Regeneration in Hydromedusae[1].

By

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With Plates IV—VII.

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I. Introduction.

Considerable work has been done on the regeneration and grafting in the Hydrozoa by LOEB, BICKFORD, DRIESCH, HARGITT, PEEBLES, MORGAN and others. The results of these experiments have been, at times, somewhat conflicting. The work among the Tubularians has been largely limited to Tubularia crocea and T. mesembryanthemum, and the histological work has not been extensive nor conclusive. The work of which this paper is a record was therefore undertaken to review certain experiments and try to throw some light on disputed or inconclusive statements. Also to carry out careful histological studies on the regenerated structures. The experimental work was done during the summer of 1901 at the Marine Laboratory at Woods Hole. The histological work was begun the next winter at Syracuse University, and completed in the spring of 1903 at the University of Nebraska.

To my father, Professor C. W. HARGITT, I am greatly indebted for supervision and helpful suggestions during the early part of the work.

II. Material.

Material for the experiments was found in Great Harbor, Vineyard Sound, and surrounding waters. Tubularia crocea, Eudendrium ramosum, and Pennaria were found in great abundance on the piles of the U. S. Fish Commission docks. Tubularia tenella and Tubularia

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larynx were dredged from a shelly bottom in Vineyard Sound. This latter species has quite a different habit from the others While T. crocea and T. tenella grow in thick bunches or clusters of a moderate height, T. larynx seems to twine about older individuals of the same or possibly other species. It attains quite a considerable height also. T. tenella and T. larynx were operated on within a few hours, the other forms within 30—60 minutes.

III. Methods.

The hydroids were cut, with a pair of sharp scissors, into the desired lengths and placed in watch glasses, petri-dishes, finger bowls etc. in fresh sea water and covered with glass plates to exclude the dust. The water was changed at least once a day, oftener if conditions demanded. At first the water was taken from the tap in the laboratory, but as this seemed to have an injurious effect on the hydroids, it was later taken directly from the open harbor. In grafting, the cut surfaces were brought into close contact and held in place by freshly shaven bits of lead. The hydranths were always removed before grafting, because if left their movements would disturb the pieces and thus destroy the contact.

Specimens were killed in GILSON's fluid and in corrosive acetic acid to which picric acid had been added. Staining was done in toto with Borax Carmine and the resulting sections were very satisfactory for general structure and form, tho detailed work on the nuclei etc. could not be done on such slides. On the slide staining was done with HEIDENHAIN's iron-haematoxylin, and also with DELAFIELD's haematoxylin, then running up to absolute alcohol and differentiating with picric acid. This latter method was very good for differentiating the entoderm granules etc., but in general the best results were obtained with iron-haematoxylin.

IV. Experimental Work.

1. Regeneration.

From his work on Antennularia antennina, LOEB (1893, p. 42) drew the following conclusions, »In Antennularia gravitation not only determines the place of origin of various organs, but also the direction of their growth«. He states that rhizoids grow from the lower side or end, and hydranths from the parts directed upward, regardless of the position of the stem. DRIESCH (1897) working on Antennularia

ramosa found that other factors beside gravity influenced the regeneration. MORGAN (1901) found for A. ramosa that roots usually form at both ends. He suggests however that »The development or presence of roots on the basal end, prevents development of roots on the apical end«. STEVENS (1902) working on the same form concludes that the kind of regeneration is determined neither by the polarity of the piece nor by »orientation with respect to gravity«, nor by condition existing at the other end of the piece; but that certain parts of the stalk show a tendency to form roots and other parts to produce stems.

To determine whether gravitation had any effect on the regeneration in Tubularia crocea, I took pieces with branches and placed the distal end of the main stalk in sand. The piece was fixed so that the ends of the branches cleared the sand and were entirely surrounded by water. In no case did stolons form, tho hydranths formed quite rapidly and readily. It was further noted that hydranths formed sooner on the ends of branches, whether they extended laterally or downward, than they did on the ends of the main stems which pointed directly upward (Fig. 5). This would seem to indicate quite clearly that gravity has no influence on the regeneration in this species, at least on the regeneration of the stems, and furthermore that regeneration of hydranths occurs more rapidly at the distal ends of stems.

LOEB (1893) also states that in Margelis and Pennaria the kind of regeneration and the direction of growth is determined by contact. If the end was surrounded by water a hydranth was formed. That contact has no effect on the regeneration in Tubularia crocea, T. tenella and T. larynx, is shown by the following examples. In several experiments the pieces were simply laid flat on the bottom of the dish, regeneration was as follows: In T. larynx hydranths almost always formed at both ends, stolons never formed. In T. crocea and T. tenella hydranths developed at the distal end first and quite often at the other end also. Stolons very rarely formed. In several pieces of T. crocea a stolon like growth along the bottom of the dish was the first indication of regeneration. This growth continued for quite a distance and then turned upward away from the dish and formed a hydranth. Experiments on Eudendrium and Pennaria confirmed LOEB's results with Margelis and Pennaria, at least to a great extent. Pieces of Pennaria when laid flat on the bottom of the dish almost invariably formed stolons from the ends of the stems

which were in contact with the glass. It was only from those branches which were completely surrounded by water that hydranths developed. This formation of stolons took place as readily from the distal as from the proximal end. In one case regeneration in Pennaria was very similar to that in Tubularia crocea, viz. the formation of a stolon like growth along the bottom of the dish and later the growth upward and the formation of a hydranth (Fig. 8). In general Eudendrium showed the same phenomena, tho the influence of contact was not so marked. Indeed in one instance the cut end of a branch in direct contact with the glass formed a hydranth. When Tubularia and Pennaria were placed in sand no stolons formed from the end in the sand, tho this may have been due to the lack of oxygen as LOEB (1892 p. 64) suggests.

DRIESCH (1897), PEEBLES (1900) and others state that when the end of Tubularia is cut obliquely, the tentacle anlagen will be laid down obliquely. PEEBLES found this variable however and in a later paper (1902) she obtained the following results. When long and short pieces are grafted by oblique surfaces, the resulting anlagen were sometimes obliquely and sometimes squarely placed. Pieces of equal length grafted by oblique surfaces developed anlagen squarely placed. While I gave no particular attention to this feature, nor investigated the conditions active in bringing about this phenomenon, in general it was noted that both long and short pieces which had been cut obliquely had the anlagen squarely placed.

The results of some of the principal experiments are summarized below.

Experiment 5. — Twenty pieces of Pennaria were placed in a dish, the bottom of which was covered with sand, in the following way: The pieces were stuck into the sand so that the main stem was vertical. The branches did not touch the sand but were entirely surrounded by water. Some pieces were fixed with the distal end in the sand, others with the proximal. Several pieces of stolons were also fixed upright in the same manner. Regeneration was very slow, tho it was never as rapid in this species as in Tubularia or Eudendrium. After several days considerable new growth had taken place and new perisarc secreted around this growth, but then the coenosarc had withdrawn leaving the perisarc empty. This growth occurred on all the pieces and was quite irregular, extending in all directions. After this had continued for several days, hydranths began to develop. The first one appeared on the proximal end of

a stem (distal end in the sand), tho very soon another one formed on the distal end of a stem (proximal end in the sand). Hydranths continued to develop for a considerable time, seeming to form as readily and at about the same rate from the proximal as from the distal ends. A hydranth also formed from the upper end of one of the stolons (Fig. 7). In several specimens hydranths formed on the distal ends of branches which pointed downward, and later turned directly upward (Fig. 6). The first indication of a developing hydranth is the formation of a knob by the coenosarc. A thin perisarc is secreted around this and the knob may increase in size a little or become elongated. The perisarc is ruptured, the knob of coenosarc pushes out and forms a hydranth, the tentacles first appearing as small buds or outgrowths and reaching mature size by new growth. In one specimen in which the distal end was in the sand a new stolon-like growth formed from it and pushed up through the sand.

Experiment 11. — This experiment with Tubularia crocea was conducted in the same manner as experiment 5. The pieces, however, were all placed with the distal ends in the sand. In 24 hours a hydranth had formed on the end of a branch (Fig. 5). In 36 hours three more hydranths had formed, all on the ends of branches, some extending laterally others downward. Twenty four hours later four hydranths had developed on other branches, one extending laterally and three pointing downward. Two hydranths had regenerated at the end of the main stem, i. e. at the proximal end. After this the pieces began to degenerate and soon died. Thus, in this experiment covering a period of about 80 hours, ten hydranths regenerated. Of these 8 developed at the distal ends of branches and 2 at the proximal ends of stems, which would seem to indicate a rather marked polarity. The formation of hydranths at the proximal ends of stems was always much slower in this species than the development at the distal ends.

Pennaria was found growing under entirely different conditions in different habitat, tho the morphological differences were slight. The most abundant was that growing on the piles of docks in the harbor. The other form was attached to floating eel grass and matured later than the previous form (cf. HARGITT 1901, p. 224). Experiments to determine regeneration were tried with both forms.

Experiment 13. — Pennaria from the piles of docks.

The pieces were simply laid flat on the bottom of the dish. Regeneration was rather slow. After some time the coenosarc emerged

from the perisarc. If a hydranth was to regenerate the coenosarc enlarged into a knob from which tentacles budded and the hydranth formed. If, on emergence from the perisarc, the coenosarc came in contact with the bottom or the side of the dish it flattened out to form holdfasts (Fig. 9). The behavior of these pieces resembles very closely that of Margelis noted by LOEB (1893). Every branch or stem which came into contact with the dish formed holdfasts, and hydranths formed only from the free ends. Moreover the holdfasts formed with equal facility from both ends. One pieces anchored itself to the bottom of the dish by the distal end and then assumed an upright position. Several hydranths formed from the branches and the proximal end of the stem (Fig. 15). As already suggested hydranths formed rather slowly. The first one was completely regenerated at the end of 42 hours and after this they formed continually for ten days. In this time 40—50 developed tho not all were present at any one time. Some would form and degenerate or be withdrawn into the perisarc and others form.

Experiment 14. — Pennaria from eel grass.

This experiment was conducted at the same time and in the same manner as experiment 13. The behavior of the pieces and the method of regeneration was the same as in the preceeding experiment, holdfasts forming at the places of contact with about equal rapidity and ease. The first hydranth was completely regenerated within 30 hours, tho after the first they did not develop as rapidly nor as abundantly. This may have been due to some harmful effect of the water, or more probably to a less healthy condition of the stems.

Experiment 16. — Pieces of Tubularia crocea placed with the distal ends in the sand as in Experiment 11. In about 18 hours a hydranth had completely regenerated on the end of a branch pointing downward. Twenty four hours later 3 more had formed, two of them on branches extending downward. After this hydranths formed on branches and on the ends of the main stems, i. e., at the proximal ends. Sometimes the hydranths on the ends of drooping branches hung downward, at other times they turned and grew upward. In this experiment, as in experiment 11, there seemed to be a strong tendency for hydranths to form at the distal ends of branches rather than at the free (proximal) ends of the stems, and when they formed on the end of the stem regeneration was much slower.

Experiment 23. — Tubularia crocea cut in pieces of about 3 mm length and placed on the bottom of the dish. No change was notice-

able for about two days and then, in several pieces, there seemed to be a collection of red pigment at one end. No further change or development took place although the pieces were kept two weeks. Several other like experiments were tried, both with T. crocea and T. tenella. In no case, however, did any growth take place, nor any sign of regeneration occur, except the collection of red pigment. This latter condition was found in perhaps 4 or 5 pieces out of 100 or more.

Experiment 24. — Tubularia crocea cut into pieces 12—20 mm in length and placed on the bottom of a dish. Some were cut obliquely some square across. In 24 hours the tentacle anlagen had developed in several pieces (Fig. 3). Thirty six hours later the anlagen had been formed in 12 more pieces, and 24 hours after this 10 new pieces showed the anlagen. After this the anlagen developed in the pieces gradually so that in 10 days all but 5 pieces out of 50 showed them. The proximal series seemed to be developed first. The general features of the development of the hydranths have been considered by other authors. First the coenosarc withdraws and the cut edges close in. Whether this closing in is a simple bending over of cut edges (MORGAN 1901), or due to amoeboid motion of the whole membrane (MORGAN 1902), or to a centripetal force acting on the cells at the cut edges (STEVENS 1902), I did not determine. After this membrane has been formed a thin perisarc is secreted over the open ends and the circulation of the enteric fluid begins, the longitudinal ridges of entoderm disappearing in the hydranth region. Then the tentacle anlagen are folded off, appearing as ridges. This folding seems to take place along the entire length of the tentacle, beginning at the distal end. The proximal anlagen are formed first. All this has taken place within the perisarc and seems to be a transformation of tissue as stated by BICKFORD (1894) for T. tenella. When the anlagen have been completely formed, the thin perisarc over the end of the piece is ruptured, the coenosarc elongates the hydranth is forced out and assumes the perfect hydranth form. The gonads were never noted as developing till later.

In several pieces this process was followed only to the formation of the tentacle anlagen, then the coenosarc was pushed out of the perisarc and the tentacles appeared as small knobs or buds, attaining their full size by further growth.

Experiment 25. — Tubularia tenella cut into pieces of 12—20 mm and arranged as in experiment 24. In about 48 hours several pieces

showed the formation of one line of tentacles. The development then proceeded rather slowly, hydranths being formed gradually. The process of development of hydranths is somewhat different from that in T. crocea. The end closes over in about the same way, circulation of fluid begins, and red pigment is deposited in the hydranth region. Then instead of the formation of the tentacle anlagen the hydranth body is separated from the rest of the stem by a constriction (Fig. 14*a*). The tentacles appear not as ridges, but as small buds (Figs. 14*b*, 14*c*), and the hydranth is pushed out of the old perisarc (Fig. 14*d*—14*f*). The tentacles continue to grow until they reach their normal length.

Experiment 27. — Tubularia larynx. The pieces were cut in about the same lenghts and arranged in the same manner as in the previous experiments. In 24 hours only one piece showed the presence of tentacle anlagen (this was at 8.00 P. M.). The next morning nine or ten fully formed hydranths were found. The next day all that had not yet developed hydranths were found to have formed them. (However, several pieces from the extreme basal region did not regenerate at all during the progress of the experiment.) Most of the pieces had hydranths at each end and there seemed to be a strong tendency to form in this way; moreover there was no noticeable difference in the time of development of hydranths of either end. All the hydranths were cut off. In 24 hours all showed the tentacle anlagen, and a little little later hydranths were fully formed. One piece which had regenerated a hydranth, cast it and formed another. This one was removed and another developed. This one was also cut off and still a new one regenerated. Thus in just a week, one piece regenerated four hydranths one after the other, the last three being formed in four days. Since I was forced to leave Woods Hole at this time, the regeneration of this piece could be followed no further.

In this species the regeneration differs from that of T. crocea and T. tenella. In every case under observation the first sign of regeneration was the emergance of the coenosarc from the old perisarc. A new perisarc was secreted about the coenosarc and a slight annulation was always present in the new perisarc at its union with the old. After the emergance of the coenosarc the red pigment was deposited near the end and tentacle anlagen were formed, resembling somewhat those in T. crocea (Figs. 13*a*, 13*b*). The anlagen were much longer however, and were usually twisted or twined spirally around the perisarc (Fig. 13*a*), and the distance between the two

series of tentacles was much greater. The proximal row was formed first as in T. crocea, but the distal tentacles seemed to form more as those of T. tenella, i. e. as buds. After the tentacles had been formed the hydranth emerged from the new perisarc, and took on the perfect hydranth form. The distal tentacles assumed their full size by further growth. Often gonads began to form before the hydranth emerged from the perisarc, or at any rate immediately afterwards (Figs. 11—13). Another difference in the laying down of anlagen is as follows. While in T. crocea the distal anlagen were formed very close to the end of the coenosarc, in T. larynx they developed a considerable distance back of the end thus leaving quite a mass of undifferentiated coenosarc between the distal row of tentacles and the distal end of the coenosarc (Figs. 13*a*, 13*b*). After the hydranth emerged from the perisarc this seemed to be absorbed into the rather short hypostome of the hydranth (Figs. 11, 12).

2. Grafting.

Experiments in grafting were tried upon Eudendrium ramosum, Eudendrium dispar, Pennaria tiarella, and Tubularia crocea. The results confirmed those of HARGITT (1899) that union of the same species takes place equally well whether grafted orally or aborally. However, I was unable to secure a union of Eudendrium ramosum and E. dispar as he did, tho the experiments along this particular line were not extensive enough to determine whether this was not due to some unfavorable condition.

The hydroids were operated upon as soon as possible after they were obtained. They were cut into the desired lengths with a pair of sharp scissors, as they left a clean surface and crushed the stem very little if at all. The cut pieces were then placed in petri-dishes, watch glasses etc. filled with fresh sea water. The cut surfaces were carefully brought into as close contact as possible and held in place by pieces of freshly shaven lead. The hydranths were removed in all cases, as the movements of hydranths and tentacles would destroy the close contact of the pieces. As soon as the pieces had stuck together, the lead was removed and the pieces allowed to develop freely. In Eudendrium and Pennaria the pieces were very soon held firmly in place without the lead, because of the holdfasts developed from the ends of the stems and branches in contact with the glass. After the coenosarcs had united a new perisarc was secreted over the point of union. In Tubularia crocea union was complete in from

18 to 24 hours. At the end of this time the circulation of fluid could be observed taking place between the two pieces. In the uniting of the coenosarcs the pieces were often pushed apart slightly (Fig. 4). However, a new perisarc was soon secreted over this and the wound entirely covered. The formation of hydranths took place quite rapidly after union was complete, sometimes forming at both ends. Eudendrium usually united in 24 hours, tho hydranths were not fully formed till 24—48 hours later. The method of union was similar to that of Tubularia and new perisarc was formed over the point of union (Fig. 1). The grafting of Eudendrium and Pennaria was was tried but no union took place, the coenosarc of Pennaria forcing itself out into stolons (Fig. 10).

In Pennaria the time necessary for complete union was from 24 to 48 hours. Hydranths developed slowly, not being fully formed till 24—48 hours later. A feature quite common in the grafting of Pennaria was the formation of stolons from the point of contact of the two pieces (Figs. 2*a*, 2*b*). Pennaria found growing on the piles of docks was successfully grafted with Pennaria from floating eel grass, tho, as already mentioned, the habits of the two were quite different.

V. Histology.

In her work on Tubularia tenella BICKFORD (1894, p. 422) says, »In the case of this hydroid . . . the regeneration appears to be largely a direct transformation of a stem portion over into the body portion of the new hydranth«. Since in her experiments no definite histological demonstration of this was undertaken, it has seemed desirable to repeat her experiments and to work out critically the histology of the processes involved. Also to determine to what extent the same processes of transformation are operative in T. crocea and T. larynx.

STEVENS (1901) who has worked out the histological changes involved in the development of the tentacles in T. mesembryanthemum, states that the proximal tentacles form by a folding of both ectoderm and entoderm, and the distal tentacles by a rod or column of entoderm being separated from the rest of the entodermal tissue, and the ectoderm folding around it and pinching off the tentacle. In regard to the increase in surface of the ectoderm made necessary by this process of folding she says, »The increase in surface of the ectoderm is due partly to cell division and partly to a change in the cells

from a more to a less columnar form«. Her drawings show mitotic figures in both ectoderm and entoderm cells. This does not seem to be the case in Tubularia crocea, T. tenella, and T. larynx. The cells do sometimes lose their typical columnar form, but not to any great extent. Therefore there must be cell division to account for this increase in surface of the ectoderm. Special attention was directed toward the staining and to the observation of mitotic figures, and yet in no case was the slightest evidence of mitotic division presented. Nuclei were found which gave evidence of amitotic division, and the various stages in this process were also presented, so that the cells probably divide directly. However, the resting nuclei very commonly contain two nucleoli and it is hard to distinguish between this resting condition and the early stages of amitosis. So it was not possible to prove definitely the presence of amitosis in all cases.

While this is a somewhat unusual histologic phenomenon and the wholly negative character of the evidence is insufficient to warrant definite conclusions, still it would seem rather remarkable that a critical examination of many hundreds of sections should have failed to reveal the presence of mitosis if at all prevalent.

Tubularia crocea.

Distal tentacles. — The distal tentacles seem to form about as STEVENS (1901) states for T. mesembryanthemum. Several of the entoderm cells are squeezed away from the enteric cavity into the entodermal tissue, forming a sort of column as shown in Figs. 22, 23. This causes the ectoderm to push outward slightly. These entoderm cells are gradually forced further away from the enteric cavity, sometimes assuming a position entirely within the ectoderm, against the outer wall of the entoderm and with their long axes at right angles to the long axes of the other cells. This of course pushes the ectoderm outward still more, forming a ridge. The ectoderm then gradually folds around this column of cells, finally enclosing it, the edges of the ectodermal folds fusing and thus pinching off the tentacle. Fig. 24 shows a condition sometimes found; a greater number of entoderm cells are pushed outward to form the entodermal column, the enclosing by the ectoderm and the completion of the tentacle being brought about in the usual way.

Proximal tentacles. — The proximal tentacles are formed somewhat differently, seeming to be the result of complex folding brought about in the following manner. The first evidence of developing tentacles

is a slight folding of both ectoderm and entoderm (Fig. 16). The folds at first rather loose and sinuous, gradually crowd closer together till the edges touch (Figs. 17, 18). By this time the folds have elongated radially and and a sort of entodermal column is thus formed, tho it is not as distinctly marked as in the distal tentacles. At this stage the whole region of the developing tentacles is composed of a series of narrow elongate folds (Fig. 19). The entodermal column is composed of two rows of cells with their edges closely dovetailed or else flattened and the edges overlapping. When this stage is reached the edges of the ectodermal folds begin to approach each other, gradually coming nearer and nearer (Fig. 20). Finally they entirely surround the entoderm cells and thus cut off the tentacle (Figs. 20, 21).

Tubularia tenella.

Distal tentacles. — The distal tentacles are developed about as in T. crocea. The entoderm cells are forced away from the enteric cavity, folding the ectoderm, which gradually encloses the entoderm cells and thus forms the tentacle. Fig. 29 shows the cells being squeezed away from the enteric cavity. In Fig. 30 later stages are shown. Quite often the first indication of a developing tentacle is a single cell (sometimes several) lying in the ectoderm close against the supporting layer and with its long axis at right angles to the long axes of the other cells. The origin of this cell can not always be definitely determined from the series of sections in which it occurs, tho it is doubtless entodermal. In other series it can be definitely traced to its original position in the entoderm. In series in which the cell can not be traced from the ectoderm into the entoderm, the hydranth had probably developed beyond the initial stage and the entoderm cell (or cells) had already undergone the migration from the normal position in the original stem. This single cell in the ectoderm seems to go through repeated divisions forming a mass of cells, after which the development proceeds in the manner already described.

Proximal tentacles. — The formation of the proximal tentacles is quite different from that of T. crocea, being accomplished by evagination in the early stages and not by folding. The evagination begins in the entoderm, the ectoderm being simply pushed outward. Figs. 25—28 show the course of development. In Fig. 25 the entoderm has begun to evaginate, pushing the ectoderm outward slightly. In Fig. 26 some of the entoderm cells have been pushed away from the enteric cavity

and the folding of the ectoderm continued. Fig. 27 shows the evagination nearly completed and the cells arranged in two rows, with edges interlocking. At about this stage the ectoderm begins to surround the evaginated entoderm cells, continuing till the folds of ectoderm meet, fuse, and thus separate the tentacles as shown in Fig. 28. In longitudinal sections the evagination is even more distinctly shown (Fig. 31). The development thus far has taken place within the perisarc as in T. crocea. When the regenerating hydranth emerges from the old perisarc the tentacles are not, as in T. crocea, fully formed (cf. Figs. 14*d*—14*f*), but only attain their final length by further growth. This would seem to indicate very clearly that, although in the beginning the formation of tentacles may be by the transformation of old tissue, the completion of the process is by new growth. BICKFORD's conclusions, already referred to, may therefore be true for the body of the hydranth, but the tentacles are at least partially of new growth.

Tubularia larynx.

Distal tentacles. — The distal tentacles form in about the same manner as in T. tenella. The single cells lying in the ectoderm, against the supporting layer, are found somewhat more commonly than in T. tenella and it is more difficult to trace their origin, tho they are doubtless entodermal. However, in several series of sections this cell has been traced to a position partly in the ectoderm and partly in the entoderm (Fig. 43). In a number of series also, entodermal cells seem to have been forced away from the enteric cavity in a manner similar to that found in T. tenella (Figs. 36—38). In these figures the appearance of early stages is shown. These cells being forced away from the enteric cavity more and more form a column of cells as shown in Figs. 39, 40. The ectoderm gradually surrounds this column and thus the tentacle is formed. Fig. 43 shows the single cells in the ectoderm. These cells seem to divide and form a column within the ectodermal layer (Fig. 41), and are gradually surrounded by the ectoderm and the tentacle pinched off (Fig. 42).

A comparison of Figs. 41—43 with Figs. 36, 39, 40 suggests the possibility that in some cases the first cell squeezed away from the enteric cavity is forced into the ectoderm immediately and by division forms a column of cells, which is surrounded by the ectoderm to form the tentacle; while in other cases the necessary number of entoderm cells are gradually forced away from the enteric cavity into the ecto-

derm and without undergoing any divisions make up the core of the tentacle, which is surrounded by the ectoderm in the usual way.

Proximal tentacles. — In the formation of the proximal tentacles, the complex folding of T. crocea does not take place, nor is the very evident evagination of T. tenella the method, but rather a combination of the two. The development begins very much the same as in T. tenella (Figs. 34, 35) by what seems to be an evagination of the entoderm. A sort of ridge is thus formed. The ectoderm folds around the entodermal cells (beginning at the distal end) very much the same as in T. crocea. By a continuation of the evagination and folding, the entoderm cells are surrounded by the ectoderm and the tentacle cut off (Figs. 32, 33). The tentacle when thus cut off is of normal length. Unlike T. crocea the gonads begin their formation before the hydranth emerges from the perisarc (cf. Figs. 13*a*, 13*b*).

Rather strange is the occurance of nematocysts in the entoderm and in the debris of the enteric cavity. This is most common in the regenerating stems of T. larynx, tho occasionally found in other forms. WEISMANN (1883) explains the presence of nettling cells in the developing male gonad of Clava squamata as a protection against a parasitic fungus. He performed a number of experiments with the result that the male form could not be infected, while the female gonad, which was not provided with the nettling cells was easily infected with the fungus. Such an explanation could scarcely account satisfactorily for the presence of the nettling cells in the entoderm. This explanation would prove to be especially inadequate since in other forms, in which the nettling cells are not found in the entoderm, the open end closes no quicker than in T. larynx.

Among the colonies of T. larynx obtained during the summer, three pieces were found to be naturally regenerating hydranths. These were killed and later sectioned. The folds in the hydranth region were extremely complex, often pinnate, and entirely unlike anything observed in the artifically regenerating hydranths of T. crocea, T. tenella or T. larynx. Owing to the very limited amount of this material no conclusions can be drawn, and no explanation offered, unless it be possible that such regeneration was the result of abnormal conditions, tho it is not easy to conceive what they might be.

Eudendrium ramosum.

Externally the first indication of a developing hydranth is the formation of a knob like protuberance by the coenosarc. A thin

layer of perisarc is secreted around this knob, tho this is later ruptured when the hydranth begins to develop further and the tentacles to form. This protuberance increases in size, elongating more or less and assuming a spherical shape. Then the tentacles bud off, at first very small, they later assume their normal length by new growth. The proboscis seems to form as a swelling or evagination at the distal end of the hydranth and rather late in the development the mouth opening breaks through.

Transverse sections of very early stages of the regenerating hydranths, i. e. sections through the knob like structure previously referred to, show the layers of ectoderm and entoderm to be much wider than in the normal stem regions, the cells being extremely elongated radially (Fig. 44). There are more cells present than in the normal stem, so that there must have been considerable cell proliferation. Indications of mitosis were found in both ectoderm and entoderm, tho not as abundant as in the entoderm of later stages. In the latter, however, mitoses were far from common. Indications of amitotic division were observed, being quite marked in some cases.

The tentacles seem to start by evagination involving both layers, and somewhat similar to the same condition in Tubularia tenella (Figs. 45—47). The further development of the tentacles takes place by new growth. The entoderm cells are arranged in a single row, being superimposed one upon another (Figs. 57*a*, 57*b*). These entoderm cells were sometimes found in the process of dividing mitotically and this phenomenon was occassionally observed in the ectoderm cells surrounding the tentacles. Amitotic division was not common among them, tho occasionally found. The shape of the cells however, was changed from columnar to cuboidal. Sometimes they were flattened even more (Fig. 60) so that their length radially was less than their dimensions in other directions. This flattening of the ectoderm cells would increase the surface to a considerable exent and thus allow growth of the entodermal core of the tentacle. It is extremely doubtful however, whether the increase in surface of ectoderm necessary to permit the growth of the entodermal column to the normal length could be accounted for entirely by the flattening of the cells. There must therefore be proliferation of the ectoderm cells, but whether this is by mitotic or amitotic division or by both could not be definitely determined, tho indications suggest mitotic division. If at all prevalent however, it seems rather strange that

mitotic figures were not found more abundantly, since sections were made of all stages of development and stained especially to bring out this feature. The occurrence of amitosis may be accounted for partially as an aid in cell proliferation, and perhaps also for another purpose to be considered a little later.

In the formation of the entodermal core of the tentacle in the manner shown in Figs. 57—60 (i. e. a single row of cells one on top of another), the question as to the method of development of this condition presents itself. Three explanations may be suggested:

1) Several cells from the primary evagination may divide and form a double row of cells as in Tubularia, and later be forced together to form a single row.

2) The single cell usually found at the apex of the evaginated fold (Figs. 45, 46, 49) may divide and the cells arising from this division continue to divide until the entodermal core is fully formed.

3) The cell at the apex of the evagination may mark the beginning of cell division, but instead of all the cells resulting from this primary division continuing to divide, only the apical cell of the developing entodermal core continues to divide till the core of the tentacle is fully formed.

That the first will not explain the facts is evident from an examination of sections of different stages, particularly of early stages. The conditions shown in Figs. 45, 46, 49, are brought about by the primary evagination as already stated. This condition is always found at the beginning of the development, and in sections of later stages there is no indication of this double row of cells, exept at the base of the tentacle (cf. Figs. 57*a*, 57*b*). If a double row of cells formed by cell division and later these were forced into a single row, the double row would undoubtedly still be present at early stages. That this is not the case is shown by a comparison of Fig. 57*a*. In this tentacle the entoderm is composed of only four cells, and these are arranged in a single row. This was always the condition after the tentacle had begun to elongate. It was more difficult to determine which of the other two suggested explanations was the more probable, because of the paucity of definite mitotic figures. The following facts however, seem to point strongly to the second as the more probable explanation. In Fig. 57*b* at »*a*« are shown two cells somewhat smaller than the others of the entodermal column. These two cells have every appearance of being the products of the division of a single large cell situated in that region, i. e. they

indicate a division of one of the middle cells of entodermal column. Furthermore, in transverse sections through about the middle portions of the developing tentacles, mitotic figures were found in the entoderm, which would not be the case if only the apical cell divided. Comparisons of some of the longitudinal sections show that the entoderm cells in the lower (proximal) end of the core of the tentacle are not of the same size, as would be the case if only the apical cell divided, but some are large and some are small as tho the result of intercalary division. These facts and observations show quite conclusively that the entodermal core of the tentacle is the result of the division of the single apical cell of the primary evagination, and then a continued division of all of the cells resulting from the first division. The entodermal core is surrounded by the ectoderm in early stages (Figs. 48, 50), and in the further development stretches the ectoderm by the division of the cells of the entoderm (Figs. 57*a*, 57*b*, 60). The increase in surface of the ectoderm is therefore partly due to the change in form of the cells from a columnar condition to a more or less flattened condition, tho there is doubtless some cell division in the ectoderm also. Figs. 52—56 show the course of development of two tentacles from the very early stage in which a single entoderm cell has been forced away from the enteric cavity, to the condition in which the tentacles are fully formed. The presence of more than one cell in sections of the tentacles as shown in some of the figures is due to the tentacle being cut somewhat obliquely instead of transversely.

In the mature hydranth the tentacles are sometimes composed of two series arranged in a single whorl. Those of one series are somewhat drooping in habit, the others more erect. The tentacles of the two series alternate in position and there is also an alternate elevation and depression of their bases. This condition of the tentacles is shown in the process of development in Fig. 51. The irregular mass of polygonal cells shown in this figure is due to the section being made across the mass of cells between the enteric cavity of the hydranth and the hypostome (cf. Figs. 58, 60).

The hypostome develops as a hollow sphere at the extreme distal end of the hydranth, beginning as an outgrowth of the layers of the regenerating hydranth. At a very early stage, however, all connexion between the enteric cavity of the hydranth and the cavity of the hypostome is blocked up by a mass of entoderm cells, probably due to a multiplication of cells in this region. The further

development is for the most part an increase in size. The shape of the cells change the ectoderm being finally composed of very much flattened cells, the result of the increase in size of the hypostome without great multiplication of cells of the ectoderm. The entoderm is made up of a large number of columnar cells very much elongated radially and very narrow. Figs. 58, 60 show the relation of the two layers and also the mass of entoderm cells blocking the passage between the enteric cavity and the hypostome. This mass of cells is at first without regular arrangement, being crowded together into a dense mass. Later in the development, these crowded cells take on a more regular arrangement as represented in Fig. 58. There is already present in this stage a sort of dividing line between the cells of the two sides, and by the separation of the layers of the two sides along the dividing line, the opening between the enteric cavity and the hypostome is formed (Fig. 59). At the angle formed by the junction of the hypostome with the hydranth proper the cells remain larger (cf. Fig. 59), a condition likewise found in the normal hydranths. The stage of development of the hydranth at which the opening forms seems to vary. In Fig. 59 the opening is shown and the tentacles were less than half their normal length. Fig. 60 shows the hydranth almost completely regenerated, tentacles of almost natural size and the opening has not yet been made. The mouth opening does not form till the hydranth is otherwise completely regenerated.

The enteric cavity of the regenerating hydranth is more or less filled with a mass of debris, (degenerating cells etc.), somewhat similar in appearance to the same condition in Tubularia. Doubtless this mass is partly composed of some of the cells blocking the opening between the hypostome and the enteric cavity at an earlier stage. The protoplasm of Eudendrium does not contain nearly as many granules as that of Tubularia, and the longitudinal entodermal ridges of the latter are not present, so that this debris is not made up of masses of granules set free by the breaking down of entodermal ridges, etc. The origin of this debris was not fully determined. Whether this debris is cast out of the hydranth, when the mouth opening is formed, as STEVENS (1902) has demonstrated for T. mesembryanthemum and T. crocea, was not determined during the course of the experimental work.

Pennaria.

The early appearance of regenerating hydranths in Pennaria is very similar to that of Eudendrium, viz. an enlarged knob or bulb-like protuberance of the coenosarc, from which tentacles seem to bud.

The material on hand was all killed in the early stages of regeneration so that the complete progress of development could not be worked out. Some points, however, in the early development seem to be sufficiently important to warrant mentioning them. One very pronounced feature is the great abundance of mematocysts in the regenerating hydranths. The ectoderm of the end and sides of the enlarged knob are very plentifully supplied with nematocysts and they are more or less abundant in the entire region of the future hydranth, as well as on the stem just below the knob. They were not found in the entoderm as in T. larynx.

There is the same thickening of the ectoderm and entoderm layers found in sections through very early stages as in Eudendrium ramosum and this condition is probably the result of cell proliferation. A careful examination of sections show mitoses to be fairly abundant, mitotic figures being found in both ectoderm and entoderm (Figs. 61, 62). Therefore in this form, as well as in Eudendrium ramosum, indications point to mitotic division as an active process in the development of the regenerating structures. However, indications of direct division were not lacking. In Fig. 63 is shown a nucleus in the process of direct division, accompanied by division of the cytoplasm, a cell wall being partly formed. In other sections nuclei were found dividing directly but the cytoplasm seemed to have taken no part in the process (Fig. 64). Furthermore amitotic division can not be considered merely as a somewhat abnormal condition brought about by artificial conditions. The same thing is found in sections made through the young hydranths developing normally on colonies growing in their natural habitat. Fig. 65 shows a considerable number of nuclei in different stages of direct division, and this is in the region of the developing tentacle. Such dividing nuclei were limited to the ectoderm.

Only a few sections through developing tentacles were obtained. Fig. 66 shows the appearance of the cells in one case. Since the core of the mature tentacle is made up of a single row of entoderm cells, as in Eudendrium, the same process of development would be expected. Whether this is the case could not be definitely determined owing to the lack of material of the later stages.

The Occurrence of Amitosis.

Other writers have referred to the occurrence of mitosis in both the ectoderm and entoderm of regenerating hydroids. Therefore on starting the histologic work, which has been described above, I expected to find approximately the same conditions. When traces of mitosis were not found, after staining particularly for mitotic figures, an explanation was sought for. As referred to under »Methods«, the hydroids were killed in GILSON's fluid or in a mixture of corrosive acetic and picric acids. The killing was not done with the particular aim of securing mitotic figures, but rather for general histologic investigation. It may be possible therefore, that the paucity of mitotic figures in most of the species considered is due to improper killing, and that if methods were employed especially to determine this phenomenon, mitosis would be fairly abundant.

However, the presence of amitosis is a condition which demands some explanation. In Tubularia crocea particularly the occurrence of amitosis was very common, and in fact in all the species studied it was more or less evident. Comparisons of some of the drawings will show nuclei in the process of direct division. In Fig. 63 this division of nucleus is being followed by a division of the cytoplasm. The rather common occurance of this phenomenon and the relatively large amount of evidence thus brought forward is very suggestive as to amitosis being an active process in the regeneration of hydroids.

WILSON (1896) in referring to amitosis says, »It is of extreme rarity, if indeed it ever occurs in embryonic cells or such as are in the course of rapid and continued multiplication. It is frequent in cells . . . which are on the way toward degeneration.« It has also been suggested that amitotic division may involve the nuclei only, and the cytoplasm does not divide; this for the purpose of increasing the nuclear surface as an aid in metabolism. WILSON further says, »In lower forms of life at least (amitosis) does not necessarily mean the approach of degeneration, but is the result of special conditions.«

In the hydroids studied the amitotic nuclear division does not seem always to be followed by cytoplasmic division, tho this may of course be due to the too early stages of the division. This suggests the possibility that the conditions here may be for the purpose of increasing the nuclear surface to aid in metabolism. Such an explanation might account for the presence of so many amitotically dividing nuclei as are shown in Fig. 65.

WILSON refers to a paper by ZIEGLER (1896) in which it is stated that amitotically dividing nuclei are usually of large size and are distinguished in many cases by a »specially intense secretory activity«. This »secretory activity« would be necessary for a time at least in Eudendrium and Pennaria, while the perisarc was being secreted around the protruding coenosarc, and amitotic nuclear division may be thus be partially explained in these two species. In Tubularia however, where the hydranths regenerate within the perisarc and only a few cells at the end secrete a new perisarc, the amitotic division could not be explained in this way. Indeed while this direct division may be partially explained in the ways already considered, it seems to be too prevalent to be accounted for by these conditions alone. Furthermore the cells do not seem to be in a degenerate condition, and are the cells from which the hydranths or tentacles are formed. It may be the result of special conditions, which as WILSON states has been conclusively proven to be the case in lower forms, tho as to just what the special conditions might be, it is difficult to conjecture. However, this phenomenon can not be fully explained till further investigations have been made.

VI. Summary and Conclusions.

1) Gravity has no apparent influence on the regeneration of Tubularia crocea and Pennaria tiarella.

2) Contact determines in some measure the kind of regeneration and the direction of growth in Eudendrium ramosum and Pennaria tiarella.

3) In Tubularia crocea there is a marked polarity, hydranths appearing more abundantly and in a shorter time at the distal ends.

4) Pieces of Tubularia crocea and Tubularia tenella of about 3—4 mm lenght failed to regenerate.

5) In Tubularia crocea the method of regeneration is about as found by other authors. The cut end closes over, the liquid in the enteric cavity begins to circulate, and red pigment is deposited in the hydranth region. Tentacles appear first as longitudinal folds or ridges and are pinched off from the hydranth body to assume the final form and size while still within the perisarc. The proximal tentacles develop first, the development beginning at the distal end.

6) In Tubularia tenella the first part of the regeneration is similar to that in T. crocea. The hydranth body is separated from the stem

by a constriction before the tentacles begin to develop. Tentacles appear as rather short buds, the final form and size being assumed by new growth after the hydranth emerges from the perisarc.

7) In Tubularia larynx there is a tendency for hydranths to regenerate at both ends of the stem, and there is no difference in the time of their development. The first stage of regeneration is the emergence of the coenosarc from the old perisarc, and the secretion of a new perisarc around the protruding part. A few annulations are always present close to the old perisarc. Circulation of fluid and the deposition of pigment is similar to that in T. crocea. Tentacle anlagen are much longer than in T. crocea, and often twined spirally around the coenosarc. Tentacles assume their final form and size while still within the perisarc. · Gonads may begin to form before the hydranth emerges from the perisarc.

8) Eudendrium ramosum, E. dispar, Pennaria tiarella, and Tubularia crocea were successfully grafted, union taking place equally well, whether the distal ends or the proximal ends were joined, or whether distal end was grafted to the proximal end. No union of Eudendrium ramosum and E. dispar was secured, nor of Eudendrium and Pennaria.

9) In Tubularia crocea the distal tentacles form by the separation from the entoderm layer of a rod or column of entoderm cells which are surrounded by the ectoderm to form the tentacle. Proximal tentacles form by a complex folding, involving both ectoderm and entoderm, the ectoderm gradually surrounding the entodermal fold and separating the tentacle from the hydranth body.

10) Distal tentacles of Tubularia tenella form as in T. crocea. Proximal tentacles develop partly by evagination of the entoderm and later by a new growth after the hydranth emerges from the perisarc.

11) The formation of the distal tentacles of Tubularia larynx is about the same as in T. crocea and T. tenella. Proximal tentacles form by a combination of evagination and folding, along the whole length, the tentacles being of mature size and form when the hydranth emerges from the perisarc. Nematocysts are found quite commonly in the entoderm and in the debris of the enteric cavity of T. tenella and T. larynx.

12) Regeneration of Eudendrium ramosum is first indicated by a knob-like protuberance from which the tentacles bud. The tentacles begin their development as evaginations involving both ectoderm and

entoderm. The entodermal cell at the apex of the evagination divides, and all cells resulting from this division may continue to divide till the entodermal core of the tentacle is formed, the cells being arranged in a single row. Proliferation of cells in the developing hydranth is by mitosis, amitosis also occuring. Increase in the surface of the ectoderm is brought about partly by a change in the form of the cells and partly by cell division. The hypostome develops as an outgrowth of the developing hydranth, cell division also taking place. The proximal end of the hypostome is blocked by a mass of entoderm cells till a comparatively late period. The mouth opening forms when the hydranth is otherwise completely regenerated.

13) The early appearance of regenerating hydranths of Pennaria is similar to that of Eudendrium. Nematocysts are very abundant in the ectoderm of the regenerating hydranths. The layers of ectoderm and entoderm are much thickened and the cells are more abundant, being the result of cell proliferation. Mitosis is quite abundant, tho amitosis is also found.

14) Amitosis is quite abundant in the tissues of the regenerating hydranths studied. When not followed by cytoplasmic division (as seems to be the case sometimes) it may be for increasing the nuclear surface as an aid in metabolism or it may be the result of special conditions. The explanation of amitosis in regenerating hydroids can not be definitely determined on the present evidence.

University of Nebraska, May 23, 1903.

Zusammenfassung.

1) Schwerkraft hat keinen sichtbaren Einfluss auf die Wiedererzeugung von Tubularia crocea und Pennaria tiarella.

2) Bei Eudendrium ramosum und Pennaria tiarella ist die Art der Berührung einigermaßen entscheidend für die Regeneration und die Richtung des Wachsthums.

3) Bei der Tubularia crocea findet sich eine ausgesprochene Polarität, Hydranten erscheinen in größerer Zahl und in kürzerer Zeit an den entgegengesetzten Enden.

4) Theilstücke der Tubularia crocea und Tubularia tenella von ca. 3—4 mm Länge zeigten sich nicht fortpflanzungsfähig.

5) Bei der Tubularia crocea ist die Art der Fortpflanzung ungefähr so, wie sie von anderen Forschern gefunden wurde. Das abgeschnittene Ende schließt sich von oben, die Flüssigkeit in der inneren Höhlung beginnt zu cirkuliren und rothes Pigment setzt sich in der Gegend der Hydranten ab. Fangarme erscheinen zuerst in Falten und Erhöhungen der Längsrichtung nach und eng bei einander, obwohl vom Hydrantenkörper entfernt, um endgültige Form

und Gestalt anzunehmen, während sie noch im Perisark sind. Die zunächststehenden Fangarme entwickeln sich zuerst, die Entwickelung beginnt an den entgegengesetzten Enden.

6) Bei der Tubularia tenella ist der erste Theil der Fortpflanzung ähnlich der bei der Tubularia crocea. Der Hydrantenkörper ist vom Stamme durch eine Verengung geschieden, bevor noch die Fangarme beginnen sich zu entwickeln. Die Fangarme erscheinen als ziemlich kurze Knospen; die endgültige Form und Gestalt nehmen sie erst nach weiterem Wachsthume an, wenn der Hydrant aus dem Perisark herausgewachsen ist.

7) Bei der Tubularia larynx besteht die Tendenz der Hydranten, sich an beiden Enden des Stammes zu entwickeln und es giebt keinen Zeitunterschied in der beiderseitigen Entwickelung. Die erste Stufe der Fortpflanzung ist die, dass der Cönosark aus dem Perisark hervorkommt und ein neuer Perisark rund um den hervortretenden Theil sich absondert. Einige wenige Ringbildungen sind stets in der Nähe des alten Perisarks zu finden. Die Cirkulation der Flüssigkeit und das Absetzen des Pigments ist ähnlich wie bei der T. crocea. Die Anlagen zu Fangarmen sind viel länger als bei der T. crocea und winden sich oft in Spiralenform um den Cönosark. Die Fangarme nehmen noch innerhalb des Perisarks ihre ursprüngliche Form und Gestalt an. Fortpflanzungsorgane (Gonaden) bilden sich wahrscheinlich, bevor der Hydrant aus dem Perisark hervortritt.

8) Eudendrium ramosum, E. dispar, Pennaria tiarella und Tubularia crocea wurden mit Erfolg aufgepfropft und die Vereinigung fand gleichmäßig gut statt, man mochte die entfernteren Enden zusammenbringen, oder ein entfernteres an ein näheres. Eine Verbindung von Eudendrium ramosum und E. dispar wurde nicht gesichert, noch die von Eudendrium und Pennaria.

9) Bei der Tubularia crocea bilden sich die entfernteren Fangarme durch Trennung der entodermen Lagen eines Stabes oder einer Säule entodermer Zellen, welche von Ektodermzellen umgeben sind, die die Fangarme bilden. Die zunächst liegenden Fangarme bilden sich in einem Komplexe durch Falten derart, dass Ektoderm und Entoderm einbegriffen sind. Das Ektoderm umschließt allmählich die entodermale Falte und trennt den Fangarm vom Hydrantenkörper.

10) Die entfernteren Fangarme der Tubularia tenella bilden sich wie bei der T. crocea. Die zunächstliegenden Fangarme entwickeln sich zum Theil durch Hervorkommen aus dem Entoderm und später durch neues Wachsthum, nachdem der Hydrant aus dem Perisark hervorgekommen ist.

11) Die Formation der entfernteren Fangarme der Tubularia larynx ist ungefähr dieselbe, wie bei der T. crocea und T. tenella. Die näherliegenden Fangarme werden gebildet durch eine Kombination von Hervortreten und Zusammenfalten der ganzen Länge nach. Diese Fangarme sind in voller Größe und Form, wenn der Hydrant aus dem Perisark hervortritt. Nesselzellen werden ganz häufig in dem Entoderm und Überreste der inneren Höhlung bei der T. tenella und T. larynx gefunden.

12) Die Fortpflanzung von Eudendrium ramosum zeigt sich zuerst durch einen knopfähnlichen Auswuchs an, aus dem die Fangarme hervorwachsen. Die Fangarme beginnen ihre Entwickelung als Evaginationen und ziehen sowohl das Ektoderm wie das Entoderm mit hinein. Die entodermale Zelle am obersten Punkte der Evagination theilt sich und alle Zellen, die aus dieser Theilung hervorgehen, können ihrerseits die Theilung fortsetzen, bis der entodermale Kern des Fangarms sich gebildet hat und die Zellen in einer Reihe sich angeordnet

haben. Die Zellentheilung bei der Entwickelung der Hydranten kommt auch vor bei Mitosis und Amitosis. Zunahme der Ektodermoberfläche wird theils durch Wechsel der Zellenform, theils durch die Theilung der Zellen zu Stande gebracht. Das Hypostom erscheint als ein Auswuchs des sich entwickelnden Hydranten, indem zugleich Zellentheilung stattfindet. Das näherliegende Ende des Hypostoms ist ziemlich lange durch eine Masse entodermaler Zellen verschlossen. Die Mundöffnung entsteht, wenn der Hydrant andererseits vollständig neu gebildet ist.

13) Die ersten Erscheinungen der Hydrantenfortpflanzung der Pennaria sind ähnlich denen bei Eudendrium. Nesselzellen sind in dem Ektoderm der sich fortpflanzenden Hydranten reichlich vorhanden. Die Schichten des Ektoderms und Entoderms sind sehr verstärkt und die Zellen treten zahlreich auf, was das Resultat der Zellenproliferation ist. Mitosis kommt oft vor, obwohl sich auch Amitosis findet.

14) Amitosis ist bei den Geweben der soeben erwähnten Hydrantenfortpflanzung sehr häufig. Wenn nicht als eine Folge cytoplasmatischer Theilung (wie es bisweilen der Fall zu sein scheint), so tritt sie auf wegen der Zunahme der Kernoberfläche als Beihilfe des Metabolismus, oder sie kann die Folge besonderer Verhältnisse sein. Über die Erklärung der Amitosis bei der Hydroid-Fortflanzung kann im vorliegenden Falle nicht endgültig entschieden werden.

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Explanation of Figures.

Plates IV—VII.

Fig. 1. Eudendrium ramosum grafted by distal ends.
Fig. 2*a*. Pennaria grafted by proximal ends.
Fig. 2*b*. Pennaria grafted by distal ends. *st* stolons.
Fig. 3. Tubularia crocea. Distal end showing the tentacle anlagen.
Fig. 4. Tubularia crocea grafted by proximal ends.
Fig. 5. Tubularia crocea, distal end in sand, new hydranth having regenerated. The unshaded portion is the old stem.
Fig. 6. Pennaria, distal end in sand showing regeneration. Unshaded portion is the old stem.
Fig. 7. Pennaria, stolon in sand showing regeneration. Unshaded portion is old stem.
Fig. 8. Pennaria lying flat on the bottom of the dish, with regenerating hydranth. Unshaded portion is old stem.
Fig. 9. Pennaria showing holdfasts and hydranths. This drawing is made from parts of two different stems.
Fig. 10. Pennaria and Eudendrium grafted. *st* stolons from Pennaria.
Figs. 11, 12. Regenerated hydranths of Tubularia larynx.
Figs. 13*a*, *b*. Regenerating hydranths of Tubularia larynx, showing the tentacle anlagen. Developing gonads in black.
Figs. 14 *a*, *b*, *c*. Early stages in the development of the hydranths of Tubularia tenella.
Figs. 14 *d*, *e*, *f*. Hydranths of Tubularia tenella after emergence from the perisarc.
Fig. 15. Pennaria anchored to the bottom of the dish by the distal end, showing regeneration. Unshaded portion is old stem.
Figs. 16—24. Tubularia crocea.
Fig. 16. Early stages in the folding of the ectoderm and entoderm to form the proximal tentacle. $\times$ 600.
Fig. 17, 18. Later stages of folding. $\times$ 600.

Figs. 19, 20. Ectoderm folding around the entoderm to cut off the proximal tentacle. $\times$ 500.

Fig. 21. Proximal tentacles separated from the hydranth body. $\times$ 500.

Figs. 22, 23. Two stages in the development of the distal tentacles. Column of entoderm cells being formed. Ectoderm folds around this column to cut off the tentacle. $\times$ 600.

Fig. 24. Separation of a mass of entoderm cells in a column. Ectoderm gradually surrounds the column to form the tentacle. Amitotically dividing nuclei shown. $\times$ 600.

Figs. 25—30. Tubularia tenella.

Figs. 25, 26. Early stages in the evagination of the entoderm to form the proximal tentacle. $\times$ 600.

Fig. 27. Evagination of the entoderm complete and the ectoderm folding around the entoderm to cut off the tentacle. $\times$ 500.

Fig. 28. One proximal tentacle cut off and another with the entoderm entirely surrounded by ectoderm but not yet separated from the hydranth body. $\times$ 500.

Fig. 29. Longitudinal section through the developing proximal tentacle, showing the evagination of the entoderm and the formation of the tentacle. $\times$ 600.

Fig. 30. Entoderm cells being forced away from the enteric cavity to form the entodermal column of the distal tentacle. $\times$ 600.

Fig. 31. Entodermal column of distal tentacle separated from the entoderm layer and the ectoderm beginning to surround it. $\times$ 600.

Figs. 32—43. Tubularia larynx.

Fig. 32. Proximal tentacles separated from the hydranth body, others in the process of separation. In one tentacle the entoderm is not completely surrounded by the ectoderm. $\times$ 600.

Fig. 33. Earlier stages in the development of the proximal tentacles. Entodermal column nearly complete, and the ectoderm just beginning to surround it. $\times$ 600.

Figs. 34, 35. Very early stages in the development of the proximal tentacles, only a few cells pushed away from the enteric cavity. $\times$ 600.

Figs. 36—38. Early stages in the formation of the distal tentacles, the entodermal column shown in different stages of development. $\times$ 600.

Figs. 39, 40. Entodermal column being surrounded by ectoderm to complete the tentacle. $\times$ 600.

Fig. 41. Entodermal column, entirely outside of the entoderm layer, being surrounded by ectoderm. $\times$ 600.

Fig. 42. Completion of the process shown in Fig. 41. $\times$ 600.

Fig. 43. Single entoderm cells lying almost entirely within the ectoderm. A condition sometimes found in the early stages of development of the distal tentacles. Figs. 41, 42 are really the completion of this process. $\times$ 600.

Figs. 44—60. Eudendrium ramosum. 44—57 $\times$ 600, 58—60 $\times$ 290.

Fig. 44. Very early stage. Layers of ectoderm and entoderm much thickened.

Fig. 45—47, 49. Different stages in the early evagination of the entoderm.

Figs. 48—50. Transverse sections thru completed or partially completed tentacles.

Fig. 51. Transverse sections thru tentacles in different stages of development. The two, more or less distinct series of anlagen, represent the development of the two series of mature tentacles whose bases are alternately elevated and depressed. The mass of polygonal cells are entoderm cells massed together in the region of the hypostome.

Figs. 52—56. Progress of development of two tentacles, from the condition in which only a single cell has been pushed away from the enteric cavity (Fig. 56), to the complete separation of the tentacles (Fig. 52).

Figs. 57, 58, 60. Longitudinal sections through the tentacles in various stages of development.

Figs. 58—60, show different stages in the development of the hypostome. In Fig. 60 a considerable amount of debris is present in the enteric cavity.

In the figures representing sections of Eudendrium ramosum there often appear to be two entoderm cells in the core of the completed tentacle. This is due to the fact that the section is not exactly transverse but has been made somewhat obliquely. Where three nuclei seem to be present this is due to the appearance of the nuclei of the underlying cells.

Figs. 61—67. Pennaria tiarella.

Figs. 61, 62. Nuclei in both ectoderm and entoderm in the early stages of mitosis. $\times 1000$.

Fig. 63. Nearly completed amitotic division of an ectodermal cell. $\times 1000$.

Fig. 64. Nucleus of ectoderm cell in an early stage of amitotic division. The cytoplasm has not yet begun to divide. $\times 600$.

Fig. 65. Transverse section through the hydranth region of a young budding hydranth, which was forming normally in its natural habitat. $\times 1000$.

Figs. 66, 67. Sections through the developing tentacles of Pennaria. $\times 600$.