On Budding in Polyzoa.

By

Alfred C. Haddon, M.A.,
Professor of Zoology in the Royal College of Science, Dublin.

With Plates XXXVII and XXXVIII.

Introductory.

Mr. Hincks, in his valuable monograph on the British Marine Polyzoa (1), after discussing the nature of the "brown body" found in the old zoœcia of Polyzoa, and its relation to the developing bud closes with these words (p. lxiii): "There seems, therefore, to be grounds (pro tanto) for desiring some further investigation of the subject." This, then, must be my excuse for offering these somewhat imperfect observations.

The investigations on Flustra carbasea were made in the month of May, 1879, whilst I was occupying the table belonging to the University of Cambridge, in Dr. Dohrn's Zoological Station at Naples. The other observations were made on species obtained from Dublin Bay during 1881-2.

Of the nature of the brown body itself I do not propose to treat, as the evidence of other observers as well as of my own studies is perfectly satisfactory in favour of its being, as Hincks says, "derived from the polypide, and is the result of its decline;" but I will limit myself solely to the origin and development of the bud.

Own Observations.

Flustra carbasea, E. and S. (The following observations, when not otherwise stated, apply to the living state only) In most of the empty zooecia which had been previously inhabited, a brown body was observed situated towards its lower end, this was surrounded by funicular tissue ("endosarc," Joliet), which sent out irregular strands to the walls of the zooecium, some of them being connected with the band of flexible endocyst which stretched across the mouth of the zooecium. In the centre of this band, and therefore connected with the endocyst on the one hand and with the funicular tissue on the other, was situated, in the earliest observed stages, a small rounded mass of cells yellowish in colour, surrounded by a sheath of transparent cells, which together constitute the nascent bud.

The bud soon acquires a well marked central cavity (Pl. XXXVII, fig. 1), then becomes oval in form, and depends from the anterior band of endocyst. A further elongation next takes place, this process being more rapid above than below, resulting in a pyriform body, of which the upper and narrower part consists of a thin double walled sac, the outer wall being the sheath and the inner one the attenuated internal layer. The lower and wider portion consists of the thin outer sheath enclosing the active internal cells (Pl. XXXVII, fig. 2). To anticipate—the inner cells will form the external layer of the tentacular sheath, the external epithelium of the tentacles, and the internal epithelium of the alimentary canal of the new polypide, while the outer layer or "sheath" will form the inner layer of the tentacular sheath, the inner epithelium of the tentacles and the tissue which surrounds the digestive tract.

A series of somewhat complicated changes now takes place in the lower moiety of the inner layer. (It should here be premised that the outer layer is perfectly passive throughout, merely adapting itself in such a manner as to wrap itself round the active inner layer.) One side of this portion of the bud protrudes, the protrusion becomes constricted off in such a manner as to produce a blind sac, depending by the side of the remainder of the bud, the constriction is quite complete except at the uppermost point, this being the spot where the rectum will be connected with the lophophore; fig. 3, Pl. XXXVII, which is drawn from a preparation, illustrates the commencement of this process. It will be noticed that the inferior portion of the area which is being constricted off is connected with funicular tissue; as a matter of fact, there is often a slight difference in the character of those cells which occupy a corresponding position in yet earlier buds. While this has been happening, the other portion, which has a plate-like form, becomes crenulated along its margin, the crenulations, which point upwards and rather inwards, increase in size and we have some twenty-two incipient oval tentacles formed. These tentacles are thus early ranged into a circular lophophore, continuous except in that region from which the above-mentioned pouch is hanging. This gives an appearance of bilateral symmetry to the lophophore, as was noticed by Allman in Paludicella (19), and Nitsche in Flustra membranacea (13) (Pl. XXXVII, figs. 4 and 5.).

The developing polypide now consists of a disc-like body (lophophore), surrounded by twenty-two oval tentacles, emarginated at one spot from which depends a cæcal pear-shaped bag—the future stomach and intestine.

A circular depression occurs towards one end of the disc of the lophophore which rapidly deepens into a rounded sack (Pl. XXXVII, figs. 6 and 7). The fundus of this sac impinges

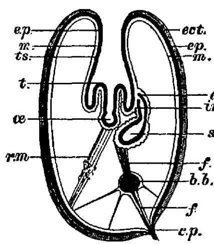

Fig. 1.—Diagram showing the relations of the devoloping bud in a Marine Polyzoan. *a*, anus; *b. b.*, brown body; *c. p.*, communication plate; *ect.*, ectocyst; *ep.*, epiblast; *f.*, funiculus; *int.*, intestine; *m.*, mesoblast; *œ.*, œsophagus; *r. m.*, retractor muscle; *st.*, stomach; *t.*, tentacles; *t. s.*, tentacular sheath.

upon the cæcal stomach, the two organs coalesce and their lumens become continuous by wall-absorption. This diverticulum is the œsophagus, and the polypide has now the characteristic form of the adult.

The woodcut (fig. 1) indicates the relation of the developing bud to the zooecium far better than a verbal description can do.

Very shortly after this, owing partly to absolute increase in size and also to the elongation of the tentacular sheath, the polypide has come into contact with the brown body, which, as was before mentioned, lies near to the bottom of the zooecium (Pl. XXXVII, fig. 8). The walls of the stomach, or more strictly, that portion of the stomach which forms the gastric cæcum, grow round and envelop the brown body, so that the brown body passes as a whole into the alimentary tract of the young Flustra, which now has the form depicted in Pl. XXXVII, fig. 9.

The brown body immediately commences to undergo disintegration, and, previously to passing into the intestine, the remains are whirled round and round within the globular pyloric portion of the stomach by the action of the minute cilia with which the latter is clothed (Pl. XXXVII, figs. 10 and 11). Ultimately all trace of the brown body, as such, is lost save a small quantity of fæcal matter in the intestine, and by this time the gastric glands become very apparent (Pl. XXXVIII, fig. 12).

The reason why these buds, at this stage, appear of a different colour from the ordinary marginal buds of the colony, as was first noticed by Hincks and animadverted upon by Joliet (17) (still later, see Hincks (1) pp. lvii, lxii), is probably owing to the digestion of the brown body with the concurrent development of digestive glands, the other buds gaining the whole of their nutriment directly from the parent tissues, and thus not requiring a distinct digestive apparatus.

Annulations of the stomach at this stage indicate the existence of circular muscles, the walls of the pylorus become muscular and much thicker, and, as before mentioned, its lumen is ciliated, the cæcum is a wide pouch lined with secretory cells. The intestine is swollen, while the rectum is a short very narrow tube still retaining its primitive connection with the tentacular crown, to which it is attached about one third from the base.

While the changes described above have been taking place, the tentacles have been gradually lengthening, at first, they are short finger-like processes from the periphery of the lophophore, closed above, open below, containing within their cavities an extension of the original outer layer of the bud which here forms an epithelial lining (Pl. XXXVII, figs. 8 and 9, *a*, and also woodcut, fig. 1); not till comparatively late do cilia arise on the outer epithelium, only certain aspects of the surface of the tentacles are clothed with cilia (see Pl. XXXVII, fig. 9, *a*).

The tentacular sheath ultimately becomes continuous with that portion of the endocyst of the zooecium which surrounds the mouth of the cell as was insisted upon by Nitsche (13, p. 463).

The retractor muscles of the body and lophophore arise, as noticed by Repiachoff (15) from the peritoneal lining of the polypide.

The funiculus early becomes prominent and is probably derived from the irregular strands of funicular tissue which occur in the parent zooecium; it appears as a thickish cord stretching from the fundus of the developing polypide to the base of the zooecium, and, almost invariably, it is in direct connection with the brown body, so that it serves to direct the developing alimentary tract to that nutritive mass, thereby ensuring the better nutrition of the growing bud.

Abnormalities extremely rarely occur in which there may be two buds developed, or more than one brown body, or the polypide may not come into contact with the brown body. The second abnormality probably being the result of the third.

It is thus clear that the bud in Flustra carbasea is developed at a distance from the brown body, that it approaches the latter, envelopes it, and extracts nutriment therefrom. As was pointed out by Repiachoff (16) the same occurs in several genera of Polyzoa (Tendra, Lepralia, Membranipora,

&c.). Joliet also witnessed the ingestion of the brown body in Eucratea chelata, which passed through the alimentary canal of the developing polypide, but owing to its resistant membrane the brown body suffered no alteration; but in Lepralia granifera the very thin envelope of the brown body is destroyed, not being able to resist the action of the juices of the stomach, the movements caused by the cilia, and the contractions of the intestinal walls: thus the brown granules which it contains are set at liberty, whirled about and shortly evacuated by the rectum. Hincks, himself (l.c. p. lxii, footnote) noticed the formation of a polypide-bud quite separate from the brown body in Bugula calathus.

After the able discussion of the subject by Joliet (17), it seems quite superfluous to reopen the controversy as to the probable origin of the bud from the brown-body ("germ-capsule"); but Hincks (l. c. p. lxiii) has still left it a slightly open question. According to Smitt (and Hincks), there would be at least two modes of bud-formation amongst the Polyzoa: 1. In the old zooecia (*a*) formed quite close to the brown-body, and arising directly from it, (*b*) formed at a distance from the brown-body and not arising from it. 2. In the new zooecia, also arising de novo. From the accounts of other observers, one method of bud-formation serves in all cases, the origin in an old or a new zooecium being always from the same tissue, though they are by no means agreed as to what that tissue is. It is merely a question as to how close to or how far from the brown-body the bud shall arise.

Taking all the evidence we possess, it seems to be quite evident that the generally received account is the correct one, but that the approximation of the undeveloped bud to the brown-body may mask its real distinctness in a few instances.

A further observation on a living specimen (Naples, Sept., 1881) is represented on Pl. XXVIII, fig. 13. The bud had reached the stage of Pl. XXXVII, fig. 1; it was suspended in the anterior band of endocyst, and was connected with the parent polypide by the tentacular sheath of the latter, and probably also by some funicular tissue. In this example the older polypide was rapidly histolysing into the brown-body; thus in this case the bud was formed before the complete degradation of the parent, and at a slight distance from it.

A prepared specimen (Pl. XXXVIII, fig. 14) indicates the origin of part at least of the bud from the endocyst of the opercular opening; the original occupant of this zooecium had scarcely commenced to decay.

Flustra securifrons, Pall.—Another prepared specimen from Naples (Pl. XXXVIII, fig. 15) shows a possible double origin for the lophophore and stomach in a young marginal zooecium. It will be seen that anterior band of endocyst has just been formed, and slung upon this is an undoubtedly epiblastic invagination or proliferation, coated by mesoblast. On one side is a mass of cells, which is continuous with what appears to be the incipient funiculus. This mass of cells, I take it, will form the future stomach and intestine; it soon ceases to exist as a distinct group of cells. I have several times noticed this stage.

Flustra papyracea, E. & S.—In new zooecia the buds may be seen to arise in close contact with the endocyst of the floor or of the wall of the cell, according to whether they may be terminal or lateral additions. Very shortly they assume a more central position, and are more or less thickly enveloped in a funicular plexus, from which latter there is every appearance of additions being made to their substance. The development of the polypide is exactly as described above.

In old zooecia the buds are developed in the anterior portion of the cell.

Pl. XXXVIII, fig. 16, shows a bud which is partly formed of columnar cells and partly of rounded. The latter appear to be produced at the expense of the funicular tissue; the former probably arose from the epiblastic layer of the endocyst.

Bugula flabellata, J. V. Thompson.—Pl. XXXVIII, fig. 17, shows a new zooecium, within which is the young bud, which has a well-marked bilobed appearance. Closely applied to the fundus of the stomach-sac is an ovary, which has been supplied ready-made to the bud. It is invested by the funicular tissue, which organically connects all the members of a Polyzoan colony. Figs. 18 and 19 are consecutive sections of a similar bud at a later stage, and illustrating the same point. Fig. 20 is a longitudinal section of a slightly later stage, showing the œsophageal invagination inpinging upon the stomach.

Eucratea chelata, L.—In old zooecia the bud is derived from a small mass of cells, which is situated just below the hinge of the operculum, and from the first is apparently in equally intimate connection with both the endocyst and strands of funicular tissue (Pl. XXXVIII, fig. 21); subsequently it occupies a central position just above the brown-body, and then it commences to go through the characteristic development. It is this stage which has, I imagine, deceived Joliet into believing that the bud arises from the funiculus itself.

In new zooecia the bud has a similar origin, only in this case from the base of the zooecium. Pl. XXXVIII, fig. 22, shows the lophophore to be quite distinct from the digestive tract, while the latter is closely connected with the funiculus.

Alcyonidium gelatinosum, L.—A portion of the bud, at all events, arises by invagination of the endocyst. Pl. XXXVIII, fig. 23, clearly shows that both the epiblastic and the mesoblastic layers of that tissue are equally implicated.

Fig. 24 is a longitudinal section, corresponding to figs. 7, 20, 22, &c.

I have, in fact, observed the distinctness of the lophophore from the alimentary tract in the following forms:—Bugula avicularia, B. flabellata, Flustra carbasea, F. papyracea, F. securifrons, Eucratea chelata, Diachoris magellanica, Alcyonidium gelatinosum, Vesicularia spinosa.

Larval Gemmation.

The phenomenon of budding is generally supposed to take place during the embryological history of a Polyzoan. The following very brief summary of what is known on the subject is abstracted from the late Prof. Balfour's 'Elements of Comparative Embryology,' vol. i.: the sentences within inverted commas being transferred from that invaluable work.

Entoprocta.

The larval gemmation of Pedicellina is, for convenience sake, noticed a few pages further on.

Ectoprocta—Gymnolæmata.

At the stage of thirty-two segmentation spheres the archenteron is formed by the invagination and subsequent sub-division of four (Barrois) or eight (Repiachoff) middle cells of the oral surface, but it does not appear that this archenteron is ever functional, and there is every probability in favour of the view that this functionless organ gives rise to a bud, the so-called "dorsal organ" (='pharynx' of Barrois), as the archenteron in Pedicellina has been shown to do by Hatschek (see below, p. 531). It is worth noticing that "according to Hatschek it develops as a solid outgrowth of the hypoblastic walls of the mesenteron shortly before the mesenteron joins the œsophagus (fig. 129, B, *x*)," p. 244. "A nearly similar organ to this is found in the embryo of Loxosoma [Vogt, 6, and Barrois, 1*]. Here, however, it is double, and forms a kind of disc connected with two eye spots," p. 245.

The greater part of the internal organs of the larva now degenerates and forms a nutritive or yolk-mass. "The skin of the larva after these changes gives rise to the ectocyst or cell of the future polype. The future polype itself appears to originate, in part at any rate, from the so-called dorsal organ."

"The first distinct rudiment of the polype appears as a white body, which gradually develops into the alimentary canal and lophophore. While this is developing the ectocyst grows rapidly larger, and the yolk in its interior separates from the walls and occupies a position in the body cavity of the future polype, usually behind the developing alimentary canal. According to Nitsche it is attached to a protoplasmic cord (funiculus) which connects the fundus of the stomach with the wall of the cell. It is probably (Nitsche, &c.) simply employed as nutritive material; but, according to Barrois, becomes converted into muscles, especially the retractor muscles."

"Adopting the hypothesis already suggested in the case of the Entoprocta, the metamorphosis just described would seem to be a case of budding accompanied by the destrucion of the original larva."

"This view of the nature of the post-embryonic metamorphosis is apparently that of Claparède and Salensky, and is supported by Claparède's statement (see below, p. 538) that the formation of the first polype 'resembles to a hair' that of the subsequent buds," p. 249.

Dr. W. Repiachoff (14) in his study of the development of Tendra zostericola, says that he cannot with certainty say how the inner epithelium of the middle and hind gut arises, but his figures clearly show that this tissue is intimately connected with the "brown-mass." Several figures in his plate viii, indicate the occurrence of an epiblastic involution at the pole of the embryo, opposite to that where the blastopore has closed up. This invagination will form the external layer of the tentacular sheath, the outer epithelium of the tentacles and the œsophagus of the primary zooid; it is in fact the stomodæum. The pedicle of invagination of the archenteron is absorbed, the latter being the rounded body, which he calls the "brown-mass." From one end of this a U-shaped prominence is produced, which is apparently hollow from the very commencement of its formation, the remainder of the mass being solid: this is the future intestine. The outer cells of the "brown mass" differentiate into the inner epithelium of the stomach, which soon acquires a free communciation with the exterior through the œsophagus. The central residual portion of the "brown-mass" is digested within the stomach like any other food-yolk. The "brown-mass" is surrounded by a delicate membrane, the splanchnopleure. To render the above account more clear, I reproduce his fig. 7, woodcut No. 2, which compare with woodcut No. 1.

It is clear, if the above be a correct interpretation, that the initial individual of a colony, in this species at all events, passes through a development which is normal in all its essentials, nor does there appear to be any histolysis of the primary larva.

To recapitulate—omitting the purely secondary phenomena of the external form and the behaviour of the body-wall—the blastopore closes up and the pedicle of invagination forms

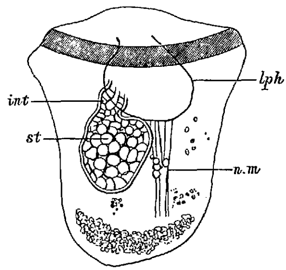

FIG. 2.—Primary zoœcium of Tendra zostericola. The tentacles, though present, are not shown. (After Repiachoff.) *lph.*, lophophore; *int.*, intestine; *n. m.*, retractor muscle; *st.*, stomach.

neither the œsophagus nor the intestine. The archenteron is at first solid; a portion of its substance is prolonged to form the intestine, which subsequently opens to the exterior outside the tentacles. The tentacular sheath and the tentacles are derived from an epiblastic depression, from the floor of which the œsophagus is evaginated, which then fuses with the stomach. The inner face of all these organs is coated with mesoblast. The details of the later development are perfectly normal.

It is possible that, in some cases, the indifferent character of the cells of the archenteron and the stomodæal invagination, have misled observers into the belief that the embryo has undergone histolysis, and that the first zooid of the colony is produced by larval gemmation, for the view of the total formation of a bud ('polypide' of authors) from the endocyst has been so firm that a well-marked involution, such as the stomodæum, would be interpreted as a bud rather than as a portion of the embryo. For myself, I am inclined to believe, with Barrois, that the occurrence of the destruction of the primitive larva is not necessarily universal amongst Polyzoa.

Ectoprocta—Phylactolæmata.

I have treated of these later on.

Other Authors' Observations on Adult Gemmation.

Entoprocta.

According to Prof. Carl Vogt, in Loxosoma phascolosomatum (6), the bud is formed by a rising of the outer cellular layer of the parent, carrying its cuticle along with it. The cavity thus produced is filled not with "cells" but with an undivided sarcodic mass, which very soon breaks up into homogeneous non-nucleated masses of protoplasm. This anomalous material at first groups itself into three masses, superiorly the hood (capuchon) or lophophore, which from the first possesses a central cavity, the vestibule; below this is a small solid mass of cells, the stomach, and inferiorly lies the pedal gland. Other differentiations of these protoplasmic masses produce the transitory pedal body between the stomach and pedal gland, the reproductive organs between the lophophore and stomach, and the general parenchyma of the body. The stomach acquires a central lumen and the intestine and rectum now make their appearance, also, by him, derived from the protoplasmic mass. They, too, are at first solid. The œsophagus is a diverticulum from the hood. The tentacles are the last organs to make their appearance, then the vestibule first opens to the outer world, and the rectum into it, and the bud becomes detached. In this form the pedal gland atrophies. The author informs us that he has tried the effect of various reagents and also section-cutting, but has "abandoned these methods, which demand so much time and care, and in the present case could give me no positive information upon points which direct observation of the living organism had failed to solve." It appears to me that the formative elements of the bud are true cells, as all other observers maintain, and that the earliest stages were incorrectly determined. The difficulties of the homologies of the parts vanish, if taking a somewhat later stage, we look upon the anterior mass with its central hollow to be an invagination from the epiblastic cells ("hypodermal layer") at the apex of the developing bud, whilst the underlying originally solid mass of cells which have primitively proliferated from the parental stomach and the pedal gland with the other internal structures, are modifications of migrated mesoblast. The development of the œsophagus as a depression from the hood also favours the interpretation, as does Vogt's account, if we except his earliest stages.

Subsequently, Prof. M. Salensky examined the gemmation of Loxosoma crassicauda (9). He describes the first stage as consisting of a small group of cells surrounding one central one. The latter by division forms a central mass which attaches itself to the anterior end of the lengthening and pedunculated outer wall of the bud. A slight longitudinal fissure appears in the ectoderm (epiblast of the bud), which is the rudiment of the orifice of the hood. The central mass becomes hollow and forms the hood and the whole of the digestive tract. "The rudiment of the digestive tube presents itself under the form of a cul-de-sac, in which two parts can be distinguished. . . . The superior part is the rudiment of the intra-tentacular depression, the inferior part is the rudiment of the digestive tube and of the rectum. . . . The superior part appears as a sac open in front. The edges of the aperture by which the sac opens now consists of ectoderm and endoderm which are completely united," p. 21. By "endoderm" Salensky means the inner layer of the double-layered bud, which tissue, according to him, forms the inner epithelium of the alimentary tract, the intra-tentacular space and the inner surfaces of the tentacles, their outer surface being formed at the expense of the ectoderm, the tentacles themselves arising just where these two layers fuse. On p. 19, he says, "the ectoderm and endoderm have arisen from the ectoderm or the integument of the mother. This fact is so clear to anyone who observes the profile of young buds of Loxosoma, that there cannot exist any doubt as to its reality. From the analogy which exists between all the species of Loxosoma, I may affirm that the described phenomena should be common to all the species."

The history of the other organs need not detain us; it is not stated from which of the two primitive tissues they are secondarily derived.

Salensky's account presents us with fewer difficulties than that of Vogt, but while agreeing with him as to the epiblastic nature of the outer layer of cells, I would suggest that the central cell, which he thinks is of the same value but does not prove it, is really derived from the alimentary canal of the parent, and is therefore hypoblastic. It is also possible that the involution which he describes, but on which he does not lay much stress, really forms the intra-tenacular space, as his account of the formation and position of that cavity appears to me to warrant that supposition, and that his (epiblastic) endoderm developes only into the stomach and intestine.

Hincks in his abstract of this paper (10), says: "I am quite unable to harmonise the account given by the author of this portion of the developmental history with that which we have from Vogt."

It will probably be found that the harmonizing of these and other accounts is possible according to the views stated above.

Prof. Oscar Schmidt (5) has propounded the original view that the bud in Loxosma cochliaris formed parthenogenetically from an egg, and that it is therefore not a true bud but an embryo! His paper is accompanied by a plate which is too sketchy to be of any value whatsoever. Nitsche and Salensky overthrow this theory, and the latter points out that buds in which no ovaries are developed may give rise to secondary buds, thus precluding any possibility of a parallelism between the bud of a Loxosoma and the ovicell of one of the Ecto procta.

Nitsche (4) has studied the gemmation of Loxosoma Kefersteinii. In this form he asserts that the bud originates from a grouping together of one or two ectoderm cells, these divide and form a single layered ring round one central cell. This latter, which he calls the "Endodermzelle," divides into two, then into four, and altimately forms a mass of cells which acquires a central lumen, and by subsequent constriction differentiates into the cavity of the hood and into the alimentary tract; the generative organs arise as a pair of lateral protuberances between the hood and the stomach; the external orifice of the hood is formed comparatively late. The muscle cells and gelatinous connective tissue of the bud are derived from two or three "Mesoderm" cells which make their first appearance when there are some half dozen "Endoderm" cells, and which are probably segmented off from ectoderm cells of the bud. He is unable to say from which layer the foot gland is derived. In this form the bud is not attached to the parent by the aboral extremity of the stem but at a spot where the body and the stem unite.

It is thus quite clear that this able investigator regards the whole bud as being derived from the epiblast of the parent.

I have cut a large number of sections to elucidate the question of the budding in Loxosoma. The form I worked at was L. tethyæ, so abundant on sponges of the genus Tethya, at Naples. Most of my specimens were killed with osmic acid and stained in picro carmine. Unfortunately my results are not so exhaustive as they might be. Pl. XXXVIII, fig. 25, shows an epiblastic down-growth from the apex of the bud, which will form the cavity of the hood; below this is a small group of cells the nature of which I am unable to state definitely; they may be mesoblastic, or they may partly or wholly be hypoblastic, for there is no reason why the closely lying hypoblast cells of the stomach should not proliferate to supply its complement towards the bud, but it must be distinctly borne in mind that I have no direct evidence at present in favour of this view. Pl. XXXVIII, fig. 26, is a slightly later stage. At a much later stage (Pl. XXXVIII, fig. 27), below the hood cavity lies the small circular stomach which contains a central cavity and which is continued into a short blind intestine which already possesses its normal curvature. I could discover no connection between the stomach and the cavity of the hood. Woodcut 3 would represent a diagram of such a stage. There is no need to point out its parallelism with a similar stage in so many other Polyzoa. The subsequent formation of an œsophagus, and the later development of the bud may be passed over.

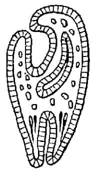

FIG. 3.—Diagram to illustrate the probable relations of the lophophore and stomach in a Loxosoma bud. The upper invagination is the lophophore cavity, the lower is the foot-gland, the compressed body within the bud is the stomach.

Uljanin (3) describes the development of the bud in Pedicellina. A protuberance of the cuticle contains some round clear cells; the outer soon arrange themselves as an epithelium, and a constriction divides off the bud from the stem; meanwhile two cavities appear in the central parenchyma; the lower and larger one he rightly regards as the stomach, the upper one he calls the "brood pouch," whereas it really is the lophophore cavity, the lumen of which is at first quite distinct from that of the stomach. There is nothing of further interest to us in his paper.

Prof. Salensky (9) has also studied the development of the bud in Pedicellina echinata, on p. 32 he says, "At the summit of the bud, several of the ectodermal cells elongate and sink within; probably these cells give rise to the endoderm." The further development of the bud follows almost precisely the same course as that which he gives for Loxosoma. As we shall immediately see, Hatschek gives a different rendering of the phenomenon, and I would point out that Salensky's figure of his earliest stage (Salensky pl. xiv, fig. 26) would very well bear the former's interpretation.

The fullest account of the budding Pedicellina is in the very careful researches of Hatschek (8), in which he shows that at the growing point of the stolon there is a single-layered tubular mass of cells lying close beneath the external epithelium, which is continually dividing into two by transverse constriction. Of these the anterior portion separates from the posterior, and becomes connected with a solid mass of cells, which have proliferated off from the external epithelium. This latter soon acquires a lumen, and we have a pair of single-layered closed sacs occupying a distinct prominence of the stolon, which are beginning to be shut off from the general cavity of the stolon by the neighbouring fusiform mesoderm cells arranging themselves into a diaphragm. Some of the scattered fusiform cells of the stolon become cut off, and so pass into the bud; but at the junction of the primitive closed sac, with the proliferating epithelium, there is one single mesoderm cell, which by division soon forms a small rounded mass, and is apparently *concerned with the formation of the generative organs.* The larger anterior sac forms the intra-tentacular space, and by the involution of its walls produces the tentacles, and of its floor the œsophagus and the hind gut. A central solid invagination, which shortly becomes a hollow sac, is the rudiment of the nervous system. The posterior smaller sac is prolonged and bent upon itself, and becomes converted into the stomach and intestine, communication taking place between the invaginations which form the fore and the hind gut.

In the embryo Hatschek has discovered that a couple of cells separate themselves from the oral side of the endoderm. These form a single-layered sac, which becomes quite detached from the alimentary tract of the embryo, and is connected with a small ciliated invagination of the lateral epiblast; it also possesses a mesoderm coating. This remarkable structure is regarded by Hatschek, with great probability, as the first bud; and it will be noticed that it contains the three germinal layers of the embryo. Unfortunately, there is a gap between this stage and the earliest of his true stolon buds; but it seems pretty evident that the primitive single-layered tubular mass of cells mentioned above is the persistent structure derived from the stomach of the embryo. Assuming this to be the case, we have then in every Pedicellina-bud the three embryonic layers, each one of which gives rise to its traditional organs, viz. the epiblast, to the external skin, the lophophore, the intra-tentacular space, the œsophagus, rectum, and nervous system; the hypoblast, to the stomach with its digestive cells, and the intestines; the mesoblast, to the muscles and general parenchyma of the body. The generative organs apparently arise from a special mass of mesoderm cells, which very early appear as a single cell, which may arise from the primitive hypoblast, or may be one of the primitive embryonic mesoderm cells. After describing the embryonic bud, Hatschek says (p. 515):—" The whole formation, which we have just studied, gives, as will be shown further on, the material for the construction of all the secondary individuals of the stock, whilst the whole of the remainder of the larva goes directly over to the primary oldest individual."

It appears that Prof. J. Reid (2) was one of the first (1845) to point out the fact that new buds form on the stems of Pedicellina echinata when the polypides die; it has also been noted by several observers since. It would be a most interesting fact if this process were found to take place when no remnant of the polypide was left. The histology and morphology of this phenomenon require to be elucidated.

Ectoprocta—Gymnolæmata
(Marine Polyzoa).

Nitsche (13) makes a distinction between the outer epithelium of the endocyst and the inner muscular layer, and he derives the outer epithelium of the lophophore and tentacles and the inner epithelium of the alimentary canal of the bud in Flustra membranacea from the former ("Epithelialschicht")—in other words, for him, the lophophore and alimentary canal of the young bud have a purely epiblastic origin. The tentacular sheath, the muscles and peritoneal lining, &c., of the polypide being derived from the inner muscular tunic of the endocyst, i. e. from mesoblastic tissue.

In Fl. membranacea all the changes in the decadence of a polyp into a brown-body can be seen; this is yet more clearly manifest in Alcyonidium hispidum. Here single zooecia likewise very frequently lose their polyps by decay, but long before the polyps have lost their characteristic form, and have become brown-bodies, the endocyst of the upper end of this zoœcium begins to form a new polyp by budding inwardly. In the same zoœcium we very frequently find a decaying polyp, which very distinctly shows its original nature, together with a new young bud, which does not differ in the least from the polyp-buds in the zoœcium-buds at the edge of the colony. Here, also, the new polyp is formed, just as the old one, by the budding of the endocyst of the zoœcium inwardly (p. 466). By "polyp" Nitsche, of course, refers to the alimentary tract, as he accepts the dual nature of the zooecium and its contained digestive apparatus.

Salensky (9) states (p. 55) that the internal tissue of the two-layered bud is derived from the external epithelium of the endocyst of the parent, and the outer from the internal layer. Here again the lophophore and digestive tract are epiblastic structures, while the mesoblast of the bud is derived from the mesoblast of the parent.

Joliet (17) refers all the buds to his "endosarc." Under the term 'endosarc' Joliet includes "all the formations which one calls under the names of colonial nervous system, funiculus, fusiform layer of the endocyst." "It is this which constitutes the muscular tunic of the fresh-water Bryozoa, the parenchyma of the stems of the stolons of the Pedicellinæ, and of the feet of Loxosoma."

He says that it is a "provisional name," "which I shall be quite disposed to change for another more general term as soon as I shall have seen, or someone has shown me, its homologue with the ectoderm or the endoderm of allied animals or of the embryo." Surely neither alternative is necessary! It will be seen from what follows that I do not regard the funiculus as a simple structure, nor the bud as entirely derived from the funiculus, therefore I cannot class all the contents of a zoœcium, save the outer layer of the endocyst, as being formed from an homologous tissue. The tissue, as he describes it, answers in position, structure, and generally in function, to the mesoblast of all other animals, and therefore it seems to be to be superfluous to coin a new term to express mesoblastic tissue.

Joliet asserts that in some cases the bud is entirely derived from the funiculus—Eucratea chelata, Vesicularia spinosa (young zoœcia), Beania mirabilis, Lepralia Martyi, and L. granifera; in others, the bud is apparently in intimate relation with the 'endocyst,' but always connected with a funiculus — Membranipora membranacea, M. pilosa, and possibly the old zoœcia of Vesicularia spinosa. It must be remembered that Joliet limits the term 'endocyst' simply to the external epithelium (epiblast) of the body wall. He says (pp. 221-2) : "When a bud forms anew upon the endocyst of an old cell, one generally sees that it is very early provided with a funiculus, which, even then, almost attains its (proper) diameter; and ever since my attention has been drawn to this point I have never seen a bud formed under these conditions which lacked this attachment. I am thus driven to believe that the buds develop by preference upon the points of the endocyst where the strands, of which I have spoken, are fixed, and thus from their earliest state they are naturally provided with a funiculus."

Thus Joliet is driven to admit, apparently against his inclination, that in some cases the 'endocyst,' outer epithelium (epiblast), may participate in the formation of the bud, he goes on to say (p. 247): "I should almost be tempted to generalise and to say, to terminate, that in all the Bryozoa the development of the polypide is made at the expense of the pretended colonial nervous system, if the Pedicellinæ did not constitute, according to Salensky, a very serious and very striking exception. This author, in a recent work (9), seeks to demonstrate that the budding of the digestive tract, which he compares to the Polypide, is made at the expense of the endocyst. I here produce a figure certainly strongly resembling his, and in which the bud is still reduced to five cells; but these cells do not appear to me to be directly united to those of the endocyst, and have always appeared to me to have more resemblance with the fusiform cells of the parenchyma.

Even supposing that the opinion of Salensky is justified, as we shall see immediately, the tissue called nervous is directly derived from the endocyst and that in the young buds of Vesicularia spinosa the granules, at the expense of which the bud is formed, belong to the colonial nervous system, and are only the cells of the endocyst recently detached from the walls, — one may say that the two cases are closely allied."

But, as in the case of Pedicellina, he begs the question by some such argument as the following :—That, according to Salensky, the new bud arises from cells proliferated from the endocyst; that the endosarc similarly arises from the endocyst; therefore we may say that in this case the bud arises from the endosarc!

It does not appear to me that Joliet's figures illustrating the proliferation of the endósarc from the "endocyst" are perfectly conclusive. The figure of the bud of Pedicellina, which he refers to above (his pl. xii, fig. 9) really proves nothing. His fig. 1, pl. xii, is possibly more to the point, but then Hatschek (accepting his statements to be true) has disposed of this most thoroughly. The only other figure he gives us is that of the vegetative extremity of a stolon of Bowerbankia imbricata (pl. xii, fig. 2) ; it remains to be proved whether this case falls with Pedicellina, or, if it exists, what is the exact interpretation of this proliferation.

Dr. E. Ehlers (16) describes the phenomena of budding in the form he has more particularly examined, Hypophorella expansa (Ehlers). In the lateral branch from the stolon which is about to form a new animal, and which we may term the bud, he finds externally a cuticle within a nucleated blastema (kernhaltiges Blastem); he does not find the two layers which Nitsche describes in Flustra, but has thought, though he cannot prove it, that the outer layer which forms the cuticle may be a Syncytium, though "I have never succeeded in showing nuclei in it," corresponding to the cylindrical layer found in Flustra. The bud increases greatly in size and early assumes a form much like the adult zoœcium. The cuticle passes into the ectocyst of the adult internally. Owing to the rapid growth there is a large central cavity, the walls are undoubtedly lined with the syncytium and with a portion of the original central blastema, while from the apex depends, icicle-like, the remainder of the blastema. This latter is to form the alimentary tract, its peritonenal lining and some of the muscles, the rest are formed as processes from the body-wall, i.e. from its inner layer. The tentacle-sheath is formed by an invagination of the tissue at the apex of the bud, so that the syncytium forms its inner lining; that is really the future outer layer of the tentacular sheath. "With regard to this homogeneous outer layer of the Hypophorella bud, in which one would like to see the homologue of a cell-layer, I must remark that I have never seen it continued into the first rudiment of the gut" (p. 109). "The endoderm appears as a separate development of the tissue of the indifferent body-wall at the spot distinguished by the above-mentioned invagination." The tentacle disc (Tentakelscheibe) or incipient lophophore is formed from the endoderm. There is very early a cavity in the formative material of the alimentary tract; this is the stomach. The tentacles grow out from the edge of the tentacle disc, at first only eight. The remaining two or three appear a little later. [It is usually stated that the permanent number of tentacles arise from the first, but in several forms, e.g. Fl. papyracea, Diachoris magellanica, &c., I have observed that four lateral ones usually appear first, the more central being the larger, but I have not yet satisfied myself as to the exact rhythm; 'lateral' has, of course, relation to the median line as marked by the mouth and anus.—A. C. H.]

As far as I can discover, Ehlers speaks of the blastema which forms the alimentary canal as "endoderm," because it does produce that structure, and naturally speaks of the remainder as mesoderm, while he really has no doubt that the outer homogeneous layer is the ectoderm. In this all critics will probably agree with him, but the exact origin of this blastema has yet to be demonstrated. I would, however, join issue with our author on one point, and that is the origin of the tentacle-disc.

On Taf. IV, fig. 34, he figures the invagination of the tentacle-sheath, and in the bottom of this depression he shows two large cells. These he imagines form the inner portion of the sheath; apparently they have the same optical character, as the incipient tentacle-disc, and from what I can make out that organ has not yet appeared. It is strange that the tentacle-sheath should so early differ in optical characters from the remainder of the alimentary tract, when it is derived, by him, from the same tissue, so I am strongly inclined to suspect that this careful observer has fallen into an error, and that the lophophore like the outer portion of the tentacular-sheath, is really an epiblastic derivative, which later on acquires continuity, as far as its cavity is concerned, with the remainder of the digestive apparatus. This correction will give morphological completeness to the whole process.

Claparède (12) derives the buds from the endocyst both in the larvæ and in the adults. In his description of the larva, he says: "From a certain spot of the endocyst an oval mass projects towards the interior, in which a cavity soon appears. This hollow structure entirely corresponds with the invaginated sac of a young Bugula-bud, the development continues henceforth in a perfect parallel with that of the bud. It is very probable that this sac arises from the primitive mouth-furrow of the larva, but I have not directly observed that it does so. I need not describe the formation of the polypide within the sac, as it resembles to a hair that of the polypides of the bud," p. 169.

From an examination of Smitt's Plates (11), it would seem that the lophophore and œsophagus are at first distinct from the digestive tract in Tubulipora serpens (pl. iv, fig. 9), and in Alcyonidium parasiticum (pl. v, fig. 13-14), and that they subsequently become united. Hincks, who is the English exponent of Smitt, clearly states Smitt's opinion that the buds are derived from the brown-body ("germ-capsule") at all events in many cases; but this view has been so fully discussed and combatted by all subsequent writers, that I need not dwell on it further.

Hincks, in his admirable Monograph (1), adds his testimony to that of Smitt, but is willing to admit that in many cases the buds may be derived from the endocyst or funicular tissue. He does not really go into the question of gemmation, nor does he give any perfectly satisfactory observations of his own, neither does he discuss the morphology of the phenomenon.

Ectoprocta—Phylactolæmata.

Freshwater Polyzoa.

Allman in his beautiful monograph (19) says: "With the exception of some peculiar forms of gemmæ (statoblasts) to be presently described, these bodies (gemmæ) always originate in the endocyst." He then goes on to describe the process of gemmation in Paludicella and in Lophopus. The figures which he gives bear out his view, but all his observations were made from living examples, and thus he has not seen the cells implicated in the process, nor verified his results by means of sections. It is thus left uncertain what exact part is played by

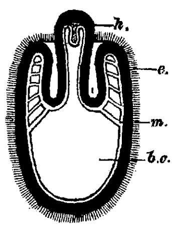

FIG. 4.—Diagram of embryo of Alcyonella, modified from Allman. *e.* Ciliated epiblast. *m.* Mesoblast. *h.* Hypoblast. *b. c.* Body-cavity.

the external cells (epiblast) and the inner network of muscular fibres (mesoblast) of the endocyst, but judging from pl. xi, figs. 5, 9, and 10—14, it would seem that the epiblast of the parent gives rise to all the alimentary organs of the bud, while the mesoblast of the mother passes into the mesoblast of the bud. I would point out that his pl. xi, figs. 7—9, and 12—15, suggest a double origin of the alimentary organs, and that the connection between the cavity of the lophophore and the lumen of the stomach occurs comparatively late.

The account of the development of Alcyonella by Dr. Allman is unfortunately far from satisfactory, and I would venture to suggest another interpretation (fig. 4) of the stage represented in pl. xi, fig. 30, and by No. 4, fig. 5, p. 34, which is, that the polypide is developed from the remains of the archenteron of the embryo, probably by a direct conversion of the walls and of the lumen of the archenteron into those of the alimentary tract of the young polypide. The lophophore and œsophagus would be derived from the overlying epiblast. The remainder of the body wall of the embryo, consisting of epiblast and peripheral mesoblast, "becomes enveloped in an ectocyst, to constitute the cell of the adult polyzoon. The subsequent changes are produced by the gemmation of new polypides, with their proper ectocysts and endocysts" (p. 35). In other words the embryo passes over entirely into the first adult of the colony. At a very early stage, between figs. 30 and 31, a second polypide makes its appearance; there is little doubt that this second bud is constricted off from the older polypide, although Allman leaves one to suppose that it, like the former, "appears to take place in a manner quite similar to that by which new polypides are produced by gemmation from the walls of the endocystal cavity in the adult" (p. 34). It is very unfortunate that Allman should have derived the alimentary canal from the epiblast, when hypoblast already was present in the embryo.

"Plumatella fruticosa presents similar developmental phenomena; the ciliated larva, however, in this species, differs from that just described, in having its polypide single."

I do not propose to discuss the morphology of the statoblasts at present. Allman (l. c. p. 38) describes how they take their origin entirely from the funiculus. Ultimately "a young polyzoan gradually emerges and floats away. . . . At the period of its escape it possesses all the essential organization of the adult. . . It loses no time, however, in developing gemmæ, which soon change it to the compound form of the adult" (p. 39).

Metschnikoff (21) in his studies on Alcyonella describes the formation of the bud in the embryo. Allman in his Presidential Address to the Linnean Society (24) thus narrates it. The segmentation of the egg produces "a central cavity surrounded by a double layer of cells. This constitutes the cyst of the well known Alcyonella-larva, within which two polypides subsequently make their appearance by budding. In this budding both lamina of the cyst-walls participate. The outer lamina serves for the formation of the outer epithelium of the tentacles, and the inner epithelium of the alimentary canal; while the central nervous system, which in the larva is very large, is also most probably derived from it. The inner lamina, on the other hand, forms all the muscles of the body, as well as the genitalia and the inner epithelium of the body cavity."

Nitsche (22) has also studied the budding in Alcyonella fungosa and in Cristatella mucedo. I again quote from Allman's address, p. 499, "He (Nitsche) had already shown that the wall of the cystid or zoœcium of Alcyonella consists of three different layers besides the externally excreted ectocyst or cuticula. These are an outer epithelium, an inner epithelium, and a tunica muscularis lying between the two and consisting of a structureless supporting membrane on which lie transverse and longitudinal muscular fibres. The first indication of the polypide-bud shows itself as a sac-like bulging inwards of the cystid wall. In this bulging the tunica muscularis, however, takes no part, but seems to be absorbed at the spot where the bud occurs. The polypide-bud consists therefore at this stage of a two-layered cellular sac, whose inner layer, bounding its central cavity, passes continuously into the outer epithelium of the cystid wall, while the outer layer is continuous with the inner epithelium of the cystid. Nitsche follows Metschnikoff in regarding the outer epithelium of the cystid as the outer germinal layer or ectoderm, the inner epithelium as the inner germinal layer or endoderm; and if we further regard the tunica muscularis as a middle germinal layer or mesoderm, we may view the young polypide-bud as composed of two concentric cellular layers, the internal derived from the ectoderm, the external from the endoderm of the cystid, while the mesoderm of the cystid takes no part in the formation of the bud. . . Folds and secondary introversions of this two-layered cellular sac give to the young polypide its definite form. . . The inner epithelium of the alimentary canal is derived from the ectoderm of the cystid, while the outer is derived from the endoderm. The two layers of the tentacular sheath have a precisely similar derivation." A detailed account of the further development is given, and on p. 501 we read, "as Nitsche suggests, we must not in the present instance lose sight of the fact that the inner layer of the bud, though arising from the ectoderm of the cystid, has fundamentally different relations from those of an ordinary ectoderm, for there proceeds from it, at the same time with the nervous substance of the ganglion, the entire epithelial lining of the intestinal tract." While the "endoderm" of the cystid behaves in all respects like an ordinary Mesoblastic tissue. What could have been the conditions which in process of time have so upset the traditional functions of the germinal layers!

Hatschek (8) describes, in Cristatella, the relations of the polypides to the colony and the increase by gemmation. In the lateral growing points he finds that there is a single-layered sac, depending within the cavity of the stolon and slung by a mesodermic layer. This sac constricts into two unequal portions: the portion constricted off becoming the inner epithelium of the alimentary tract of a new polypide, the tentacular portions being supplied by an epiblastic involution (Hatschek, fig. 3, p. 539). This process being repeated, Hatschek says that Nitsche's figures (22 and 23) do not prove his statement that in Alcyonella the inner layer of the polypide sac is derived from the ectoderm of the cystid, and goes on to say that Nitsche's figures will bear his (Hatschek's) interpretation of the similar process in Cristatella. This continually constricting sac described by Hatschek lies between the epiblast and the mesoblast of the stolon, and it is quite open to us to discuss its morphological value. If we look upon this sac as hypoblastic tissue derived from the archenteron of the embryo, the budding of these fresh-water Polyzoa would present no difficulty. Allman describes the initiatory steps of the formation of a colony. If we accept a different (i.e. a hypoblastic) origin of the internal epithelium of the alimentary tract of the earliest polypides than that which Allman indicates, then the two accounts mutually assist one another.

Reinhardt (25) states that in Alcyonella fungosa and Cristatella mucedo: "After segmentation the mass is converted into a gastrula by invagination; the gastrula-mouth closes and the segmentation cavity disappears." . . .

"The cystid (in Cristatella) consists, as in Alcyonella, of an ectoderm, a median layer (the tunica muscularis), and an entoderm. Thus, Hatsckek must be wrong when he names the inner layer of the bud mesoderm, and his description of the budding is inexplicable by comparison with the abovementioned details, though these may perhaps correspond with his second unknown process of budding. The bud develops by a thickening of the ectoderm into which the entodermic cells are pushed; there is no indentation of the former. The tunica-muscularis is very early formed; the cavity of the tentacle-sheath is separated later from the alimentary canal, and the lophophore is formed by an invagination into this tentacle-sheath. The later development of the buds corresponds with that described by Nitsche in Alcyonella." (From the English abstract.)

Reinhardt clearly gives us the three germinal layers, it is difficult to understand him perfectly as he gives no figures, but, accepting his statements, we apparently have an embryo in which the alimentary canal has a retarded development, an embryo which is, in fact, all body-cavity, such an embryo can easily result from an ordinary enterocoelous form, such as Argiope, Sagitta, &c., by an exaggeration of the cœlomic diverticula and a simultaneous arrest in the formation of the permanent alimentary canal. The following diagram (fig. 5) will sufficiently explain my meaning. This would make the body-cavity of these forms an enterocoel. The musculature of the body-wall appears to develop prior to the formation of these diverticula.

This author brings into harmony the observations of Allman, Metschnikoff and Nitsche, for we have only to concede that the epithelial lining of the body-cavity of the embryo (cystid) in my interpretation of Allman, (which, by the bye, was made before I had seen the accounts by Nitsche and Reinhardt), is derived from archenteric diverticula; an earlier or later development of the tunica muscularis between the two layers, is really of little consequence.

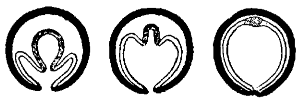

Fig. 5.—Diagram representing a possible degradation in the formation of the alimentary tract from an originally enterocoelous larva.

Salensky (9) states that his own observations on Paludicella have convinced him that the superior layer of the zooecium gives rise to the lophophore and the internal epithelium of the polypide, while the inferior layer is transformed into the interior layer of the zoœcium, the tentacular sheath, and, at the same time, into the muscles. He says (p. 56):—"It is impossible not to remark the interesting analogy existing between these two layers and the embryonic layers of other animals." . . . . "It is acknowledged by several recent embryological researches that the endoderm of many animals forms from the ectoderm, sometimes as a thickening of this latter, sometimes as an invagination."

This "analogy," which other authors have remarked, must not be relied on as giving any true insight into the nature of the phenomenon of budding, for we cannot look upon the epiblastic layer of the endocyst as being morphologically equivalent to the embryonic cells (segmentation spheres) of larvæ in the blastula stage. Moreover, the invaginated or grown-over hypoblast cells of the gastrula stage are not derived from the epiblast (ectoderm). Before this difference in position these two layers are usually optically, and they certainly are morphologically and physiologically, quite distinct—apparent optical similarity can never constitute morphological identity. The fundamental difference between the epiblast and the hypoblast is shown by the usually very early distinction between these two layers. For instance, the two layers are often practically distinguishable in the stage of eight segmentation spheres, and even in some cases the first segmentation furrow marks their distinctness. We must, therefore, disallow the use of the term "endoderm" for that mass of cells derived from the epiblast of the parent zooecium, which is supposed to give rise to the alimentary canal of the new polypide.

We can agree with Salensky when he continues:—"From its position and from the formations which it produces, the inferior layer of the zoœcium presents a great resemblance to the mesoderm." He might have added that they are one and the same.

This method of bud-formation he believes to be common to the whole group of the Polyzoa.

A. Hyatt, in his elaborate memoir on the 'Sub-order Phylactolæmata' (20), scarcely alludes to the phenomenon of gemmation. On p. 221 he says:—"The free part of the endocyst of the cell on the abdominal side, bringing forth true buds." And on p. 218 he gives a sketchy account of how "the statoblastic polypide begins to multiply by the process of budding. An internal swelling of the endocyst, on the lower side, in the vicinity of the bases of the anterior retractor muscles, first shows the position of the coming polypide. This elongates into a little hollow sac with a thickened rim, upon the upper edge of which, in the Hippocrepian Polyzoa, a slight notch is formed by the duplication and pushing out of its sides into two loops joined along the centre" (the lophophore). . . . "A transverse constriction of the body of the little sac draws the line between the œsophagus and the stomach; and the subsequent deepening of this constriction divides off the internal cavity, establishing the cardiac and pyloric valves." The figures which Mr. Hyatt gives are most unsatisfactory, nor does he appear to have checked his observations by means of sections. The minute outlines which he gives of Cristatella ophidioidea (Hyatt) will equally well bear Hatschek's interpretation. The incipient bud of Fredericella regina (Leidy) (pl. vii, fig. 5, v) is made to depend from the endocyst, but we are not informed as to the significance of the two layers which he there depicts. The figure of Plumatella arethusa (Hyatt) may have any interpretation. The only point which is quite clear is that Hyatt believes that the polypide buds are entirely derived from the endocyst of the parent.

Dumortier (18) made some interesting observations on Lophopus crystallinus. He states that he has seen balls of mucus floating in the general fluid of the body become attached to the body-wall. "I have said that these globules appear to be of the nature of mucus, for, besides that they are formed by the stomach, an eminently mucous organ, their substance does not permit the supposition that they are endowed with any organised tissue" (p. 49). "The adventitious bud once disposed of, it soon establishes a focus of irritation in that place, which will excite the development in the interior of its mass, and a protuberance at the exterior so as to make a bump." Dumortier states that the alimentary tract is developed from this ball of mucus, while the tentacles are developed from the body-wall. The tendency of his statements is towards the view of the hypoblastic origin of the digestive portion and an epiblastic origin of the lophophore, though, of course, this view of the case could not present itself to him (1836).

General Conclusions.

In all cases of budding in the animal kingdom, so far as I am aware, it has been shown that representatives of the three primary germinal layers enter into the bud, and there form corresponding tissues, but, strangely enough, the Polyzoa form an apparent exception to this rule, as the buds are said to arise solely from the endocyst (Nitsche, &c.), or from the endosarc (Joliet). Assuming the generally received opinion of the nature of these tissues, in neither case would the bud have any hypoblast in its composition. It is inconceivable to me how a bud could originate unless it possessed an offshoot from all the essential organs of its parent: that is to say, the bud should possess a portion of the parental epiblast, mesoblast, and hypoblast; for how could either the epiblast or the mesoblast suddenly depart from its ancestral traditions and take upon itself the function of digestion? It is conceivable that, in process of time, the method of gemmation should be considerably modified, but hardly that one of the most important of the three primary embryonic tissues should not be represented at all. Embryologists are fully conversant with variations in the development of organs, and with the masking of the origin of certain organs, as in the case of "precocious segregation," but they nevertheless have firm faith in the essential "conservatism" of the layers themselves.

The question now before us is: Are the three germinal layers represented in the buds of Polyzoa? The following are my reasons for answering this in the affirmative.

Nitsche and others, as we have noticed above, would derive the whole of the bud from the endocyst—that is, from the epiblast and peripheral mesoblast. Joliet, in combating this view, points out that in Eucratea chelata and in all the Cheilostomata which he has studied he has reason to believe that the bud is really formed on some portion of the endosarc, and not on the endocyst. In Hypophorella, Ehlers, the bud "is produced on the funiculus in the centre of the cell, as in Eucratea. In many cases it is developed at the very base of the zoœcium, immediately over the communication plate or septum and the orifices through which the connective threads pass, and therefore probably in connexion with the endosarc. I [Hincks] have observed it in this position in the young cell of Beania mirabilis; and in this species Joliet has convinced himself that the polypide is actually derived from the endosarcal cord. In the rudimentary zooecium of Victorella pavida the forming polypide seems to me [Hincks] to be enveloped in the endosarcal plexus, and to be (in all probability) produced by it. . . . It may be, as Joliet suggests, that the authors who have referred it to the endocyst have not been sufficiently alive to the distinction between these two tissues. It may be that the function is to some extent shared by the endocyst" (Hincks, pp. l, li).

Joliet thus clearly believes in the endosarcal origin of the bud. This 'endosarc' is, by him, derived from the endocyst. In his use of the term 'endocyst' one must not understand both layers, but only the outer. This seems to be clear from Joliet's account, and from his deriving migratory cells also from the growing end of the outer layer. The inner layer of the endocyst (peripheral mesoblast or somatopleure) is composed, according to all authors, of fusiform cells—i. e. cells similar to the characteristic cells of Joliet's endosarc. It is thus certain that Joliet would consider the buds of the Polyzoa as composed solely of mesoblastic tissue, or possibly of some modified epiblast as well.

We have seen that in Flustra carbasea the tentacles and the mouth area arise from one mass of tissue, and from the latter an invagination takes place forming the mouth and œsophagus (Stomodæum); whereas the stomach and intestine arise from another mass of tissue. These two closed sacs (Stomodæum and stomach) later on unite to form a continuous tube. It was this well marked double origin of the digestive tract which first led me, when in Naples, in 1879, to study the question of Polyzoan gemmation. I have already enumerated some of the forms in which I have since seen the same phenomenon.

The resemblance of the above to the formation of similar structures in the embryos of so many animals is most striking, and seems to suggest that we have here to deal with an epiblastic derivative which forms the outer layer of the tentacular sheath, the outer epithelium of the tentacles, the mouth area, and the lining of the œsophagus; and with a hypoblastic derivative which occupies itself with the inner lining of the stomach and intestine. We may safely assert that the outer layer of the incipient polypide is mesoblastic as it develops into the inner layer of the tentacular sheath, the inner epithelium of the tentacles (somatopleure), and into the investing sheath of the alimentary canal (splanchnopleure), as well as into the muscles of the future polypide. Nitsche and Hatschek show for the Phylactolæmata, and the latter for Pedicellina, that the nervous system is derived from an epiblastic invagination. There are no observations for the Gymnolæmata, as to the ganglion, but it is in such close contact with the lophophore that we may safely assume its origin from that body. This would, of course, give it an epiblastic derivation.

Prof. G. J. Allman was, I believe, the first to promulgate the view that the zooecium and the polypide are distinct individuals, at all events this statement is very generally accepted; but it seems rather incredible that generations of individuals solely composed of a digestive canal and its appurtenances, such as muscles and nerve ganglion, segmental organ, and possibly generative organs, should live within the body cavity of one persistent individual which lacks these organs and only possesses a body wall, funiculus (?) and body cavity.[1]

[1] The analogies which have been drawn between this supposed phenomenon, and the undoubted cases of physiological and structural differentiation amongst the Hydrozoa, will not really hold good; for in these the buds, though still connected, are all external, and their specialisation can readily be accounted for; whereas, in the other case, each successive internal so-called bud develops within the body-cavity of its parent in such a manner as to have precisely the same relations as if it really was its alimentary tract, and not a bud. It is not easy to conceive how this could come about, nor is it rendered any easier if we yet farther follow the distinguished author of this view, and regard the zooecium as the host not only of a nutritive polypide, but also of male and female individuals; for Prof. Allman suggests that even the testis and the ovary are, save sexually, aborted polypides!

It is impossible to regard the body-wall and the alimentary canal of the Entoprocta as distinct individuals, and their gemmation resembles, in its essentials, that of those animals which can multiply by budding (e.g. Ascidians). The budding of some of the Phylactolæmata, too, does not necessitate this strange commensalism. Why, then, should it only occur amongst the Gymnolæmata?

Let us admit that the previous inhabitant of a zoœcium dies away altogether, but before doing so gives rise to a bud in a normal manner, which bud is primitively located on the oral wall of the zoœcium of its parent. The future history of the bud would present no startling peculiarities if its growth were to take place in two directions; if some of the epiblastic and mesoblastic portions of the bud tended to form the body-wall of the new Polyzoan: as it is already provided with an ectocyst there would be no need to form a new one, so the new body-wall would simply be applied to the dead cyst. Meanwhile, an epiblastic involution depends into the body cavity of the newly-formed individual, carrying down with it the hypoblastic derivation from the parent, both being coated with a mesoblastic sheath. This is the structure which has been regarded by authors as a whole bud, and which has been variously termed "bud," "zooid," "polypide," and "polyp," but which I make bold to say is merely a portion of the new bud. (It will be noticed that in the preceding pages I refer to this structure under the generally received terminology, I purposely do so to prevent any confusion.) I have already detailed the future history of this part of the bud, so it would be superfluous to repeat it again here, and that of the body-wall has no especial interest.

It might be objected that the funicular tissue extends throughout the entire colony, and that it does not die with the temporary inhabitants of the zoœcium; assuming this to be the case, there is nothing to prevent this tissue being enclosed by the body-wall of the growing bud, without its being a primitive portion of that bud, after being thus enclosed it would serve to connect the new member with the rest of the colony, and by this means the bud would be engrafted into the life of the whole, for, undoubtedly, without being histologically nervous, this tissue can transmit stimuli, and it certainly possesses other important functions. It is difficult to conceive of portions dying and being renewed de novo, besides, having such undifferentiated functions it would à priori have greater vitality and be less likely to die with each individual, especially as it is all the time protected from external damage by the walls of the zoœcium.

I have shown that in Eucratea chelata the bud partly arises from the endocyst, and therefore we must be cautious in accepting Joliet's statement as to the universality of the origin of the buds from the endosarc.

We, have, however, just seen that Joliet and Hincks lead one to imagine the possiblity of different tissues, the endocyst and the endosarc (funicular tissue) being implicated in the gemmation of certain forms, and my own observations very strongly incline me to this view. Hatschek's beautiful investigations are very clear as to the complex origin of the bud, and practically prove that all the three germinal layers are concerned in the budding of Pedicellina and Cristatella.

To recapitulate:—In the Entoprocta, Hatschek's observations prove the process of gemmation to be normal in Pedicellina. My own on Loxosoma indicate that no real anomaly exists in that form.

The discrepancies of most observers, combined with the errors of some in their interpretation of the phenomena in Pedicellina, will allow us some latitude in dealing with the generally received views on the budding in Loxosoma.

In the Phylactolematous Ectoprocta, Hatschek's account of Cristatella gives a clue as to what will possibly prove to be the characteristic method of gemmation in the group, and it is one which has every morphological probability.

The absence (?) of statoblasts in Paludicella may perhaps be accounted for by supposing that, compared with the true Phylactolæmata, this form is a late immigrant into fresh water, and that it still retains most of the structural characteristics of the Marine Ectoprocta. If this be the case, it is probable that the mode of gemmation in this Polyzoan will be found to resemble that in the latter rather than that in the former.

The Gymnolæmatous Ectoprocta present us with the greatest difficulty, and it must be remembered that we have here to deal with a highly specialised and at the same time degraded group—the degradation being mainly caused by the sessile habits and by the secretion of a strong protective covering, resulting not only in the loss or diminution of certain organs, such as a muscular body-wall, nervous system, sense organs, excretory ogans, &c., but also in the simplication of certain tissues. This is especially noticeable in the body-wall and in the mesoblastic tissues generally, the tendency apparently being for these tissues to lose their distinctive cellular character and to form syncytia or even plasmodia; for the vagrant protean funiculus is more comparable with a plasmodium, in which the fusiform cells described by Joliet are immersed, than with an ordinary cellular tissue.

In many forms of this group both the endocyst and the funiculus appear to take part in the gemmation. I would again draw attention to the marginal buds of Flustra (Pl. XXXVIII, fig. 16) and Bugula flabellata (Pl. XXXVIII, fig. 17), in the latter of which the ovary lies in such close contact with the fundus of the developing stomach that it suggests something more than a secondary attachment. The ovary (shown by Huxley to be developed from the funiculus in Bugula avicularia), as is well known, passes ready formed into some buds imbedded in certain funicular tissue. Might we not assume that the stomach tissue also has a similar origin? Indeed, some still earlier buds exhibit a very close connection between the stomach and the funiculus. In most of the forms enumerated on p. 523 I have seen the stomach intimately united with the funiculus in early buds, and, though I have not yet been able to prove that the stomach mass does absolutely and entirely arise from the funicular tissue, yet the evidence in favour of that view is, to my mind, very strong.

There is, however, a certain amount of direct evidence that a portion of the bud is derived either from an invagination or from a proliferation from the outer layer of the endocyst—in other words, from the epiblast of the parent organism (see Pl. XXXVIII, fig. 23, &c.).

Every one will agree that the bud contains mesoblastic elements directly derived from the parent.

Assuming, then, that the digestive tissue of the bud is derived from the funiculus of the parent, a new construction must be put upon this important organ of the Polyzoa, necessitating a hypoblastic origin for a part at least of this much discussed tissue. I would venture to suggest that, at all events in the Gymnolæmata, a portion of the cord is indirectly derived from the archenteron of the embryo which initiated the colony. This derivative may be plasmodic rather than cellular, and probably is more or less clothed with degenerate mesoblast. If subsequent investigations can demonstrate this, then the anomalous character of Polyzoan gemmation will be taken away, and the phenomenon reduced to a more normal method.

Whatever value the suggestions just put forth may possess, this paper will at least indicate the lines upon which this question must be approached in the future.

Bibliography.

General.

(1) T. Hincks.—"A History of the British Marine Polyzoa." London, Van Voorst, 1880.

(1*) J, Barrois.—"Recherches sur l'emb. des Bryozoaires," Lille, 1877.

Entoprocta.

(2) J. Reid.—"On the Anatomy and Physiology of some Zoophytes," "Ann. and Mag. Nat. Hist.,' vol. xvi, 1845.

(3) B. Uljanin.—"Zur Anatomie u. Entwickl. der Pedicellina," No. 2, 'Bull. Soc. Imp. des Natural,' Moscow, 1870.

(4) H. Nitsche.—"Beiträge zur Kenntniss der Bryozoen," "Ueber den Bau u. d. Knospung v. Loxosoma Kefersteinii," 'Zeit. f. Wiss. Zool.,' Bd. xxv, Suppl. Bd., p. 361, 1875.

(5) O. SCHMIDT.—"Die Gattung Loxosoma," 'Archiv. f. Mik. Anat.,' xiii, 1876.

(6) C. VOGT.—"Sur le Loxosome des Phascolosomes," 'Archiv. de Zool. expér. et génér.,' Tom. v, 1876.

(7) T. HINCKS.—"Loxosoma, by Carl Vogt," Translation and Condensation of above, 'Quart. Journ. of Mic. Sci.,' vol. xvii, New Ser., 1877.

(8) B. HATSCHEK.—"Embryonalentwick. u. Knospung d. Pedicellina echinata," 'Zeit. f. Wiss. Zool.,' Bd. xxix, 1877.

(9) M. SALENSKY.—"Études sur les Bryozoaires Entoproctes," 'Ann. des Sci. Nat.,' 6 sér. Zool., Tom. v, 1877.

(10) T. HINCKS.—"Salensky on the Polyzoa Entoprocta," Abstract of above, "Quart. Journ. of Mic. Sci.,' vol. xviii, New Ser., 1878.

Ectoprocta Gymnolæmata.

(11) F. A. SMITT.—"Om Hafs-Bryozoernas Utveckling och Fettkroppar," 'Æfvers af Kongl. Vet.-Akad. Förh.,' 1865, No. 1. Stockholm.

(12) E. CLAPARÈDE.—"Beiträge zur Anat. u. Entwick. d. Seebryzoen," 'Zeit. f. Wiss. Zool.,' Bd. xxi, 1871.

(13) H. NITSCHE.—"Beiträge zur Kenntniss der Bryozoen," 'Zeit. f. Wiss. Zool.,' Bd. xxi, 1871.

(14) W. REPIACHOFF.—"Zur Entwick. der Tendra zostericola," 'Zeit. f. Wiss. Zool.,' Bd. xxv, 1875.

(15) W. REPIACHOFF.—"Zur Naturgeschichte der chilostomen Seebryozoen," 'Zeit. f. Wiss. Zool.,' Bd. xxvi, 1876.

(16) E. EHLERS.—"Hypophorella expansa. Ein Beitrag. zur Kenntniss der minirenden Bryozoen." Göttingen, 1876. ('Abhandl. d. Königl. Gessells. d. Wiss. zu Göttingen,' Bd. xxi, 1876.)

(17) L. JOLIET.—"Contributions a l'hist. nat. d. Bryozoaires des côtes de France," 'Arch. d. Zool. Exp.,' vol. vi, 1877.

Ectoprocta Phylactolæmata.

(18) B. C. DUMORTIER.—'Mém. sur l'anat. et la phys. des Polypiers composés d'eau douce nommés Lophopodes.' Tournay, 1836.

(19) G. J. ALLMAN.—'A Monograph of the Fresh-water Polyzoa,' Ray Society, London, 1856.

(20) A. HYATT.—"Observations on Polyzoa, Suborder Phylactolæmata," 'Proc. of the Essex Inst.,' vol. iv, No. 8, 1865. Salem, Mass.

(21) METSCHNIKOFF.—'Bull. de l'Acad. de St. Pétersbourg,' Tom. xv, 1871.

(22) H. Nitsche.—"Untersuch. u. d. Knospung d. Süsswasserbryozoen, insbesondere der Alcyonella," 'Sitzungsberichte der naturforschenden Gesellschaft zu Leipzig,' 1874.

(23) H. Nitsche.—"Ueb. d. Knospung der Polypide d. Phylactolæmen Süsswasserbryozoen," 'Zeit. f. Wiss. Zool.,' Bd. xxv, Suppl. Bd., p. 343, 1875.

(24) Allman.—"Recent Progress in our Knowledge of the Structure and Development of the Phylactolæmatous Polyzoa," Anniversary Address, 'Jour. Linn. Soc.—Zool.,' vol. xiv, 1879.

(25) W. Reinhardt.—"Zur Kenntnis der Süswasser-Bryozoen," 'Zool. Anzieger,' iii, No. 54, 1880, Abstract in 'Journ. of Roy. Mic. Soc.,' iii, No. 4, 1880.

EXPLANATION OF PLATES XXXVII, XXXVIII.

Illustrating Prof. Haddon's paper "On Budding in Polyzoa."

The letters in all the figures have the same signification.

b. b. Brown body. *bd.* Bud. *coe.* Coecum of stomach. *c. p.* Communication plate. *ct.* Cuticle. *ec.* Ectocyst. *en.* Endocyst. *ep.* Epiblast. *f.* Funiculus. *f. t.* Funicular tissue. *hy.* Hypoblast. *int.* Intestine. *lph.* Lophophore. *m.* Mesoblast. *msc.* Muscle. *œ.* Œsophagus. *op.* Operculum. *ov.* Ovary. *p. gl.* Pedal gland. *p. m.* Parietal muscles. *p. v.* Pyloric vestibule. *r.* Rectum. *r. m.* Retractor muscle. *st.* Stomach. *t.* Tentacles. *t. s.* Tentacular sheath. *x.* Cells of doubtful origin. *z.* Zoœcium.

FIG. 1.—Two layered bud of Fl. carbasea attached to anterior band of endocyst of an old zoœcium.

FIG. 2.—Bud in which the tentacle sheath has become differentiated.

FIG. 3.—Side view of a later stage (from a prepared specimen), showing the earliest appearance of the stomach-mass and the incipient tentacles.

FIGS. 4 and 5.—Front and back view of the same bud. The bud now consists of an anterior disc produced at its margin into a number of tentacles, from one side of which depends the digestive canal. The latter is directly connected with the brown body by means of the funiculus.

FIG. 6.—Later stage, showing the commencement of the œsophagus as a diverticulum from the disc of the lophophore; the digestive canal has indications of its division into stomach, intestine, and rectum, the cæcum of the stomach is also making its appearance. The retractor muscle is now well developed, each muscle fibre possessing a well-marked nucleus.

FIG. 7.—Optical section of a stage slightly earlier than that of Fig. 6, showing the structure of the bud, and the relation of the latter to the brown body and to the zoœcium. The fibres of the retractor muscle are not yet differentiated.

FIG. 8.—Stage in which the cæcum of the stomach has come into contact with the brown body.

FIG. 9.—A later stage, in which the brown body has become surrounded by

the cæcum of the stomach of the bud. The style-like body in the intestine probably indicates that a certain amount of digestion has already commenced. Circular muscles have appeared in the walls of the rectum, giving it an annulate appearance.

FIG. 9*a*.—Portion of tentacle of above, the central epithelium has shrunk away from the outer as the animal was dying.

FIG. 10.—A yet later stage, in which the cæcum of the stomach has attained its adult position, it having passed through an angle of 90°, and having now an upward instead of a downward direction. The contained brown body has commenced to be disintegrated.

FIG. 11.—The brown body has almost entirely disappeared, its remains now being found in the intestine; before passing thence they are whirled about in the richly ciliated pyloric vestibule. The intestine is greatly distended by its contents; it would appear that the mass nearest to the rectum is purely fæcal, while those portions nearest to the stomach are in process of digestion and absorption. As the polypide is retracted close to the bottom of the zoœcium, the retractor muscle is shortened and coiled.

FIG. 12.—The adult bud, from which the brown body has entirely disappeared, and in which the walls of the stomach and its cæcum are provided with numerous digestive cells. Circular muscles have also made their appearance in the walls of the stomach. The mouth is ciliated.

FIG. 13 shows a zoœcium, within which the polypide is rapidly histyolising to form a brown body. The upper portion of the zoœcium already contains a young two-layered bud of about the same age as that of Fig. 1.

FIG. 14.—A portion of a zoœcium, showing the operculum and the tentacular sheath of the contained polypide. A prolongation depends from the anterior band of endocyst which will form a part at least of a new bud.

FIG. 15.—Early stage of bud-formation in Fl. securifrons. The new zoœcium is lined with a delicate endocyst, which superiorly is forming the operculum.

FIG. 16.—A zoœcium of Fl. papyracea with a developing bud, which suggests a possible double origin from the endocyst and funicular tissue.

FIG. 17.—Longitudinal section through a young marginal zoœcium of Bugula flabellata, showing the mass of cells which will develop into the lophophore and the more or less distinct stomach; to the fundus of the latter the funiculus and ovary are very closely applied, these two structures being supplied to the new zoœcium from the old zoœcium, of which the new one is a bud.

FIGS. 18 and 19.—Successive sections through a similar terminal zoœcium, illustrating the same points.

FIG. 20.—Longitudinal section through a slightly older bud, demonstrating

the separate lophophore and digestive tract. The œsophagus is arising from the former as a diverticulum.

Fig. 21.—Origin of the bud in Eucratea chelata, as a thickening of the endocyst below the mouth of the zoœcium, and possibly also from the funicular tissue.

Fig. 22.—Young terminal zoœcium, showing the double nature of the lophophore, and the stomach, &c.; also the intimate relation of the latter to the funiculus.

Fig. 23.—Origin of a portion at least of the bud of Alcyonidium gelatinosum as a diverticulum of the endocyst, in which the epiblast of that structure forms the inner layer and the mesoblast a muscular layer, &c.

Fig. 24.—Section showing the double origin of the lophophore and digestive tract in the same.

Fig. 25.—Section of a young bud of Loxosoma tethyæ, with the epiblastic invagination and underlying group of cells of doubtful origin.

Fig. 26.—Slightly older stage of ditto, with incipient mesoblastic muscle-cells.

Fig. 27.—Still later stage, with the pedal-gland as an epiblastic invagination. The stomach and intestine are separate from the lophophore. The intestine is in reality in a lower optical plane than the other organs.

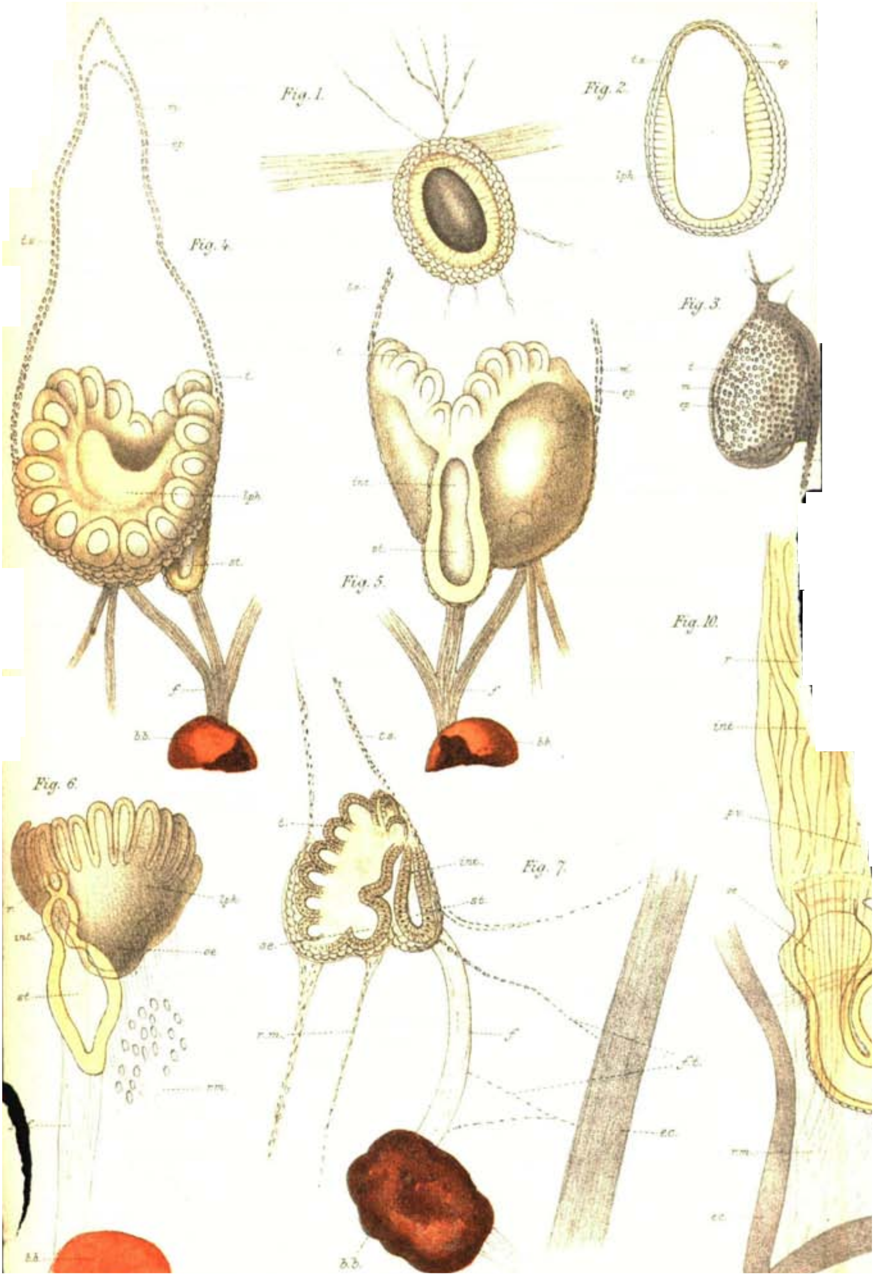

Fig. 1.
Fig. 2.
Fig. 3.
Fig. 4.
Fig. 5.
Fig. 6.
Fig. 7.
Fig. 10.

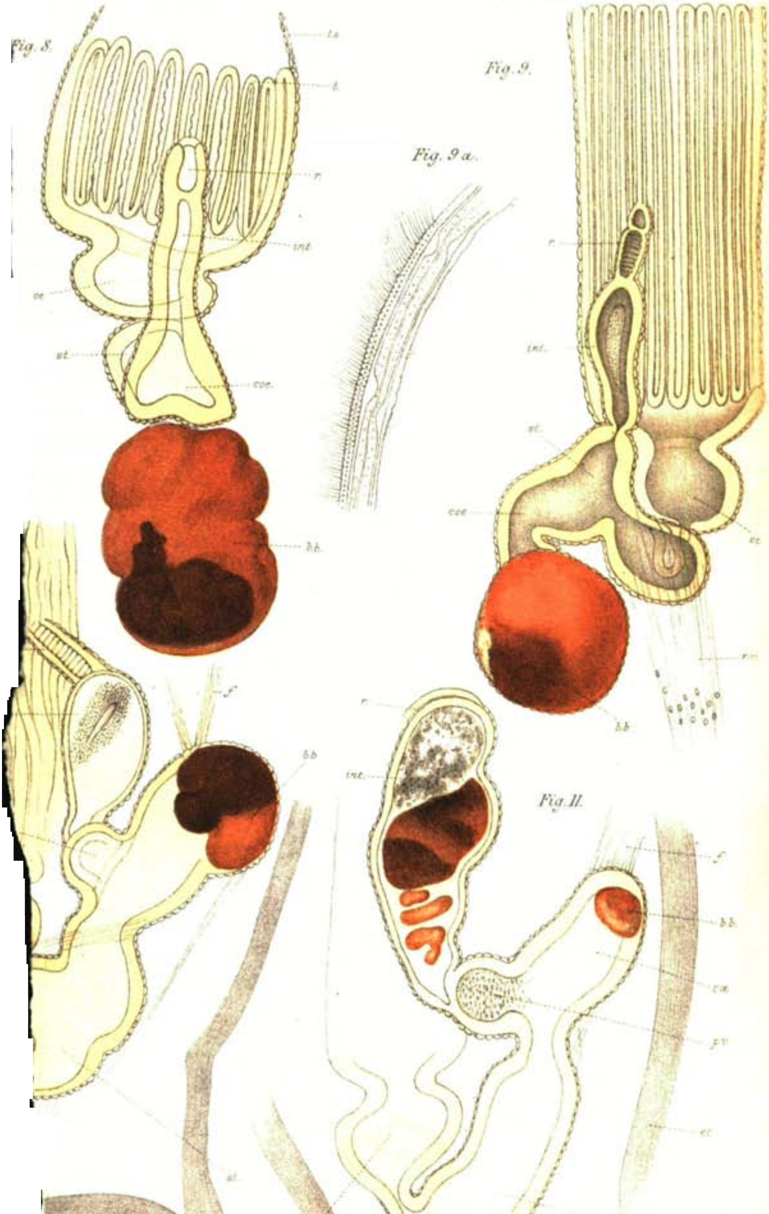
Fig. 8.
ts
t
r
int
oe
st
coe
b.b.
f
bb
Fig. 9.
r
int
st
coe
ec
rm
bb
Fig. 9 a.
Fig. 11.
r
int
f
b.b.
cm
pv
ec
st

Fig. 12.

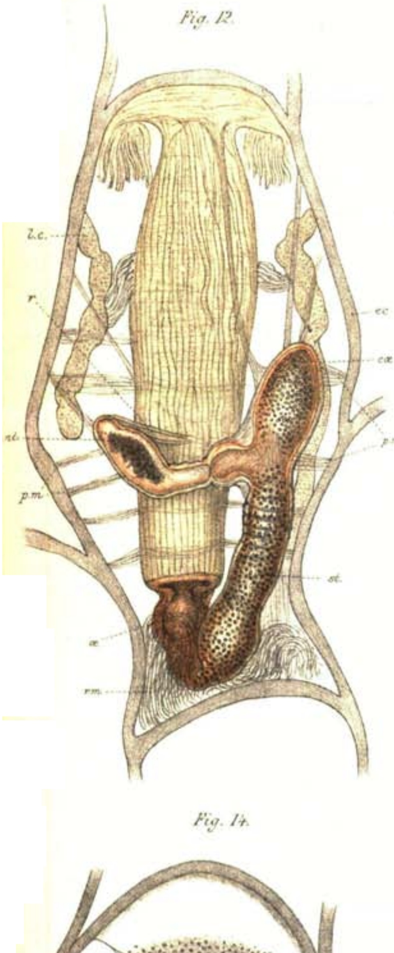

Fig. 13.

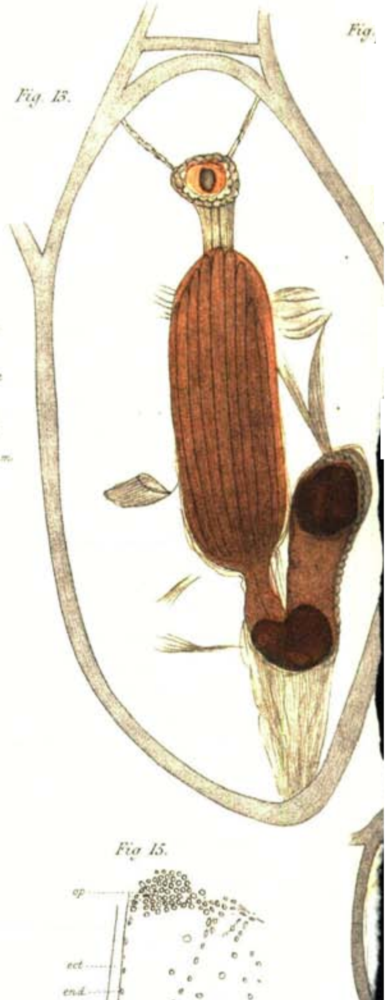

Fig.

Fig. 14.

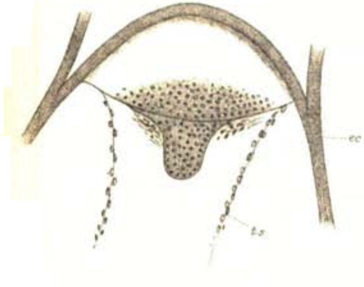

Fig. 15.

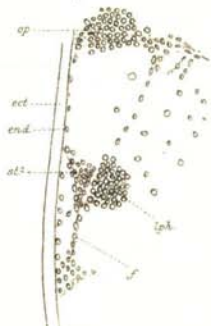

Fig. 17.

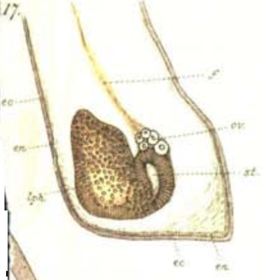

Fig. 18.

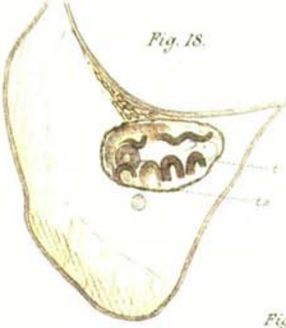

Fig. 19.

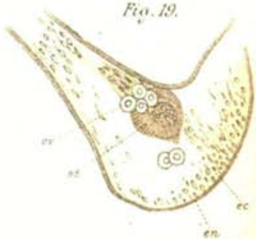

Fig. 20.

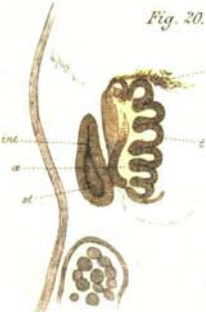

Fig. 21.

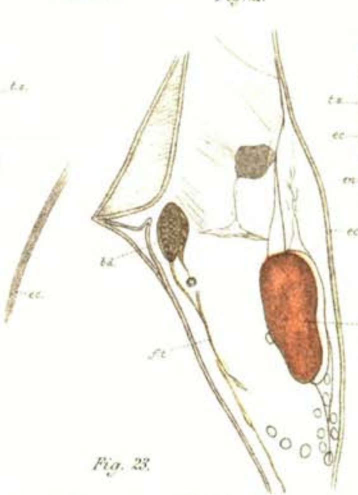

Fig. 22.

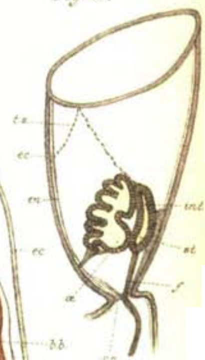

Fig. 16.

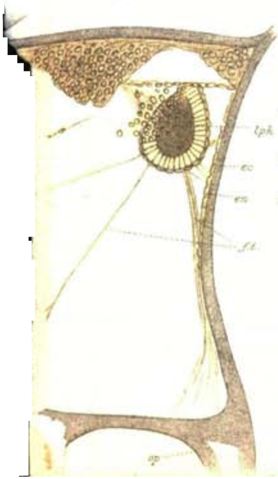

Fig. 23.

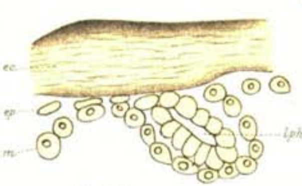

Fig. 25.

Fig. 24.

Fig. 26.

Fig. 27.