians*.

Although I must regard the question as a very open one, the conclusion which appears to me to be suggested by the above facts is that one is not justified in assuming that the budding of the *Polyzoa*, for instance, commenced with the acquirement of a habit of embryonic fission like that found in *Crisia*, but that the embryonic fission may be the consequence of the previously acquired power of adult budding. It may be pointed out that the embryonic fission of *Crisia* gives rise to numerous larvæ, each of which may form the starting-point of a new colony. In the case of adult *Polyzoa*, the result of budding is merely to increase the number of individuals in a colony, with the exception of *Loxosoma* (in which the bud normally becomes free) and of certain dendritic forms of colony, in which the decay of the proximal part of the colony leads to the separation as distinct colonies of what were at first merely branches, or of cases like that of *Crisia* itself, where new colonies are formed by the upgrowth of new stems from a creeping rootlet, which acts as a stolon for the production of

new colonies.¹ It should be further noted that the production of new polypides in old zoœcia is one of the most characteristic ways in which the property of budding manifests itself in Ectoprocta, and that this process is most easily interpreted as a process of regeneration of lost parts.

The provisional conclusion may therefore be stated as follows:—That the process of embryonic fission, which may appear abnormally in certain individuals in so many groups of animals which do not multiply by fission, has in *Crisia* become a normal phenomenon of the development; and that this process is correlated with the tendency which is so strongly marked in the Polyzoa to produce buds in the adult condition.

Giard (11) has recently published a note on what he terms "poecilogonie," i. e. the phenomenon exhibited by certain animals of developing in a more or less "condensed" manner, in correlation with the amount of nutritive reserves in the egg, or with the conditions under which the parent is living. As examples of this process are mentioned, *inter alia*, the following cases:—In *Leptoclinum lacazii*, Gd., the same colony may produce two sorts of eggs; of these, one is poor in yolk, and gives rise to small larvæ, whose tail is absorbed early, and which do not begin to bud even on the third day. The other kind is rich in yolk, and produces larvæ which are still free-swimming on the fourth day, and which then already contain a colony of three individuals. *Ophiothrix fragilis*, Müll., lays eggs which develop, according to the conditions, either into perfect or into imperfect Plutei, or into embryos incapable of swimming, and which develop directly. The remarkable variations in the development of *Aurelia aurita* and of *Palæmonetes varians* are also included in this category; in the latter form the size and number of the eggs, as well as the rapidity of the metamorphoses, varying according as the animal lives in the brackish waters of the North or in the fresh-water lakes of the South.

Giard's observations suggest that the acquirement of em-

The statoblasts of the Phylactolemata are indeed a further exception since each of these bodies is able to give rise to a new colony.

bryonic fission in Cyclostomes may have been connected with the presence of the nutritive conditions which are suited to induce the precocious formation of buds. Nothing can be more striking than the obvious continuity of protoplasm between the several units of the colony in a decalcified branch of *Crisia*. In the individuals which are modified as ovicells the protoplasmic network is particularly well developed. The embryo is thus surrounded by a rich nutritive material; and just as the presence of a nutritive placenta in a Placental Mammal has resulted in the diminution of the size of the ovum, and in various abnormalities in its early segmentation, so in *Crisia* the size of the egg is reduced to a minimum, the whole of the nutritive substance being retained in the parental tissues and handed on to the egg or embryos as required, while the segmentation is entirely abnormal. Further, while the Mammalian embryo becomes easily comparable with that of any other Vertebrate embryo after a certain number of the early stages have been passed through, so the *Crisia* larva becomes, to some extent at least, comparable with the free larva of any other Polyzoan, although with this difference from other Polyzoa, viz. that the primary embryo has given rise to numerous larvæ, a process comparable with the artificial production of a complete embryo from a single blastomere of the two-cell stage in the experiments of Driesch (9) and of Fiedler (10).

Attention has already been called to the similarity between the early stages of the development in *Crisia* and those in *Salpa*. The latter is another example of the modification of the first processes in the development, associated with the presence of special maternal nutritive arrangements. The embryo of *Salpa* develops, as is well known, in close connection with a kind of placenta; and its early stages are, compared with those of most other animals, highly abnormal. The formation of buds from the individual developed from the egg does not take place at once, as in *Crisia*, but is deferred until the animal is mature, when buds are produced in very large numbers from the stolon.

Similarly the egg of *Pyrosoma*, like that of *Salpa*, makes its appearance in the same precocious manner as that of *Crisia*, being formed very early from the so-called "genital string" (Salensky, 29). The early development, which is modified by the presence of yolk, takes place in the interior of the old colony, and is very abnormal, the blastomeres being for a time completely separated from one another (Salensky, p. 443). The result of the development is the formation of the well-known "Cyathozoid," with its colony of four "Ascidiozooids," the formation of which is compared by Salensky (30, p. 92) with the embryonic fusion of *Lumbricus trapezoides*. The formation of a stolon (represented by the chain of four Ascidiozooids) in the *Pyrosoma*-embryo is further regarded as the precocious acquirement by the embryo of the power of budding already possessed by the Synascidians.

*Peripatus*¹ is well known to be viviparous, and the extraordinary character of the segmentation of its ovum may have some relation to the presence of external sources of nutriment.

The cases already quoted may be taken as showing that some of the abnormalities in the development of *Crisia* may be due to the nutritive conditions in which the development takes place. Just as the presence of food-yolk within the egg modifies the character of the segmentation and of the formation of the layers, so the presence of copious stores of nutrient material in the maternal tissues outside the egg may also affect the early developmental processes. Thus the large number of relatively large larvæ which develop from the minute egg of a *Crisia* could not be produced if the egg were not supplied with nutriment from outside itself. While some of the irregularity in the segmentation of the egg may be due to this cause, the extreme independence of the blastomeres at an early stage may be connected with the acquirement by the embryo of a habit of forming buds in the embryonic condition.

¹ See Sedgwick, No. 31.

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It hardly falls within the province of this paper to discuss the details of the normal budding in Polyzoa. Both Braem (5) and Davenport (8a) have shown that polypide-buds in general are derived from a mass of "embryonic" tissue, handed down from the beginning of the formation of the colony, some part of this tissue being left over for the production of fresh buds on each occasion when a polypide-bud is formed. Braem's account of the formation of the statoblasts in Phylactolaemata more nearly resembles the development of the "secondary embryos" in Crisia than any other process as yet described in Polyzoa. The funiculus is, indeed, not an embryo; but the young statoblasts are formed from it in much the same way as that in which the larvae are developed from the "primary embryo" in Crisia. The funiculus consists of a core of ectoderm surrounded by a sheath of mesoderm (both kinds of cells having an "embryonic" character). The statoblasts are formed by a process which is to all intents and purposes a transverse segmentation of the funiculus.

EXPLANATION OF PLATES XXII, XXIII, & XXIV,

Illustrating Mr. Sidney F. Harmer's paper "On the Occurrence of Embryonic Fission in Cyclostomatous Polyzoa."

PLATE XXII.

FIG. 1.—*C. eburnea*. Median longitudinal section through a young ovicell, showing the egg, which is already partially surrounded by the polypide-bud (Zeiss, DD).

FIG. 2.—*C. ramosa*. Part of a similar section at a more advanced age, showing the complete inclusion of the ovum (Zeiss, DD).

FIG. 3.—*C. ramosa*. Similar section at an older stage. The polypide-bud has become the "follicle." The tentacle-sheath and the aperture are well developed. Ovicell at the "funnel-stage" (Zeiss, DD).

FIG. 4.—*C. eburnea*. Spermatozoa. To the right, three mature spermatozoa, drawn in the living condition (Zeiss, F'); to the left, three stages in the development of the spermatozoa, treated with osmic acid and picro-carmine (Zeiss, F); in the middle, four immature groups consisting of four spermatozoa each, killed with osmio vapour (Zeiss, $\frac{1}{2}$ immersion, 4 oc.).

Figs. 5—7.—*C. ramosa* (Zeiss, DD).

Fig. 5. (Combined from several sections of the same ovicell.) The egg has divided into three blastomeres; the valve is developing, and the distal end of the tentacle-sheath has become thickened.

Fig. 6. Proximal end of a longitudinal section (more advanced). The embryo consists of a compact rounded mass lying in a large follicle, which projects freely into the tentacle-sheath.

Fig. 7. A similar preparation at a stage when the vacuolation of the follicle is commencing.

PLATE XXIII.

FIG. 8.—*C. eburnea*. Ovicell at a stage corresponding to Fig. 6 in *C. ramosa*. The embryo is larger and the follicle is much smaller than in that species (Zeiss, DD).

Figs. 9—14.—*C. ramosa* (Zeiss, D D).

Fig. 9. The vacuolation of the follicle is nearly complete. The tubular aperture is formed, and the structures connected with its base are well developed.

Fig. 10. Invagination (?) in a "primary embryo," at about the same stage as Fig. 9.

Fig. 11. Considerably later stage. The follicle has become a dense protoplasmic reticulum, containing the massive "primary embryo," now transformed into a budding organ, which is giving rise to numerous secondary embryos, three of which are seen lying freely in the reticulum. At the upper end a giant-cell, derived from the thickened distal end of the tentacle-sheath (cf. Figs. 5, 13 and 9).

Figs. 12—14. Illustrating the development of the aperture of the ovicell. In Fig. 12, the primary aperture still remains open, the distal end of the tentacle-sheath being thickened. In Fig. 13, the tubular aperture of the adult ovicell is developing; it contains the remains of the primary aperture. The thickening of the distal end of the tentacle-sheath has increased in size, and the valve is well developed. In Fig. 14, the tubular aperture is almost complete. The thickening of the tentacle-sheath still extends into its base. At its distal end a depression occurs, which is possibly the remains of the primary aperture. The tube ends in a cap prolonged into a narrow tube, of unknown significance.

PLATE XXIV.

FIG. 15.—*C. eburnea*. A young internode decalcified, with a developing ovicell. For explanation of the letters (A, B and C) see p. 205 (Zeiss, A).

Figs. 16—23.—*C. ramosa*.

Fig. 16. The aperture of the same ovicell from which Fig. 6 was taken. The origin of the giant-cells from the thickened distal end of the tentacle-sheath is shown (Zeiss, F).

Fig. 17. Longitudinal section of an ovicell which is filled with secondary embryos. To the left, the primary embryo (Zeiss, A).

Fig. 18. Protoplasmic reticulum with giant-cells, from an ovicell at about the stage of Fig. 11 (Zeiss, F).

Figs. 19—21. Development of ovicell (Zeiss, A). The ovicell has in each case been numbered 4, in order to admit of ready comparison between the three stages.

Fig. 22. Young secondary embryo, in longitudinal section (Zeiss, F).

Fig. 23. Older embryo, in longitudinal section (Zeiss, F).

Fig. 1.

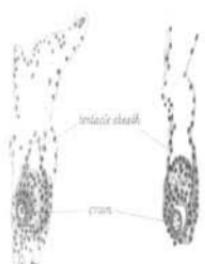


Fig. 2.

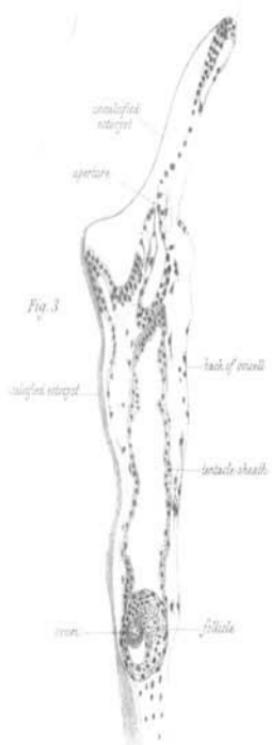


Fig. 4.



Fig. 6.

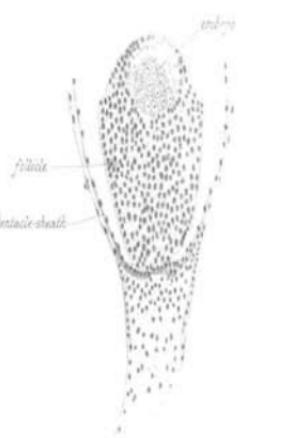


Fig. 5.

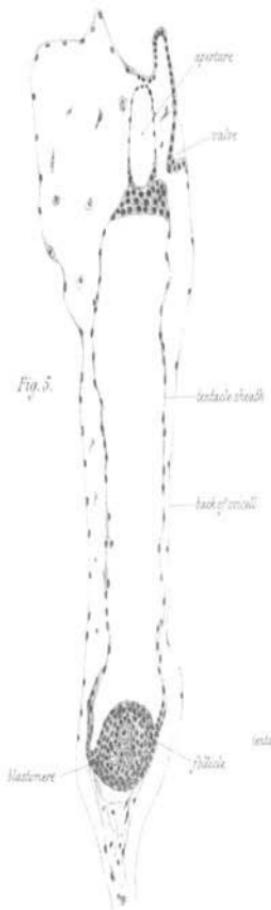
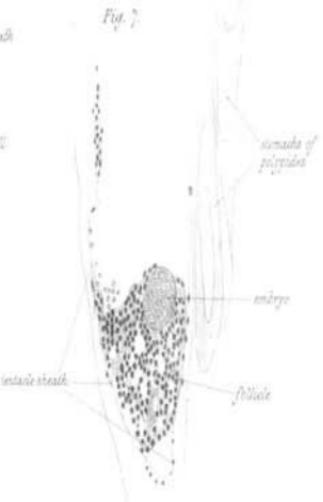


Fig. 7.



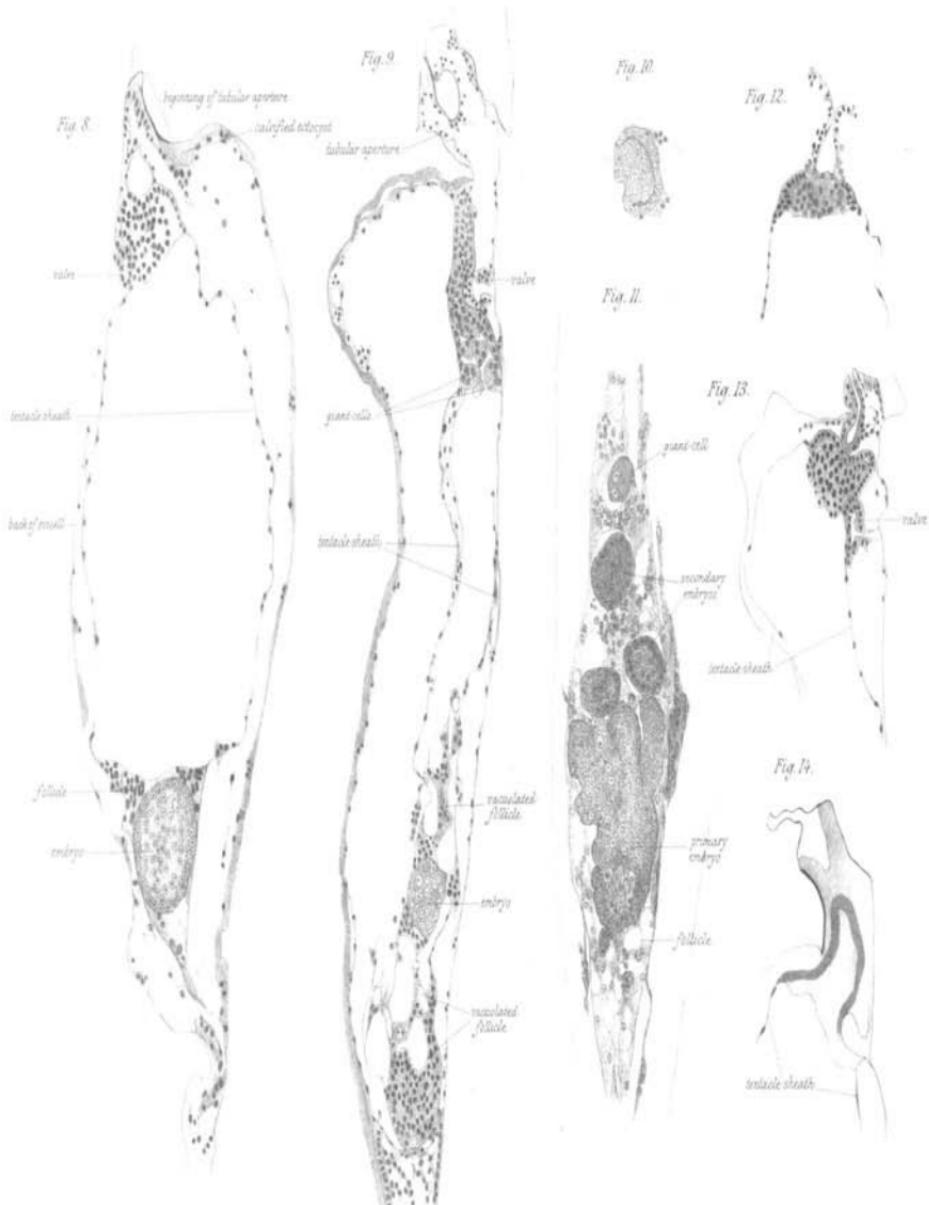


Fig. 5.



Fig. 6.



Fig. 9.



Fig. 20.



Fig. 21.



Fig. 17.



Fig. 18.

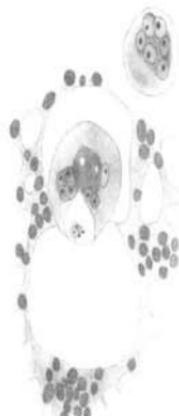


Fig. 23.



Fig. 22.

