Regeneration in *Antennularia*.

By

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With 2 figures in text.

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In 1892 LOEB published an account of a series of experiments on the hydroid, *Antennularia antennina*. The colony in this species consists of long unbranched stalks with numerous lateral pinnae bearing the hydranths. Pieces of the stalk were suspended in an aquarium and variously oriented. It was found that in every case new »stems«¹⁾ grew vertically upward, and new »roots«¹⁾ vertically downward or nearly so. The stems were negatively and the roots positively geotropic. By inverting some of the pieces he caused the newly regenerated stems to cease their growth as stems and produce roots. By changing the angle of a piece he changed the direction of a regenerating stem. LOEB therefore described regeneration in *Antennularia antennina* as a case where man can control regeneration; i. e., determine the kind of structure regenerated, because of the sensitiveness of the organism to the influence of gravity.

DRIESCH, in 1897, performed a few experiments on *Antennularia ramosa*, a branched form. He seems to have confined his observations to regeneration at a basal cut end, which was repeatedly cut. At first roots only were produced. When these were removed, roots grew again and also a delicate stem. When these were cut off, two or three stems and sometimes a few roots developed. After cutting

¹⁾ The terms »stems« and »roots« have been substituted for hydrocauli and hydrorhizae in all of the papers on regeneration of *Antennularia*. The so-called »roots« are in reality holdfasts and in many cases behave more like stolons than like roots.

again only one or two stems appeared. The stems were negatively geotropic.

MORGAN in 1901 also worked with *Antennularia ramosa*. He found that pieces from various parts of the colony usually produced a bunch of roots at both ends without regard to orientation. Pieces remaining attached to stones, if placed at the bottom of the tank, grew stems; but when suspended with the cut ends directed downward, they did not regenerate. He attempted to test the relation of regeneration in *Antennularia* to gravity by fastening pieces of a colony to a wheel turned by a stream of water from the tap and making $5\frac{1}{2}$ revolutions in a minute. No regeneration occurred.

At Prof. MORGAN's suggestion I made a long series of experiments on *Antennularia* at Naples in 1901 and 1902. I also had *Antennularia ramosa* and was unable to obtain *A. antennina* after I found that the different results obtained by LOEB, DRIESCH and MORGAN were probably to be attributed to the different habit of growth of the two species. My experiments showed that in *Antennularia ramosa* the kind of regeneration at a cut end is determined neither by the polarity of the piece nor by its orientation with respect to gravity, but by the location of the piece in the colony and its condition, — vigorous or otherwise. Pieces from the basal part of the stalk, below where living hydranths are found, usually produced only stems at both ends without regard to orientation, and did this repeatedly if cut. Pieces taken from more distal regions of the colony produced roots at both ends whatever might be the orientation of the piece, and did this three or four times when repeatedly cut, but finally developed one or more stems. If an end that had begun to produce stems were cut back a centimeter or more, it would again produce roots. Thus it appeared that an isolated, hydranth-bearing piece of a vigorous colony would first regenerate roots at both ends by which to attach itself if possible, and would continue to do this until somewhat exhausted, and then would produce one or more stems with hydranths as a last resort to save the colony. Practically the same result was observed when the roots were not cut off. Fresh roots were produced repeatedly and finally a stem.

Stems grew from cut ends of colonies left attached to stones on the bottom of the aquarium and also when suspended in an inverted position. Roots, either attached to pieces of cork or free in the water, produced stems. All of the stems, if given time enough, eventually oriented themselves with respect to gravity, but it was evident that

the kind of regeneration in *A. ramosa* was not determined by the influence of gravity. Exactly the same results were obtained when pieces were attached to the cork blades of a wheel placed in filtered sea water and turned by clockwork, at a rate of one revolution in 20 minutes, as when the same kind of pieces were suspended free in the aquarium or were attached with cactus spines to squares of cork strung on glass rods. The kind of regeneration depended upon the part of the colony from which the piece was taken, and changed with repeated cutting in the case of median and apical pieces. Histological examination of regenerating stalks together with observations on the behavior of the living coenosarc showed that regeneration was not due to cell multiplication, but to withdrawal of coenosarc from one part and putting it forth in a new form, — roots, or stems with hydranths; i. e., an extreme case of morpholaxis.

Naturally I was curious to see what *Antennularia antennina* would do on the wheel, and the opportunity to try it came while I was working at the Naples Station last spring ('09). The same wheel and clockwork were used as before. As control, pieces were also fastened to squares of cork on glass rods in the same aquarium. Regeneration in these pieces occurred exactly as described by LOEB.

A brief description of the wheel will be necessary in order to understand the orientation of the pieces of *Antennularia*. It consisted of a small hub and six spokes to each of which was attached a piece of cork about 5×10 cm. and 6 or 7 mm. thick (Fig. 1). The cork blades were so placed that their length (10 cm.) was at right angles to the plane of movement of the wheel. Pieces of the hydroid colony were attached 1) at right angles to the radii and parallel with the plane of movement (Fig. 1, $a_1 a_2 b_1 b_2$), 2) parallel with the radii ($c_1 c_2$), and 3) perpendicular to the plane of motion (Fig. 2, $d_1 d_2$).

The first lot of material was brought into the laboratory on Mar. 18th and kept in running water until the wheel could be put in order. On Mar. 23d, after examination under the microscope to be sure that the colonies were still alive, pieces 5 to 6 cm. long were fastened to the blades of the wheel with sea-urchin spines. These pieces were all attached to the outer edges of the blades, tangential to the circumference of the wheel (Fig. 1, $a_1 a_2$). A second lot of better material, brought in on Mar. 27th, was fastened to the ends of the blades at right angles to the radii and parallel with the plane of motion (Fig. 1, $b_1 b_2$). Some of the pieces were placed so

that the distal end pointed in the direction in which the wheel was turning, others with the opposite orientation. On Apr. 12th, 10 out of 16 pieces of the first lot showed regenerating stems at one end;

Fig. 1.

Fig. 2.

Fig. 1. Diagram of wheel used in the experiments, showing position of pieces of *Antennularia*, a_1 a_2 , b_1 b_2 , c_1 c_2 .

Fig. 2. Diagram of one blade of the wheel, showing the d_1 and d_2 positions.

9 of these new stems grew straight out in line with the axis of the original piece (Fig. 1, a_1), and 1 nearly at right angles to the old stalk and pointing toward the center of the wheel. Some of the pieces had regenerated at the distal end, others at the proximal end, but more at the proximal end.

On Apr. 16th the regenerating stems of the two lots cut on Mar. 23d and Mar. 27th were described in my notebook as follows:

11 pieces had a regenerated stem at one end, tangential to the circumference of the wheel and parallel with the plane of motion.

1 piece had a new stem at each end.

1 piece had a regenerated stem nearly at right angles to the original stalk and pointing toward the center of the wheel.

1 piece had a new stem at right angles to the old stalk and to the plane of movement. This and the preceding were cases of anterior regeneration.

2 pieces had two new stems diverging nearly at right angles, both posterior.

There were 6 cases of posterior regeneration, 8 of anterior and 1 of regeneration at both ends. Eight pieces regenerated in the direction in which the wheel was turning, 6 in the opposite direction and one at both ends.

Another lot of *Antennularia*, placed on the wheel Apr. 25th, showed the following results on May 10th.

Regenerated stem at the forward end only (<i>a b</i>) . . .	8
- - - rear - - (<i>a b</i>) . . .	5
- - - both ends (<i>a</i> or <i>b</i>)	3
- - radial (<i>c</i> ₁ or <i>c</i> ₂)	3
- - at rt. angles to plane of motion (<i>d</i>) . . .	3
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Regeneration at distal end	4
- - proximal end	15
- - both ends	3
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The pieces were kept on the wheel until about the first of June and various peculiarities were made note of. In one case where a piece was fastened in the *a* position, a new stem grew radially outward where the stalk was fastened to the cork. Several pieces produced two or three diverging stems at one end. The most peculiar result was observed in the case of several new stems which at first grew straight out from pieces in the *a* position, then later turned toward the circumference of the wheel, and after a time made a second turn toward the circumference (Fig. 1, *a*₂). This and the following case were the only ones where the direction of growth seemed to bear any definite relation to the direction or plane of

movement of the wheel. In most cases a new stem grew out in line with the axis of the old stalk. A few cases were observed where a piece in the α position was accidentally turned at an angle with the plane of motion of the wheel, and the new growth was at an angle with the old part and parallel with the plane of motion.

Taking the experiments as a whole we find a fair proportion of regenerated stems. These stems point in a variety of directions, — radial, both outward and inward; tangential; at right angles to the plane of motion, and at various other angles, indicating that under the conditions of the experiment, regeneration of stems in *Antennularia antennina* occurred independent of the influence of gravity or centrifugal force. It would therefore seem to be a fair conclusion that, although, as LOEB states, one can control the kind of regeneration in *Antennularia antennina* by suitable orientation, the influence of gravity is not a necessary factor for the regeneration of stems.

With the regeneration of roots it is otherwise. A few days after the first material was placed on the wheel I noticed two short roots, but these died and no more appeared. However, I supposed that the pieces were fastened to the cork by roots acting as holdfasts, as had been the case with *Antennularia ramosa*, but as soon as the spines were removed the pieces of *Antennularia* floated free in the water, showing that no holdfasts had been produced. I recalled LOEB's remark that roots were more sensitive to the influence of gravity than the regenerated stems and was obliged to conclude that so far as these experiments go, *Antennularia antennina* seems for the most part to be incapable of producing »roots« when the influence of gravity is eliminated.

The cases in which the direction of growth of new stems seemed to be affected by the direction of motion of the wheel suggest that it might be worth while to arrange a more carefully controlled series of experiments to test this point, using a wheel which could be turned at several different rates.

In conclusion I wish to express my appreciation of the facilities afforded me for carrying on these experiments at the Naples Station.

Summary.

- 1) When pieces of a colony of *Antennularia antennina*, 5 to 6 cm. long and variously oriented, are attached to the cork blades of a wheel (radius 7 cm.), making one revolution in 20 minutes, they

regenerate stems in various directions, apparently independent of the influence of gravity.

2) In these experiments the pieces of *Antennularia* produced practically no roots. It is therefore possible that regeneration of roots in *A. antennina* is dependent on the influence of gravity.

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Zusammenfassung.

1) Werden Stücke einer Kolonie von *Antennularia antennina*, 5—6 cm lang und verschieden orientiert, an die Korkschaufeln eines Rades (7 cm Radius) angeheftet, welches in 20 Minuten eine Umdrehung macht, so regenerieren sie Stämme in verschiedenen Richtungen, anscheinend unabhängig vom Einfluß der Schwerkraft.

2) Bei diesen Versuchen brachten die Stücke von *Antennularia* so gut wie keine Wurzeln hervor. Es ist daher möglich, daß die Regeneration von Wurzeln bei *A. antennina* von dem Einfluß der Schwerkraft abhängig ist.

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