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Regeneration of the Alimentary Canal of *Stichopus mollis* (Hutton) across a Mesenteric Adhesion

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Summary.

Three specimens of *Stichopus mollis* from 299 examined, showed that the regenerating alimentary canal had followed an abbreviated course by crossing from the dorsal to the ventral mesentery along an adhesion of the two mesenteries. This method of regeneration, unusual for *S. mollis*, shows features resembling those normally found in *Holothuria tubulosa*. There was evidence that the regenerating alimentary canal was formed from mesenteric tissue only, as in normal regeneration of *S. mollis*, without contributions from other tissue. The segment of mesentery omitted from the course of the regenerating alimentary canal did not show any increase in depth from body wall to its edge, nor any proliferation and differentiation of tissue at the mesenteric edge in this region. It is suggested that a gradient of activity with a strictly anterior-posterior polarity is established unusually early along the alimentary canal. Those parts of the mesenteric sheet which lie laterally to the gradient are therefore not activated.

Description.

Several accounts of regeneration of viscera in holothurians have shown that it occurs along the whole length of the free edge of the mesenteric remnants remaining after autoevisceration in *Thyone* (Scott, 1914; Kille, 1935) and in *Stichopus* (Bertolini, 1930; Dawbin, 1949). Only part of the total length of the mesentery is involved during regeneration in *Holothuria tubulosa* Bertolini (1932). The two modes of regeneration are quite distinct and apparently constant for each species. There is no evidence showing that different methods of regeneration occur in the same species.

Regeneration of viscera in *Stichopus mollis* normally occurs along the complete length of the mesenteric remnants (Dawbin, 1949), but three specimens showed regeneration on a different pattern. In these specimens, the alimentary canal passes from one portion of the mesentery across an adhesion to a more posterior portion, thus omitting a length of the mesentery.

The methods used for inducing autoevisceration and for the keeping of specimens during regeneration are those previously described. For the examination of stages reached in regeneration Bouin's fluid was injected to harden structures, and an incision was first made along the right dorsal interambulacrum. The state of the organs and the distribution of mesenteries was first determined by gently reflecting the flaps on either side, and removing portions of the body wall which were clearly not in contact with mesenteric attachments. In each of the three cases considered in this study, there was an adhesion between the dorsal and ventral mesenteries (Fig. 2). This would have been torn if the flaps had been completely reflected in the usual manner. A new incision was therefore made along the left dorsal interambulacrum and the flaps were then reflected. This left the mesenteric edge in a looped condition (Fig. 2) instead of the S-shaped condition found after opening along the right dorsal interambulacrum. (Fig. 1.)

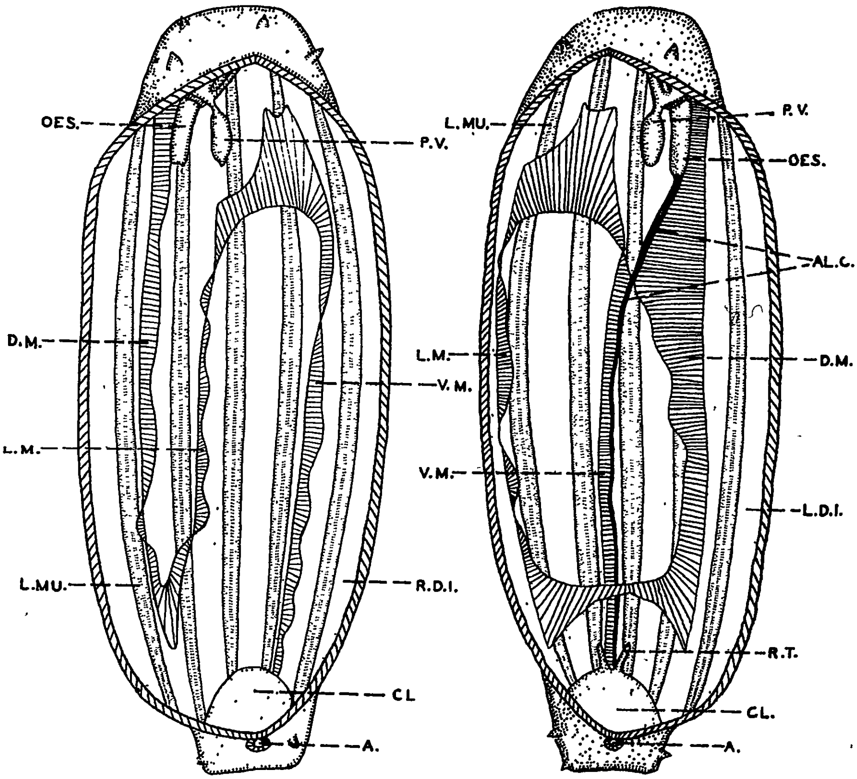

FIG. 1. FIG. 2.

FIG. 1.—Dissection of an eviscerate specimen opened along the right dorsal interambulacrum, showing the S-shaped course of the mesentery.

FIG. 2.—Dissection opened along the left dorsal interambulacrum, showing the relationship of the regenerating alimentary canal to the mesenteries. A., anus; AL.C., regenerating alimentary canal; CL., cloaca; D.M., dorsal mesentery; L.D.I., left dorsal interambulacrum; L.M., lateral mesentery; L.MU., longitudinal muscle band; OES., oesophagus; P.V., polian vesicle; R.D.I., right dorsal interambulacrum; R.T., regenerating respiratory tree; V.M., ventral mesentery.

The organs remaining and the distribution of the mesenteric remnants after autoevisceration in *Stichopus mollis* have been described previously (Dawbin, 1949). Usually there is no cross connection between any portions of the mesentery from the oesophageal remnant to the cloaca, as the mesentery follows its S-shaped course along the dorsal, lateral and ventral loops (Fig. 1.).

Before reflexion of any portion of the body wall, the dorsal remnant of the mesentery hangs freely into the body cavity from the mid-dorsal interambulacrum, and its free edge can closely approach the free edge of the ventral mesentery. The length of the mesenteric remnants from the point of attachment at the body wall to its free edge was found to vary. In three cases out of 299 examined, it was found that the dorsal mesentery had contacted and fused with the ventral mesentery at a point almost level with the anterior free edge of the lateral mesentery (Fig. 2). This established a direct connection of the mesentery from the oesophageal remnant to the cloaca, without the necessity of following the whole length of the dorsal, lateral and ventral borders. The posterior portion of the dorsal mesentery and the whole of the lateral mesentery is thus eliminated from the direct course. Unfortunately the three specimens were all at a relatively similar stage of regeneration on examination, having undergone 44, 47 and 50 days' regeneration respectively. It was therefore not possible to determine whether the adhesion had taken place immediately after autoevisceration, or whether there had been a later increase in depth of the mesenteries permitting contact between the dorsal and ventral loops. From the state of the regenerating tissue along its edges (see below) it is apparent that contact was made at some early stage in regeneration.

Regeneration occurred along the dorsal mesentery between the oesophageal remnant and the point of contact with the ventral mesentery, and along the edge of the latter to the cloaca. Along this length the lining epithelium on either side of the mesentery had fused over the torn edge, and the mesenchyme cells between had increased to form a rod-like thickening. This is the primordium of the alimentary canal, and its structure showed that its formation followed the same course as that described previously for *Stichopus mollis* (Dawbin, 1949). The alimentary canal of the specimens examined after 47 and 50 days' regeneration tapered from a diameter 1·5 mm. at the oesophageal remnant to 0·8 mm. at the cloaca. Histological study showed that an irregular lumen was present between the mesenchyme cells along the whole length. A distinct inner epithelium had not been formed, but there was a concentration of nuclei around the lumen indicating its initial development. Longitudinal and circular muscle fibres had not been developed at this stage.

The specimen examined after 44 days' regeneration showed the greatest diameter of the alimentary canal anteriorly, being 0·5 mm. wide at the oesophageal remnant and 0·4 mm. wide at the point of contact with the ventral mesentery. From this level it tapered posteriorly towards the cloaca. Just anterior to the junction with the cloaca, it narrowed to a width of 0·1 mm. This specimen was smaller than the preceding, being 47 gms. as compared with 150 gms. and 170 gms., and the alimentary canal was correspondingly of smaller diameter. A lumen was present from the oesophageal remnant to the junction of the dorsal and the ventral mesenteries. There were irregular spaces among the mesenchyme cells along the anterior third of the ventral mesentery, followed by a solid cord of cells from this region to the cloaca.

In all three specimens, the mesenteric edge not included in the direct connection from the oesophageal remnant to the cloaca remained unthickened, and showed no evidence of cell proliferation at any point. In addition there was no forward growth of the mesentery to form the pocket-like extension normally present in the angle between the dorsal and lateral mesenteries (see Fig. 1) at this stage of regeneration.

The separation of the dorsal and ventral intestinal haemal vessels as extensions from the regenerating alimentary canal was similar to that described previously (Dawbin, 1949). The transverse connecting vessel had not been split off in any of the specimens.

In these three specimens the respiratory trees were $2 \cdot 0 \times 1 \cdot 0$ mm., $3 \cdot 0 \times 1 \cdot 0$ mm., and $10 \cdot 0 \times 1 \cdot 5$ mm. in size respectively. Similar variability in dimensions during the early stages of regeneration has already been recorded (Dawbin, 1949) and there was no difference in the mode of origin and the stages of differentiation of the layers.

Discussion.

The relationships of the organs found after regeneration in the three specimens examined in the present study may be compared with those found by Bertolini (1932), in *Holothuria tubulosa* after 60 days' regeneration. In the latter, a thin transparent tube grows posteriorly from the oesophagus and crosses the coelomic cavity to the mesentery which previously held the intestine, omitting all the mesentery which formerly held the loop of the stomach. At the same time another thin tube with a blind end starts from the cloaca and extends forward along the mesentery which formerly held the last portion of the original intestine. After about 60 days the two tubes meet and unite to form a single tube extending from oesophagus to cloaca. *Holothuria floridana* and *H. impatiens* also show the growth of rudiments from the anterior and posterior ends to meet and form the regenerating alimentary canal, but in these species the rudiments involve the edge of the mesentery throughout their entire length (Kille, 1936). Scott (1914) noted that the regenerating alimentary canal of *Thyone briareus* was situated along the mesenteric margin, but Kille (1935) was the first to show that it was developed by proliferation of the mesenteric tissue and that only the intestinal epithelium was derived from two centres at opposite ends of the animal. To test the possibility of the regenerating alimentary canal growing across the coelom from one section of mesentery to another in *Thyone,* he removed sections of mesentery, but found that they rapidly redeveloped from minute fragments which remained close to the body wall, and a typical regeneration followed.

The condition found in the three specimens of *Stichopus mollis* thus most closely resembles that found in *Holothuria tubulosa* after 60 days' regeneration. In both species a certain region of the original mesentery is avoided and the alimentary canal follows an almost direct course from the oesophagus to the cloaca.

The stages in reaching this condition, however, differ in the two species. While it is reached in *Holothuria tubulosa* by the union of rudiments growing towards each other from anterior and posterior ends, this is not the case in the present specimens of *Stichopus mollis.*

The alimentary canal of a specimen examined after 44 days' regeneration tapered markedly posteriorly, with the narrowest diameter and least histological differentiation just anterior to the cloaca. If there had been a forward growth from the cloaca the diameter and state of differentiation in this region would be as least as great as farther anteriorly along the mesenteric edge, and would be expected to be actually greater. The condition found in the specimens studied showed clearly that there had been no forward growth of a tubular rudiment from the cloaca. All three specimens showed an abrupt decrease in diameter from the oesophageal remnant to the regenerating alimentary canal, with no gradual transition between the two regions. None of the specimens gave evidence of posterior growth of the regenerating alimentary canal from the remnant of the oesophagus. In normal regeneration in *Stichopus mollis,* the whole of the regenerating alimentary canal is formed along the mesenteric edge from mesenteric tissue, without any contributions from remnants of the alimentary canal at the anterior and posterior ends. There is no evidence to suggest otherwise in the three specimens examined in this present study.

While the connection between the anterior and posterior portions of the alimentary canal in *Holothuria tubulosa* is made after the growth of a rudiment across the coelomic cavity, the alimentary canal in *S. mollis* follows the mesenteric edge along its length over a bridge formed by the adhesion of part of the dorsal mesentery to the ventral mesentery.

The section of the mesentery omitted from the course of the regenerating alimentary canal in the three specimens of *S. mollis* is the region which in normal regeneration shows the greatest increase in depth as it grows forward to eliminate the angle between the edge of the dorsal and lateral mesenteries (See Fig. 1.). After 60 days' regeneration, the mesentery in this angle normally undergoes a tenfold increase in depth from its point of attachment at the body wall, to the free edge supporting the regenerating alimentary canal. In these three cases, where this region did not have regenerating tissue along its edge, there was no evidence of any increase in length of the mesentery after 50 days' regeneration. This may indicate that some interaction between the mesentery and regenerating tissue at its edge causes an extension of the mesenteric sheet in this region. It seems highly probable that the proliferating tissue of the regenerating alimentary canal, normally present in this region, develops a local growth stimulator. In its absence. no stimulation for increase in depth of the mesentery occurs.

The excluded segment of the mesentery shows no regenerating tissue along its edge. This could be due to loss of potency of the mesenteric edge, or a lack of some inducing agent necessary for proliferation and differentiation in the region omitted. As regeneration can occur in all specimens of *Stichopus mollis,* including the largest sizes obtainable, the mesentery appears to retain its potency throughout life, so it is to be concluded that regeneration was absent because no inducing agent was present.

The inducing agent may be absent because an anterior-posterior polarity has already been established in a direct course from the oesophagus to the cloaca. In normal regeneration the new tissue follows an S-shaped course initially with some of the anterior parts of the alimentary canal situated behind other parts which are morphologically posterior. The state of differentiation of the regenerating alimentary canal then bears no constant relationship to position along the mesenteric edge (Dawbin, 1949). In later development the extension of the mesenteries results in a straightening of the alimentary canal towards the anterior-posterior axis. At this stage the regenerating alimentary canal tapers from a more advanced condition of regeneration anteriorly, to a less advanced condition posteriorly, and a continuous anterior-posterior gradient is then first apparent, and has been reached slowly by activity involving the total length of the mesenteric edge.

A direct route for the regenerating alimentary canal is provided along the anterior-posterior axis at a much earlier stage in the cases of adhesion of the dorsal to the ventral mesentery, and it provides the substratum for a gradient of activity in the axis of polarity of the whole animal. Once this gradient is established, it is suggested that the mesenteric edge not included in the axis is then outside the gradient field of organising activity, so proliferation and differentiation of tissue is not induced in this region.

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