The Regeneration of an Oesophagus in the Anemone, Sagartia luciae.

By

Annah Putnam Hazen.

With Plate XXXI.

Eingegangen am 18. April 1902.

During the summer of 1901, while at the Marine Biological Laboratory, Woods Holl, Mass., I undertook, at the suggestion of Prof. T. H. MORGAN, a study of the regeneration of Sagartia. I attempted (1) to follow the regeneration of a new oesophagus in small pieces cut from the base of the column and pedal disk and containing no part of the old oesophagus, (2) to determine the orientation of the regenerating individual and its relation, in this respect, to the one from which it was cut, and (3) to discover, as far as possible, if the axis were changed after the cut, what the determining factors were which regulated the position of the new axis.

The experiments were made on Sagartia luciae, a small anemone which is found in great abundance at low tide at Woods Holl. The anemones were allowed to fully expand after collecting and then a quick cut was made with either a sharp scalpel or scissors, removing a small piece from the base, as indicated in Fig. 1 by the line *a—b*. Owing to the rapid contraction of the anemones a small part of the oesophagus was occasionally cut off, but, as it was necessary for the success of the experiment that this should not occur, the pieces were carefully examined after being allowed to expand and most of the suspicious ones were rejected. Only a few, which contained a small part of the oesophagus projecting through the cut, were retained. The pieces were placed in shallow dishes of sea water and allowed to regenerate for vaying periods of time, then, after being narcotized with magnesium sulphate, were preserved in 5% formalin for study.

Regeneration of an oesophagus.

After the operation the pieces roll up, the cut walls turn in and in a short time join together. The walls gradually expand after the wound has healed and the cylindrical shape is again assumed. In some cases, probably where the operation was particularly severe, the entire piece was covered by a loose, thin membrane. This, as well as the gastrovascular space, is shown, by the sections, to contain degenerating cells. The recovery requires more time in these specimens and the tentacles and oesophagus are relatively late in appearing. When, as frequently occurs, mesenteries and filaments project through the cut, the walls close slowly around them, until they finally break off and disappear. Generally the wall closes completely before tentacles, or the oesophagus develop, but in those cases where a number of mesenteries project, some of them may remain fastened to the body wall or the cut may continue to be open until after the regeneration of the oesophagus has begun. In this way, the position of the regenerating oesophagus may be determined in its relation to the cut. An illustration of this is given in Fig. 2 which was drawn from a series of diagonal sections. Although a period of five days had elapsed since the operation, the opening made by the cut had not completely closed around the filaments (*f*) which project outside the body cavity. A free communication was thus maintained between the gastrovascular space and the exterior, yet tentacles and a new oesophagus have been regenerated at a different place, indicated in the figure by *a* and shown by sections farther along in the series. As far as I have been able to determine, only in those cases where a part of the wall of the old oesophagus was cut off with the piece and projected outside the body has the cut been kept open and modified into a mouth.

The position of the scar is marked, for sometime, in living and preserved specimens, by the light, unpigmented appearance of the regenerating tissue at that point. In sections, also, the new cells can be distinguished in the early stages without difficulty, as the regenerating part does not stain as deeply as, and contains less pigment than, the old tissue. At first there are no gland cells at the point of growth, but they soon appear as long slender cells which gradually thicken. In Figs. 3, 4 and 5 the old tissue filled with gland cells is shown at the right beneath the tentacles and may be compared with the regenerating tissue containing fewer gland cells at the left. There is no indication, that I have been able to find, that the cells migrate from the old tissue into the new, but rather, the wall appears to regenerate by morphallaxis.

While following the experiments a good deal of discrepency was noticed in the time which elapsed before the tentacles appeared in the different pieces. This seemed to be governed largely by the length of time which was necessary for the piece to become attached to the disk. A piece cut so that a considerable portion of the pedal disk was removed with it become quickly attached and tentacles appeared often by the second day. If, however, the pieces were cut from the column and a whole new disk had to be regenerated, the tentacles did not appear until after the piece was fixed, and were therefore relatively late in appearing. In these regenerating pieces which were watched from day to day, it was evident that the development of the tentacles depended largely upon the attachment, yet it is impossible to say until more experiments are made what other factors are involved or how important they may be. Sections of the early stages show that the tentacles are first developed as evaginations of the body wall (see Fig. 7 *t*). Later, at one side of the base of the tentacles, the regenerating oesophagus appears. The tentacles increase in number and surround the oral disk, but when as few as two or three tentacles have developed, the oesophagus is at one side, not between them.

The oesophagus is formed by an invagination of the mesogloea and of the inner layer of cells, which, in its early development, has the form of a circle and which, after farther growth, appears as an inverted cup or funnel. The section represented in Fig. 3 passed longitudinally through the middle of one of the early stages and shows the endoderm (*en*) and mesogloea (*me*) invaginated at two points (*o*). As the invagination continues, the growth is caused by the activity of these two layers (see Fig. 4) and the inverted cup or oesophagus is seen to be lined by the endoderm as well as covered with it on the outside. The mesogloea forms a loosely fibrous tissue between the two layers of endoderm: while the ectoderm (see Fig. 3) remains as an unaltered, continuous sheet over the surface of the small anemone and takes no part in the formation of the new oesophagus.

This is particularly interesting when it is remembered that in the development of the embryo, the ectoderm invaginates with the endoderm and mesogloea and forms the entire inner wall of the oesophagus. Often, in the regenerating specimens, the cup is less symmetrical than the one shown in Fig. 4 and one side is longer than the opposite (see Fig. 8).

In following the subsequent development, the next stage of importance occurs when the ectoderm breaks over the oesophagus and becomes continuous with the endodermal lining (see Fig. 5 *a*). Even here, the ectoderm does not push in, but the point of union between it and the endoderm is plainly visible within the circle of tentacles. Not only is this clear in the early stages, but also in later ones (I have examined a series of specimens including those which have regenerated twenty-two days) and the division between the two layers (ectoderm and endoderm) is always distinctly outside the oesophagus and within the ring of tentacles.

The endoderm cells lining the oesophagus lose their pigment granules (see Fig. 5) and become slender; in contrast to those found elsewhere. New mesenteries regenerate by folds of endoderm and mesogloea of the body wall which appear in the region of the regenerating oesophagus (see Figs. 4 and 5 *m*) and they are often early connected with it.

A variation from this way of regenerating an oesophagus is found in those specimens in which a mesentery was left in one particular region opposite the pedal disk. Here, one of the endodermal layers of the mesentery, near the oral end, invaginates through the mesogloea as a funnel, that opens into the gastrovascular cavity at the aboral end. At the opposite end of the invagination the funnel breaks finally through the ectoderm forming a mouth, much as in the other case. Fig. 6 is drawn from a series which shows the development of the oesophagus in the middle of a mesentery. The section passed across the invagination and parallel to the mesentery — as the developing oesophagus was slightly diagonal. Since the mesenteries are composed of endoderm and mesogloea and are regenerated by ingrowths of these two layers from the body wall, this variation from the more usual regeneration of an oesophagus does not seem fundamental. The later development shows that both old and new mesenteries become attached later to the oesophagus. Gastric filaments also are joined to the open end of the oesophagus and some observations would seem to indicate that they — and perhaps mesenteries also — sometimes form the lower portion of the wall of the oesophagus itself (see Fig. 5 *f*).

Orientation of small pieces cut from the base.

As the mesenteries are placed perpendicularly in the sea anemone, their position in these regenerating pieces, — especially the younger ones — will show the relation of the piece to that of the individual from which it was cut. If the mesenteries are placed perpendicularly in the piece, it has the same orientation as the one from which it was cut: if not, a new axis has been formed. A careful study of this point indicates that the old axis has little if anything, to do with determining the position of the new axis and that a piece can adapt itself to a change of orientation and regenerate as quickly as if there had been no change of its axis.

Fig. 7 was drawn from a specimen in which the single mesentery (*m*) passed nearly horizontally across the piece, thus showing that the new individual had assumed an axis approximately at right angles to the one from which it was cut. There is, however, evidence of a marked tendency, shown after the piece has become attached, towards rearranging the old mesenteries in a perpendicular position. This effort toward a readjustment is seen first in the region of the new oesophagus and later extends over the whole piece. If the mesenteries are attached at one end only, as shown by the mesenteries in the left of Fig. 8 *m*, the free end is raised and probably fastens to the oesophagus as the two mesenteries at the right of the figure have.

Although I have not been able, as yet, to follow all the phases in the rearrangement of the mesenteries in these small regenerating pieces, I am convinced that the transformation is complete and that all the mesenteries become finally perpendicular. The way in which mesenteries which are fastened at either end to the body wall (as in Fig. 7) can more and more adapt themselves to a new axis of the body is not clear at present, but the suggestion may be made that unequal growth in different regions might allow one end to push up and the other to settle down.

Factors which determine the orientation of the piece.

In this connection, the question of the factors which determine the orientation of the piece comes up and may, it seems to me, be explained in part as follows. When the piece is cut and dropped in a dish of water, the heaviest part sinks to the bottom and becomes attached at that point. In cases where a part of the pedal disk and enough muscular tissue are left undisturbed to allow the piece to turn over, the old disk was retained, even if the piece were at first placed so that the disk was not at the bottom. If, however, the muscular activities are interfered with or the pedal disk is absent, the piece remains at it falls, on which ever side that may be, and a new disk develops and a new axis is established. Even in the youngest specimens, the sections show that the filaments and mesenteries are collected at the base and that the upper half contains only those which are attached to the body wall. As the growth of the piece, after attachment, is mainly upward in the region of the tentacles and oesophagus, this difference is more decidedly marked in specimens which have regenerated several days. The point of contact, therefore, which is determined by gravity, marks the position of the new, pedal disk. The attachment once formed, the greatest activity of regeneration is concentrated at the opposite end where the tentacles and the oesophagus appear. It is possible that the position of the axis is determined by a geotropic influence or by a combination of geotropism and stereotropism (the stimulus of contact). Renewed experiments which I hape to make during the coming summer, will doubtless throw more light on this point.

Summary.

1) An oesophagus regenerates in small pieces cut from the base of Sagartia luciae as an invagination of the mesogloea and endoderm in the shape of an inverted cup in which the mesogloea forms the middle layer and is covered on both outside and inside by endoderm. Later the ectoderm breaks above the cup and becomes continuous with the endoderm lining it. The ectoderm takes no part in the regeneration of the oesophagus.

2) Tentacles develop as outgrowths of the old body wall (including ectoderm, mesogloea and endoderm).

3) New mesenteries regenerate as infoldings of the mesogloea and the endoderm which become attached to the oesophagus.

4) The orientation of the regenerating piece may, or may not, be the same as that of the individual from which it was cut.

5) The fixation of the piece to the bottom of the dish takes

place before the oesophagus regenerates. The contact, in some ways, stimulates the regeneration of an oesophagus at the opposite end. If the piece is cut so that it contains a small part of the old pedal disk or if it has lost the power of turning itself over, it must remain in the position in which it falls and its orientation is therefore determined by gravity.

Smith College, Northampton, Mass.

Zusammenfassung.

1) An kleinen Stücken von der Basis von Sagartia luciae bildet sich der Ösophagus aufs Neue als eine Einstülpung von Mesogloea und Entoderm in Gestalt eines umgekehrten Bechers, in welchem die Mesogloea die mittlere Lage bildet, während sie innen und außen vom Entoderm bedeckt ist. Später trennt sich das Ektoderm über dem Becher und tritt mit dem Entoderm, welches jenen überzieht, in kontinuirliche Verbindung. Das Ektoderm bildet bei der Regeneration des Ösophagus keine Tasche.

2) Tentakeln entwickeln sich als Auswüche an der alten Körperwand. (Sie enthalten Entoderm, Mesogloea und Ektoderm.)

3) Es regeneriren sich auch neue Mesenterien in Gestalt von Einfaltungen der Mesogloea und des Entoderms, welche sich am Ösophagus anheften.

4) Die Orientirung des in Regeneration begriffenen Stückes kann dieselbe sein, wie die des Individuums, von dem es abgeschnitten wurde, oder auch nicht.

5) Das Stück befestigt sich auf dem Boden der Schale, ehe noch die Regeneration des Ösophagus eintritt. Die Berührung mit dem Gefäßboden scheint in mancher Beziehung für die Regeneration eines Ösophagus auf der ihm abgekehrten Seite einen Reiz abzugeben. Wird das Stück so abgeschnitten, dass es einen Theil der alten Fußscheibe enthält, oder hat es seine Fähigkeit, sich umzudrehen eingebüßt, so muss es in der gerade erhaltenen Stellung verbleiben und seine Orientirung wird in Folge dessen von der Schwerkraft bestimmt.

Explanation of Figures.

Plate XXXI.

Fig. 1. Diagram in which the line *a*—*b*, indicates the position of the cut. *o* oesophagus.

Fig. 2. × 40. Section cut somewhat diagonally through an individual which had regenerated 5 days. The cut walls had not completely closed around the filaments (*f*) which project outside the body. In other sections in this series the tentacles and oesophagus are found at a place corresponding to *a*. *m* mesenteries.

Fig. 3. × 60. A longitudinal section cut through a piece which had regenerated 2 days. *ec* ectoderm containing gland cells. *en* endoderm with pigment granules. *me* mesogloea, *m* mesentery, *o* oesophagus, *t* tentacles.

Fig. 4. $\times$ 60. Longitudinal section through a piece which had been regenerating 3 days. *m* regenerating mesentery, *o* oesophagus, *t* base of tentacle.

Fig. 5. $\times$ 60. Longitudinal section through a piece which had regenerated for 3 days. *a* union of ectoderm with the endoderm lining the oesophagus (*o*), *f* filaments, *m* regenerating mesentery, *t* tentacles.

Fig. 6. $\times$ 60. Specimen killed 5 days after the cut. Section through an oesophagus (*o*) which has regenerated in a mesentery (*m*). In other sections in the series, the oesophagus opens into the gastrovascular space, but it does not connect with the ectoderm.

Fig. 7. $\times$ 40. Longitudinal section through a piece which had regenerated 4 days. *m* mesentery, *t* tentacle.

Fig. 8. $\times$ 40. Longitudinal section through a piece which had regenerated 4 days. *m* mesentery, *o* oesophagus, *t* tentacle.

Fig. 1.

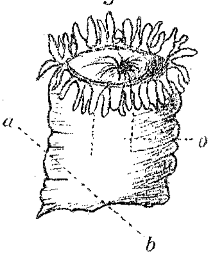


Fig. 2.

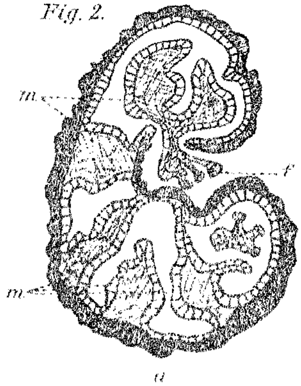


Fig. 3.

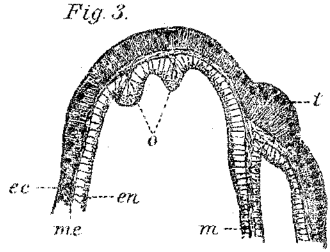


Fig. 4.

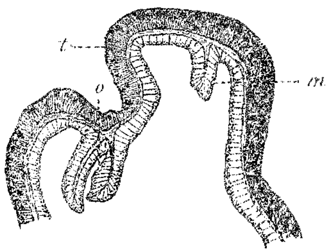


Fig. 5.

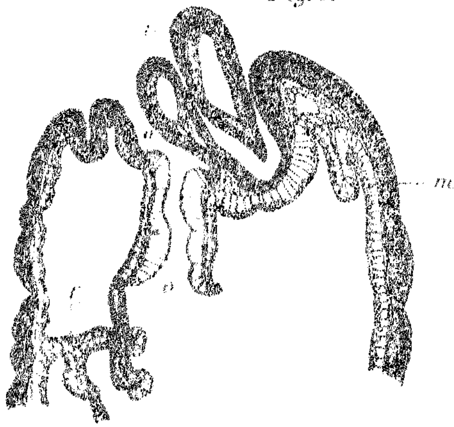


Fig. 6.

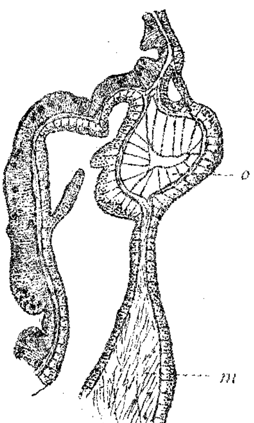


Fig. 7.

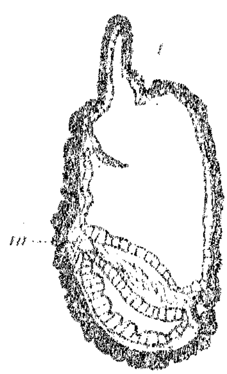
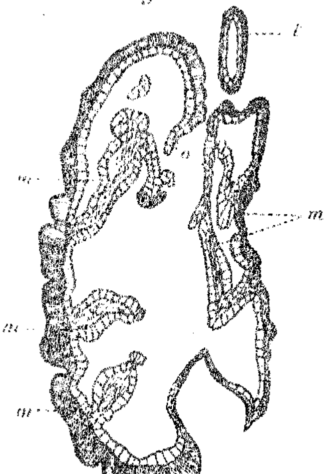


Fig. 8.



A. F. K. del.
Lith. Anst. Julius Klinkhardt, Leipzig.
Verlag v. Wilhelm Engelmann in Leipzig.