

Observations on Fresh-water Polyzoa.

(*Pectinatella gelatinosa*, nov. sp.)

by

A. Oka.

Imperial University, Tōkyō.

with Plates XVII—XX.

The present paper embodies the results of my investigations on a new species of Fresh-water Polyzoa that lives in a large pond in the grounds of the Imperial University, Tōkyō, and is published with the hope that it may throw some light on certain points in the structure and development of the order Phylactolaemata, which have hitherto remained obscure in spite of many efforts of former investigators. The researches were begun, in the spring of 1888, at the suggestion of Prof. I. Ijima, and I am indebted both to him and to Prof. K. Mitsukuri for useful advice. My thanks are also due to Mr. S. Watase, now of the Johns Hopkins University, who, while here some years ago, studied the same species, for kindly sending me his drawings showing the formation of the statoblast.

Although the species which I have studied does not agree in some points with the generic description of *Pectinatella* in Hyatt's Observations (5), it can belong to no other genus. The statements given there were made when only one species, viz *Pect. magnifica*, was known, and must certainly be modified to receive the new one. The

diagnostic characters of the present species to which I give the name of *Pect. gelatinosa*, are as follows :

Colony oval, hyaline; branches of cœnœcium dichotomous; no septa between the cells; ectocyst gelatinous, fills up the space between the branches, forms a common base for many colonies; invaginated fold obsolete; alimentary canal straight when retracted; tentacles 90-98; statoblast saddle-shaped, curved in two axes; marginal spines minute, only seen under a moderate power of microscope.

The colonies grow among aqueous plants and on the underside of floating logs just below the surface of water, and seem to flourish in direct sunshine as well as in shadow. They are found together in a large number forming a luxuriant mass of gelatine, sometimes two metres in length. The outline of each colony is irregularly hexagonal on account of mutual pressure. The gelatinous ectocyst of neighboring colonies coalesce, and form a common base 2-3 cm. thick.

This species furnishes very favorable materials for the student of this group of animals, the transparency of its gelatinous ectocyst, the unequalled large size of the polypide and the promptness with which they evaginate, giving great facility for investigation.

The general appearance of a group of colonies is represented in natural size in fig. 1, Pl. XVII. The color of the cœnœcium and the lophophore is slightly yellowish, the œsophagus and the stomach are brown, and the rectum usually contains dark grayish refuse matter, otherwise of light brown color.

The largest colony that I have seen measured 7 cm. in diameter. The polypides are most crowded and in fullest vigor along the margin of a colony, and much less crowded in the middle portion,

say about one in each four square millimetres. The more centrally situated polypides being older are the first to die, so that in old colonies, the polypides are found only on the outer part, leaving the inner part bare and only marked with dark spots, the remains of dead polypides. When agitated the polypides retract only for a short time, and soon expand their tentacular crown again. Even in being transferred from one vessel to another, some of the polypides of a colony do not retract at all. In confinement, however, they seem to become more timid and, once retracted, remain in that state for a longer time than when free.

Each colony originates from a single individual that comes out of the statoblast in the first weeks of July, becomes larger and larger by successive budding, attains its full growth in October, and continues to live until the end of December. Compared with a species of *Plumatella* living in the same pond, the times of the first appearance and of the total disappearance are each about two months later. As I have not found this species anywhere else, I can say nothing about its geographical distribution.

Methods of Investigation.

Before proceeding further, I may here give a brief account of the methods of investigation employed. To kill the animal in a fully expanded condition was in this case very easy, although it is the principal difficulty met with in the preservation of all other genera. When 70% alcohol is gradually poured into a vessel containing the colonies, more than half the polypides die protruded. If we use such stupefying reagents as chloral hydrate or cocain chlorhydrate, every one of the polypides dies in a fully expanded condition.

The colonies after being killed were put into alcohol to be hardened. Some of them were fixed with a saturated solution of corros-

sive sublimate or a weak solution (0.1%) of chromic acid, previous to hardening. For staining, borax-carmin and picro-carmin were chiefly used. In cutting sections, I imbedded sometimes a whole colony, sometimes separate polypides, in celloidin and paraffin.

In studying the development of the polypide within the statoblast, I proceeded in the following way. First, a statoblast was put into alcohol to harden its contents which in the fresh state consist of a thick milky fluid. Then it was held between two pieces of elder pith, and the edge was cut with a sharp razor so as to make an opening in the chitinous shell. Next, it was stained and kept in alcohol until it was to be cut. In cutting the statoblast, celloidin was indispensable, for, the shell being too hard, it was impossible to get good sections with paraffin only.

For examining fresh specimens, the only thing I had to do was to put a colony (stupefied with cocain) or a part of it on a slide, and cover it, putting a wire ring under the cover-glass to prevent overpressure. In this condition, the polypides had no power to retract, and the ciliae were in vigorous motion.

To study their habits, I kept colonies alive in a glass vessel. I kept also the statoblast in a vessel, in which a contrivance was made to have water always flowing. At last the shells burst, and the little polypides peeped out of the sutures, carrying about the shells like a tiny bivalve. Each of them floats about for a very short time, and then attaches itself by means of the gelatinous ectocyst to any object it may meet with, and gives rise to a new colony.

A. Anatomy.

The branched membranaceous tube (cœnœcial endocyst) forming the greater part of the mass of a colony, together with the gelatinous covering (ectocyst) over it, constitutes the cœnœcium. The terminal

portion of each branch is turned nearly vertical to the plane of the colony and is capped by another short tube (polypidal endocyst), through the pellucid wall of which is seen the alimentary canal contained within. This terminal tube, with the tentaculate lophophore at its free end, and several delicate organs in its cavity, is called the polypide (fig. 2, Pl. XVII.).

Besides this division of the colony into the cœnœcium and the polypides, we may divide it into a number of equal parts, each consisting of a polypide and a portion of the cœnœcium. For the sake of convenience I shall call such a part "*polyzooid*," and the portion of the cœnœcium belonging to it "*cystid*." We thus consider a colony as being made up of as many polyzooids, all structurally alike, as there are polypides.

In all genera with chitinous ectocyst, the cœnœcium is divided by more or less developed septa into a number of compartments or cells, each destined to receive a polypide when the latter is retracted. Such septa are not found in forms with gelatinous ectocyst, and the cystidal cavities stand in open connection with one another.

When a polypide is retracted by the contraction of the muscles that connect it with the bottom of the cystid, its tubular wall invaginates and becomes a sort of sheath for the tentacles, known as the tentacular sheath. In the process of evagination, the tentacular sheath begins to reflect upon itself from the lower end. The evagination generally stops when the lower end of the polypide is still within the cystid. In other words, the evagination is incomplete, thus leaving a permanent fold at the boundary between the cystidal and the polypidal endocyst. In this genus, however, the polypides are often stretched out their whole length, and then no such fold is to be seen.

The shape of the polyzoan colony is different in different genera and species, but it is characteristic for each species. The manner of

branching of the cœcœcium in *Pect. gelatinosa* is shown in fig. 3, Pl. XVII. It is dichotomous with a short branch at each axil. The branches are so bent that all the polypides stand upright and as the plumous tentacles cover the whole surface of the colony, their regular symmetrical arrangement cannot be discerned without close examination.

The general plan of the structure of a polypide and its relation to the cystid are shown in fig. 4, Pl. XVII. The alimentary canal is bent in the shape of the letter V, and hangs freely in the perigastric cavity. The mouth guarded by a tongue-like epistome (*Epist.*) is surrounded by a number of tentacles (*Tent.*) arranged along the entire margin of a horse-shoe shaped lophophore (*Loph.*). The anus opens outside the tentacular area near the mouth, on that side of the body on which the arms of the lophophore stretch out. A nervous ganglion (*N. Gang.*) is seen on the anal side of the œsophagus. A thin hollow tube, called funiculus, in which the statoblasts are developed, joins the angle of the alimentary canal with the cystidal wall. An ovary (*Ocr.*) is seen inside the tip of the cœcœcial branch. The length of a polypide from the tip of the tentacles of the angle of flexure of the alimentary canal is about 4 mm.

Although the term "individual" as applied to such forms as polyzoa is very difficult to define, yet homologously with its nearest relative, the Brachiopods, each polyzoöid might be regarded as an individual in the ordinary sense of the word. Polyzoan individuals show a close analogy to "phytons" of plants.

The polypide and the cystid that constitute a polyzoöid, are respectively vegetative and reproductive in function. As will be seen further on, all the functions for the preservation of the species are performed by the latter, the funiculus being regarded as a part of it, while the former serves to procure nourishment to the cystid.

All fresh-water Polyzoa are annuals, the vegetative and the reproductive portions undergoing entire decomposition every year, while in marine forms, several generations of the vegetative portion, i.e., the polypides, form and decompose themselves on the perennial cystid, like leaves on the branches of a tree. This singular phenomenon led many naturalists (Allman and others) to regard the polypide and the cystid as two distinct individuals. In the present species also, the duration of vitality of the two portions is by no means the same. The polypides invariably die after a certain period of existence, usually after the formation of younger polyzooids of the fourth or the fifth order, but the cystids remain until the colony itself disintegrates in winter. In the central portion of a large colony, therefore, we often see only bare cystids, each with a dark grayish mass, the remains of the dead polypide, hanging in its cavity, and yet with statoblasts continuing their development in the funiculus.

About the application of the terms "anterior," "posterior," "dorsal," "ventral," &c., there is much diversity of opinion. For instance Allman calls the free end of the polypide "anterior," and the fixed end "posterior"; while Hyatt, following E. S. Morse, calls the fixed end "anterior," and the free end "posterior." Huxley homologizes Polyzoa with Tunicata, and names that side on which the anus opens "neural," and the side opposite to it "haemal," although there exists no heart. Again, if we were to compare this animal with Phoronis, we should have to call the narrow space between the mouth and the anus "dorsal," and all other parts "ventral." In fact, every one might give different sets of names in orienting the animal, according to his conception of the homology which exists between Polyzoa and other animals in which the anterior and posterior, or the dorsal and ventral poles are universally recognized. In the following pages, I shall call the fixed end

“lower,” and the free end “upper,” the side on which the anus opens “anal,” and the side opposite the anus “oral.”

The organs that constitute the Polyzoan body may be classified in the following way.

A. Organs for the preservation of the polyzooids or the colony.

1. Dermal System, consisting of the ectocyst and the endocyst.
2. Digestive System, consisting of the epistome, the œsophagus, the stomach, and the intestine.
3. Tentacles.
4. Excretory Organs (?), consisting of two short ciliated tubes.
5. Muscular System, consisting of five groups of muscles.
6. Nervous System, consisting of a ganglion with two arms for the lophophore.

B. Organs for the Preservation of the species.

7. Ovary and Testis.
8. Funiculus, in which the statoblasts are developed.
9. The part of the endocyst that produces buds.

1. Dermal System.

The integument of Polyzoa consists of two layers, quite different in their nature, the outer “ectocyst” and the inner “endocyst” (see fig. 4). The latter is not everywhere covered by the former, but is exposed on the polypides.

The ectocyst is gelatinous in this species. It fills up the space between the branches of the cœncœcial endocyst, whereas in *Pect. magnifica*, Leidy, there is no ectocyst between them. In this respect

as well as in the erect position of polypides, this species comes nearer the genus *Lophopus*. The gelatinous substance is formed by the secretion of cells of the outer layer of the endocyst. Numerous cells, some oval, others irregular in their shape, are scattered in it (fig. 6, Pl. XVIII.). Their nucleus and nucleolus are distinctly visible. These cells seem to have wandered out of the outer layer of the endocyst, and may have helped in producing the gelatinous substance, reminding us of the cells in the test of the Tunicates. The gelatinous substance is adhesive and without taste; it serves apparently to protect the colony. On drying, it shrinks almost to nothing.

The endocyst consists of four layers (fig. 7, Pl. XVIII.)

- a. Outer cell layer (*Out. lay.*).
- b. Basement membrane (*Bas. membr.*).
- c. Muscular layer (*L. mus. and Tr. mus.*)
- d. Inner lining epithelium (*Lin. epith.*).

All of these layers are not present everywhere, nor is each of them of the same structure throughout its distribution.

The cells of the outer layer, which represents the ectoderm, are everywhere distinctly bounded, columnar on the cœnœcium, flat and horizontally elongated on the polypide, except on the tentacles and the upper surface of the lophophore. In the former, they are cubical, in the latter hexagonally prismatic, and distinctly ciliated in either case. Many of the cells on the cœnœcium contain a vacuole (fig. 7, *vac.*) filled with a very refractile fluid. The number of these vacuolated cells increases as we approach the tip of cœnœcial branches, where every cell shows a large vacuole, almost filling up the whole cell (fig. 33, Pl. XIX. *Out. lay.*).

In preserved specimens, the cells are more or less shrunk, often leaving spaces between them. The nuclei are oval, and have a distinct, well-staining nucleolus. The cells on the cœnœcium are

0.02–0.04 mm. high. The nucleus measures about 0.007×0.004 mm.

The basement membrane situated directly beneath the outer cell-layer is secreted either by this, or by the internal lining epithelium, or by both. In the greater part of the cœnœcium where this membrane is separated from the inner epithelium by the muscular layer, it would be natural to refer its origin to the outer cell-layer alone, but where the muscular layer is deficient, it is difficult to decide. On the other hand, in the wall of the funiculus into which this membrane and the inner epithelium, but not the outer cell-layer, are continued, it cannot but be the product of the inner epithelium only. Generally, the basement membrane and the muscular coat are treated as one layer under the name of *Tunica muscularis*, but as they are in reality quite distinct from each other, it will be better to regard them as two distinct layers. When a colony is treated with a weak solution of acetic acid, the basement membrane separates from the rest of layers. It is thin, tough, transparent, and homogeneous.

Next to the basement membrane comes the muscular layer, consisting of transverse and longitudinal fibres. The former run external to the latter. They are not very densely set, so that in a surface view they cross one another as in coarse linen. On the main part of the polypide, only the longitudinal fibres are present. In such genus as *Cristatella*, the muscular layer gives the colony the power of slow locomotion, but what function it has to discharge in fixed *Pectinatella*, I am not prepared to say. In the cœnœcium where this layer is best developed, it is 0.005 mm. thick. It is not found in that part of the endocyst where buds are formed, and is also absent in the walls of the lophophore and the tentacles.

The internal epithelial layer lines the endocyst everywhere. It is thickest in the cœnœcium, especially at those points where budding

takes place, and is thinnest in the tentacles with nuclei scattered widely apart (figs. 16 and 17, Pl. XVIII.). The cells of this layer are fused, hence cell boundaries cannot be distinguished. The nucleus is oval, but I am unable to detect distinct nucleoli. The size of the nucleus is nearly the same everywhere, and is about 0.008×0.004 mm. This layer is furnished with short cilia, which set the perigastic fluid in motion. Average thickness of the layer in the cœcœcium is 0.008 mm.

2. Digestive System.

Minute algae and infusoria that pass by are caught in the whirlpool caused by the vibrating cilia of the tentacles, and sent into the œsophagus. The epistome that guards the mouth is furnished with special muscles which enable it to shut the oral aperture now and then. Perhaps the entrance of non-nutritive matters is prevented by this contrivance. The food, after staying for a short time in the œsophagus, pushes open the funnel-like valve (fig. 4, Pl. XVII. *funnel-like v.*) that intercepts free communication between the œsophagus and the stomach, and enters the gastric cavity where it is moved about by the peristaltic contraction of the wall of that organ. After being fully digested, the residue mainly composed of the cell-wall of diatoms and other algae, passes through the pyloric valve little by little, and accumulates in the intestine. Here, the refuse matter, usually of a dark-grayish color, is cemented together into a mass by a transparent gelatinous secretion of the intestinal wall. When the intestine is full, the contents are pushed out of the anus by the agency of the muscles of that part. The form of the excremental mass, characteristic of each genus, is the same in form as the lumen of the intestine which in our species is an elongated oval tapering toward the anus.

There are often certain amœboid cells to be found in the intestinal cavity. They stain very well, and are on that account very conspicuous among a mass of unstained matter. Judging from their shape and size, it is very probable that they are parasitic Protozoa.

The process of digestion is carried on very rapidly. When fresh colonies are brought from the pond and kept alive, all the polypides discharge their dark intestinal contents in a few hours. Gradually, new refuse matters begin to accumulate in that organ, but they are always a good deal lighter in color. These are again excreted in the next three or four hours. As the amount of food that these animals consume is considerable, it was impossible to keep them alive more than a week without furnishing them very often with water from the pond, which contained minute organisms.

The layers that constitute the walls of the alimentary canal are the same as those of the endocyst. In fact, they are direct continuations of the latter only slightly modified to serve special purposes.

The epistome is a tongue-like prolongation of the disc of the lophophore on the anal side of the mouth. Its cavity (see fig. 8, Pl. XVIII.) communicates with the general perigastric cavity by a comparatively narrow passage on the anal side of the cerebral ganglion. The cells of the outer layer of its wall are similar in appearance to those of the lophophore. They are prismatic, and the height increases nearer the mouth. The oval nucleus with distinct nucleolus lies near the base. The whole external surface is furnished with cilia. This organ has no muscular layer in the wall, but is furnished with special muscular fibres which traverse its internal cavity. These fibres are simply elongated cells with the nucleus at about the middle of their length. They are separate and never form bundles. The length of the epistome is about $\frac{1}{4}$ mm.

The œsophagus is that portion of the alimentary canal that lies

between the mouth and the funnel-like valve at the cardiac opening of the stomach. Its upper and lower sections are lined by epithelia of quite different appearance. The cells of the upper section (fig. 9, Pl. XVIII) have cilia, and their nucleus lies near the base. Verworn says that the cells of this section do not come in to any contact with one another throughout their whole length, being separated by a narrow intervening space but I cannot find any such space in *Pect. gelatinosa*, except such as is in all probability due to the post-mortem contraction of cells. In the lower section, the lining cells have no cilia, and the nuclei lie irregularly near the middle (fig. 10, Pl. XVIII). In the upper section, the free end of cells is flat; in the lower, it is rounded. In both the nucleus has a distinct nucleolus. The cells of the lower section do not stain well, and seem to contain a secretive substance, which may be comparable with the saliva of higher animals.

The length of the œsophagus is about 1mm. and its diameter 0.3 mm. The lumen of the œsophagus when expanded is round in section throughout its entire length, but in its upper section contraction changes it into a stellate shape. The muscular layer is but scantily developed in the œsophageal wall. The outer covering is the continuation of the lining epithelium of the endocyst with which it agrees in all respects.

The œsophagus in its downward course occupies an excentric position in the tubular body of the polypide, and where the latter is externally marked off from the lophophore by a slight constriction it actually comes in contact with the body-wall on the oral side. At this point, the lining epithelium of the polypidal wall is continuous with the outer covering of the œsophagus, and forms a sort of mesentery (fig. 15, Pl. XVIII.). This mesentery extends horizontally on both side for a short distance, and prevents over-invagination

of the body-wall when the polypide is retracted. Thus the alimentary canal is attached to the body-wall at four points, viz. the mouth, the anus, the funiculus and the above mentioned mesentery.

At the entrance of the stomach there is as already mentioned a funnel shaped valve, with the free end pointing into the cavity of the stomach (fig. 11, Pl. XVIII.). It consists of a funnel-like prolongation of the basement membrane, on the œsophageal side of which are arranged the characteristic cells of the œsophagus, and on the gastric side, the pyramidal cells of the stomach. This valve, whose length is about 0.2 mm., prevents the passage of food from the stomach back into the œsophagus.

The stomach is a spacious saccular organ whose long axis is bent in the shape of V, bringing the pyloric opening near the cardiac. It measures 2 mm. in length, and 0.6 mm. in breadth at the widest part. The inequality of the length of the arms of V brings the cardiac opening about 0.5 mm. nearer the free end of the polypide than the pyloric.

The inner layer of the stomach has two kinds of cells ; the long club-like cells (fig. 13, Pl. XVIII. *cl. c.*) and the short pyramidal cells (*pyr. c.*). As they are arranged in groups forming alternate longitudinal rows, the lumen of the stomach is stellate in cross-section. The number of the rows of each kind is generally twelve or more (fig. 12, Pl. XVIII.). In both, the nuclei lie at the base and the nucleoli are distinctly visible. The long club-like cells do not stain well, while the short pyramidal cells freely take up the coloring matter. In the fresh state, the longer cells contain a yellowish brown fluid and the shorter cells are of a light yellowish color, so that the stomach appears longitudinally striped with yellow and brown bands. As the alimentary canal has no distinct glandular appendage, the brown fluid contained in the longer cells probably performs the

function of the digestive fluid. Hence they have been called hepatic cells by Allman. The function of absorption seems to be performed by the shorter cells. The length of the longer cells is various, the longest measuring 0.06 mm., while the shorter pyramidal cells measure approximately 0.02 mm. On the gastric side of the cardiac valve, and at the blind end where the stomach is continuous with the funiculus, the rows of the longer cells stop short, and only the short pyramidal cells are present.

The muscular layer of the gastric wall, composed only of the transverse fibres, is well developed, especially below. At the thickest part this layer is 0.007 mm. in thickness. At the blind end of the stomach, however, there is no muscle, and here the inner cell-layer comes in direct contact with the fluid contents of the funicular cavity (fig. 32, Pl. XIX.). At this point, the wall is generally pushed inward in the form of a shallow pit.

The outer epithelium does not differ from the corresponding layer of the œsophagus and the endocyst.

The pyloric valve is represented by a simple constriction of the entire wall of the alimentary canal. Its opening is very narrow, allowing the passage of only a small quantity of indigestible matter at a time.

The intestine is a tubular organ tapering toward the anus. It is about 1.2 mm. in length, and 0.3 mm. in width. The inner layer is composed of only one kind of cells, which are much shorter but somewhat broader than the longer cells of the stomach. The height of these cells is about 0.025–0.03 mm. The nucleus is at the base and the nucleolus is distinct (fig. 14, Pl. XVIII.). These cells do not stain well; the gelatinous fluid they contain is probably the medium by which the excrement is cemented into a compact mass. The muscular layer of this part, in which only ring fibres are present, is weakly

developed except near the anus, where it forms a sort of sphincter. The anus when expanded is as wide as the widest part of the intestine, but when contracted it closes altogether. The outer cell-layer is similar in all respect with that of other parts of the alimentary canal. At the point where the intestine is tightly pressed against the œsophagus, the outer layer of the former passes directly into that of the latter, bringing the cells of the inner layers of both organs in contact.

3. Tentacles.

The tentacles are arranged in one continuous series along the outer and the inner margin of a horse-shoe shaped lophophore, as mentioned before. They are hollow cylindrical organs measuring 1 mm. in length, and 0.03 mm. in breadth. They are to be considered as prolongations drawn out, as it were, from the endocyst. In the living state, they are freely movable in every direction at the will of the animal, but I have never seen them coil or contract. Generally, they stand nearly parallel to one another in graceful curves (fig. 2, Pl. XVII.).

The cross section of a lophophoral arm (fig. 30, Pl. XIX.) is almost semicircular in outline, slightly convex above and rounded below, measuring 0.3 mm. in breadth, and nearly as much in depth. The ciliation on the upper surface is distinctly visible on sections.

The cells of the outer layer of the tentacular wall have all the essential characters of those of the endocyst. They rest on a fine basement membrane and are furnished each with a long cilium (fig. 16, Pl. XVIII. Out. lay.), constantly vibrating in a certain fixed direction. The ciliation of that side of the tentacles turned away from the mouth drives the water upward, while that on the opposite side tends to drive it toward the mouth below. The inner layer of the tentacles

(fig. 16, Pl. XVIII. Lin. epith.) is very thin and has the nuclei scattered at great intervals. I was not able to detect any trace of cilia on the lining epithelium, but the rapid motion of the perigastric fluid, going toward the tip along one side and coming back along the other in the narrow tentacular cavity, indicates their existence. The lumen is a little more than 0.01 mm. in diameter.

The account, given by Verworm, of the manner of junction of the tentacles with the lophophore and the tentacular membrane in *Cristatella* applies equally well to the species investigated by me. In fig. 18, Pl. XVIII, I have endeavored to show diagrammatically the relation of several parts at the bases of tentacles.

Externally to the row of tentacles there is a thin membrane, the tentacular membrane, 0.3–0.4 mm. in breadth, formed by a duplicature of the outer layer of the lophophoral wall along its outer edge. It consists of a basement membrane covered on both sides by a layer of flat cells, the direct continuation of the outer layer. The basal portion of each tentacle is joined to the tentacular membrane by another narrow triangular membrane.

Alternating with the bases of the tentacles, a series of duplicatures on each side of the lophophoral cavity is produced in the inner layer, so that if we were to cut across the arm and look into it, we should see a series of vault-like arches. The tentacular cavity opens into that of the lophophore between each two of such folds of the inner layer. These folds descend almost to the floor of the lophophoral cavity, and have been reckoned as part of the muscular system by Hyatt, under the name of "brachial contractors," but I see no ground for regarding them as such, since they consists simply of flat cells.

The bases of the tentacles are not in one plane. Those on the anal side near the epistome are the most elevated. The number of the tentacles is generally even, but in some individuals there is a

median tentacle on the anal side, making the total number odd.

There can be no doubt that the function of the tentacles is three-fold, serving for respiration, for collecting food and for feeling. Of these, however, the first seems to be their principal office, when we consider the large extent of their surface exposed to water, and the constant current kept up in the latter by a special contrivance, as well as the perigastric fluid that circulates within their lumen. The Tentacles thus bear a close resemblance to the fringed arms of Brachiopods.

Circulation. The perigastric fluid contained in the general body-cavity may justly be regarded as representing the blood. Of its nature and the mechanism of circulation, little was known before. There are no special organs, such as heart and blood vessels, and the only means of driving the perigastric fluid is the supposed ciliation on the lining epithelium of the general body-cavity. The nutritive part of the food taken up by the alimentary canal is conveyed to all parts of the body by this fluid. It is transparent, colorless, and has no taste. Water seems to constitute the greater part of its constituents.

The fluid contains, floating in it, numerous round cells, each with a large vacuole almost filling up its body and filled with a refractile fluid (fig. 20, Pl. XVIII). The nucleus is pushed against the wall by the vacuole. The study of the development of polypides in the statoblast shows that these free cells are derived directly from the granular mass that constitutes the main contents of the statoblast, and in young stages they contain similar granules instead of the vacuole. It is therefore plain that they are, at any rate, nutriment carrying cells, which might be regarded as blood corpuscles.

Besides these, there are generally present a greater or smaller number of cells or fragments of cells of a quite different appearance, which have probably detached themselves from some part of the body.

The floating elements were observed by previous investigators (Allman, Hyatt, Verworn), but no great importance was attributed to what were probably either parasitic organisms or detached cells. Hyatt, for instance, observed "numerous organisms, many of which probably parasitic, which float in the fluid, sometimes in such a number as to interfere with the examination of the internal structure." It is probable that at least some of these "organisms" were what I regard as the blood corpuscles.

The direction of the blood currents as observed in the natural state is shown in fig. 21. On the anal side of the body cavity the fluid is driven toward the free end of the polypide, evidently by ciliary action, which however could never be actually brought to view. In the lophophoral arms, the corpuscles travel along the floor to their ends, and either return directly along the ceiling, or enter the tentacles, in which they ascend on the side nearer the tip of the lophophore, and descend along the opposite side. In the cavity of the epistome, the fluid streams along the ceiling to its tip and coming back along the floor of that organ, either enters the epistome again, or goes to the tip of the lophophoral arm along its lower side. On the oral side of the polypide, the fluid is always seen flowing downward.

Allman and Hyatt deny the presence of cilia on the external wall of the alimentary canal, but Verworn saw them at the end of the stomach in *Cristatella*. My observations in living specimens of *Pectinatella* confirms the statements of the last author.

Both Allman and Hyatt observed that the cœncœcia of *Lophopus*, *Cristatella*, and *Plumatella vitrea*, readily emptied themselves of

their perigastric fluid when taken out of the water. They assumed that the fluid passed out through pores in the endocyst, but they searched in vain for such communications.

It is certain that when a polypide retracts, a portion of the fluid contained must of necessity pass out at some place, since the cœcæal wall does not expand beyond a very limited extent. Notwithstanding my special attention to this point, *Pect. gelatinosa* also gave no result, and I should prefer to go no further than to assume the presence of external openings in connection with the excretory organs.

4. Excretory Organ (?)

Joliet (6), in a paper entitled "Organe segmentaire des Bryozoaires Endoproctes," gives a pretty full description of two short funnel-shaped tubes in *Pedicellina* and *Loxosoma*, first noticed by Hatschek. In the division Ectoprocta, however, our knowledge on this subject is very limited. As far as I know, the two figures given by Farre, and the remarks by Hincks and Smitt, both of whom do not go beyond conforming the observation of the first, constitute the whole bibliography on this subject. They all noticed a ciliated pipe that opens between the mouth and the anus in *Aleyonidium* and *Membranipora*, both of which are gymnotæmatous. In regard to the order Phylactolæmata, if we except the short account given by Verworn, illustrated with two semi-diagrammatic figures, there exists no literature known to me. Verworn left the terminations of that organ undetermined, confining his attention to only the middle portion where it is most conspicuous. Braem touches on this subject in his note in the *Zoologischer Anzeiger*, but he too could not determine how the tubes terminate. Such being the case, I have investigated this organ with special attention.

There are two ciliated tubes just beneath the outer layer, on the anal side of the body, between the anus and the bases of the median tentacles of the inner row. The walls of these tubes are continuations of the epithelial lining of the invaginable portion of the endocyst. They open below into the body-cavity by funnel-shaped openings. They measure 0.15–0.19 mm. in length, though the portion where the wall is entire is much shorter (fig. 26 bis, Pl. XVIII). The shape of the funnel-like openings may be compared most appropriately with the obliquely cut end of a hollow tube.

The exact form of these tubes and their relation with other organs will be best understood by referring to figs. 21–26, Pl. XVIII, which show their cross sections with the neighboring parts at various levels.

In a cross section passing through the middle part of the tubes, we see them as two oval sections lying side by side (figs. 24 and 24 A). The ciliated epithelial wall consists of cells which are cubical near the median plane of the polypide and flat on the opposite side. Consequently both the nuclei and cilia are densely set in the portion nearest the median plane of the polypide and scattered at some distance from one another on the outer side. The tubes are closely enveloped on the anal side by the outer layer of the invaginable tube (*Out. lay.*), and on the oral side by the lining epithelium (*Lin. epith.*) of the body-cavity. The diameter of the tubes measures about 0.03 mm.

Tracing these tubes downward, that part of the wall farther removed from the median line soon disappears, i. e., the tubes open into the body-cavity on that side (fig. 26). As the two tubes deviate from each other below, a part of the perigastric space appears between them (fig. 26, *epistom. cav.*) This is the passage by which the cavity of the epistome communicates with the perigastric.

The median side of the wall ends abruptly on the anal side of the ganglion; below this point cross sections show only one continuous body-cavity. Thus, the body cavity is divided into three branches on the upper part of the polypide. The middle one (fig. 26, *epistom. cav.*), passing along the anal side of the ganglion, extends into the epistome, while the lateral ones are prolonged into the lophophoral cavity. The inner walls of these lateral branches pass gradually into the ciliated tubes.

If we now trace the tubes upwards, they are found gradually to approach each other, and their walls soon coalesce. A little higher the cavities of both open into each other, and there is seen a single flattened tube (figs. 23, *Nephr.* & 23 A). The whole inner surface of this part as well as that of the two deviating tubes below, show distinct ciliation in sections, the cilia being always directed toward the perigastric opening. If we trace this flat tube still further upwards, it again becomes divided in most individuals into two, in some into three tubes (figs 22 *t* & 22 A), each of which is continuous with tentacular lumen. In this part, the ciliation is no longer visible, but compared with the inner layer of the tentacles, there are more nuclei. But, further upwards, the nuclei are fewer in number and the lining epithelium presents similar appearance as that of ordinary tentacles (figs. 21 *t* & 21 A). What can be the function of these ciliated tubes? The fact that they open into the perigastric cavity by ciliated funnel-shaped openings naturally reminds us of the segmental organs of certain worms. And thus many observers have been induced to regard the function of these tubes as being of an excretory nature. If such is really the case, there should be some orifice by which they open outwards, for the high degree of development they attain prove that they are not useless remnants. This makes me venture to assume the existence of minute apertures, at least on the two or three innermost

tentacles of the anal side, presumably at their tips, although I am unable to produce any positive proof. The pores, if ever present, must be of very minute size, indiscernible by ordinary methods in a manner analogous to the pores at the tip of Actinian tentacles.

5. Muscular System.

The muscular system consists of five groups of muscles. They are :

- a.* Muscles of the funiculus.
- b.* Parieto-vaginal muscles.
- c.* Retractor of the polypide.
- d.* Muscular layer of the alimentary canal.
- e.* Muscular layer of the endocyst.

To these may be added the muscles of the epistome.

The first three are, as development shows, modifications of the last two, which in turn may be regarded as only locally differentiated forms of one and the same layer.

In the development of the polypide in the statoblast, the muscular fibres are formed from certain cells of the granular mass, and, in the process of budding, from the lining epithelium. In either case, the cells elongate, and become spindle shaped, with the nucleus at the middle. They lengthen more and more and the nuclei become indiscernible, although these can often be made visible by the aid of acetic acid. Excepting some fibres of the parieto-vaginal muscles, which remain in this state to the end, the muscular fibres are extremely thin, and do not show nuclei in their interior. It seems that these fine fibres arise by the longitudinal splitting of the original muscle-cells, as is known to take place in many other animals.

The muscles are never striated. Even in the retractor of the

polypide, which is obviously of greatest physiological importance, the fibres are smooth. In marine Polyzoa, however, I have observed that the muscles of the avicularia and the vibracula are striated.

The muscular fibres belonging to the funiculus run longitudinally on the inner surface of the basement membrane, on which the cellular wall of the funiculus rests (fig. 31, Pl. XIX). They run separately without forming bundles, and present the same appearance as those of other parts. Their extreme fineness as well as their small number agrees with the fact that the funiculus contracts, if ever, in a very limited degree.

The muscles running between the cystidal wall and the bottom of the invaginated fold (at the junction of the cystidal and the polypidal endocyst) are called the Parieto-vaginal muscles (fig. 4, Pl. XVII, *M''*). Their fibres run either solitary or in bundles, forming on an average 13-14 sets arranged somewhat radially. Their points of attachment to the cystidal wall is irregular. These sets of muscles cause the presence of the invaginated fold of the body-wall. In *Pect. gelatinosa* when the polypides fully expand, this fold, which is otherwise distinctly present, disappears, the muscles relaxing to their full extent.

The great retractors of the polypide consist of a pair of well developed muscular bundles, right and left in the perigastric cavity (fig. 4, Pl. XVII, *M'''*). The fibres are modifications of the muscular layer of the endocyst, extraordinarily developed to serve their special purpose. The point of attachment of each bundle to the bottom of the cystid is single, but the upper portion is split into a large number of smaller bundles which are inserted into the walls of the œsophagus and the stomach at various places, but most numerous at the upper part of the former. The bundle is ensheathed in a sort of fine sarcolemma, which could distinctly be demonstrated at such places

where the fibres were mechanically torn away leaving the sheath uninjured.

In the muscular layer of the alimentary canal, only transverse fibres are well developed, and the longitudinal fibres, if ever there be any, are very scanty. The layer becomes thicker as we approach the blind end of the stomach. The musculature in question performs peristaltic movements, periodically on the œsophageal and fairly constantly on the gastric wall. The blind end of the latter is subject to stronger constrictions in accordance with the thickened muscle-layer of this part. The peristaltic movement of the gastric wall helps not only to move about the contents of that organ, but also to send the residue into the intestine. The muscular fibres of the intestinal wall are especially well developed near the anal opening; they serve to discharge the excrements out of the body and to keep the anus tightly closed. At the point where the blind end of the stomach joins the funiculus, there is no muscular layer (fig. 33, Pl. XIX).

The muscular layer of the endocyst has already been treated under the body-wall. The outer ring fibres are especially well developed around the orifice of the cœncœcial branches and form a sort of sphincter to close the opening produced when the polypides retract. When the polypide is extended, the cœncœcial branch becomes slender by the contraction of the ring fibres, but apparently it is not by their agency that the polypides are pushed out, for this process takes place even in a cœncœcial branch with its wall cut open, so that the fluid contained can transmit no pressure upon the invaginated polypide.

The muscles that move the epistome remain in a very primitive state of development, consisting of loosely distributed fibres which, as already mentioned, are mere elongated cells with the nucleus at the middle. They traverse the cavity of the epistome, joining its

underside with the ceiling. As seen in cross sections, they are more closely set near the edge and almost entirely wanting in the central part of the epistome.

6. Nervous System.

This system has been described more or less fully in all works on Polyzoa, but the accounts given are very different from my own observations. Nearly all investigators describe the cerebral ganglion as a solid cellular mass. Nitsche, studying the process of gemmation, states that the ganglion has at first a ventricle, which, however, obliterates with the growth of the animal. Contrary to this statement, Saeffitgen (10) recently discovered that in *Cristatella* and *Plumatella*, the cavity of the ganglion persists throughout life, and further that the ganglionic wall is not everywhere of the same thickness, being at some parts as thin as the lining epithelium of the body-wall. I have observed that in *Pectinatella* also the cavity exists in the mature state; it is so very large that at first sight it might be mistaken in sections for a part of the body-cavity.

In fig. 28, Pl. XIX, I have represented the form of the ganglion in *Pectinatella gelatinosa*. It may be compared with a spindle bent somewhat in the form of U, and fitted with its concavity to the anal side of the œsophagus, in rather an oblique position with the arms turned slightly upward. The end of each arm again makes a sharp bend in the anal direction and is continuous with a large nerve trunk which proceeds into each lophophoral arm. The ganglion is in direct contact with the inner cell-layer of the œsophagus, the outer layer of that tube enveloping it on all other sides; the ganglion is in fact situated between the two layers of the œsophagus (fig. 29, Pl. XIX). The lophophoral nerve trunks are likewise located between

the outer and the inner cell-layers of the body-wall; they run, namely, immediately beneath the outer layer of the lophophoral ceiling, covered below by the lining epithelium.

As mentioned above, the ganglion is not a solid cell-mass as has been described by nearly all investigators. On the contrary, it contains a spacious ventricle, extending to the end of the arms, or horns, as is diagrammatically shown in fig. 30 *a, b, c*, Pl. XIX. The wall of the ventricle is very thin and of an epithelial nature on all sides except at the bottom somewhat on the anal side, where it is very thick, forming the ganglion *sensu stricto*,—a condition which reminds us of the Teleostian cerebrum.

This thick portion is distinctly bounded from the thin epithelial part of the wall, and is well seen in the fresh state as a somewhat reddish mass, with a slight constriction in the median plane of the polypide. It is this part that Hyatt took for the ganglion which he describes as composed of two lateral masses united by a very thick commissure. It is no wonder that he overlooked the thin epithelial portion, since this is hardly recognizable in surface views. As can readily be imagined by combining the three sections given in fig. 30, passing through the brain in different directions, the thick portion is a transversely elongate rounded mass, with a transverse slit-like depression, looking orally and upward. The whole mass is not of the same structure throughout, but shows a differentiation into peripheral and central portions. In the former, the nuclei (of ganglion-cells) are densely crowded, while in the latter we see a faintly stained granular mass (Punktsubstanz) containing only a few or no nuclei. The cell outline to each nucleus is not to be seen.

The thin part of the wall of the ventricle differs in nothing from an ordinary epithelium, being composed of a layer of flattened cells. It is continuous with the peripheral portion of the proper ganglionic

part. How the nerve fibres, if there be any, pass out from the latter into the nerve-trunks, I have been unable to elucidate.

The cross-section of the lophophoral nerve trunk is kidney-shaped, with the concavity turned above (fig. 31, Pl. XIX. *nerve*). In it the nuclei of nerve-cells are seen much crowded. Longitudinal sections show that the nerve-cells in question are spindle-shaped (bipolar) with the nucleus at the middle, and closely packed together. A few fibres run amongst them ; these are probably to be regarded as nerve-fibres. The trunks themselves are very thick and large in comparison with the mass of the central ganglion, and their structure gives the impression of an elongated ganglionic mass rather than of a nerve. The trunk gives off on each side a branch into each tentacle. Such a branch is of fibrous appearance and could be traced only for a very short distance after its departure from the trunk.

The presence of a circumoesophageal nervous commissure in fresh-water Polyzoa is a matter of obscurity, having been accepted by a few and denied by many. My observations on *Pect. gelatinosa* convinced me of its absence.

The colonial nervous system present in many marine Polyzoa, which keeps the action of the members of a colony in harmony, seems to be altogether wanting in this species, as is probably the case in all other fresh-water Polyzoa. Special attention to this point showed no trace of nervous connections between the polypides in preparations of sectioned colonies. The fact agrees with the behavior of the polypides in a living colony, in which only directly disturbed polypides retract, while all the rest remain protruded as if nothing had happened.

7. Ovary and Testis.

Pectinatella gelatinosa has a distinct ovary, although it develops only in very rare instances. When present, it is situated inside the cystid near its tip on the oral side. It is a solid club-shaped outgrowth of the internal lining epithelium, and usually contains ten or more ova in different stages of development (fig. 33, Pl. XIX), the space between them being filled with connective tissue stroma. Ripe eggs can fall into the perigastric cavity only by the rupture of the ovarian wall. The largest ovarian ovum measured 0.35 mm. The length of the ovary is about 0.9 mm., and the breadth 0.5 mm. No doubt can ever be entertained about the ovarian nature of the body in question. That the funiculus has nothing to do with the production of eggs has also been ascertained by Braem for *Cristatella* and *Plumatella*.

As to the testis, my investigations gave no result. I searched for it in hundreds of polypides, but in vain. I once saw something like spermatozoa within the tip of a cystidal branch, but I failed to make it sure. At any rate, true sexual organs are very imperfectly developed in accordance with their secondary importance in the reproduction of this species.

8. Funiculus.

The funiculus is a hollow tubular organ, about 5-6 mm. long, which connects the blind end of the stomach with an opposite point of the cystidal wall. Its wall is composed of three layers, but the innermost one, consisting of a few longitudinal muscular fibres, hardly deserves to be called a layer (fig. 32, Pl. XIX). The outermost layer is the continuation of the outer lining of the alimentary canal or the lining epithelium of the endocyst, from either of which

it differs in nothing. The cells of this layer rest on the outside of a tube of basement membrane, which forms the middle layer, and are rather thickly set, every cross section of the tube showing from nine to twelve nuclei. Thus my observations on this organ are identical with and only confirm Nitsche's. Verworn denies the existence of muscular fibres in *Cristatella*. In *Pectinatella* they are decidedly present, although few in number and isolated, so that they are liable to be overlooked if not specially searched for. The outermost layer is the only cell-layer in the wall of this organ. I cannot but assume that Kræpelin had fallen into error in describing the funiculus as made up of two cell-layers, the equivalents of the outer layer and the lining epithelium of the endocyst respectively.

The diameter of the lumen is about 0.02 mm., and the thickness of the wall about half as much.

The narrow lumen of this tubular organ, whose wall must be regarded as entirely mesodermal, is bounded at its upper end by the inner cell-layer (entoderm) of the stomach, and at the lower end, by the outer layer (ectoderm) of the endocyst (figs. 33 and 34, Pl. XIX). It is in this organ that the statoblasts are developed. That the funiculus should not be regarded as the ovary, as was done by some former investigators, is self-evident, at least in the present species as well as in those in which a distinct ovary has been demonstrated in quite another region of the body.

9. The part of the Endocyst that produces buds.

Budding takes place at a certain fixed position as Braem asserts, namely, at a definite area on the oral side of the cystidal endocyst. Here the endocyst is somewhat thicker than other parts of the same

wall, and the outer cell-layer and the lining epithelium are clearly distinguishable from each other, as at other places, although no muscular layer intervenes between them. At this place, the cells of the outer layer are wanting in vacuoles and both layers stain more deeply than anywhere else. The area is comparable to the growing point in plants. How the buds arise, shall be treated under a special chapter later on.

B. Reproduction.

In fresh-water Polyzoa, reproduction may take place sexually or asexually in three different ways, as tabulated below :

	Reproduction by	
	1	2 3
	Ovum	Statoblast Budding
Nature :	sexual	asexual
Function :	to form primary poly- zoïd giving rise to a new colony.	to form a number of new polyzooids, thus increasing the extent of a colony.
New individual originates from :	one cell	many cells
The number of body-layers that enter into the formation of the new individual :	one	two.

By the first mode, an ovum should undergo segmentation, and passing through a series of metamorphosis give rise to a new primary

polyzoöid. This mode, however, seems to take place very rarely, if ever, in the present species.

By the second mode, germs enclosed in hard chitinous cases (statoblasts) are produced in the funicular cavity of polyzoöids. They are set free by the decay of the parent colony, and float on the surface of water during winter, sometimes packed in ice. Next summer a primary polyzoöid is developed in each, serving as a foundation for a new colony. Thus, this and the first mode perform the same purpose, in so far as both serve to establish new colonies, and the withdrawal of the latter is supplanted by the great activity of the former.

By the third mode, a certain part of the endocyst adds, by growth in a certain definite manner, new polyzoöids to the primary polyzoöid. This mode of reproduction increases the size and determines the form of the colony.

In certain cases the colony may propagate itself by simple division. For instance, Allman and Hyatt observed that in old colonies of such genera as *Cristatella*, *Lophopus* and *Pectinatella*, all of which have gelatinous ectocyst, the branches separate themselves from the cœnœcial trunk by constriction. In *Pect. gelatinosa*, however, I have never met with the same phenomena. On the contrary, all the colonies collected by me showed no sign of such fissiparity, all of them being entire and of the form characteristic to this species. In most of them, the shell-halves of the statoblast in which the primary polyzoöid has developed were seen sticking to the underside somewhere about the centre.

With regard to the first mode of reproduction, I had no chance of making observations any further than determining the presence of ovaries in certain polyzoöids. The phenomena of reproduction by the remaining two modes shall be treated, for sake of convenience, under the following four heads:

- 1, Statoblast,
- 2, Development of the Statoblast in the Funiculus,
- 3, Development of the Polypide in the Statoblast, and
- 4, Budding.

1. Statoblast.

The general structure of this seed-like body, differing in shape and size in different species, is now well-known and the following description refers specially to the statoblast of *Pect. gelatinosa*. In winter the dead colony is soon decomposed and the statoblasts contained in it are set free. During winter and spring months, they may be found on the surface of the water in large numbers, clinging to floating logs, bamboo sticks or trunks of aquatic plants. They are of a dark brownish color with a wide marginal zone of a lighter tinge.

Let us take one of them and examine it more minutely. Its shape is, properly speaking, like a flat lens. The outline, as it lies flat, is quadrate-oblong, about 1.5×1.3 mm., and about 0.3 mm. in thickness. I may here mention that this species has the largest statoblast among all known Phylactolematous Polyzoa. It presents double curvature after the manner of a saddle (fig. 5, Pl. XVII). For conveniences sake, we may call that side on which the longer axis is convex as the "convex surface," and the opposite side as the "concave surface," although these terms do not hold good with regard to the shorter axis. On both sides, the whole surface is beautifully marked into hexagonal areas, more distinct in the marginal zone than in the central portion. The extent of the central darker area is various in different statoblasts, and it may also differ on different sides of the same statoblast. Generally it ranges from 0.5 mm. to 0.6 mm. in diameter.

Closer examination shows that what appeared as a distinctly reticulated marginal area is a sort of broad rim around a chitinous body of compact nature. This rim consists of a number of prismatic caskets filled with gas, the diameter of the caskets increasing as we approach the margin. The hollow caskets have their axis vertical to the plane of the statoblast and are arranged in two horizontal layers. They serve as a buoy to float the central body, which is the most important part of the statoblast.

If the free edge of this rim, or the annulus as it is called, be examined under a strong power, we see a great number of minute hooks projecting from it (fig. 35, Pl. XIX). They are found most abundantly where the margin is somewhat angular. Some of them are complex, while others are simpler, but all are formed by the combination of simple hooks in various ways. They are mere outgrowths of the edge of the annulus, and have no direct connection with the central body, as is the case in *Cristatella* and *Pect. magnifica*. They are short and stout, and the tips are rounded. They measure about 0.02–0.03 mm. in length and are too minute to be of much functional importance. When the annulus splits horizontally, as it does of itself when the polypide begins to develop within, these spines are found only on the margin of the concave side.

The curvature of the annulus and the presence of hooks on the free edge seem to be worth careful consideration. In all statoblasts, the annulus serving as buoy performs an effectual service in distributing the species as well as in enabling the establishment of colonies near the surface of water. Where the annulus shows curvature, the distributive power is evidently enhanced, exposing its curved surface to the influence of the motion of water, or, if dried up, of wind. As to the hooks I have no doubt that, as Kraepelin has said, they serve as anchors to secure attachment for

the colony that is to grow. At the same time they must be looked upon as assisting distribution to a great extent. By their means, for instance, the statoblasts have a chance of clinging to the feathers of some water-birds or to floating logs or weeds and of being carried away to distant localities. The strongly developed hooks on the statoblasts of *Cristatella* or of *Pect. magnifica*, in which the annulus is but weakly developed and cannot serve more than as a mere buoy, may perhaps have in this respect a great importance. In the present species, the extreme insignificance of the hooks as distributing organs is probably sufficiently counterbalanced by the extensively developed annulus with its double curvature, so marked a feature of this as compared with all other species. Braem regards the hooks as protective organs, but as such they can have no great value in the case of *Pect. gelatinosa*.

The annulus, as studied on sections (fig. 46, Pl. XIX), is made up as usual of two horizontal strata, each consisting of a single layer of upright hollow prisms which remind us of cells in a honeycomb. The central part showing indistinct reticulation in surface-views proves to be the exposed surface of a thick chitinous capsule of spheroidal shape (*centr. caps.*). This central capsule is made up of two watch-glass like valves tightly apposed with rims, the demarcation between them being visible as a faint line. The wall is composed of two distinct layers of chitin, which may be called the outer and the inner stratum respectively. The outer is darker in color, and by far the thinner of the two. This stratum is the continuation of the chitinous wall of the annulus, and its exposed surface is raised into low ridges that form a network with hexagonal meshes.

The thick inner stratum of the chitinous capsule looks bright yellow on sections. Directly beneath this capsule, there is a membranous envelope (*env. membr.*) distinctly composed of flat hexagonal

cells with centrally situated small nuclei. This cellular envelope completely encloses a granular mass of protoplasm (*gran. mass.*) in which are scattered minute nuclei. These nuclei measure only 0.001×0.003 mm. in average, and are thus several times smaller than the nuclei of body tissues. They are very flat with their plane parallel to that of the statoblast.

The granular contents and the cellular envelope form the essential part of the statoblast, while the chitinous capsule, the annulus and the marginal spines are all accessory organs for its preservation and distribution.

2. Development of the Statoblast.

The knowledge of the origin of statoblast is certainly of vital importance in determining the true nature of this gemmule-like body, but in the rather scanty literature on this subject the statements given are widely different from one another. As to my own observations, I have seen in the lumen of the funiculus sometimes a single cell and at other times a loose group of two or more cells, representing the earliest stages of development of statoblasts. They are round in outline, and each supplied with an oval nucleus. Neither in size nor in general appearance do they perceptibly differ among themselves, or from those of neighboring tissues. This circumstance deprives me of all grounds to share Verworn's view that the increase of cells is due to continued division of an originally single cell. This author sums up the earliest steps in the development of a statoblast in the following words: An einer bestimmten Stelle des Funiculus vermehren sich die Epithelzellen desselben zu einer kleinen Aufschwellung and drängen dadurch gegen das Lumen. Eine Zelle davon tritt in das Lumen hinein and wird zur Eizelle, während die anderen

sich zu einen Follikel formiren. Die Eizelle macht einen regelmässigen Furchungsprocess durch, dessen Resultate eine solide Morula ist. Wie man sieht, wird also auch durch diesen Furchungsvorgang die Knospennatur der Statoblast widerlegt." Hence he concludes: "Die Statoblasten sind als parthenogenetische Wintereier aufzufassen welche sich im Gegensatze zu befruchteten Eiern am Funiculus entwickeln." I did not find this view corroborated by facts. Neither the thickening of the funicular epithelium nor an "Eizelle," which to judge from his figures must have been several times larger than any ordinary cell of the funiculus, could be found.

On the contrary, what I have seen in *Pectinatella gelatinosa* leads me to the conclusion that each statoblast originates from at least eight cells of separate derivation. Where they come from is a question which I cannot answer from direct observation. However, that it receives no element from the entoderm is evident from the fact that where there are many statoblasts in the same funiculus, the older ones always lie nearer the stomach, completely shutting up the passage. The question then reduces itself to whether the original cells are derived from either the funicular wall (mesoblast) or the outer layer of the endocyst (ectoblast) at the point where it bounds the funicular cavity below, or from both. As will be seen later on, the intrastatoblastic development of a polyzoöid essentially agrees with the process of development by budding, differing only in such points as are necessitated by the mechanical conditions of each case. We should then expect similar elements in the "anlage" of a statoblast as in a bud, that is, both the funicular wall and the outer layer of the endocyst should *a priori* give their contingents to form a statoblast. The correctness of this assumption is proved by the observations of Braem (Zool. Anz. 1889.). According to this author, the primitive statoblast consists of two kinds of cells, which are genetically different, one deriving

itself from the funiculus and the other from the ectoderm. It is needless to say that in the above light, a statoblast cannot be anything else than a specially modified form of bud, in other words, a portion of both layers of the endocyst protected against severe climate by special contrivances for the preservation of the species.

But to return to the process of development, a certain number of cells, probably from the two sources referred above to, assemble in the funicular lumen and arrange themselves into a group at first loose and irregular. During this early stage, the funicular wall nowhere shows thickening, contrary to Verworn's observations. Very soon the group becomes compact and assumes a morula-like form. It can now be safely asserted that new additions of cells no longer take place, but that the morula henceforth increases in size by multiplication of its own cells. The mass bulges out the funicular wall as it enlarges.

Arrived at a stage when the morula measures about 0.05 mm. in diameter, a certain number of cells (8-12 as seen in equatorial sections) on one side of it form a special group (fig. 38, Pl. XIX), at first very indistinctly distinguishable from the rest of the cells. Gradually, a small cavity appears in the centre of that spherical group of cells which are steadily increasing, changing it into a hollow, rather flattened sphere with distinct epithelial wall. This hollow sphere is the "cystogene Hälfte" of German authors, so called on account of its giving rise to the chitinous covering of the statoblast, and the remaining mass of cells constitutes the "Bildungsmasse." According to my observations, these two portions are not morphologically distinguishable from each other at a very early stage, but become secondarily distinct. This is also the view held by Nitsche and Verworn, while Braem saw them originate sharply separated from the outset in *Cristatella*. According to the last-mentioned

author the cystogenous sphere, which consists solely of cells of ectodermal origin, is the first to form and to this is added the Bildungs-masse by proliferation of (mesodermal) cells of the funicular wall. Provided that in either case the two portions are respectively ectodermal and mesodermal products, it would be of but secondary importance whether they are distinct from the beginning or become outwardly indistinguishable for a time. More study of this point is exceedingly desirable.

Further history of the development corresponds in the main with what is already known. The cells of the two portions are constantly increasing in number and the entire mass in size. Meanwhile, the cystogenous cells attain the character of columnar epithelium; the whole cystogenous sphere flattens, and soon takes the form of a shallow watch-glass, the internal cavity disappearing (fig. 40, Pl. XIX. *cyst. c.*). We may speak of it as the cystogenous cup. The concavity of the cup grows deeper, always closely clasping the mass of the remaining cells, i.e., the "Bildungs-masse." The cells of the latter begin to present a granular appearance by the deposition of refractile spherules in the protoplasm, comparable in nature to the deutoplasm of eggs or of yolk-cells in Plathelminthes. Braem could not convince himself of the truth of Nitsche's and Verworu's opinion that the granules are direct products of the nuclei; nor could I find any support to this view. About this stage, the cells in question assume a spindle-shape, the axis standing vertical to the cavity of the cystogenous cup (fig. 39, Pl. XIX, *gr. m.*). This state was also noticed by Braem in *Cristatella*. However, as the granulation advances, they become rounder again, until each cell is represented by a globular mass of granules with a nucleus at the centre (fig. 41, Pl. XIX, *gr. m.*).

As the cystogenous cup grows in size, its rim begins to close around the granular cell mass. This occurs after the latter has

almost attained its maximum size. In the meantime, a thin sheet of chitin is secreted between the two layers of the cystogenous cup; it is difficult to say whether it is the product of one or of both layers. This chitinous sheet subsequently attains considerable thickness. We may speak of it as the chitinous cup, as it has that shape along with the cystogenous cup. As the latter expands, its mouth narrows and the whole body of the young statoblast somewhat flattens, taking the form of a spheroid, the axis of which corresponds with that of the cystogenous cup. The two layers of the cystogenous cup were at first of the same thickness, but now the outer begins to thicken by the increase in height of its cells while the inner undergoes a contrary change. The cells of the latter begin to flatten first at the bottom-portion of the cystogenous cup.

Along the equatorial line of the spheroidal mass, the outer layer of the cystogenous cup is thrown into a fold, which encircles the young statoblast belt-like all around. The belt becomes more and more extensive, and consists, as seen in sections, of two closely opposed strata of cylindrical cells.

Meanwhile, a second chitinous layer is formed over the chitinous cup already present. Thus, the chitinous cup comes to consist of two layers; the outer of which is by far the thinner. Simultaneously and directly continuous with this outer chitinous layer a thin plate of the same nature is also deposited between the two epithelial strata of the belt. It may conveniently be designated the belt plate.

The elongated prismatic cells of the outer cystogenous layer, secrete around their basal ends thin chitinous wall continuous with the belt plate or the outer chitinous cup, on which they all sit. They thus bring forth hexagonal caskets open at one end, into which every one of them abuts with their bases. But the wall of these cells does not develop everywhere to the same extent. It keeps very low on

the exterior of the bottom of the cystogenous cup, and when the cup closes into a complete capsule, as it does later, the same condition is also seen on the opposite side, so that on a mature statoblast the polar surfaces show only a network of very low ridges. However, on both sides of the belt plate and of the capsular surface immediately adjoining them, the chitinous wall of cells attains considerable height, but never reaches the surface of the cell-layer. The open ends of chitinous tubes thus formed are finally closed by the formation of what is called the lid-plates. This process proceeds on the one hand centrifugally from the outer layer of the chitinous cup, at a line which circumscribes the reticulated polar area, and on the other in the opposite direction starting from the marginal edge of the belt-plate, so that the tubes on the midway are closed last. A glance on figs. 43-16 will make the matter clear at once. Moreover, the lid-plates divide the prismatic cells on either side of the belt-plate into an outer and an inner portion. The latter is completely enclosed in chitinous caskets, while the former conjointly with the epithelium covering the polar area invests the entire outer surface of the young statoblast. This investment is to be seen as long as the statoblast remains at the place of its development, but decays when the latter is set free by the dissolution of mother-polypides. As the lid-plates are developing, the marginal spines appear. Also at about this stage, the closure of the mouth of the cystogenous cup takes place. It thus completely encloses the granular cell-mass, followed by closure of the two layers of the chitinous cup, which then is turned into a perfect capsule. After this, the two polar areas present no point of structural difference.

The portion of the prismatic cells that are enclosed within the chitinous wall soon undergoes decomposition and gives place to a gas filling up the caskets. Thus the formation of the swimming-belt is

complete. Nitsche's statement that the cells evacuate the caskets before their closure is probably an error.

As already said, the inner cystogenous layer thins out by the flattening of its cells, and when the chitinous plates completely inclose the granular mass it forms a thin epithelial covering to the latter directly within the central capsule (figs. 42-46, Pl. XIX, *Enc. m.*). The size of the nuclei becomes smaller as the height of cells decreases, and reaches at last the dimensions given before when the mature statoblast was described. The cells of this membrane are distinctly bounded and hexagonal in shape.

Returning to the stage represented in fig. 41, the granular spheres, composing the mass contained within the cystogenous cup, have each a centrally placed nucleus, and growing larger (fig. 42, Pl. XIX.) press upon one another so that they assume a polyhedral form. They remain distinctly bounded as long as the rim of the chitinous cup remains open, but fuse together after the latter closes. It is a singular fact, that in some statoblasts, either the granular mass is produced in over-quantity, or the capsule formed is too small, so that a portion of the mass is left outside the statoblast as the capsule closes, afterwards disappearing.

The nuclei of the granular mass become smaller as the development of the statoblast advances. Arrived at a stage represented in fig. 42, Pl. XIX, the nuclei almost lose their peculiar chromatin reaction, and stain very faintly, so that in some preparations it is very difficult to detect them. This condition, however, lasts for a very short interval, and in all the later stages the nuclei are again distinctly visible. This peculiar behavior of the nuclei may have lead Verworn to assume that the granules are the product of the splitting of nuclei and that the latter as such are not found after the complete development of the granules.

The statoblast at the earliest stage of its development is of a milky-white color. The chitinous parts as they form themselves at first present light yellow color, which, as the development advances, darkens to the characteristic hue of the mature statoblast.

On attaining a certain size, the statoblast bulges out the funicular wall chiefly on one side, with its plane always parallel to the axis of the funiculus. When many statoblasts develop in the same funiculus, they generally lie alternately disposed, by which means economy of space is effected. It is on that side of the statoblast with which it joins the funicular tube that the cystogenous cup closes.

The number of statoblasts that develop in a single polyzoöid is usually five or six, in some cases as much as eight. Of these, the uppermost one is the oldest and the lowest the latest formed, so that at a certain period statoblasts in various stages of development in serial order may be seen in the same funiculus. In those old polyzoides that occupy the central part of a colony all the statoblasts usually attain maturity, while in the peripherally situated younger polyzoides the latest formed statoblast is generally still in quite an early stage of development at the time when the colony begins to dissolve away. These immature statoblasts undoubtedly suffer common decomposition with the mother-colony. As every polyzoöid produces statoblasts, their number in the entire colony is really very great. Once I counted no less than 870 statoblasts in a very small colony of about 1.5 cm. in diameter.

3. Development of the Polyzoöid in the Statoblast. *

As the mature statoblast floats on the surface of water, the belt-plate of the annulus splits horizontally, so that the shell may now be said as being composed of two valves. These however remain tightly apposed during winter. On the arrival of warm temperature, they separate from each other, but holding the whitish contents between. The two valves have then very much the appearance of a pair of cymbals. The separation takes place at a stage when no change is yet perceptible in the contents; hence I am inclined to ascribe its cause to some external influence rather than to internal pressure.

The contents of the statoblast, i. e., the granular mass with its enveloping epithelium, form a spheroidal mass. All along the outer margin or the equator of the spheroid, where the separation of the shell-valves has brought it in direct contact with water, the enveloping epithelium becomes thicker (figs. 48, *Out. lay.* and 48 A, Pl. XX.), owing to increase in height of cells, accompanied by great increase in size of the nuclei, which are now as large as those of grown-up polypides. The process of thickening thus begun at the equator proceeds gradually toward the two poles of the spheroidal mass, so that the membrane thickens latest at these places.

Meanwhile, the cells at two opposite areas on the equator become especially taller, so that the enveloping membrane acquires a marked thickness at these places. The areas in question are oblong

* After finishing the manuscript of this paper, I received No. 324 (1889) of the *Zoologischer Anzeiger* containing Braem's preliminary report entitled "Die Entwickl. d. Bryozoen-colonie im keimenden Statobl." His statements differ in many fundamental points from mine. There is sufficient ground to assume that very considerable variation of development obtains among different species of Polyzoa.

in shape, lying with their long axes along the equator, although no sharp boundary can be fixed. From an early stage, they show differences in the appearance of their cells and take quite different directions in their future development. The axis joining the centres of these areas corresponds, as will be seen later on, with the longitudinal axis of the future polyzooid. With regard to the relation of this axis with the longer or shorter axis of the statoblast, there seems to be no constant rule, although in the majority of cases the former corresponded with the shorter axis of the statoblast.

In one of the two areas, the cells acquire distinctly cylindrical form, and vacuoles are formed in some of them. In fact, they soon take the form and character of the cells of the outer layer of the endocyst. They begin to secrete gelatinous ectocyst of a sticky nature, by which means the germinating statoblast attaches itself to anything it may meet with, be it the wall of an aquarium, floating wood, or shells of other statoblasts.

The other area gives rise to the polyzooid. Its cells are of less height and vacuoles develop in them later than in the other area. At about the middle point of the area, the cells multiply, and a group of them sinks into the granular mass below, forming a solid club-shaped body, which a little later on becomes hollow by the retreat of its cells toward the periphery. We have now a hollow closed sac bounded by an epithelial layer of cells and connected with the superficial thickened area by means of a very short solid stalk (fig. 49, Pl. XX). Soon after, the latter also acquires a lumen, and the cavity (fig. 49, Pl. XX. *prim. l.*) of the hitherto closed sac comes to communicate with the exterior. Some cells of the granular mass lose a part of their granules, and arrange themselves into a sort of layer on the outside of the sac (fig. 49, Pl. XX. *lin. epith.*). The nuclei of these cells become larger as the granules lessen in quantity, and

approach those of ordinary cells in size and appearance. The outer limit of this layer is by no means definite, gradually losing itself in the granular mass.

As the sac elongates, it becomes constricted at the middle, dividing into an outer and an inner chamber. The constriction between the two chambers is the future mouth, and the inner chamber represents the future oesophagus and the stomach. The outer chamber soon acquires the form of a hollow cone, at the base of which the mouth opens and which tapers towards the outer opening. At the base of this conical chamber the epithelium is especially thickened and eventually gives rise to the lophophore and the tentacles, the chamber itself being the tentacular sheath. The investing layer derived from the granular cells (*lin. epith.*) become more and more conspicuous, and lines the entire outer surface of both chambers.

The lophophore is at first a semicircular ridge, clasping the mouth on that side which corresponds to the original bottom of the cystogenous cup (convex side). The ridge arises by the folding of the wall, in which process both layers are concerned. The ends of this semicircular ridge are prolonged in the form of free finger-like processes, the rudiments of lophophoral arms. The interior of the lophophoral rudiment is occupied by the granular mass as soon as it is formed. The developing polypide lies on its anal side when the statoblast is placed on its concave side.

Another constriction divides the lower chamber into the oesophagus and stomach. The stomach begins to send a hollow process upward to form the intestine (fig. 51, Pl. XX. *Intest.*).

The free edge of the lophophoral rudiment is divided into a series of knobs, which are conspicuous nearer the median line, becoming gradually smaller towards the tips of the arms. These knobs are the origin of the outer row of tentacles. In the meanwhile, a second

ridge running parallel with, but less extensive than, the first one, develops on the anal side of the mouth. Its extremities soon meet and fuse together with the limbs of the first-formed semicircular ridge. Tentacles are formed on the new ridge in the same way as described above; the range of their row extending on either side to tips of lophophoral processes. Thus the inner row of tentacles is established on the lophophore.

The hollow process sent up by the stomach grows larger, and finally its cavity opens into the upper chamber or the tentacular sheath, which, when evaginated, forms the tubular body of the polypide.

The account given above may suffice to show how the general shape of a polypide is formed in the contents of a statoblast. In the meantime rudiments of many other organs, of which the brain, the muscles, and the funiculus are the most important, have begun their development.

The cerebral ganglion arises as a pit-like invagination of the inner layer of the oesophageal wall, which is continuous with the outer layer of the body-wall. The process begins to take place at a stage when the stomach sends up the process that afterwards becomes the intestine, on the anal side of the oesophagus, just inside the mouth. The invagination is soon constricted off, turning it into a closed sac, which as it is being formed, carries with it the outer layer of the oesophageal wall, so that the latter invests it externally, at the same time connecting it with the oesophagus. The cavity of the sac persists as a sort of ventricle. The lower portion of the wall of the sac early begins to thicken, which process does not of course concern the investing layer, and finally develops itself into that portion which constitutes the main ganglionic mass (vide p. 115). The remaining portion of the sac-wall, except at two points, becomes thinner and

thinner as the entire ganglion increases in size. The two exceptional points just referred to, are where the sac-wall produces a pair of solid horn-like processes, each of which gradually elongates towards the tip of the lophophoral arms, passing between the two layers of their ceiling. The position of the lophophoral nerve-trunks directly beneath the outer layer led me at first to assume their origin from the latter, in a way analogous to the development of the central nervous system in vertebrates. A careful study, however, convinced me that such is not the case.

At the time when the intestinal cavity becomes continuous with the exterior at the anus, the whole body-cavity is still filled up with the granular mass. Some of the cells of the latter are seen to differentiate themselves from the rest, at two regions as seen in a median sagittal section (fig. 51, Pl. XX.), the one extending between the involuted tentacular sheath and the cystidal wall, and the other between the lower part of the oral side of the oesophagus and the part of the cystidal wall opposite to it. At these places, the cells lose their granules, elongate, and become spindle-shaped joining the two points between which they lie. Their further development has been already treated under the muscular system. The muscles that develop in the above mentioned regions are the parieto-vaginal and the retractors of the adult polypide respectively. The muscular layer of the endocyst and the alimentary canal develops itself later, probably from the cells of the lining epithelium in a similar way.

Almost simultaneously with the first appearance of muscles, the cells of the granular mass lying between the blind end of the stomach and the coenoecial wall opposite to it, lose a portion of their granules, and aggregate into a solid rod, which is, in sections of stained specimens, readily recognizable on account of the deeper coloring of its cells in contrast with the surrounding faintly colored granules.

Afterwards, what remains of the granules in these cells is entirely absorbed, and a lumen is formed inside the rod, converting it into a tube, the rudiment of the funiculus. Thus, it will be noticed that both the muscles and the funiculus are produced *in situ* from the granular mass in the statoblast.

When the development of the polypide is complete, two buds are already present on the oral side of the cystidal wall, one on each side of the median plane. These buds are first seen in the stage when the intestine is still blind. The manner of their development will be treated under the budding.

As noticed before, the granular cell mass compactly fills up the entire body-cavity until after the formation of all the important organs of the polypide. The cells then loosen themselves, as the consequence of the decrease of granules, which are being constantly used up, while the enhanced growth of the cystidal wall makes the body-cavity more and more spacious. When the young polypide begins to evaginate and expand their tentacular crown, naked conglomerates of granules, each with a nucleus at the centre, are seen scattered in the body-cavity. Mixed with these conglomerates, we see some others which have obtained a distinct wall, with the nucleus pressed against it. In a somewhat later stage, the granules are no longer visible in those cells with peripherally situated nuclei; instead of them we see a large vacuole in each cell, which has thus acquired the characters of what I have proposed to call blood-corpuscles.

It is perhaps worth noticing that the developing polypide carries the shell halves on the anal and the oral side of its body, presenting an appearance comparable to the condition of shells in Brachiopods.

4. Budding.

This mode of reproduction in Polyzoa has been studied by numerous investigators, but their opinions are more or less divided, especially as to the origin from which the bud receives its hypoblastic elements, and consequently, with regard to the relations of the germinal layers. Most of them derive the hypoblast from the outer layer of the endocyst, while a few are inclined to believe that the bud receives it from the gastric organ of the mother polypide.

According to Allman (1), who describes the process of budding in *Lophopus* and *Alcyonella*, the outer layer of the endocyst gives rise to all the lining cells of the alimentary canal, while the lining epithelium of the mother polypide becomes also the lining epithelium of the bud.

Metschnikoff (7) gives an account of budding in the embryo of *Alcyonella*. He found that after continued segmentation of the egg, the cells arrange themselves into a two layered hollow sphere, both layers of which enter into the constitution of the bud, the outer giving rise to the outer layer of the tentacles, the inner lining of the alimentary canal and probably also to the nervous ganglion, and the inner, to the lining epithelium and all the muscles.

Nitsche (8) studied the process of gemmation in *Alcyonella fungosa* and *Cristatella mucedo*. In both species, the wall of the alimentary canal is formed from a part of the endocystic invagination of the mother polyzooid. In other words, the lining layer of the alimentary canal is derived from the outer layer of the body-wall. Both Metschnikoff and Nitsche regard the outer layer of the endocyst as the ectoderm and the inner as the entoderm.

Hatschek's (4) account of budding in *Cristatella* is as follows. A hollow sac lies directly beneath the outer layer, invested by

the inner layer of the body-wall on its inner side, at the position in which the buds are constantly developing. When a bud is to be produced, a portion of this sac is constricted off and gives rise to the inner layer of the alimentary canal, while all other parts of the young polypide are formed by an invagination of the body wall. Thus, the sac is being constantly constricted off, as long as new buds are added to the colony.

Reinhard (9) studied the first budding in the embryo of *Acyonella fungosa* and *Cristatella mucedo*. The cells formed by the segmentation of the ovum produce a true gastrula by invagination. The blastopore, however, soon closes. The gastrula is comparable in all respects to the type of some other animals, and, therefore, he regards the inner layer as the entoderm. In the development of the bud, the entodermal cells seem to push into a certain thickened portion of the ectoderm, and form a part at least of the wall of the alimentary canal.

Salensky (11) also states that the outer layer of the zoëcium (cystid) gives rise to the lophophore and to the internal cells of the digestive tract, while the inner layer becomes the lining epithelium of the new polypide. He believes that the entoderm of the alimentary canal originates from the ectoderm of the zoëcium.

Haddon (3) who studied the gemmation of some marine Polyzoa, came to the conclusion, on theoretical grounds rather than from actual observation, that the alimentary canal is derived from the entodermic tissue of mother polypides.

To the position and the order of budding, the previous workers seem to have paid but little attention, except Bræm who dwells on the matter at some length. To this author we owe much of the exact knowledge of the process of budding. As will directly be seen, the process of budding takes place at certain definite polyzooids and in a certain definite manner, thus determining the shape

of the colony so characteristic for each species. What Bræm describes for *Cristatella* on this point does not apply in all its details to the present species.

At the place where buds appear, there is no muscular layer, as already observed by Nitsche, and the endocyst may here be represented as consisting of only two layers, viz. the outer cell layer and the inner lining epithelium. The latter, in direct contact with the former, passively follows all the changes in form undergone by the outer layer of the endocyst. So, it must be borne in mind, that when in describing different stages of budding, the changes of the outer layer (which is the inner layer of the bud as will be seen further on) alone are mentioned, similar changes are repeated by the lining epithelium (the outer layer of the bud).

At first, some cells of the outer layer push their way inward in the form of a solid knob covered by the lining epithelium (fig. 57. Pl. XX). At a certain observed stage in which the knob consisted of eighteen cells, many more were on their way of entering.

A cavity ultimately appears in the centre of the knob (fig. 58, Pl. XX.) and the cells arrange themselves regularly around it in epithelial order. The cavity soon comes to communicate with the exterior by means of a canal formed by the gradual retreat of cells at that part (fig. 59. Pl. XX.). The bud now represents a double-walled sac whose inner and outer layers are respectively continuations of the outer and inner layers of the endocyst. Thus it is plain, that the bud originates not by direct invagination of the two layers of the endocyst, but by the formation of a closed sac which secondarily opens outward.

As the bud grows in size, it inclines downwards and its oral side is connected to the cœnœcial wall along its whole length by a mesentary-like membrane which is the continuation of the lining epi-

thelium. A glance at fig. 56, Pl. XX. will make this clear. The middle portion of this mesentery-like membrane becomes thinner, and is finally perforated as shown in fig. 57, Pl. XX. The sac is then joined to the cystidal endocyst at two points, viz. at its opening and at the bottom. Rudiments of new buds are produced in the region lying between these two points, which separate more and more from each other.

The solid rod-like part of the lining epithelium which now joins the bottom of the sac-shaped bud with the cystidal wall, is the rudiment of the funiculus. It gradually lengthens, and a lumen is secondarily formed in it, turning it into a tubular organ. It grows in size, and with the early appearance of scanty muscular fibres inside its cavity the development of the funiculus is complete. Thus the result of my observations on the formation of this organ seems to agree essentially with that of Bräm (2) who describes the process in the following words: "In der Mediane erheben sich die Zellen des äusseren Blattes in Gestalt einer an der Oralseite der Primärknospe herablaufenden Längsleiste, welche seitlich von den Fortsetzungen der Magenfalten begrenzt erscheint. Indem sich die Zellen des Knospenhalses dann nach vorn umschlagen und an der Bildung des Integuments betheiligen, löst diese Leiste sich von dem Muttergewebe, welches hinter ihn zusammenfliesst, als selbstständige Strang los und verbindet einen oral und median vor dem Primärknospe gelegenen Punct der Leibeswand mit dem Grunde des Knospensackes."

A constriction is formed in the middle part of the sac-like bud, dividing it into two chambers. The constricted opening is the mouth of the future polypide, and the lower chamber develops into the alimentary canal. The upper chamber becomes somewhat conical in shape tapering toward the orifice of the bud. At the basal disc of

this chamber, where the mouth is situated, the cells of the inner wall are prismatic while elsewhere they are flat.

We now recognize all the parts that we have seen at a certain stage of intrastatoblastic development. The lophophore with its tentacles, the nervous system and the intestine, all develop just in the same way as described in the previous chapter. One important difference exists in this, that in the one case the lining epithelium is produced from cells of granular mass, while in the other it is the result of the increase in extent of the same layer of the mother cystid. It will be noticed from above statements, that the entire inner layer of the alimentary tract is derived from the solid knob sunk in from the outer layer of the endocyst. The hollow process (the intestine) sent up from the stomach meets with and opens into a pit sent in from above outside the tentacular area, on the side turned toward the centre of the colony. The lophophoral arms of every individual always project toward the anal side of the polypide; consequently they are all directed toward the centre of the colony.

While new polypides are thus being developed, their cystids are also growing in size, and some cells of its lining epithelium gradually give rise to the muscular layer. At first, when the young polypide is still represented by a simple sac, the portion of the mother cœncœcium around its orifice is only slightly elevated above the rest of the wall, but as the growth of the polypide advances, it becomes more and more prominent, growing in such a manner as to form at last a cell for the young polypide.

The retractor muscles of the polypide begin to appear when the bud is still a simple sac, shortly after the formation of the rudimentary funiculus. At the point of junction of the rudimentary polypide and the cœncœcium, some cells of the lining epithelium becomes differen-

tiated from the rest by assuming a spindle-shape. These cells gradually separate from their mother-layer and form two loose bundles which join the cœnœcium with the middle portions of the now two-chambered bud. The parieto-vaginal muscles also originate in a similar way, but at a considerably later stage, when the lophophore already shows a certain number of knob-like tentacles at its median portion. Thus, in the process of budding, both the funiculus and the muscles are developed as differentiations of the lining epithelium.

The young polypide, as it first evaginates, is a very pretty little animal with less than thirty tentacles. The more medianly situated tentacles are best developed, while they are yet knob-like nearer the tip of the lophophoral arms, where new tentacles are being added by degrees.

The buds arise on the marginal cœnœcial branch alone, on the side facing away from the centre of the colony, i.e., on the oral side when we take the polypide into consideration. They always develop in pairs, one on each side of the median plane. Hence the dichotomy of the cœnœcium, with a polypide-bearing branchlet at each axil. The colony as a whole is consequently fan-shaped at first. With continued budding, it grows toward the periphery, its radius lengthening in arithmetical, and the marginal line in geometrical ratio. The two extremities of the latter soon touch each other in a complete circle; after this the growth of the colony throws its marginal line as well as its hitherto flatly expanded surface into folds, which make the regular arrangement of polypides unrecognizable at a glance.

The upper series of diagrams in fig. 62. Pl. XX, show early stages in the development of a colony, each circle indicating an individual. These figures represent for sake of simplicity each individual as giving off only two buds at a time, and each of these buds again performing gemmation after some time. In reality, however,

such is not the case. On the contrary, we usually see in an actively budding individual at the margin of a colony, not only buds of the first order but also those of second and third order already formed. Buds of the first order are present, as already stated, in a single pair, while those of the second occur in two pairs, and the next order, the most rudimentary, in four pairs. When the buds of the first order have grown sufficiently to be regarded as new individuals, those of the second and the third order occupy the grade of the first and the second order, while those of the third order arise anew. A comparison of the lower series of diagrams in fig. 62. with the upper series will help to make the matter clear. The blackened spots in the lower diagrams show the gemmiparous portion of the endocyst. This spot might appropriately be compared with the growing point of plants. With the growth of the colony, it advances centrifugally, splitting dichotomously at regular intervals. In this way, the colony grows as long as the condition is favorable.

It need scarcely be pointed out that the development of the first polyzooid in a statoblast essentially agrees in process and condition with that of later polyzooids by means of budding. In fact the first polyzooid is similarly budded off from the statoblastic contents, the whole of which is to be seen in the light of a primary cystid derived of and containing all the essential elements of cystids of the previous year. Whereas in marine forms the cystids winter as such, those of fresh-water forms persist only in the form of statoblasts to germinate in the following year as do the perennial cystids of the former. In the budding of fresh-water Polyzoa, a cystid and a polypide are formed simultaneously and an intrastatoblastic primary cystid is to

be considered as a particular sort of bud in which the formation of a apygid remains latent until the next year.

With regard to relations of germinal layers in a primary cystid, all the granular cells of the "Bildungsmasse" might with propriety be called the mesoblast on grounds of their genesis and of their future history. For the same reasons, the enveloping epithelium might be looked upon as the ectoblast except at the growing point, i.e. where the buds are formed. At this point the cells are still in undifferentiated embryonal condition comparable to cells of a blastula which differentiates into Ectoblast and Entoblast for the first time at its invagination. As the colony grows, the growing point of the primary cystid is split and transmitted into each succeeding bud, very much like the growing point of a plant; in other words all the growing points seen in marginal polyzooids of a polyzoan colony have started. I believe Bram is of the same opinion. Considering, on the contrary, the outer layer of the ectocyst at the growing point as strictly epiblastic, the conclusions, to which Nitsche, Joliet, Salensky, &c. were led, that no hypoblast enters into the bud and that it is formed as a secondary product of the epiblast, are certainly unavoidable. But such a conclusion does not accord, as was pointed out by Haddon, with the generally accepted nature of budding in the animal kingdom. In my opinion the budding in Polyzoa is only so far exceptional as the Epiblast and hypoblast take part in an undifferentiated embryonal condition.



Works referred to.

1. G. J. Allman.—A Monograph of the Freshwater Polyzoa. 1856.
2. Fr. Brém.—Untersuchungen über die Bryozoen des süßsen Wassers. Zool. Anz. XI. 1888.
3. A. C. Haddon.—On budding in Polyzoa. Quart. Journ. Mic. Sc. XXIII. 1883.
4. B. Hatschek.—Embryonalentwicklung und Knospung d. *Pedicellina echinata*. Zeit. f. w. Zool. XXIX. 1877.
5. A. Hyatt.—Observations on Polyzoa, Suborder Phylactolamata. 1865.
6. L. Joliet.—Organe segmentaire des Bryozoaires endoproctes. Arch. de Zool. expér. et gén. VIII. 1880.
7. E. Metschnikoff.—Bull. de l'Acad. de St. Pétersbourg. XX. 1871.
8. H. Nitsche.—Beiträge zur Kenntniss der Bryozoen. Zeit. f. w. Zool. XXV. Suppl. 1875.
9. W. Reinhard.—Zur Kenntniss der Süßwasser-Bryozoen. Zool. Anz. III. 1880.
10. A. Sæftigen.—Das Nervensystem der Phylactolamen süßwasser Bryozoen. Zool. Anz. XI. 1888.
11. M. Salensky.—Études sur les Bryozoaires endoproctes. Ann. des Sc. Nat. 6 sér. Zool. V. 1877.
12. M. Verworn.—Beiträge zur Kenntniss der Süßwasser Bryozoen. Zeit. f. w. Zool. XLVI. 1888.



Explanation of Plates.

Plate XVII.

- Fig.* 1. A small group of colonies. nat. size.
Fig. 2. A polypide. $\times 10$.
Fig. 3. Shape of the cœnœcial endocyst.
Fig. 4. Diagrammatic representation of a polypide and a portion of the cœnœcial endocyst.

Tent. Tentacles. Epist. Epistome.

N. Gang. Nervous ganglion.

Oesoph. Oesophagus.

Invag. tube. Invaginable tube.

Over. Ovary. Stato. Statoblast.

M. I. Muscles of the funiculus.

M. II. Parieto-vaginal muscles.

M. III. Retractor of the polypide.

M. IV. Muscles of the gastric wall.

M. V. Muscular layer of the endocyst.

M. VI. Muscular fibres of the epistome.

Neph. Nephridia. Loph. Lophophore.

Tent. membr. Tentacular membrane.

- Fig.* 5. Statoblast. *a.* Front view. *b.* View in profile.

Plate XVIII.

Fig. 6. Cells in the ectocyst. $F \times 2$.*

Fig. 7. Section of the endocyst. $F \times 2$.

Out. lay. Outer layer.

* Zeiss' powers.

- Bas. membr. Basement membrane.
 Tr. mus. Transverse muscular fibres.
 L. mus. Longitudinal muscular fibres.
 Lin. epith. Lining epithelium.
 Vac. Vacuole.

Fig. 8. Longitudinal section of the epistome, with the ganglion and the excretory organs. B \times 4.

Gang. cav. Ganglion cavity.

Fig. 9. Cells of the upper half of the œsophagus. F \times 2.

Fig. 10. Cells of the lower half of the œsophagus. F \times 2.

Fig. 11. Section of the cardiac valve. B \times 4.

Fig. 12. Cross section of stomach.

Fig. 13. Cells of the inner layer of the gastric wall. F \times 2.

pyr. c. pyramidal cells.

cl. c. club-shaped cells.

Fig. 14. Cells of the rectum. F \times 2.

Fig. 15. a. Diagram showing the extent of the mesentery.

b. Section of the mesentery. D \times 2.

Fig. 16. Cross section of a tentacle. F \times 2.

Fig. 17. Longitudinal section of the tentacle. F \times 2.

Fig. 18. Diagram showing the base of tentacles.

Fig. 19. Diagram showing the direction of the currents of the perigastric fluid.

Fig. 20. Cells floating in the perigastric fluid. F \times 2.

Figs. 21, 22, 23, 24, 25, 26. Sections at various levels of the upper portion of a polypide. B \times 4.

Figs. 21A, 22A, 23A, 24A. Sections of the excretory organs. F \times 2.

Fig. 26 bis. Entire form of the excretory organs.

Plate XIX.

Fig. 27. Nervous ganglion.

Fig. 28. Saggittal section of the ganglion. E \times 2.

Fig. 29, a, b, c. Diagrammatic Sections of the ganglion, showing the extent of the ganglion cavity.

a, sagittal, *b,* horizontal, *c,* frontal, sections.

Fig. 30. Cross section of a lophophoral arm. D \times 2.

Fig. 31. Cross section of the funiculus. F \times 2.

Fig. 32. Longitudinal section of the upper extremity of the funiculus. D \times 2.

Fig. 33. Longitudinal section of the lower extremity of the funiculus. D \times 2.

Fig. 34. Section of Ovary. F \times 2.

Fig. 35. Marginal spines of statoblast. F \times 2.

Fig. 36. The Enveloping cell-layer of the statoblastic content. F \times 2.

Figs. 37-45. Various stages in the development of the statoblast.
37-39. F \times 2. 40. D \times 2. 41-45. B \times 4.

Fun. Funicular wall. Cyst. c. Cystogenous cells. Gr. m. Granular cell-mass. Caps. chitinous capsule. Env. m. Enveloping cellular membrane.

Fig. 46. Section of a mature statoblast. B \times 4.

Fig. 46A. A portion of the statoblastic content. F \times 2.

Plate XX.

Figs. 47-52. Various stages in the development of Polypide in the statoblast. B \times 4.

Prim. l. Primitive lumen.

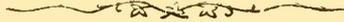
Bl. c. Floating cells.

Figs. 47A, 48A. Portions of the statoblastic content in the stages corresponding to Figs. 47 and 48. $F \times 2$.

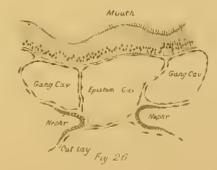
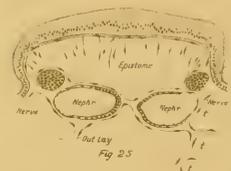
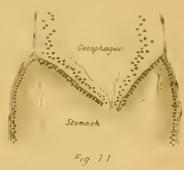
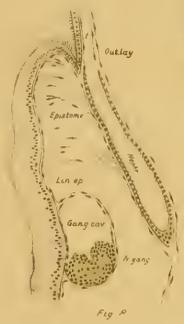
Fig. 52. Floating cells. $F \times 2$.

Figs. 53-60. Stages in Budding. 53, $F \times 2$. 54-56, $D \times 2$.
57-60, $B \times 4$.

Fig. 61. Diagrams showing the manner of budding. The Roman numerals show the order of the individuals.







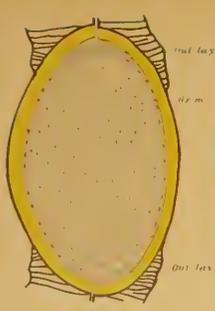


Fig. 1

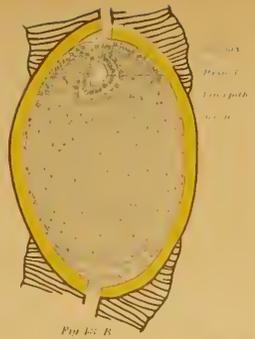


Fig. 2

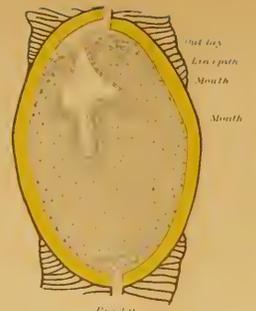


Fig. 3

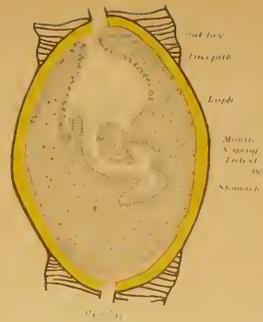


Fig. 4

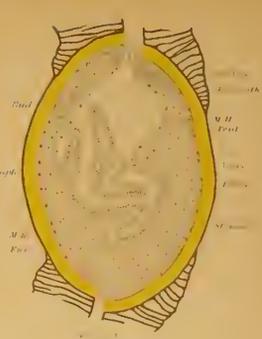


Fig. 5



Fig. 6

Fig. 6



Fig. 7



Fig. 8



Fig. 9



Fig. 10



Fig. 11

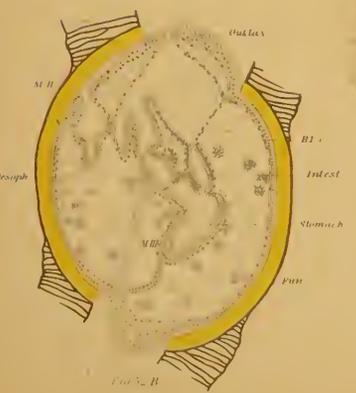


Fig. 12



Fig. 13



Fig. 14



Fig. 15



Fig. 16



Fig. 17



Fig. 18



Fig. 19