Auto-evisceration and the Regeneration of Viscera in the Holothurian *Stichopus mollis* (Hutton)

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**Summary.**

In *Stichopus mollis* autotomy can be induced by various artificial methods, but the process rarely occurs naturally. The injection of distilled water into the body cavity was effective in all cases, and favourable to survival of the animal for the regeneration of expelled organs. Autotomy can be induced in specimens of all sizes from 8-300 gm. and in regenerating animals after the organs approach normal size. There is no evidence indicating any periodic seasonal autotomy. The points of rupture in *S. mollis* are constant in position. The organs expelled are the alimentary canal between the oesophagus and cloaca, the associated haemal vessels, the respiratory trees and in some cases the gonads.

The rate of regeneration in *S. mollis* is slower than that recorded for other holothurians. The alimentary canal is regenerated along the free edge of the mesenteries originating as a solid cord of cells, with no evidence of contributions from other than mesodermal tissue. The lumen appears at any region and is not formed simultaneously along the length of the alimentary canal. Autodifferentiation in different regions of the alimentary canal occurs during early regeneration. It is later followed by stages which show a close relationship to position in the body. A straightening and a reduction in length of the alimentary canal is achieved by the extension of mesenteries which tend to eliminate the angles in the looping of the gut. After differentiation of tissues and increase in diameter of the alimentary canal, feeding re-commences. Lengthening of the alimentary canal and the development of regional differentiation as found in normal animals does not occur until after four months regeneration. The haemal vessels originate as cords of cells along the alimentary canal. The transverse connecting vessel is split off longitudinally from the ventral intestinal vessel while the alimentary canal is still relatively straight. Respiratory trees arise as rudiments from the anterior end of the dorsal wall of the cloaca. They grow forwards as tube-like structures and initially are perforated. Branches commence to appear when a length of 2 cm. is reached.

Autotomy in *S. mollis* is compared with that of other species, and the lack of applicability to *S. mollis* of hypotheses suggesting a possible utility of the process in other species is discussed. The method of regeneration in *S. mollis* is compared with that of other holothurians.

**Introduction.**

*Stichopus mollis* is known from the littoral zone of Central and Southern New Zealand, and from New South Wales to Southern West Australia. It is found on coarse sand and mud in sheltered regions from low-tide level to a depth of 1,530 metres. Previous work on this species, listed by Mortensen (1925), has dealt mainly with the systematic features of the external appearance and of the calcareous spicules which form the internal skeleton. In his systematic account
of New Zealand holothurians, Dendy (1896) gave a brief account of the gross internal anatomy of S. mollis. He observed that "it is very difficult to prevent the animal from discharging its viscera when one attempts to preserve it." No further observations on auto-evisceration in this species, or investigations of the possible regeneration of the organs expelled were made.

Autotomy has frequently been reported in holothurians. The expulsion of internal organs has been demonstrated in most cases as a response to artificial stimuli (Pearse, 1909; Scott, 1914; Kille, 1931, 1935) with fewer cases of its definite occurrence in natural conditions (Minchin, 1892; Bertolini, 1932a; Kille, 1936). Transverse fission of the whole body has been recorded in specimens kept in aquaria (Dalyell, 1851; Chadwick, 1891), but it has also been found to occur in natural conditions (Monticelli, 1896; Crozier, 1917; Deichmann, 1922; Kille, 1936). As far as auto-evisceration is reported, there is considerable variation in the points of rupture, and consequently of the organs expelled by members of different genera of holothurians.

While it has been recognised that regeneration occurs after these cases of autotomy, there are fewer accounts giving precise details of the stages in the reconstitution of organs lost. For Thyone, Scott (1914) and Kille (1935) described stages of regeneration after induced autotomy, while Torelle (1909) described the restoration of parts experimentally removed. Regeneration in Holothuria is described by Bertolini (1932) and Kille (1937). The only accounts of regeneration in Stichopus are those by Bertolini (1930, 1931), describing the condition observed in specimens of S. regalus which were in stages of regeneration when collected from natural conditions, as it was not found possible to keep S. regalus alive for more than a day or so in the laboratory. There has been no account of regeneration in Stichopus at known intervals after autotomy.

The problems of the mechanism of autotomy, and the regeneration of the organs and organ-systems expelled from holothurians have not been fully studied. In some instances, this may be related to experimental difficulties or to insufficiency of material examined. In the case of S. mollis there is ample material available. Evisceration by various stimuli can be induced, and experimentally the animal is a good subject excepting that the rate of regeneration has proven remarkably slow.

In the course of this study it was found necessary to study the normal anatomy and histology of S. mollis. A description of the results is beyond the scope of this paper, but in details essential for a comparison of regenerated with normal tissues, it was found similar to S. chloronotus as described by Sivickis and Domantay (1928). Reference to this paper can be made for a comparison of the regenerated organs of S. mollis with those of normal specimens of Stichopus.

ACKNOWLEDGMENTS.

The greater part of this work was carried out at the Zoology Department, Victoria University College, and the writer wishes to acknowledge the late Professor H. B. Kirk's encouragement and advice during the first part of this study, and Professor L. R. Richardson's helpful advice and criticism of the manuscript. I have also
to thank Mr. P. Maclean, curator of ‘‘Te Aro’’ Baths, Wellington, and Messrs. Adams and Aitken, of Portobello Marine Aquarium, Otago Harbour, for their careful attention to specimens kept during regeneration.

**Methods.**

A number of methods were tested for artificially inducing the expulsion of internal organs. The use of 1 part 7N ammonia in 800 parts of sea water as described by Kille (1931) for *Thyone* was unsatisfactory for *S. mollis*. Some specimens died without expelling viscera, while in the remainder autotomy did not occur in less than several hours and the specimens died within 14 days. A series of specimens were induced to undergo autotomy by placing them in just sufficient sea-water to cover the specimen. This method was effective within from 8–24 hours, without further treatment. It was successful in 85% of the specimens treated in this way, but had the disadvantage that autotomy would frequently occur during the night and specimens would then remain for several more hours in a small volume of polluted sea-water. This contributed to a high mortality rate.

When injecting distilled water into the body cavity of holothurians as a control in experiments for testing the effect of drugs on autotomy and behaviour, Domantay (1931) found that it produced no effect in small amounts. Four specimens of *Holothuria sanguinolenta* injected with 10–14 cc. of distilled water, however, expelled viscera. These results suggested the use of distilled water for inducing autotomy in *S. mollis*. The injection of 1 cc. per 10 gm. body weight induced autotomy in all specimens treated. The response occurred within 30 seconds to 5 minutes after injection, which greatly facilitated observations on the process of autotomy, the weighing of specimens before sea-water was drawn into the body cavity, and the rapid transference of autotomised specimens to fresh sea-water.

It was not found possible to maintain autotomised specimens under the available aquarium conditions. They could be kept satisfactorily in wooden crates with perforated sides and with rocks, fine sand and mud in the bottom. The boxes were submerged to a depth of 1½ metres in “Te Aro” salt-water baths, in Wellington Harbour. Sea-water from the harbour circulated freely through the boxes, which were nevertheless protected from the effect of heavy seas. While many specimens died in less than six weeks after autotomy, sufficient numbers remained in good condition up to 145 days, to permit studies in regeneration.

Specimens for examination were anaesthetised by adding magnesium sulphate to the sea-water. When extended and unresponsive, Bouin’s fluid was injected into the body cavity to fix and harden mesenteries and regenerating tissue sufficiently to prevent any tearing on opening the specimen. An incision was made along the right dorsal interambulacrum, and the flaps of the body wall were pinned out. The specimen was then immersed in fresh Bouin’s fluid, which completed fixation and rendered the structures opaque for closer examination with a dissecting microscope. Before fixation, the regenerating tissues and mesenteries are very soft and transparent, which makes it difficult to determine their limits accurately.
Transactions

As the alimentary canal of *S. mollis* is inconveniently long for complete serial sectioning, 2 cm. lengths of regenerated alimentary canal, together with the attaching mesentery and a part of the body wall were selected for histological study from the following positions:—At the junction of the original oesophagus remnant and the anterior portion of the regenerating alimentary canal; at the posterior end of the dorso-median (dorsal) mesentery; the edge of the middle portion of the left dorso-lateral (lateral) mesentery; the anterior portion of the right ventro-lateral (ventral) mesentery; the edge of the ventral mesentery at the junction with the cloaca; the respiratory trees if present. A variety of stains were used, including Delafeld's haematoxylin and eosin, Mallory's triple, and Heidenhain's iron haematoxylin, which proved most satisfactory for nuclear staining.

AUTOTOMY.

*Stichopus mollis* can be induced to eject its internal organs by a number of artificial conditions, but very rarely does so in its natural habitat. Only six specimens out of 330 examined from the Cook Strait region were eviscerate at the time of collection. All these were found cast up after storms. In Otago, *S. mollis* inhabits deeper water, and can be obtained only by trawling. Of 90 specimens examined after collection in this way, 62 were found to be devoid of viscera. All these had apparently lost the viscera owing to the effects of trawling, as the breaks in the alimentary canal were quite new, and none showed any signs of regeneration. In 420 specimens collected during all months of the year, there has been no evidence of spontaneous auto-evisceration under natural conditions. On the other hand, autotomy could be readily induced artificially in all specimens tested, at any season of the year. Specimens only 8 gm. in weight had the capacity for autotomy as well marked as the largest examined—i.e., up to 300 gm. After regeneration of 100 or more days it was found that autotomy of the regenerated organs can again be induced.

PROCESS OF AUTO-EVISERATION.

After the injection of distilled water *S. mollis* remains quiescent in a half-extended condition for approximately one minute. A distention of the posterior half of the animal follows, accompanied by contraction of the longitudinal muscle bands and a decrease in volume of the whole animal. The dorsal wall of the cloaca then ruptures immediately posterior to its junction with the base of the respiratory trees. Through this rupture the right respiratory tree is reflected backwards and protrudes through the anus with the tips appearing first. Both respiratory trees appear when the left branch is not connected with the haemal plexus, which is a condition frequently found in smaller specimens. The alimentary canal protrudes in the form of two loops, the first to appear being the stomach and part of the intestine immediately posterior to it. The second loop contains the intestine with the haemal plexus and usually the left respiratory tree is entangled in this. The first stage in the expulsion of viscera is relatively violent, accompanied by a rapid outflow of coelomic fluid and occupies about 15-20 seconds. A slight relaxation of the body follows and viscera flows slowly, or remains in the same state of extrusion for 30-40 seconds. Another con-
traction then commences and the remaining portions of the alimentary canal are forcibly expelled with the ruptured anterior end appearing last. The anterior break occurs in the oesophagus about 2 cm. posterior to the water vascular ring. Specimens killed during autotomy showed this region intact, although most of the internal organs were protruding through the cloaca. The rupture in this region apparently occurs late in the process. Separation of visceræ from the mesenteries occurs close to the line of junction between the two, which leaves the mesenteries intact. When the branches of gonads are sufficiently long to become entangled among the visceræ, they will be carried out with the alimentary canal.

The process of auto-evisceration is the same, irrespective of the type of inducing agent. It is normally completed two minutes after the first appearance of tips of the respiratory trees.

When gonads are insufficiently large to become entangled in the visceræ, branches are frequently expelled separately several hours later. In one case a contraction of the body followed by expulsion of branches of the gonads occurred 23 hours after the other organs had been expelled. Gonads less than 2 cm. in length are not expelled. In all specimens examined, it was found that the regions of rupture were constant, so the same organs were always expelled, except for variations in the case of gonads. All the alimentary canal:

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**Fig. 1.** Dissection opened along right dorsal interambulacrum showing structures remaining after auto-evisceration.
between the regions of rupture, the haemal vessels supplying it, and both respiratory trees are lost.

The body wall, with its attached musculature, the haemal and nerve rings with their radial branches, and the complete water vascular system is left intact. (Fig. 1.) A short length of oesophagus with the corresponding length of the dorsal and ventral intestinal vessels remains, and the cloaca and its retractor muscle fibres are left intact except for the rupture at the anterior end. Short lengths of gonad branches remain on either side of the dorsal mesentery. There is a continuous connection of mesenteries from the lantern to the cloaca, with the free edge delimiting the position of the original alimentary canal.

The dorsal mesentery (Fig. 1) originates along the dorsal interambulacrum from its anterior end and continues posteriorly almost to the cloaca. Along its inner edge the lantern and oesophagus remnant remains supported. The remainder of the oesophagus and the muscular stomach were suspended along it posteriorly. From the posterior end the dorsal mesentery crosses over a longitudinal muscle band and continues anteriorly along the left dorso-lateral interambulacrum. The posterior portion of the stomach and first portion of the intestine were suspended by this section. At the anterior end, the mesentery crosses to the right ventral interambulacrum close to the ventral longitudinal muscle and continues posteriorly to the cloaca. There is a considerable distance between the intestinal attachment and origin of the mesentery from the body wall at the region of its anterior angle. The intestine was therefore suspended considerably further posterior to the lantern than the point of mesentery origin. At this point a distinct junction occurred between small and large intestine distinguishable by colour and texture rather than change in size. The large intestine was suspended from that point to its junction with the cloaca. Regeneration of the alimentary canal occurs along the continuous edge of the mesenteries.

**BEHAVIOUR AFTER AUTOTOMY.**

Autotomy appears to act as a stimulant to *S. mollis* for several hours. Extension of the body is followed by that of tentacles to such an extent that the mouth is clearly visible. Protrusion of podia and locomotion follows, which agrees with the finding that the water vascular system is uninjured. Sensitivity to touch is as acute as in normal animals because of lack of injury to the nervous system. Cloacal pulsations continue with a regular uninterrupted rhythm. In normal animals the rhythm is regular for an interval which varies with the size of the animal. It is then interrupted by a spouting action, which forces water out of the respiratory trees with sufficient force to cause a distinct current of sea-water away from the cloaca. While such a spouting action has been observed after the contraction of the body seven days after autotomy, it is not usually present. A considerable volume of sea-water is taken into the body cavity through the ruptured end of the cloaca, within a few hours of autotomy. It is shown by a marked increase in volume and weight of the autotomised specimen. During regeneration the power of active movement, sensitivity, the general
appearance and cloacal pulsations persist unchanged in specimens which do not become moribund.

In groups of specimens collected at the same time and which received the same treatment, it was found that some would survive in excellent condition after auto-evisceration but that a large percentage would die. In some it occurred even after stages of regeneration had well commenced. From Table I it can be seen that *S. mollis* kept in aquarium tanks with non-running sea-water all died. Of 45 specimens, 38 died without any signs of regeneration and seven were killed for study as soon as they showed a sustained contraction and any sloughing of the body wall. One survived for 15 days before reaching this state. At Dunedin no specimens could be found close to low-tide level along the coast. All specimens studied there were obtained from trawlings at about 40 metres. Many of these were partly crushed by the weight of material in the trawl. Sixty-two such specimens had autotomised by the time they could be transferred to a container of fresh sea-water. None of these survived more than 20 days at Portobello Marine Aquarium, where they were kept in tanks supplied by running sea-water. Those collected near the end of a trawling were uncrushed and autotomy in these did not occur until treatment in the laboratory. Of 28 such specimens, 13 died within 20 days, but eight survived from 20–50 days and showed definite evidence that regeneration had commenced before they became moribund. Seven were in excellent condition when killed for study of regenerated tissues. Those collected by hand from near low-tide level in the Cook Strait area and kept in crates at Te Aro baths showed a similar high mortality rate up till 20 days after autotomy. Of 122 specimens, 65 died in less than 20 days, and 21 were becoming moribund when killed for study. The remaining 26 were in excellent condition when killed for study. The preceding specimens were treated before the arrival of Domantay’s (1930) paper from U.S.A. and autotomy in all, except those crushed during trawling, was induced by standing them in a small volume of sea-water until autotomy occurred.

Those in which autotomy was induced by the injection of distilled water showed a much higher survival rate than any treated by other methods. Of 52 specimens only 3 died within 20 days, and the 12 which eventually became moribund survived 30–80 days after autotomy. The remaining 37 were all in excellent condition when killed for study at intervals up to 145 days after autotomy. Some specimens not yet examined are still active after longer than this time.

Although autotomy in *S. mollis* can be induced by a variety of stimuli, survival of the animal after the process depends greatly on the method used and the rapid transference of autotomised specimens to fresh circulating sea-water. The difficulty in achieving a rapid transference after methods which require prolonged action, probably accounts for the much higher mortality rate of specimens treated by standing in a small volume of sea-water, than for those treated by the injection of distilled water. Even in the latter cases, which were transferred to fresh sea-water within one minute of auto-
### TABLE I.

<table>
<thead>
<tr>
<th>Where specimens kept after autotomy</th>
<th>Method of inducing autotomy</th>
<th>In excellent condition when killed for study</th>
<th>Moribund when killed for study</th>
<th>Died within 20 days of autotomy</th>
<th>Total</th>
<th>Percentage died or moribund</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory aquarium tanks</td>
<td>Small vol. seawater</td>
<td>—</td>
<td>7</td>
<td>38</td>
<td>45</td>
<td>100</td>
</tr>
<tr>
<td>Portobello marine aquarium</td>
<td>Eviscerated during trawling</td>
<td>—</td>
<td>—</td>
<td>62</td>
<td>62</td>
<td>100</td>
</tr>
<tr>
<td>Portobello marine aquarium</td>
<td>Small vol. of sea-water</td>
<td>7</td>
<td>8</td>
<td>13</td>
<td>28</td>
<td>75</td>
</tr>
<tr>
<td>Te Aro baths</td>
<td>Small vol. seawater</td>
<td>26</td>
<td>21</td>
<td>65</td>
<td>112</td>
<td>73</td>
</tr>
<tr>
<td>Te Aro baths</td>
<td>Injection distilled water</td>
<td>37</td>
<td>12</td>
<td>3</td>
<td>52</td>
<td>29</td>
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<tr>
<td></td>
<td></td>
<td>70</td>
<td>48</td>
<td>181</td>
<td>290</td>
<td></td>
</tr>
</tbody>
</table>

Specimens eviscerated when cast up after storms

Specimens examined for other purposes and not kept for regeneration

<table>
<thead>
<tr>
<th></th>
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<tr>
<td></td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>420</td>
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</table>
tomy 15 of 52 specimens had died or become moribund before examination.

It appears that *Stichopus mollis* is more sensitive to unfavourable conditions before and after autotomy, than most other holothurians. Bertolini (1930) working at Naples on the related *S. regalis*, found that specimens of this species which had just undergone autotomy, could not be kept alive in the laboratory for more than a day or so, whereas *Thyonome briareus* Scott (1914), Kille (1935) and other species of holothurians have been kept alive very successfully in aquaria after autotomy. A particular sensitivity to adverse conditions after autotomy may be a characteristic of the genus *Stichopus*.

**Stages in Regeneration.**

The stages in regeneration were studied by the examination of specimens at known intervals after autotomy. Until it was evident that the rate of regeneration in *S. mollis* is much slower than that found in previously studied species, specimens were killed for examination at 2–4 day intervals. Later the time between examinations was extended to 8–12 days.

During the first 12 days there is little evidence of regeneration visible to the unaided eye. The mesenteric remnants are still approximately the same size as those found immediately after autotomy, and the ruptured anterior end of the cloaca remains unclosed. Histological study shows closure of the ruptured end of the oesophagus. No changes in the mesenteric edge could be detected up to this stage.

Specimens examined after 12 days' regeneration showed a progressive increase in the distance from body wall to mesenteric edge in certain regions. Greatest extension occurs around the angle formed by the crossing of the dorsal mesentery over the right dorsal interambulacrum before it travels forward as the lateral mesentery. Extension in this angle lessens the distance of the free mesenteric edge by decreasing the angles of the loops, resulting in a more direct connection between oesophagus and cloaca. If the distance from the anterior end of the lateral mesentery to the posterior point of origin of the dorsal mesentery (*x* — *x'*, Fig. 1) is taken as unity, it is found immediately after autotomy that the mesenteric remnant from its point of origin at the angle (*x' — x''*, Fig. 1) traverses only 10% of this distance (Figs. 1 and 2). Thus the free edge of the mesenteric remnant travels posteriorly and then forward for 90% of this distance.

The extent of the growth of the mesentery after various intervals of regeneration is shown in Fig. 2 for 18 specimens which had undergone autotomy at the same time, and regenerated under identical conditions. After 17 days' regeneration (Fig. 3) the mesentery extends forward 20–25% of the distance, reaching 65% after 33 days' regeneration (Fig. 4), 75% in 41 days' regeneration (Fig. 5), and by 65 days' regeneration it has elongated to form a pocket-like structure, of which the lip bearing the regenerating alimentary canal is parallel to the anterior end of the ventral loop. When this stage is reached there is no longer any looping of the free edge in its traverse from the dorsal to the ventral mesentery (Figs. 3–6). The connection from one to the other is then in a straight line. From Fig. 2 it can be seen that the rate of increase in depth is almost con-
Fig. 2.—Growth increment of $X'-X''$ (Fig. 1) as a percentage of $X-X''$, during regeneration.

stant (except for one specimen) instead of showing a logarithmic curve as found in most growth processes. This is probably due to extension occurring in one plane as a sheet which does not increase in thickness. Growth is, therefore, restricted to two dimensions. At the mesenteric edge, the slight thickening which is the primordium of the regenerating alimentary canal, is not sufficiently large appreciably to slow down the extension of the mesenteric sheet.

At the same time there is a lesser increase in depth of the mesentery in the angle between the lateral and ventral loops. This causes a posterior movement of the position at which the free mesentery edge crosses from the lateral to the ventral loop. There is no increase in depth of the mesentery along the right ventral inter-radius from the level at which the free edge crosses from the lateral mesentery, to the junction with the cloaca.

Changes in the depth of parts of the mesentery, continuously decrease the length of the free edge from oesophagus to cloaca. This gradually eliminates the angles of looping, which are so characteristic of the alimentary canal and its mesenteric attachments in normal animals. After 65 days' regeneration there is almost a straight course from oesophagus to cloaca.

There is a series of changes along the free mesenteric edges during the alterations in lengths of the mesenteries. The normal mesentery consists of a thin connective-tissue sheet covered by an epithelial layer continuous with that lining the body cavity. The lining epithelium on each side of the mesentery meet across the torn edge after 15 days' regeneration, and fuse in the mid-line. Mesenchyme cells between the epithelial layers increase in number to form a slight thickening along the mesenteric edge. It is from this collection of mesenchyme cells that the alimentary canal is regenerated.
FIG. 3.  FIG 4.  FIG 5.  FIG 6.

Figs. 3–6.—Four stages in regeneration showing the regions of growth of the mesentery, and the restoration of organs.

A.C., alimentary canal; CL., cloaca; D.M., dorsal mesentery; L., alimentary canal at the stage where a lumen is forming; L.M., lateral mesentery; O., incompletely closed rupture of cloaca; O.E.S., remnant of original oesophagus; R.T., developing respiratory tree; S., alimentary canal appearing as a solid cord of cells; T.C.V., transverse connecting vessel; V.M., ventral mesentery; V.I.V., ventral intestinal vessel.

**Alimentary Canal.**

Up till 25 days after autotomy there is simply an increase in the number of mesenchyme cells, which gradually form a solid, cord-like swelling along all the mesenteric edges. Its most anterior point is adjacent to the closed oesophageal remnant with which it is in contact. The junctional zone remains distinct with no gradation between the oesophagus and the regenerating alimentary canal, at early stages. There is no evidence of cell proliferation from the original oesophageal remnant contributing to the formation of the regenerating alimentary canal. From the oesophagus the cord of cells is continuous along the mesenteric edge to the cloaca. It therefore develops in its early stages with the same loops as the original alimentary canal, and then becomes shorter and more direct as the length of the mesentery edge decreases.

Proliferation of mesenchyme cells does not occur at the same rate along the whole distance. There is considerable variation between individual specimens, but the most active proliferation is usually found in the anterior portions of the regenerating alimentary canal. Localised swellings may also occur at any region along
### TABLE II.

<table>
<thead>
<tr>
<th></th>
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<td>68</td>
<td>25</td>
<td>0.2 × 0.1 S</td>
<td>0.3 × 0.4 S</td>
<td>0.2 × 0.1 S</td>
<td>0.3 × 0.3 L</td>
<td>0.5 × 0.3 S</td>
<td>absent.</td>
<td></td>
</tr>
<tr>
<td>78</td>
<td>26</td>
<td>0.7 × 0.5 D</td>
<td>Remaining mesentery edge unthickened.</td>
<td>1.0 × 1.0</td>
<td>Exceptional Condition</td>
<td>1.6 × 1.0 Branching commencing.</td>
<td>absent.</td>
<td></td>
</tr>
<tr>
<td>67</td>
<td>33</td>
<td>0.4 × 0.4 S</td>
<td>1.5 × 0.4 S</td>
<td>0.6 × 0.3 S</td>
<td>0.3 × 0.2 S</td>
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<tr>
<td>87</td>
<td>41</td>
<td>0.2 × 0.2 S</td>
<td>0.4 × 0.15 D</td>
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<tr>
<td>80</td>
<td>50</td>
<td>1.3 × 0.7 L</td>
<td>1.0 × 0.5 L</td>
<td>0.6 × 0.5 L</td>
<td>0.5 × 0.4 L</td>
<td>0.4 × 0.3 S</td>
<td>14.0 × 1.5</td>
<td>Large specimen (250 gm.)</td>
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<td>65</td>
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<td>3.0 × 2.0 D</td>
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<td>2.0 × 2.0 D</td>
<td>1.2 × 0.8 S</td>
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<td>1.7 × 1.5 D</td>
<td>1.2 × 1.0 D</td>
<td>16.0 × 1.5 Branching commencing.</td>
<td>28.0 × 3.0</td>
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</tr>
<tr>
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<td>110</td>
<td>2.2 × 2.0 D</td>
<td>3.0 × 2.0 D</td>
<td>2.1 × 2.0 D</td>
<td>2.5 × 2.4 D</td>
<td>2.0 × 1.8 D</td>
<td>25.0 × 2.5</td>
<td>Distinct branches.</td>
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<tr>
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<td>145</td>
<td>3.3 × 3.0 D</td>
<td>3.0 × 3.0 D</td>
<td>2.8 × 2.6 D</td>
<td>3.4 × 3.4 D</td>
<td>2.1 × 2.0 D</td>
<td>28.0 × 3.0</td>
<td>Distinct branches.</td>
</tr>
</tbody>
</table>

S Solid.

L Unlined lumen appearing.

D Lumen lined by differentiated epithelium.
the mesenteric edge, occurring least frequently along the ventral mesentery. After 25 days' regeneration an unlined lumen commences to appear in parts of the previously solid cord of cells. The lumen does not necessarily appear in those parts of the regenerating alimentary canal which have reached greatest thickness. From Table II it can be seen that in Specimen 68 a lumen had appeared at the anterior end of the ventral mesenteric edge in a cord of 0·3 mm. diameter, while the more posterior region of greater diameter was still solid. Specimen 78, examined after 26 days' regeneration, was unusual in having the anterior portion of the regenerating alimentary canal merging with, and reaching almost the same diameter as, the original oesophageal remnant. The most anterior portion showed well differentiated layers such as are found in the normal oesophagus. This was followed by a region showing an unlined lumen amongst the mesenchyme cells and absence of a muscle layer, then by a solid cord of mesenchyme cells which was of the same diameter as that showing normal layers. The diameter of the solid cord in Specimen 78 was much greater than that found in many specimens which were developing a lumen, or even showing differentiation of the cells lining the lumen, e.g., Specimen 87 after 41 days' (Table II) regeneration. Specimen 67 after 33 days' regeneration shows this even more clearly. The thickest portion of the regenerating alimentary canal (1·5 × 0·4 mm.) was still solid, although a lumen was commencing to appear in a region of only 0·2 mm. diameter. The lumen appears without any constant relation with the position along the regenerating alimentary canal, or with its diameter.

Differentiation of the mesenchyme cells lining the lumen was found only in specimens examined after 40 days' regeneration. Specimen 87, examined after 41 days' regeneration, showed a region at the posterior end of the dorsal mesentery which had developed a lumen lined by a well-developed inner epithelium. It commenced at the posterior end of the dorsal mesenteric edge as a cavity in a cord of 0·4 × 0·15 mm. and continued across to approximately half-way up the lateral mesentery, increasing in diameter to this point where the cord had reached 1·4 × 0·3 mm. Along this segment the lumen was lined by a very well-developed inner epithelium (Fig. 7). Farther posteriorly the regenerating alimentary canal constricted sharply to a much smaller diameter, and continued as a solid cord of cells only 0·1 mm. in diameter. The lumen and its lining cells had appeared in a median segment, with only a solid cord of cells connecting it to the oesophagus anteriorly and the cloaca posteriorly.

After 50 days' regeneration the alimentary canal is of greater diameter along its whole length. In some specimens the development of a lumen and the differentiation of its lining epithelium occupied a greater length of the alimentary canal. In others, e.g., Specimen 80 (Table II), the lumen was appearing in all regions of the alimentary canal, but showed strands of mesenchyme cells extending across the lumen and dividing it into several adjacent spaces instead of forming a single cavity. In spite of its increased diameter, the differentiation of a lining epithelium had not become evident.
An extensive lumen lined by a well differentiated lining epithelium was found in all specimens (except one) examined after 60 days' regeneration. Large irregularities in the diameter of the gut and its state of development were no longer apparent. The increase in diameter of the regenerating alimentary canal in the anterior region was sufficiently great to make the point of junction between it and the original oesophageal remnant difficult to locate. The lumen from one to the other was continuous. Histological study showed that the muscle layer in the regenerating alimentary canal had not yet formed while that in the original oesophageal remnant was distinct. Posteriorly the regenerating alimentary canal contained a continuous lumen until it reached the portion suspended by the anterior third of its ventral mesentery. There was a gradual decrease in diameter to this point, from which it extended posteriorly as a solid cord of cells to a point just before its junction with the cloaca. A slight dilatation may occur a few millimeters before the cloaca with a lumen continuous with the cloacal chamber.

A continuous lumen lined by an inner epithelium from the oesophagus to the cloaca was found for the first time in a specimen which had regenerated for 80 days. The alimentary canal had still not reached the same diameter along its whole length, but showed a gradual decrease from 2.0 × 1.5 mm. in size anteriorly to 1.2 × 1.0 mm. along the posterior half of the ventral mesentery. There was an enlargement to 2.2 × 2.0 mm. at the region crossing from the anterior end of the lateral mesentery to the ventral mesentery. In normal animals this portion of the mesentery suspends the junction of the small and large intestine. Owing to the extension of the mesenteries during regeneration and the shortening and straightening of the alimentary canal regenerating along its edge, the gut now follows an almost straight course from
oesophagus to cloaca, and this region now represents its anterior third to half. It seems likely, therefore, that this enlargement represents the commencement of the stomach, which has altered its relative position following the mesentery rearrangement. The extensive folding of the lining epithelium characteristic of the normal stomach had not developed sufficiently in any specimen up to 145 days' regeneration to establish definitely this enlargement as the stomach. From Table II it can be seen that the enlargement was present in this region in each case after 80 days' regeneration.

Traces of a muscle layer appeared in the anterior portion of the alimentary canal after 80 days' regeneration. Circular muscle fibres were distributed in the mesenchyme layer close to the outer epithelium. At this stage the mesenchyme cells between the muscle fibres and the inner epithelium were formed into the inner connective tissue as found in the normal gut. The narrow outer connective tissue layer, normally present between the muscle layer and the outer epithelium was not clearly distinguishable in the regenerating alimentary canal at this stage. Large oval "wandering" cells which are intensely eosinophilic occurred in the inner connective tissue layer and can be found in the mesentery among the mesenchyme cells. The latter are continuous with the inner connective tissue layer of the regenerating alimentary canal. The "wandering" cells resemble the oval and spheruliferous corpuscles described from the body wall and mesentery of S. chloronotus by Sivickis and Domantay (1928). They are present also in the connective tissue layer of the body wall of S. mollis. It seems possible that these cells are the carriers of nutritive reserves from the body wall via the mesentery to supply the proliferating cells which form the regenerating alimentary canal.

A study of the relative concentration of mitotic figures was made in an effort to determine the regions of greatest proliferation. Preparations showing mitotic figures were not obtained during early stages with the developing alimentary canal present as a solid cord of cells. After 40 days' regeneration, in regions which had developed a lumen, there was a great concentration of nuclei occurring around the lumen, and distinct mitotic figures were found as these cells proliferated to form the inner epithelium. The few observed in the inner connective tissue layer may be partly due to the much greater dispersal of nuclei in the matrix of the connective tissue. (Fig. 7.) In addition, although the connective tissue increases in total volume with enlargement of the alimentary canal, its relative thickness decreases with enlargement of the lumen in the alimentary canal, and with the differentiation of more cells to form the inner epithelium. Increase in the diameter of the alimentary canal necessitates some increase in the outer epithelial cells, to contain it, but division occurs here only in the plane parallel to the surface, and is much less prominent than that of the inner epithelium.

The alimentary canal contains all the normal histological layers after 110 days' regeneration. Before 145 days' regeneration, the layers differ in the poorer development of the muscle layer (especially of the longitudinal muscle fibres), and in the presence of a narrower outer connective tissue than that found in normal animals.
The first evidence of growth in length of the regenerating alimentary canal is shown after 110 days' regeneration by an increase in the angulation between the region along the dorsal mesentery and that along the ventral mesentery (cf. Fig. 6 and Fig. 9). After 145 days' regeneration this angle is further increased, and indicates that any further elongation would probably occur by a gradual return to the original condition, with a loop formed between the dorsal and lateral mesenteries.

The first specimen with food material in the alimentary canal was one examined after 110 days' regeneration. Small amounts of food material were present in all specimens examined after more than this period. Although feeding had become possible, the alimentary canal after 145 days' regeneration still differed from that of normal specimens. It showed no constant difference in the relative proportions of the tissue layers corresponding to different regions of the length of the alimentary canal. The diameter of the alimentary canal was less, especially in the more posterior portions, and the length and condition of gross regional differentiation was much less than that found in normal animals. Stages later than 145 days' regeneration have not yet been examined.

Haemal System.

The ventral intestinal vessel commences after 30 days' regeneration as an extension of the mesenchyme cells with covering epithelium from along the ventral side of the alimentary canal. At intervals the outer epithelium fuses in the mid-line between the gut and the developing vessel. At these points the mesenchyme in the vessel is isolated from that of the gut and separation of the ventral vessel commences. The region in which the two mesenchyme masses remain in continuity marks the origin of the small connecting vessels between the gut and the ventral vessel. This separation was first observed after 33 days' regeneration in Specimen 67, where it was restricted to the posterior two-thirds of the gut forming along the dorsal mesentery. It was not then continuous with the original haemal vessel remnants along the oesophagus, and did not extend up the lateral mesentery or further posteriorly. Other specimens examined up to 60 days showed no further development, some showing no separation of the vessel, even from alimentary canals of greater thickness. After 65 days' regeneration the ventral vessel was found continuous as a solid cord of cells connected with the original remnant along the oesophagus, but separation from the posterior portion of the alimentary canal had not occurred. Specimen 98, after 80 days' regeneration, showed a continuous ventral vessel widest anteriorly at its point of junction with the original vessel remnant, and continuing conspicuously but narrowing until half-way along the ventral mesentery, where it ceased.

The dorsal intestinal vessel develops close to the junction of the mesentery and gut. It is formed later than the ventral vessel but follows the same stages in its formation. After 80 days regeneration it could be traced from the connection with the original vessel at the oesophageal remnant to the anterior portion of the gut along the ventral mesentery. At this stage it was less prominent than, and
did not extend so far posteriorly as, the ventral vessel. After 110 days regeneration, the dorsal intestinal vessel shows an enlargement in the region of the anterior end of the ventral mesentery (Fig. 9) to a diameter greater than that of any part of the ventral intestinal vessel. Numerous short cross vessels connect the larger dorsal vessel to the alimentary canal, and form the commencement of the plexus or "rete mirabile," which is such a conspicuous feature in normal specimens. An unlined irregular lumen appears amongst the mesenchyme cells of both intestinal vessels. No lining epithelium is present in the haemal vessels of normal animals, so the appearance of a lumen completes the histological differentiation of the vessels.

**Figures 8 and 9.** — Later stages in the regeneration of the haemal vessels.

D.I.V., dorsal intestinal vessel; R.M., developing "rete mirabile"; T.C.V., transverse connecting vessel; V.I.V., ventral intestinal vessel.

In normal specimens, a large transverse vessel connects the middle portion of the ventral vessel along the part of the alimentary canal attached to the dorsal mesentery, with the middle of the ventral vessel along the alimentary canal which is attached to the lateral mesentery. It thus crosses a large portion of the body cavity with connections only at its origin and termination. No such vessel is present during early stages of regeneration. When the alimentary canal is almost straight, the developing ventral vessel along the region from the middle of the dorsal mesentery to the anterior end of the ventral mesentery constricts longitudinally, parallel to the surface of the alimentary canal, and splits off the outer portion, which then becomes the transverse connecting vessel (Fig. 6). This was present in specimen 98 after 80 days' regeneration (Fig. 8). It showed a greater length of separation and increase in thickness in specimens examined
from 110-145 days after regeneration (Fig. 9). By a lengthening of
the regenerating alimentary canal to restore the length and looping
found in normal specimens, the developing transverse vessel could
readily resume the same relative position across the body cavity as
found in normal specimens.

Respiratory Trees.

The time of origin and rate of growth of the respiratory trees was
found to vary considerably between specimens. The earliest appear-
ance was recognisable as two small solid projections from the anterior
dorsal portion of the ruptured end of the cloaca. They were apparent
after 11 days' regeneration, being formed as solid forward projections
of the connective tissue layer of the cloaca, with a covering outer
epithelium and were 0.5 mm. in length. In some cases the ruptured
end of the cloaca healed over without immediately giving rise to buds
of the respiratory trees which were found absent even after 33 days'
regeneration in 67, a specimen which showed excellent regeneration
of the alimentary canal.

After 26 days' regeneration specimen 78 showed projections
1 mm. in length with spaces in the mesenchyme continuous with that
of the cloaca. The development of a lumen and the differentiation of
the inner epithelium followed a similar course to that found in
the alimentary canal. The lumen, however, occupied most of the diameter
of the respiratory tree at an early stage and the lining epithelium
was much narrower and less clearly defined than that of the alimentary
canal. A layer of muscle fibres was not found in any specimens
until after 100 days' regeneration.

The rate of growth in length and diameter of the respiratory
trees can be seen from Table II. It increases slowly; a specimen
after 41 days' regeneration having respiratory trees 4 mm. in length
and 1 mm. diameter, and those in a specimen after 145 days' 28 mm.
X 3 mm. The dimensions for intermediate stages are shown in
Table II. Evidence of branching was first apparent after 80 days'
regeneration (Fig. 6), and was more pronounced in later stages.
After 145 days, the branches were 1 mm. in length, very numerous,
and irregularly spaced from the tip of the respiratory trees to the
point of junction of the trunks with the cloaca. Subdivision of the
branches into smaller ampullae did not occur until after this stage.
In some specimens the left respiratory tree was found longer than the
right, but in no case had it extended sufficiently far to make contact
with the developing rete mirabile as found in normal specimens.

Distinct pores, placing the lumen of the respiratory trees in com-
munication with the body cavity, were found in some specimens.
These pores were situated near the tip of the respiratory tree and
were approximately 0.1 mm. in diameter. A thin epithelium con-
tinuous from the outer to the inner epithelium of the tree lined the
pore, showing clearly that they were not accidental perforations.
None were found in specimens of less than 50 days' regeneration. No
such pores are found in the respiratory trees of normal specimens.
It is possible that they are an adaptation for taking sea-water into
the body cavity to assist respiratory exchange until a sufficiently large
area of respiratory tree is present for adequate supply by diffusion
alone.
REPRODUCTIVE SYSTEM.

In many specimens no branches of gonads were observed. Those which had branches showed normal structure; so these branches are probably portions not expelled during autotomy. As there is great seasonal variation in the size of gonads in normal animals, the appearance and growth of branches after autotomy could also be a normal seasonal development quite unassociated with the regenerative process.

DISCUSSION.

Autotomy.

Autotomy occurs in some holothurians as a method of transverse fission into two parts, both of which survive. This has been shown by Dalyell (1851) in Oucumaria lactea (suggested to be really C. planci by Deichmann (1922); in C. planci (Chadwick, 1891; Monticelli, 1896): in Holothuria parvula (syn. H. captiva, Actinopyga parvula by Crozier, 1914, 1917; Deichmann, 1922; Kille, 1936; 1937): in Actinopyga difficilis Deichmann (1922): and in Holothuria surinamensis Crozier (1917).

Autotomy in the sense of explosion of viscera could be deduced as occurring in natural conditions from Minchin's (1892) observation that the viscera of Holothuria were frequently fished up by fishermen. He states that evisceration thus seems to be almost a normal habit. It is stated by Bertolini (1932a) that autotomy is a normal and periodic occurrence in Stichopus regalis. She found that of 119 specimens examined during September and October, 110 were regenerating and 7 had recently eviscerated, while of 50 animals collected during spring none was in a state of regeneration. Kille (1936) found specimens of Holothuria floridana already in a state of regeneration when they were collected from their natural environment.

A number of other holothurians have been found capable of autotomy as a result of certain chemical and physical stimuli, but with no definite evidence that the process occurs in these species in natural conditions. In Thyone briareus autotomy was induced by the injection of chemicals of which strychnine was most satisfactory (7 out of 20), Pearse (1909); by allowing the animal to stand in stagnant sea-water and alternating this with running water containing much oxygen which proved effective in 65 per cent. of the animals treated, Scott (1914); by the use of dilute ammonia, Kille (1931); and by electrical stimuli, Kille (1935). Both the latter methods were almost invariably successful. The injection of various chemicals induced autotomy in Holothuria atra, H. sanguinolenta, and Stichopus chloronotus, and it was induced in Synapta maculata by placing specimens in a small volume of sea-water, Domantay (1931). There appears to be no evidence that autotomy occurs in any of these species in natural conditions. On the contrary Kille (1935) found that among some 400 medium-sized specimens of Thyone briareus which were examined, there was none in a natural state of regeneration. This indicates that autoviscerisation had not occurred in nature in any of these specimens. Of 420 specimens of Stichopus mollis examined during all seasons of the year, none showed any trace of regeneration, and the only specimens which were eviscerate on collection were 6 cast up after storms,
and some specimens collected by trawling. All the eviscerate specimens showed clearly by the state of the mesentery edges that autotomy had just occurred, and can be presumed to be due to the severe mechanical effect of wave action in one case and of trawling in the other. Autovisceration could, however, be artificially induced in all specimens tested, which included a size range from 8 gm. to 300 gm. Although there is ample evidence to show the widespread ability of holothurians to autotomise certain organs under special conditions, there are fewer recorded cases of the occurrence of this process under natural conditions. Each stimulus varies greatly in effectiveness from species to species, e.g., the methods used by Kille (1931 and 1935) for inducing autotomy in *Thyonoe* were found quite ineffective for *Holothuria pervula* Kille (1937). The use of dilute ammonia was found unsatisfactory for *S. mollis*, whereas the injection of distilled water was effective in all specimens of this species. In view of the special conditions sometimes needed to induce autotomy in holothurians, it is surprising to find the property apparently present in all specimens when suitable stimuli are used.

The organs expelled by different holothurians vary among genera but are very constant within members of the genus. In *Thyonoe* the entire length of the alimentary canal, and its haemal vessels, the introvert and the lantern with its associated structures are autotomised, Kille (1935). The respiratory trees and the gonads are retained. *Phyllophorus magnus* under some conditions autotomises the whole anterior part together with the tentacles, mouth, calcareous ring and other portions "snap off from the body," Domantay (1931). *Synapta* appears to have less clearly defined points of autotomy and responds to inducing stimuli by constriction of the body into fragments. Species of *Holothuria* lose the part of the alimentary canal between the oesophagus and the cloaca, the rete mirabile and the left respiratory tree (Semper, 1861; Kille, 1936). This differs from *Stichopus* mainly in the retention of the right respiratory tree. In *Stichopus mollis* branches of gonads are also frequently expelled. Although there is a considerable variation in the organs expelled by different genera of holothurians, there is very great constancy in the points of rupture and in the organs expelled in each genus. There appear to be constant breaking points present within each genus. Lukas (1905), in a work not available to the writer, is stated by Pearse (1909) to interpret the different results as indicating that the place where self-mutilation takes place in an animal is usually determined by certain structural characteristics of the animal concerned. There is, however, insufficient data on the morphology of the regions at which rupture occurs, to show the reason for separation at these points. Histological examination of the regions of rupture in *S. mollis* did not show any distinct discontinuity or constriction of any of the layers in the alimentary canal. It is understandable that the point of junction of respiratory trees and rectum with the cloaca might be structurally weaker than other regions of the alimentary canal, but the reason for the constancy in position of the rupture in the oesophagus is not clear. All specimens of *Stichopus mollis* examined showed identical points of rupture,
It seems rather surprising, in view of the widespread occurrence of autotomy in Holothurians and in the constancy of breaking points in each genus, that possible utility of the process is suggested only in certain species. Those which undergo binary fission probably use the process as a method of asexual reproduction. Species which show regeneration in natural conditions must be capable of responding to some inducing stimulus in the environment, and it is possible that this stimulus is predator attack. The process has been suggested as protective by Minchin (1892) for Holothuria and by Domantay (1931) for the genera Holothuria, Stichopus, Theleotida and Actinopyga, which are stated to react to irritation or strong stimulation by ejecting their viscera. A series of experiments was performed on Stichopus mollis to determine its rate of response to the type of mechanical stimuli likely to be involved in predator attack. It was found that violent agitation accompanied by extensive prick- ing with tacks projecting from plates which held the specimens, was effective only after ten or more minutes. Crushing, although effective after prolonged action, was also very slow in inducing autotomy. The treatments used were much more severe than would be expected from a predator, yet the response was far too slow for autotomy to be of any value in diverting the attention of a possible predator to the expelled viscera. There is also no evidence of any fish or other organism which preys on S. mollis. It is difficult to visualise a protective function for the process in this species.

There is much evidence, as previously mentioned, to show that autotomy can be induced easily in many holothurians by certain chemical and physical stimuli. It is difficult to relate the response to these stimuli with any conditions which would be encountered by holothurians in their natural environment. A number of holothurians, including S. mollis, undergo autotomy when the sea-water becomes sufficiently foul. Experiments on S. mollis were carried out to determine the relative effects of oxygen deficiency and that of the accumulation of excretory products in inducing autotomy. It was found that specimens in previously boiled and cooled sea-water would not undergo autotomy any sooner than those kept in a similar volume of fresh sea-water. Of eight specimens kept separately in sea-water in which the stirred-up faces of Stichopus mollis was added, six eviscerated within three hours, while the eight control specimens took 15–20 hours. Two specimens died without undergoing autotomy. This shows that in S. mollis the accumulation of excretory products is the main factor in inducing autotomy in specimens contained in a small volume of sea-water. It has been suggested (Pearse, 1909; Domantay, 1931) that the ejection of the visceral organs usually decreases the total amount of metabolism in holothurians, and thus they are able to survive until better environmental conditions again arise. S. mollis was found to be even more sensitive to foulness of the water after autotomy than before the process. Specimens which had eviscerated and were not immediately transferred to fresh water showed a high mortality rate. Specimens which remained intact under the same conditions showed no after-effects. Those which eviscerated and were quickly transferred to fresh sea-water showed a higher survival rate than
those left in the fouled sea-water for a longer time. Even if autotomy could be a response to certain adverse environmental conditions, the survival of *S. mollis* is dependent on rapid transference to normal conditions. The habitat of *S. mollis* in sheltered regions below tide levels in any case presents no opportunities for the accumulation of excretory products. It is therefore difficult to see how autotomy could be of benefit to *S. mollis* as an adaptation against adverse environmental conditions of a chemical nature.

It has been suggested that autotomy in *Synapta* is pathological, and that other species in which autotomy is due to foulness of the water also employ "pathologic autotomy" (Domantay, 1931). While the lack of evidence of autotomy in natural conditions in many species including *Stichopus mollis* supports this view, the fact that it can be artificially induced, with quite constant points of rupture in all specimens of *S. mollis*, shows that the potentiality for the process is universally present in this species. For that reason the term "pathologic" can hardly be used in its strict sense. The process appears to be a normal response to certain stimuli, which rarely occur in natural conditions, and there is at present no satisfactory hypothesis to suggest a possible utility of the process to this species.

Except for holothurians in which autotomy is a process of self-division and multiplication, there is at present insufficient evidence to demonstrate clearly the function of this process in any of the holothurians known to have this property.

**Regeneration:**

The alimentary canal and haemal system regenerated in *S. mollis* originate along the entire free edge of the mesenteric remnant. They are formed by the proliferation and differentiation of mesenchyme cells. There is no evidence of contributions from the oesophageal remnant or from the cloaca.

In *Thyone briareus*, Torelle (1909) describes the origin of the intestine as:—"The new intestine is always formed as a bud from one side of the old intestine at a point near the cloaca. The new intestine grows forward as a solid rod of cells one to two millimetres in diameter." She describes its attachment to the anterior closed end of the body wall, and "until reunion with the body wall takes place, the new intestine is a straight tube. As soon as attachment to the body wall is effected it elongates and turns on itself, forming the loops characteristic of the normal animal." Scott (1914), studying the same species, noted after nine days' regeneration that the beginning of a new "stomach-intestine" was seen in the mesentery. This appears to be the first demonstration of the importance of the mesentery in regeneration.

Although the alimentary canal is described by Scott as following the mesentery edge, no suggestion is made as to whether the alimentary canal grows from the cloaca forward along the mesentery edge or arises from the edge itself. More detailed studies made by Kille (1935) showed conclusively that regeneration actively began along the entire free edge of the original mesentery and not by a forward growth from the cloacal region. Bertolini (1930, a and b, 1931) had first noted this method of origin in *Stichopus regalis*. 
In *Holothuria tubulosa*, however, Bertolini (1932b) found that the alimentary canal was formed by the growth of blind tubular elements along the edge of the mesentery, one growing anteriorly from the cloaca and one posteriorly from the oesophagus until they unite to form a continuous structure. As this method was also described in *H. floridana* and *H. impatiens* (Kille, 1936), it seems to be general for the genus *Holothuria*.

Regeneration along the whole free mesenteric edge occurs in *S. mollis*. If there are no contributions from the oesophageal remnant and cloaca, the alimentary canal and haemal system must be formed from purely mesodermal tissue. The examination of sections of the junctional region between oesophageal remnant and regenerating alimentary canal showed no evidence of growth from the oesophagus. Specimen 87 showed the posterior end of oesophageal remnant separated for about 2 mm. from the dorsal mesentery. A solid cord of cells was present along the dorsal mesentery and continued to the point of junction laterally to the oesophagus. It was not continuous with oesophagus and there was no evidence of proliferation from the oesophageal remnant. Two other specimens showed a similar condition. In all specimens examined up to 33 days' regeneration, the junction of the two regions was distinct. In later stages of regeneration the more rapid increase in diameter of the anterior half of the alimentary canal tends to lessen the distinction between the original and the regenerating alimentary canal, but a slight constriction at the junctional region was still present after 110 days' regeneration.

The junction between the ventral mesentery and the cloaca occurs on the ventral side of the cloaca just posterior to the point of rupture. Growth in thickness of the alimentary canal along the ventral mesenteric edge is slower than along any other region of the mesentery. The alimentary canal is narrowest just anterior to the cloaca. If there were contributions from the cloaca, the alimentary canal would be expected to be thicker along the posterior end of the ventral mesentery than elsewhere, instead of the reverse. In no specimen studied was there any evidence of forward contributions to the alimentary canal from the cloaca.

There remains the possibility that the lumen and inner epithelium may extend forward from the cloaca into the solid cord of cells along the mesentery edge. Kille (1935) showed that this occurred in *Thyone briareus*, and at the anterior end cells invade the solid rudiment from the regenerating lantern. As no anterior remnant of the alimentary canal is left in *Thyone*, these cells cannot arise from the alimentary canal. *Stichopus mollis* shows no regular progressive invasion of lumen or intestinal epithelium either anteriorly or posteriorly. The lumen appeared first in most cases among the cells along the edge of the angle between the dorsal and lateral mesenteries and in a number of cases it appeared independently in other regions (e.g., specimen 87). Thus all the regenerated alimentary canal, including the intestinal epithelium (normally endodermal in origin), is formed from mesenchyme cells and is mesodermal in origin. Such plasticity of the mesoderm is also found in *S. regalis* (Bertolini, 1930b), and less completely so in *Thyone briareus*. It is known to occur in certain cases in other groups, such as that shown by Penner's (1937) defect experiments in *Tubifex* where, after the removal of the
embryonic ectodermal germ band, the mesoderm can later form all the structures normally ectodermal in origin. Plasticity in embryonic forms is normally greater than that found in adult animals. There appears to be no record of adult animals with the complexity of structure found in holothurians, retaining into the adult state such marked plasticity of the mesoderm, as that found in Stichopus.

Autodifferentiation within the mesenchyme cells forming the regenerating alimentary canal, has not been demonstrated in other holothurians. The lining epithelium in the regenerating alimentary canal of S. regalus appears in the whole canal at the same time (Bertolini, 1931). In S. mollis it is shown that the development of a lumen and the differentiation of cells to form a lining epithelium can occur in regions with a solid cord of undifferentiated cells anteriorly and posteriorly. It is therefore not controlled rigidly in early stages of regeneration by the position on the axis of the animal. After the mesenchyme cells separate to form a lumen, the latter becomes lined by a concentration of nuclei without distinct boundaries, i.e., a synecytium. Presumably the nuclei are formed by rapid division of the mesenchyme cells adjacent to the lumen. Definite cellular structure then appears, and the cells, which are at first short, elongate to form a typical columnar epithelium. A remarkable co-ordination must be exercised for these structures to form in their appropriate positions in the mesentery edge, but there is no close relationship to location in the body during early stages. A gradation in diameter from the anterior to the posterior end later develops, and a definite relationship to location is then established.

In S. regalus, Bertolini (1930, 1931) states that the alimentary canal arises as a thin transparent tube which is at first perfectly straight ("perfetamente retillineo"). It later thickens and begins the formation of the loop. This is very different from the stages found in S. mollis. In the latter species, the alimentary canal commences development along the mesenteric edge relatively soon after auto-evisceration. At this stage, the course of the mesenteric edge is still similar to that followed in normal animals. The developing alimentary canal must therefore necessarily first follow the looped course of the mesenteric edge at this stage, and only becomes straight after a later extension of the mesenteries to eliminate the angles of the loops. The method by which a straight tube can arise in S. regalus is not described by Bertolini. It is very difficult to visualise a method by which regenerating tissue along a looped mesentery edge could arise first as a straight tube. It seems highly probable to the writer that the straight tube was not the earliest stage in regeneration in S. regalus, but had been developed before the specimens were collected from the sea, by a method of development similar to that of S. mollis.

The lengthening of the mesentery in the angle between the dorsal and lateral mesenteries in S. mollis achieves two main results. It reduces the length of the free mesenteric edge to less than half the distance. The alimentary canal functions normally before again lengthening. This enables the animal to commence feeding much earlier, with smaller demands on body reserves than would be the case if the alimentary canal had to regenerate along the whole length to the state of differentiation and diameter necessary for feeding to be possible. In addition the straightening of the alimentary canal
provides the base from which the transverse connecting haemal vessel is separated off. If looping of the alimentary canal were retained in regeneration a much more complex series of events would be necessary for this vessel to develop and regain its normal relationships across the body cavity. There appears to be no record of the method of development of the transverse connecting vessel in other holothurians.

The dorsal and ventral intestinal vessels in *S. mollis* were found to develop from the extension of a cord-like swelling which is budded off longitudinally along the length of the alimentary canal with local condensations separating the connecting strands between the vessel and gut. In the developing haemal vessel a further longitudinal splitting parallel to the alimentary canal separates off the transverse vessel as previously described, and leaves the intestinal vessel adjacent to the gut. In *Thyone briareus*, Kille (1935) shows that concurrently with growth in length of the intestine in the angle between the dorsal and lateral mesentery "the intestinal haemal plexus is established within the first major loop," . . . as "a crescent-shaped membrane which in texture resembles the mesentery." This membrane at first shows its greatest distance from the edge to the intestine in the angle of the first major loop and tapers to merge with the intestine anteriorly and posteriorly. With increase in length of the intestine posteriorly in the angle between dorsal and lateral mesentery, the membrane does not keep pace and separates from the gut. Condensations form a plexus of strands, and it is left as a bridge-like plexus connecting the first two major sections of the intestine. This is described as the fore-runner of the intestinal haemal plexus.

The method of development followed by *S. mollis* differs in a number of details. There is evidence that the ventral intestinal vessel separates at a relatively much earlier state of development, appearing before the lengthening of the mesenteries has achieved the straightening of the alimentary canal. It arises as a cord of cells adjacent to the alimentary canal, and extends in either direction until it connects with the haemal vessel remaining along the oesophageal remnant, and posteriorly it extends towards the cloaca. The dorsal intestinal vessel arises in a similar manner, but develops later, while the transverse connecting vessel is separated off from the ventral vessel by a similar process which, however, occurs only after straightening of the alimentary canal. Except for the series of events in the establishment of the intestinal haemal plexus of *Thyone* across the body cavity, which partly resemble those followed by *S. mollis* in the establishment of the transverse connecting vessel across the body cavity, the method and relative time of origin of the haemal vessels in *S. mollis* differs markedly from that found in *Thyone*.

Rudiments of the respiratory trees in *S. mollis* appear first after 25–35 days' regeneration and then grow forwards as tube-like structures from the dorsal wall of the cloaca. Respiratory movements of the cloaca continue after autotomy during the period of regeneration.

Before the development of respiratory trees, sea-water is taken into the body cavity, as closure of the cloaca is sometimes delayed up till 20 days after autotomy. The respiratory trees in their early stages show distinct perforations in the elongating tubes which still allow direct communication between sea-water and coelomic fluid,
The exact time of closure of these pores has not been determined, but it appears that such direct communication persists until the respiratory trees have elongated sufficiently to provide a large enough area for the supplying of respiratory requirements by diffusion alone.

The rate of regeneration of viscera in *S. mollis* was found to be slower than that described in any other species of holothurian. The viscera are far from completely developed after 145 days, which is a longer period than any recorded for the complete restoration of organs lost after autotomy in other species. On the basis of the state of regeneration of specimens examined from successive weekly hauls, Bertolini (1930b) estimated that *S. regalus* regenerates completely in about 15 days during the warmth of August at Naples. Scott (1914) found that *Thyone briareus*, after 41 days' regeneration, was practically normal, except that the organs had not yet reached full size. *Holothuria scabra* from the Philippine Island seas, was found with all the organs except the gonads regenerated after 9 days, Semper (1861). Herouard, however, found no evidence of regeneration in *H. forskali* at Roscoff two months after autotomy (Delage and Herouard, 1903). The alimentary canal of *H. tubulosa* appeared to be normal after four months' regeneration (Bertolini, 1932).

Temperature differences between the localities at which studies were made probably contribute to the differences in regenerative rate shown by members of the same genus. The range of sea temperature during the period in which *S. mollis* was studied was 8–13°C, which would be considerably lower than that of waters at Naples in August, and may account for part of the difference between this species and *S. regalus*. As metabolic rate shows only a two- to three-fold increase for each 10°C rise in temperature by van t'Hoff's law, the temperature disparity alone could not account for the total difference in regeneration time shown in the genus *Stichopus*, or in separate species of *Holothuria*. There must be large inherent variations in regenerative rate, even between species of the same genus.

It is of interest that difficulty was found in keeping *S. regalus* alive in the laboratory (Bertolini, 1930b). The mortality rate of *S. mollis* after auto-vasceraion was also found to be very high. None kept in the laboratory survived more than 14 days, and a large number of those kept in running sea-water in Portobello aquarium and in the circulating sea-water of "Te Aro" baths also died. The experimental ablation of parts of the body wall always causes protrusion of the organs or complete auto-vasceraion. All such specimens and those in which ablation was performed after auto-vasceraion died within 14 days. This appears to be in agreement with the findings of Torelle (1909), that species of the Order Aspidochirota can less readily regenerate parts of the body operatively removed than can species belonging to the Order Dendrochirotta. Specimens which have not had parts removed in addition to those expelled during auto-vasceraion, and which survive, have as marked a regenerative capacity as other holothurians for the restoration of those organs which are expelled.
DAWBIN—Auto-evisceration and Regeneration of Viscera. 528

LITERATURE CITED.


