GREEN

Budding in the Larva of Pectinatella

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BUDDING IN THE LARVA OF PECTINATELLA

BY

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Bessie Rose Green

ENTITLED Budding in the Larva of Pectinatella

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF **Master of Arts**

Henry B. Ward
In Charge of Major Work

Henry B. Ward
Head of Department

Recommendation concurred in:

Committee
on
Final Examination

I. INTRODUCTION.

During the first week of August, 1909, while working at the Illinois Biological Station at Havana, Illinois, Professor Smith, who had charge of the station, called my attention to the free swimming larva of Pectinatella.

The larvae were first seen in a jar containing a little moss, and partly filled with water which had been dipped from Quiver lake near its east shore. There were about a dozen of these forms swimming about in the water. After a careful examination, under the low power of the microscope, it was plainly seen that this free swimming form was a Bryozoan larva. In detail, it differed from any Bryozoan larvae found in that region, previously. The next thing to determine was to what Bryozoan this larva belonged. All of the collections made that day were examined, and those in which Pectinatella colonies were present contained more of the free swimming larvae than others. The possibility of their being the larvae of this form was suggested, but their identity had to be established for certain. The colonies were thoroughly washed, and placed in a jar of clear water, thus getting rid of the larvae that might have adhered to the colony. After a few minutes just as many larvae were seen swimming about, as had been observed before. By watching closely, they could be seen emerging from the colony. These observations established the fact, beyond a doubt, that the form under consideration was the free swimming larva of Pectinatella.

Several excursions were made to the same region, the east shore of Quiver lake, where larvae and colonies were collected in great abundance. Before making the collections, the water in the

neighborhood of the colonies was examined, and, as was expected, numerous larvae were seen swimming about. Evidence points toward the fact that these larvae spend a part of their existence as free swimming organisms.

The Pectinatella colonies were exceedingly numerous along the east shore of the lake in places over shadowed by trees. They grew on the trunks of trees and stems of water plants just a few inches below the surface of the water. The **size** of the colonies varied from small ones to those having a diameter of eight or ten inches. In this same region the **statoblasts** of Pectinatella were floating on the surface of the water in large numbers.

The larvae were collected by straining the water through a fine net. The colonies, especially the older ones, were placed in jars of clear water, and by shaking them gently the larvae that adhered to the surface, or had actually emerged from the colony, were soon free in the water. By straining, and repeating the process several times, larvae were collected in large quantities.

The method of killing and fixing was very simple. To prevent contractions caused by the killing fluid, the larvae were placed first into a dilute solution of chloretone. After a few minutes all movements had ceased. They were then transferred to a 0.1 % solution of hot corrosive sublimate in which they remained for about five minutes. Other killing agents were used, but this proved most successful. The stains that were most satisfactory for my work were Delafield's and Ehrlich's Haematoxylin with Acid Fuchsin used as a counter stain. Paracarmine was used for staining in toto.

A large number of the larvae were preserved, and brought to the University, where, under Dr. Ward's charge, I made a study of

their structure, and also of the process of budding.

The terminology is similar to that used by Davenport. Polypide is a term applied to the organs, taken as a whole, which are formed from a bud. If a plane be passed through the long axis of a polypide between the mouth and anus, that part in which the mouth is located is the oral side, and the opposite one, containing the anus, is the anal side. Coenocoel is the cavity of the larval sac. As far as I have been able to find out, the free swimming larva of Pectinatella has not been described. In fact very little has been written concerning the Pectinatella colony.

Kraepelin refers to the larva, and figures the ring fold. The larvae of other Bryozoans which are nearly related forms show structures that bear a decided resemblance to those of Pectinatella. The movement of this larva compares almost exactly to that of Plumatella as described by Braem.

Davenport's papers are useful for comparison in studying the process of budding. Although the process is not exactly the same in the two forms, yet it is comparable in many respects.

II. THE LARVA.

The free swimming larva of Pectinatella is a small whitish, pear shaped sac which is dome like at one end and more elongated and narrower at the other. It measures from 1 to 2 m m in length and 0,85 m m in width. The wall of the sac is comparatively thin and somewhat transparent so the polypides may easily be seen through it. The larva looks like a tiny balloon when it is quiet in the water. The rounded dome is uppermost, while the narrower end which contains the polypides and buds is lowermost. The surface of the dome is covered with cilia which vibrate very rapidly. At the edge of the dome is a ring like fold which engirdles the larva. This fold had approximately the same position, and was of the same general appearance, in all of the larvae collected. The rest of the larva was without cilia.

In studying the preserved material, it was found that the size and stage of development of the larvae varied considerably. Size cannot be considered as a criterion for the determination of the age of the larva. In many cases some of the smaller larvae contain two or even four fully formed polypides, while larger larvae have only a few small buds developed. One or two larvae of average size were collected and, on examination, proved to be larval sacs without any buds. The majority of the larvae contained four well developed polypides besides several buds in various stages of development. There were four openings at the non-ciliated end of these larvae, through which the lophophores of the polypides could be protruded and retracted. A very conspicuous reddish-brown spot could be seen at the base of the lophophores in the living specimen.

Fig. 1 represents a larva in which two polypides and two buds could easily be seen. This is a drawing of a typical larva, and the general relation of parts is well represented here. A single polypide is represented in Fig. 2.

Movement.

The larva progresses by a slow uniform movement which is accomplished by the action of the cilia that cover the dome shaped part of the larval sac. This part goes first in movement. When the larva remains quiet for a time the ciliated part is uppermost, and its long axis is in a vertical position. In moving in a horizontal plane the long axis is at a slight angle to that plane. The ciliated part is just a little higher than the opposite end. It swims about here and there as if in search of a place for attachment. The movement in general is in straight lines. Some of the larvae rested on the bottom of the jar for a few minutes at a time and then proceeded, as before, in their random movements. The exact length of the period during which the larva remains free swimming was not determined, but all larvae under my observation had become attached at the end of twenty-four hours. Only a few larvae that had become attached were collected, because conditions were unfavorable for their existence. The plan was to obtain a series of the earliest stages of development, taken a few hours apart. Only two steps in the series were obtained, for conditions were such that all of the larvae had disappeared at the end of the first day after they had become attached.

Wall of the Larva.

The wall of the larval sac is made up of two layers of

cells, the outer, ectoderm layer, and an inner layer which lines the coenocoel. In the dome shaped part the ectoderm is composed of ciliated, columnar cells which stain rather heavily. In the young larva, as is shown in Fig. 3, these cells are all of about the same size, thus forming a smooth, even layer. The nuclei are rather large, and are located near the base of the cells. In the older larva, on the other hand, this layer presents several peculiarities, as is represented in Fig. 6. In the first place, the cells have become very much elongated, thus making the layer wider. The inner half of the cells have expanded due to growth, and by so doing has increased the inner surface of this layer to such an extent that it forms many folds. A few vacuoles are found in the expanded part of the cells. Peculiar knoblike structures are found on the outer surface of this layer in the older larvae. They are very small having a height of about one-sixth that of the cilia. I was unable to make out the relation of these structures to the cells, but it was quite evident that they were projections from the surface of the ectoderm. They took on a reddish stain while the other structures stained blue. In some places, at least, it appeared as if a cilium passed through each little projection, and extended down into the cell. They are found on the entire ciliated surface of these older larvae. The ectoderm is composed of ciliated, columnar cells only as far as the edge of the ring fold. Here the character of the layer changes. There are no cilia present, and the part of this layer that forms the inner wall of the fold is composed of flattened cells. In what is termed the non-ciliated region, the layer gradually becomes very much wider. The cells are very much elongated, and the entire layer is highly vacuolated.

The ring fold is a peculiar structure. Its formation could not be traced in the free swimming larva, since all of the material collected showed the ring fold in the same stage as that represented in Fig. 4. This figure corresponds very closely with Kraepelin's figure of the ring fold of Pectinatella.

Fig. 5 represents a section of the ectoderm in the non-ciliated region of the larval wall. The cell outlines are indistinct, and the nuclei are not arranged in any definite order. In some cases the vacuoles have little strands of tissue passing through them, while in others there seems to be a substance, which stains lightly entirely filling the vacuoles.

The inner layer of the wall of the larva lines the coenocoel and also the cavity of the ring fold. In the dome shaped part the cells are flattened, making the layer very narrow. After tracing it beyond the ring fold a short distance, the cells appear more rounded and closer together. The layer becomes more or less irregular where the cells are so numerous. This increase in the number of cells of this layer is more evident in young larvae than in older ones. It bears some relation to the formation of buds, for it is only present in the region in which buds are formed.

Origin of Buds.

Buds originate in the non-ciliated region of the larval sac, and extend into the cavity. The dome shaped part of the larval wall does not contribute, in any way, toward the formation of the buds. The youngest stage in the development of the primary buds that was observed was in a larva where only two buds were present. They were located a short distance apart, and one was just a little smaller than the other. For the want of more data, I am unable to

say whether they originated as a double bud or not. Kraepelin has advanced the theory of double budding as the way in which the primary polypides originate. Braem takes up the opposite view. It is quite evident that each bud formed bears a definite relation to the bud near which it arises. Buds may arise in three ways, as a single bud some distance from an older one, as is represented in Fig. 9, as double buds of the same or different ages as shown in Figs. 5 and 7, or they may arise from the neck of an older bud, as shown in Fig. 8. Some buds arise very close to older ones, but still in the larval wall. This can be considered as a modification of the last way. It appears as if the buds are formed from both layers of the larval wall. Fig. 12 represents a very young bud. The inner layer of the larval wall by growth is folded in to form the bud. The cells of this layer are now cubical in form, and the protoplasm stains heavily showing that the cells are active. Beneath this layer is a mass of protoplasm containing a number of nuclei, but the cell boundaries are not distinct. This mass of cells is formed by the division of the ectoderm cells. The ectoderm cells are vacuolated, and, since division takes place through the center of the protoplasm, the result would be the formation of a vacuolated and a non-vacuolated cell. Further division of the latter forms the mass of cells. The nuclei are slightly larger than those in other cells, and the nucleoli are larger and appear branched. There is every indication of activity in these cells also. As development goes on these cells become enclosed within the bud where they form a layer of columnar cells, as is shown in Fig. 8. Sometimes vacuoles appear in the bud, but this is due, perhaps, to a very rapid growth of the inner layer of the larval sac in forming the bud, and a slow-

er development of that part derived from the ectoderm. These vacuoles soon disappear.

After the buds are formed, they gradually separate from the older bud near which or from which they originated. Their final position is usually to the side of the older bud and a little farther up on the larval wall. The larger and consequently the older polypides or buds are located closer to the pole of the non-ciliated region. The oral side of the polypide is always toward the larval wall, while the anal side is directed away, and points toward the center of the larval sac. In fully formed polypides their long axis is about parallel with the long axis of the larval sac.

All of the buds observed, formed on the oral side of the polypide or older bud. They arise either to the left, to the right, or directly above the neck of the older bud. In two or three cases, certain structures were developed on the anal side which had the appearance of buds, but were very small.

The primary buds, which are two in number, separate very rapidly, and take positions either 180 or 90 degrees apart. but in the same plane. The secondary buds arise close to the primaries. The secondaries may be found, one to the left of either primary, or one to the right of either primary. In some larvae one secondary arises to the right of one primary and the other to the left of the other primary. In this last type the primaries separate only 90 degrees, while in the other two, the primaries separate 180 degrees. In the two former cases the secondaries separate 180 degrees from each other, thus making their positions 90 degrees from the primaries. In the latter case, the secondaries are located 90 degrees from the primaries, but are only 90 de-

grees from each other. The two primaries and two secondaries are situated almost in the same plane, and their positions are such that each of the four polypides represents the corner of a square. In some cases the secondaries arise before the primaries are 180 degrees apart, and sometimes afterward. This statement applies where the primaries separate only 90 degrees as well. The same final relations obtain in either case. In some cases two buds are formed one on either side of the primaries, but one is always smaller than the other.

In the same larva, in general, the third pair of buds bear the same relation to the secondaries as the secondaries bear to the primaries. This budding in pairs continues for a time, and then four buds arise at about the same time, one near each of the primaries and secondaries.

The buds are regular in their development, and bear definite relations to the buds next older, near which they originate. When the buds become full grown, there appears an opening on the non-ciliated surface of the larva just at the place where the buds are attached. The outer layer of the larva becomes invaginated, and its cells in the invaginated part are filled with protoplasm, and arranged in the form of a ring. This invagination is not deep, and the cells on the summit are vacuolated, and at last separate, making the passage to the outside complete. Figs. 13 and 14 represent the formation of this opening. The sections were cut a little obliquely, and so the entire passage does not appear in one section. Four of these openings were present in the older larvae.

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EXPLANATION OF FIGURES.

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All figures were drawn with the aid of a camera lucida from preparations of Pectinatella.

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Abbreviations .

An. = anus

b. = bud

b_1. = daughter bud

c. = cilia

ca. = caecum

c.f. = cavity of fold

coen. = coenocoel

c.p. = ciliated part of larval sac

i. l. = inner layer

inv. = invagination

kmp. drm. = kamptoderm

loph. = lophophore

lu.gm. = lumen of bud

n.p. = non-ciliated part

o. = external opening in larval sac

oe. = oesophagus

P = Primary bud

p = Polypide

r. = rectum

r.f. = ring fold

S = secondary bud

St. = stomach

T = Third bud

t. = tentacle

w. = wall of larval sac

v. = vacuole

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Fig. 1. The entire larva. An optical section, showing the relation of parts of the larval sac, and also the position of polypides. x 81.

Fig. 2. A polypide in position with a part of larval wall to the right. x 270.

Fig. 3. A section of the larval sac in the dome-shaped portion. Cross section, 14μ in thickness. x 570.

Fig. 4. A longitudinal section through the ring fold. 14 x 570.

Fig. 5. A section of the larval wall in the non-ciliated portion, showing the formation of two buds at once. x 570.

Fig. 6. A section of the larval sac in the dome-shaped portion of an older larva. Longitudinal section, showing the modified ectoderm, and the peculiar structures at the bases of the cilia. x 570.

Fig. 7. Two buds forming at the same place. One is just a little older than the other. x 570.

Fig. 8. A section showing the formation of a young bud from the neck region of the older one. x 570.

Fig. 9. A young bud developing directly from the wall of the larva at some distance from an older bud. x 570.

Fig. 12. A very young bud developing very close to an older one. This shows clearly the way in which buds arise. x 570.

Fig. 13. Shows the invagination forming the opening through which the lophophores may be protruded. The summit of the invaginated part is vacuolated. The entire passage way does not show here because the sections were cut obliquely. x 570.

Fig. 14. A section of the same larva as that represented in Fig. 13. Here the opening at the outside does not show, but the opening at the summit of the invagination does. The lophophores are represented diagrammatically in this figure. x 570.

Figs. 15 & 16. A diagrammatic representation of the buds in a single larva. The relative size and position of buds are represented by the circles on the inside of the large circle.

Fig. 17. A younger stage in the development of the buds. In this larva the secondaries originated to the right of the primaries.

Fig. 18. Represents the secondaries arising to the left of the primaries.

Fig. 19 & 20. One secondary arises to the right of the primary and one to the left of the other primary.

Fig. 1.

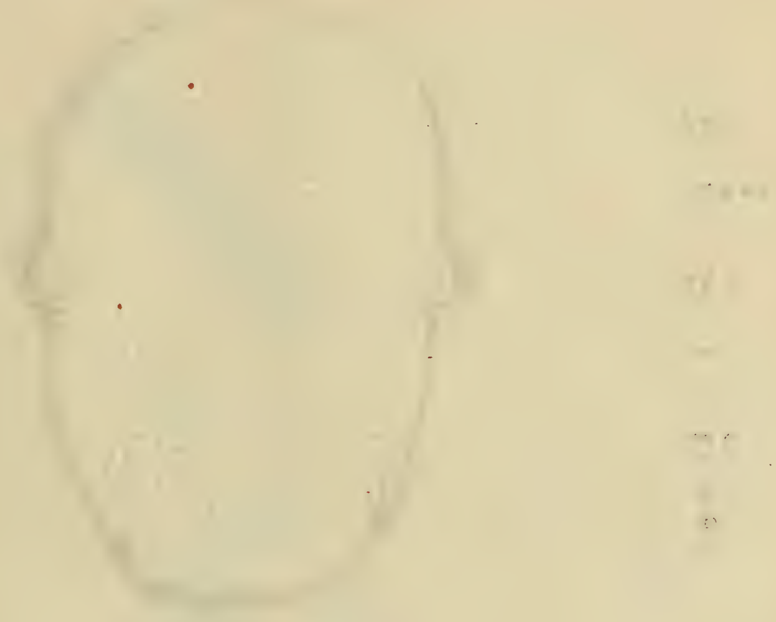

Fig. 2.

Fig. 3.

Fig. 4.

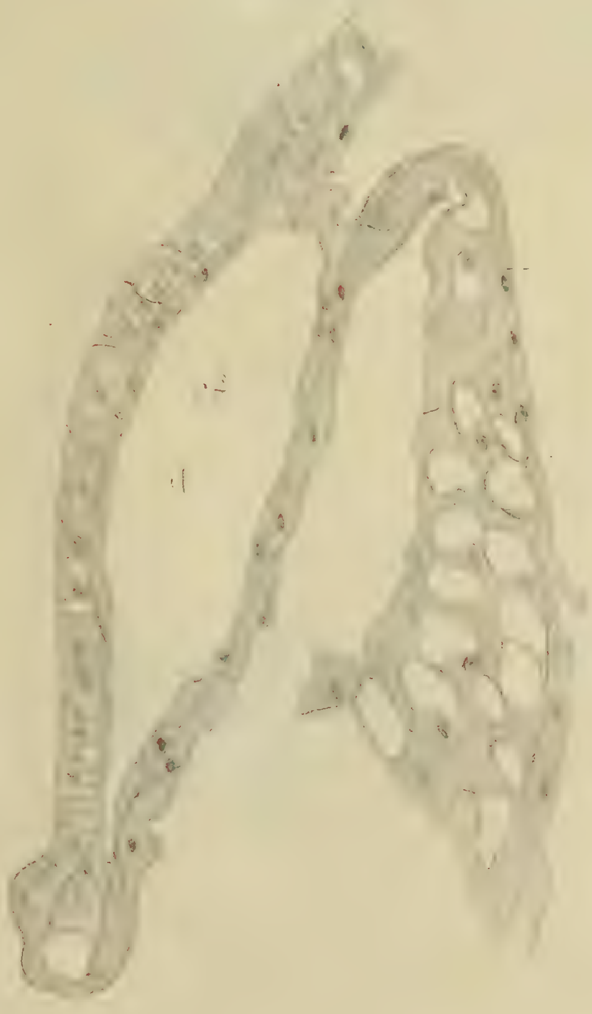

Fig. 5.

Fig. 6.

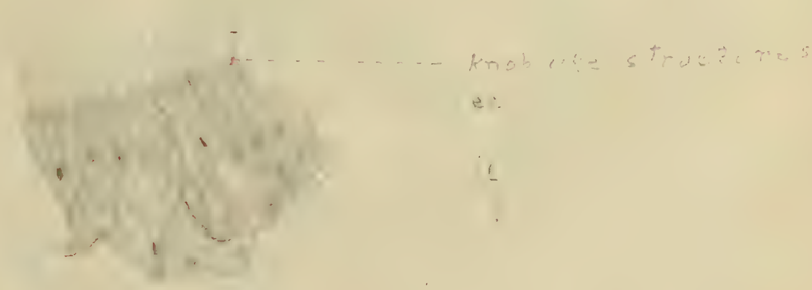

Fig. 7.

Fig. 8.

Fig. 9.

Fig. 12.

Fig. 13.

Fig. 14.

Fig. 15.

Fig. 16.

Fig. 17.

Fig. 18.

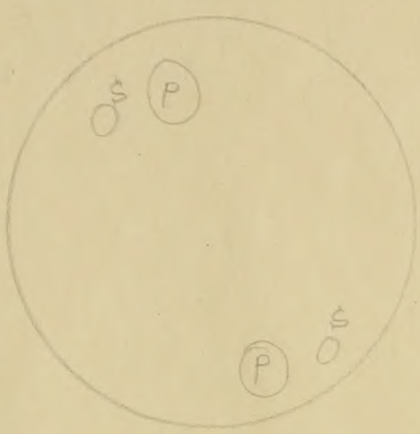

Fig. 19.

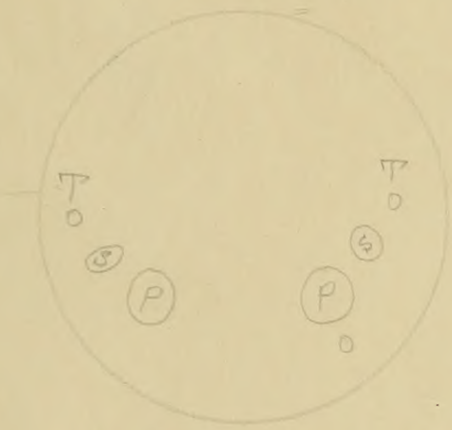

Fig. 20.