AUTOTOMY OF THE APODID SEA CUCUMBER POLYCHEIRA FUSCA (RUFESCENS): OBSERVATIONS AND SURGICAL EXPERIMENTS*

AUTOTOMY OF THE APODID SEA CUCUMBER *POLYCHEIRA FUSCA* (*RUFESCENS*): OBSERVATIONS AND SURGICAL EXPERIMENTS*

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ABSTRACT

In the holothurian *P. fusca*, with a thin body wall, autotomy was induced at a variable site by tapping with the tip of a forceps. Autotomy occurred irrespective of the presence of the head, and began at the longitudinal body wall muscle (LBWM). The produced anterior piece was capable of autotomizing repeatedly but the posterior could autotomize rarely. However the autotomic capacity of the posterior recovered considerably in two hr. The majority of anteriors lived but the posteriors died in culture (20℃), a month without food supply. Furthermore the surgical experiments were made for the opened and extended body wall with LBWMs. By tapping on a LBWM, an autotomy-inducing signal was produced at the tap site, and conducted anteriorly along the radial nerve embedded in the stimulated LBWM as the main route and along the adjacent transverse and then radial nerves as a bypass. An autotomy-inhibiting signal was produced at the autotomy site and conducted posteriorly along radial nerves and then sideward along transverse nerves, thus setting up autotomy inhibition over the posterior region. Autotomy inhibition was based on an interruption of breakage processes. The relationship between an increase in the tensility of LBWMs following stimulation and initiation of autotomy is discussed.

INTRODUCTION

Self-mutilation is a phenomenon recognized widely among echinoderms, and is divided into autotomy as a defensive mechanism and fission as a method of asexual reproduction (Emson & Wilkie, 1980). In holothurians two types of autotomies and fission have been observed: autotomy by ejection of the internal organ (eviscertion) and by transverse split of the whole body, and transverse fission followed by regeneration (Emson & Wilkie, 1980). These events result from a softening of connective tissue components (Wilkie, 1984), and is part of the phenomenon of variable tensility of echinoderm connective tissues (Motokawa, 1984, Birenheide et al., 1998). Recently research on the endogenous factor inducing evisceration has been pursued (Smith & Greenberg, 1973; Byrne, 1985; Byrne, 1986), but not that inducing transverse autotomy and fission (Mladenov, 1996).

The occurrence of autotomy by a transverse split in holothurians has been recorded in apodids alone, and its investigation in the laboratory has been made only for synaptids by a few workers (Pearse, 1909; Domantay, 1931).

The present paper reports that, in *Polycheira fusca* (apodid, no synaptid), the behaviors during autotomy were observed, and surgical experiments were conducted for intact and dissected specimens to confirm and elucidate the autotomy processes.

MATERIALS AND METHODS

In this studies the apodid sea cucumber *Polycheira fusca* (Brandt) [synonym: *P. rufescens* (Brandt)] was used. Most of them were collected at Iso Beach of Kagoshima, Japan, and part of them at Sakurajima Beach on the opposite side of Kagoshima Bay (for the map of collection sites, see Kubota &

Tomari, 1998), and maintained in aerated seawater at room temperatures of 16-27°C for maximally two weeks before use. To prepare a flag of the body wall, an individual was taken out from a bowl, the head and tail ends were pinned on a thick rubber sheet in the air, the body wall was slit with a scissors first at the place lying between the pins and between the longitudinal body wall muscles (LBWMs) and then sideward at both ends of the formed slit, the cut wall area was laterally spread to make a flag, and pinned at its periphery. Subsequently the unopened head and tail parts were cut off, and the intestine attached to the wall was removed. The flag thus prepared was of the sizes six to ten by three to four cm, and bore five LBWMs in parallel (see Fig. 2B). In some experiments, LBWMs with the radial nerve or a combination of the LBWMs and the skin-like part of the body wall was cut with a pointed knife made of a thin razor blade of stainless steel.

To induce autotomy, a definite site on the body surface of the intact animal or on the inner surface of the flag was repeatedly tapped at a rate of one to two times a second with the tip of a watch-maker's forceps. When no autotomy occurred within three min of consecutive tapping, the tapped sample was judged as no response, because in most cases autotomy occurred within two min of tapping.

The fixative used, Bouin fluid, hardly penetrated the outer cuticle layer of the body wall. Therefore the fixative was injected into the body cavity. In this case the intact animal was previously cooled with ice, and the tail end was clipped to prevent the fixative from extruding from the anus. After two to three hr the body wall of about two cm square was excised, and transferred to the same fixative in a dish. In the animals which were either during autotomy or immediately after autotomy, the fixative was injected near the autotomy site without clipping, and soon a small piece of the body wall was excised and dipped in a sufficient amount of the fixative. In both cases, the excised body walls were placed in the fixative for one or two days, and then preserved in 70% ethyl alcohol. For histological examination specimens were dehydrated with alcohol, embedded in paraffin, sectioned, and stained with Azan using Azocarmine, Aniline

Blue, and Orange G.

RESULTS
Histological and general observations

The body architecture of *P. fusca* is essentially the same as many of other sea cucumbers except for the absence of a respiratory tree. The skin-like part of the body wall is composed of the epidermis covered
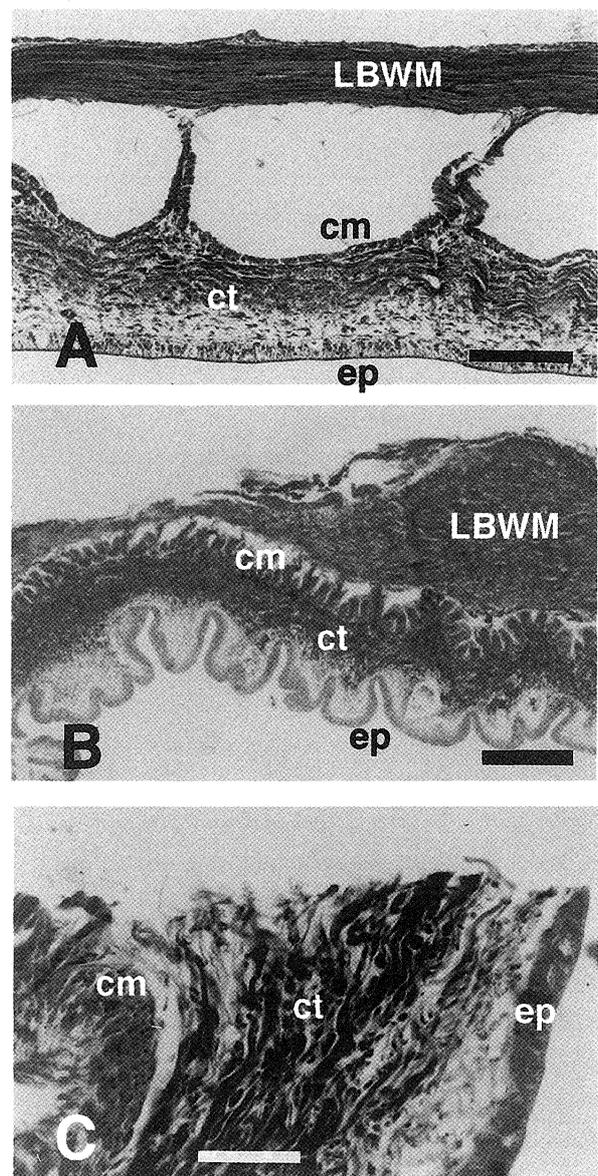

Fig. 1.   Longitudinal sections of the body wall before, during, and after autotomy (A, B & C, respectively). In A, the radial nerve is invisible because the section plane is not through the nerve. B shows that autotomy begins at the longitudinal body wall muscle (LBWM). The outline of the broken part is smooth. C shows that the free end of broken connective tissue is also smooth in contour. ep: epidermis; cm: circular muscle; ct: connective tissue. Scale bars: A, 1 mm; B, 500µm; C, 50µm.

with cuticle, the dermis, and the innermost circular muscle layer, to which are attached the longitudinal body wall muscles (LBWMs) and the radial nerves embedded in LBWMs. The skin-like body wall is thin in depth due to poor development of the dermal tissue consisting mainly of collagenous fibers (Fig. 1A).

P. fusca autotomized by transverse division of the whole body. The division occurred at a single plane between the head and the tap site; that is, at a place anterior to the stimulation point, but its accurate localization was unpredictable. Autotomy of the body wall preceded that of the intestine: separated body pieces were jointed only by the intestine tube which also eventually broke. Some longitudinal section of
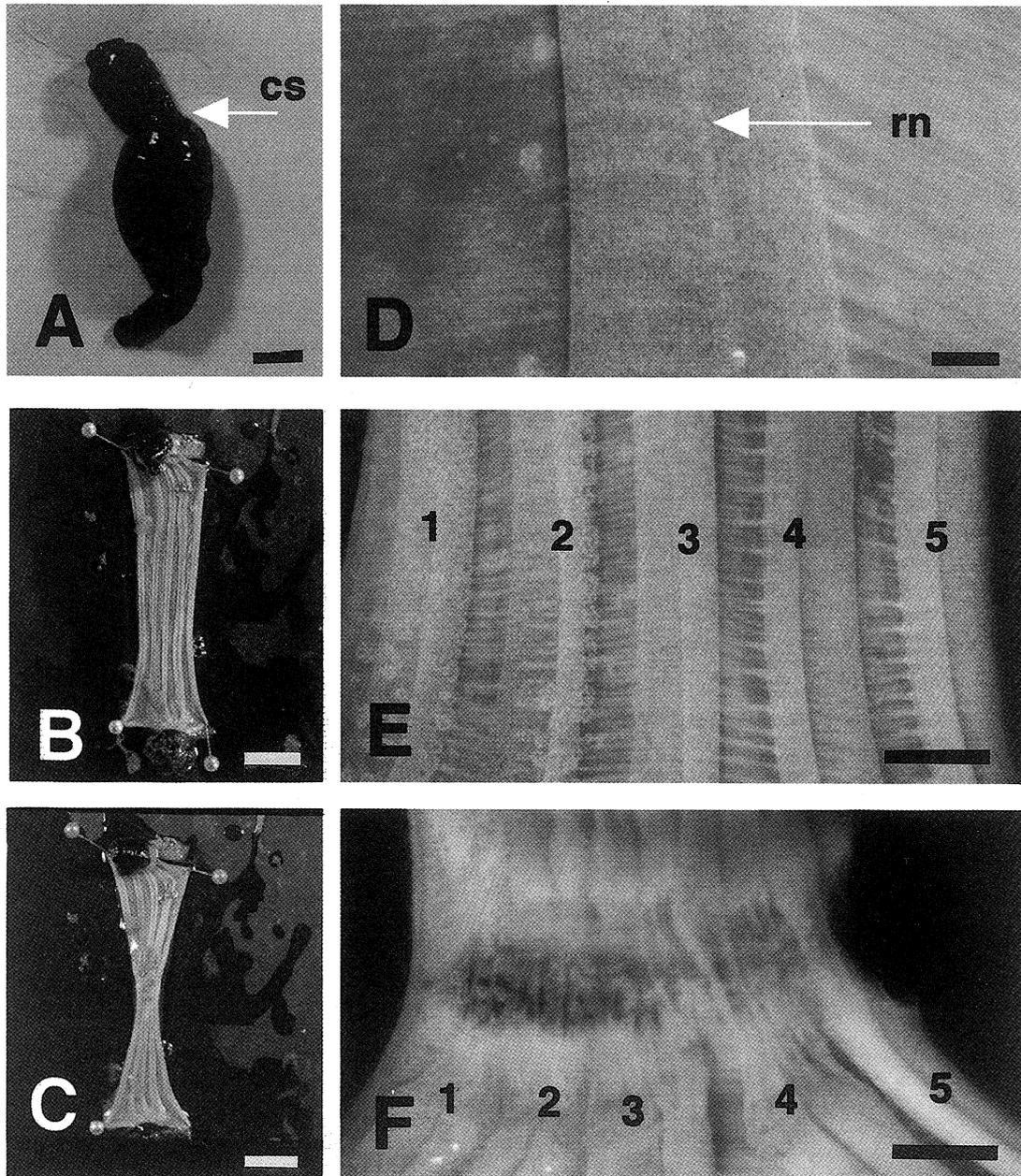

Fig. 2. Autotomy in an intact animal (A) and in opened body walls with five LBWMs (B & C, E & F), and an enlarged LBWM (D). A: a constricting animal with withdrawn tentacles at top. The formed furrow is not obvious in the photograph but indirectly seen from the contour of the left shadow. The body around the stimulation site is narrowed (region near tail). B & C: a survey of opened and extended body walls (flags) before and during autotomy (B & C, respectively). Each corner of a flag is pinned on a board. D shows the radial nerve embedded in a LMWM. The nerve is discernible as a central, diffuse streak. E & F: part of flags at an early stage of autotomy (E) and at a final separation stage (F). cs: constriction site; rn: radial nerve; 1-5: five LBWMs. Scale bars: A, 1 cm; B & C, 2 cm; D, 1 mm; E & F, 5 mm.

the body wall fixed at an early stage of autotomy showed that autotomy began at LBWM (Fig. 1B). The free ends of the autotomized muscle (Fig. 1B) and dermis (Fig. 1C) indicated a smooth outline at a low power as if cut with a razor blade.

Autotomy in intact sea cucumbers

Autotomy-inducing effect Tapping with a tweezers tip was effective for inducing autotomy. In the present studies, neither pinning for fixing the flag on a rubber sheet nor cutting with a scissors or a razor-blade knife provoked autotomy during these operations and the subsequent experiments. The sixty-cycle vibration for five min which was generated by an air pump for culturing fishes at home was of no effect, also. Autotomy happened to occur, when animals were long exposed to temperatures exceeding 30℃, or injected with Bouin fluid without precooling the body. The injected animals rapidly pinched off the posterior portion containing the fixative.

In spite of the ease of autotomy induction in the laboratory, there is no evidence that *P. fusca* divides the body in the field. For the purpose of using this animal in the present and previous studies, in total more than seven thousand individuals were collected at Iso Beach during the breeding season and at least fifty every month during the nonbreeding season. But a sea cucumber with a highly unbalanced body proportion and/or a body portion of distinctly different colors was not found in these collected specimens.

Morphological changes during autotomy Sea cucumbers, as soon as tapped, rapidly rounded and changed from relaxed forms to an elongated oval. Then the animals began to narrow considerably around the stimulation points, and before long constricted deeply at the site where division will occur. During these changes in form, the whole body anterior to the constriction site began to decrease in both length and diameter. There was no ejection of the body-cavity fluid, so that the total body volume was kept unchanged during the autotomic deformation. As a result the shrunk anterior part and the swelled posterior were formed (Fig. 2A). Separating bodies were accompanied by crawling with tentacles and/or wriggling; when the head was fixed with a pin, two pieces produced by autotomy were unable to separate until one of the two began to wriggle.

Autotomy without head. A large body piece was made by cutting off the head with scissors. In another case, three pieces of similar sizes were made: the anterior with head, the posterior with tail, and the middle. All these pieces tightly closed their open ends by instant, local contraction of the circular muscle of the body wall, and each formed a bag-like body piece. All of them could autotomize with high frequencies.

Responsiveness The responsiveness to tapping differed between the anterior and posterior pieces generated by autotomy. The anterior still retained responsiveness. The intact sea cucumber, when tapped near the posterior end, produced a large anterior piece with the head. Tapping in the anterior induced the second autotomy, and yielded a smaller anterior piece. Such repetition of autotomy was possible a few times, whereas the posterior piece was barely responsive (see Table 1, the column of 'just after').

Recovery from low responsiveness Weak responsiveness of the posterior recovered after autotomy at a slow pace. Table 1 shows that in the posterior pieces formed by the first autotomy, the second autotomy was induced at room temperature of about 18℃ with the following percentages: only 9.2% just after the first autotomy; 32.4% after 30 min; 75.0% after 120 min.

Survival Most of the anterior pieces cultured at

Table 1. The frequency with which the second autotomy was induced by tapping the posterior piece resultant from the first autotomy.

Time after division (min)	just after	30	60	90	120
Autotomy frequency (%)	9.2	32.4	51.3	60.0	75.0
(N/N)	7/76*	12/37	19/37	24/40	15/20

(*): for example, 7/76 indicates that autotomy occurred in 7 of 76 pieces stimulated. Experiments were performed at room temperature of about 18℃ during Mar. 21 to Apr. 4.

Table 2. The survival period of the anterior and posterior pieces produced by autotomy.

Days after division	0	3	7	12	33
Survival N of anterior	10	10	10	10	9
Survival N of posterior	5	3*	3	2*	0

After division, each group of 10 anteriors and 5 posteriors was placed in about 2 ℓ of aerated water at room temperature of 20℃, and observed from Mar. 21 to Apr. 23. The culture water was exchanged on the days described in the table and, besides, on days 14, 16 and 18. No food was supplied for the culture period, so that the whole size of pieces decreased extremely during it. (*): a piece which newly autotomized was found alive on the 3rd day and dead on the 12th day (broken into fragments).

Table 3. Relative stimulation times (M ± SD) to elicit autotomy: in a set of experiments, autotomy was repeated three times (Series A) or two times (Series B) in an individual and the piece(s) derived from it. They were pinned at one point of the head except for the case of Series A, the second autotomy, in which two points of the head and the posterior ends were pinned. Autotomy was induced in all by tapping the site near the posterior end.

Autotomy	1st autotomy	2nd autotomy	3rd autotomy
Series A	1(1) ⎫ (105.4 ±42.5*)	0.38 ± 0.16(2)	0.49 ± 0.18(1)
Series B	1(1) ⎭	0.56 ± 0.19(1)	

(*): M ± SD of stimulation times (s) of Series A and B.
(1) and (2) indicate that data were gained from specimens of one pin and two pins respectively.

20℃ lived for a month, whereas all the posteriors died during the same period (Table 2). The shorter survival period of the posteriors is not ascribed to the absence of the mouth and the tentacular crown necessary for eating, because no food was supplied for the culture period. No sign of regenerating the lost parts was detected during the culture period.

Relationship of muscular tensility to autotomy As mentioned above, stimulated sea cucumbers rounded the whole body without volume change, and thereafter began to autotomize. These observations made us suppose that stimulation elicits an increase in the tensile strength of the LBWM, which in turn may trigger autotomy. To examine this possibility three experiments were performed. First, sea cucumbers were passively stretched by pulling away the tail from the pinned head for five min with thirty g weight, but none of ten stretched individuals autotomized. These animals, as soon as freed from weighing, returned to the body length before weighing, and bore no sign of damage as observed over two days. On the other hand, the weight of thirty g used was considered to be at a sufficient strength, because when tentacles were tapped during stretch, the extended animals could not shrink the body. Second, the mouth and tentacles were cut off, and the remained pieces were pinned at

the tail. After cutting, exceptionally the opening of the body wall did not close, so that almost the body-cavity fluid gushed out. As a result the body extremely shortened and wrinkled. All ten shrunken pieces autotomized by stimulation (M ± SD of stimulation times to elicit autotomy: 76.9 ± 18.1 s) in spite of no occurrence of tensility increase. In the third experiments animals or their pieces were pinned at the tail end (one pin) or at the head and tail ends (two pins), and autotomy was repeated in them (for details, see the explanation of Table 3). Since the degree of reduction in body length by rounding was always larger in specimens of one pin than in those of two pins, the tensility increase evoking rounding was considered to be smaller in specimens of one pin than in those of two pins. Comparison of the stimulation times to elicit autotomy showed that the time of the two pin-specimens was shorter on average than that of the one pin-specimens (Table 3). Thus the results of the first and second experiments did not support the supposition that an increase in the tensility may provoke autotomy, but the result of the third implied that the tensility increase is concerned with initiation of autotomy. All experiments described in the present section were conducted using specimens collected at Sakurajima.

Autotomy in opened body-walls

Muscular contraction before and during autotomy
When a LBWM on the flag (or the surface between LBWMs) was tapped, the shortening of the flag in both longitudinal and transverse directions was induced in the region not fixed with pins, showing that the longitudinal and circular muscles contracted by tapping. Then autotomy commenced around the site where the circular muscle contracted most strongly (Fig. 2B & C, 2E & F). These observed processes corresponded roughly with those in intact animals.

Conduction pathway of autotomy-inducing signal
Autotomy is under nervous control, and therefore autotomy-inducing signal (AIS) conducts along the nerve. The tapped flag autotomized always at a variable site anterior to the stimulated point. When previously a LBWM was transversely cut at a halfway point together with the embedded radial nerve (Fig. 2D), and the LBWM was tapped at a distance of more than two cm behind the cut, then autotomy took place between the tap and the cut sites, never across the cut (Fig. 3), indicating that AIS was propagated upwards along the tapped radial nerve. However, when tapping was given at a site three mm behind the cut, autotomy occurred at a position across the cut point in 40% of

stimulated flags (20/50) in spite of a deep cut ranging to the underlying skin-like body wall (Fig. 4). In the unresponsive remainder (60%, 30/50), further tapping at a site anterior to the cut elicited autotomy in 83% (25/30). Since it is apparent that in the experiments of tapping three mm behind, the occurrence of the first autotomy (20/50) is not due to failure of the nerve block; the result suggests the existence of a bypass in AIS conduction in addition to the main route by way of the tapped radial nerve. AIS seems to have been propagated from the blocked radial nerve to the adjacent, intact one via the transverse nerve.

Autotomy inhibition in opened body wall

Inhibition of autotomy The flag provoked not only autotomy but showed its inhibition as seen in the intact animal. Fig. 5 illustrates that when autotomy took place on one side of a longitudinally long slit, further autotomy was inhibited in the area which was on the same side and posterior to the autotomy site (0/48), although the second autotomy was induced (47/48) in the area of the other side where the first autotomy did not occur.

Conduction pathway of autotomy-inhibiting signal From the above events the autotomy-inhibiting signal (AIHS) was considered to conduct from the autotomy site in the posterior direction. This idea was

🟦 LBWM-radial nerve
▬ Cut site of a LBWM-radial
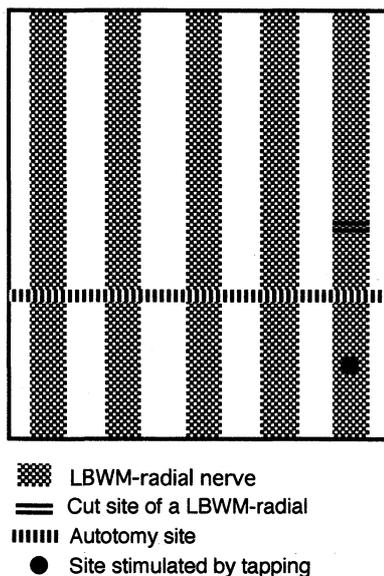ⅡⅡⅡ Autotomy site
● Site stimulated by tapping

Fig. 3. Autotomy induced by tapping on one LBWM of a flag, demonstrating that autotomy occurs between tap and cut sites. This and the following figures (Figs. 3-8) are a schematic representation, and in these figures pins used for fixing the flag are not drawn. For details, refer to text.

🟦 LBWM-radial nerve
ⅡⅡⅡ Autotomy site
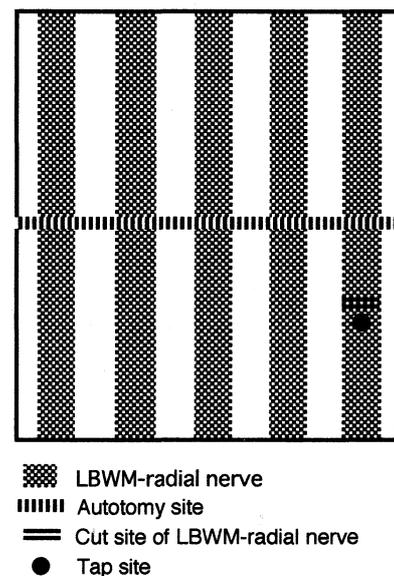▬ Cut site of LBWM-radial nerve
● Tap site

Fig. 4. Autotomy induced by tapping on a LBWM at the point of three mm behind a cut, demonstrating that autotomy occurs at the site across the cut. Refer to text for details.

LBWM-radial nerve
Slit site between LBWMs
Autotomy site in 1st tap (①)
and in 3rd (③)
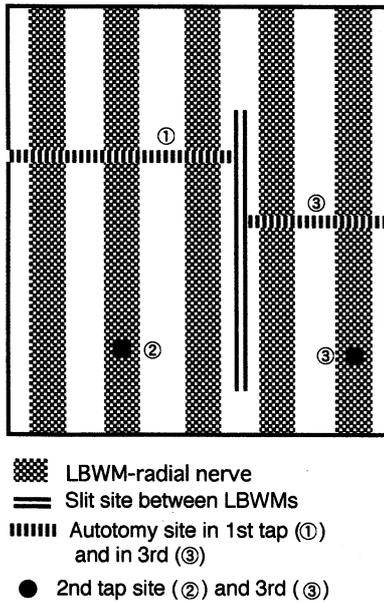2nd tap site (②) and 3rd (③)

Fig. 5. Autotomy inhibition set up in the region posterior to an autotomy site (①). Establishment of inhibition is shown by no occurrence of the second autotomy in this region. The occurrence of autotomy by the third tap indicates that inhibition did not spread to the other side of slit. Refer to text for details.

LBWM-radial nerve
Site of transverse slit
Autotomy sites in 1st tap (①)
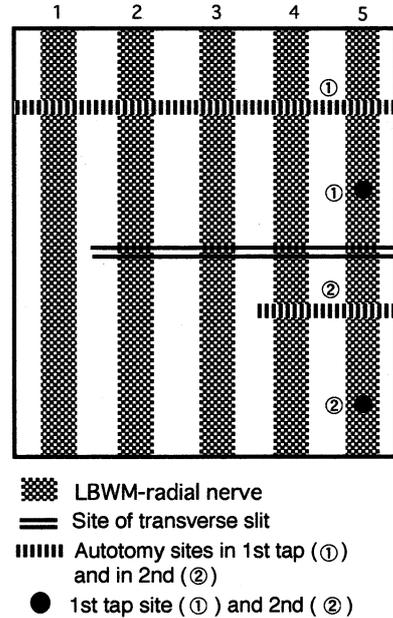and in 2nd (②)
1st tap site (①) and 2nd (②)

Fig. 7. An example of partial autotomies (breakage of LBWMs 4 and 5) induced in the posterior half by such procedures as illustrated in the figure, indicating that AIHS conducts along transverse nerves with decrement. Refer to text.

LBWM-radial nerve
Cut site between LBWMs
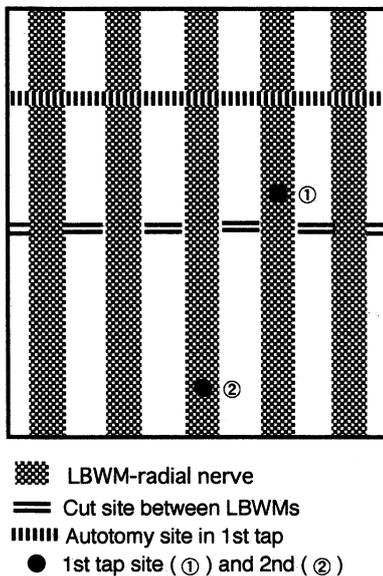Autotomy site in 1st tap
1st tap site (①) and 2nd (②)

Fig. 6. Autotomy inhibition set up in the posterior half, indicating that AIHS conducts from the autotomy site in the anterior region to the posterior half along uncut radial nerves.

LBWM-radial nerve
Slit site between LBWMs
Autotomy sites in 1st tap (①)
and in 2nd (②)
1st tap site (①) and 2nd (②)
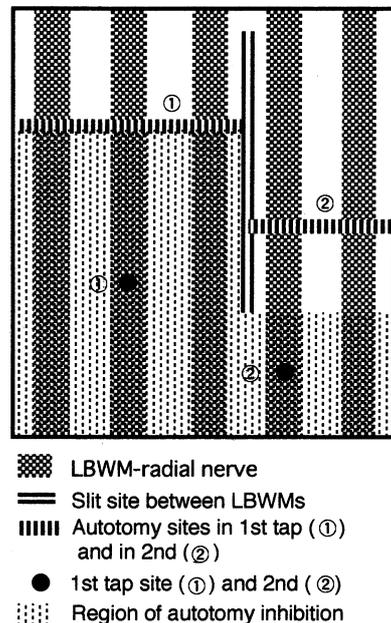Region of autotomy inhibition

Fig. 8. Autotomy (②) induced by tapping a LBWM lying in the region of autotomy inhibition, indicating that it is a breakage process to be inhibited, neither generation nor conduction of AIS. Refer to text.

ascertained by another experiment illustrated in Fig. 6. At the middle of a flag a linear series of transverse cuts was made in the body wall between LBWMs. When the first autotomy was induced in the anterior half, the second autotomy in the posterior half was

inhibited (0/26).

Further experiments were conducted on participation of the transverse nerve in propagation of AIHS. As illustrated in Fig. 7, four LBWMs, except for one of

the most peripheral LBWMs, were cut at the middle of the flag by a transverse slit, and subsequently the first autotomy was induced in the upper half of the area. If AIHS spreads over the lower area along the radial nerve of the peripheral, the uncut LBWM and then along the transverse nerves, it is expected that the second autotomy in the lower area is inhibited. To examine this possibility, another peripheral LBWM that had been cut was tapped in the lower area with the following results: in 45% of the tapped specimens (23/51), the second autotomy in the lower area was completely inhibited, namely, none of five LBWMs broke; in 47.1% (24/51), partially inhibited, namely, one to four LBWMs broke; only in the remainder 7.8% (4/51), not inhibited at all, namely, all five LBWMs broke. The breakage of the body wall by the partial autotomy occurred always on the side remote from the uncut, peripheral LBWM. These results show that in the lower area AIHS spread sideward along the transverse nerves with decrement.

The last experiment (Fig. 8) was designed to understand the nature of inhibition. By inducing the first autotomy on one side of a longitudinally long slit, an inhibitory area such as shown with a dotted patch in the figure was introduced in part of the posterior area (refer to the preceding experiment). Subsequent tapping at the inhibitory area provoked the second autotomy in the space anterior to the inhibitory area (93%, 42/45). This fact indicates that the inhibition is involved in the breakage process itself in the body wall.

DISCUSSION

The sea cucumber used in this studies, *Polycheira fusca* (Apoda), has wheel-shaped ossicles in the body wall, and autotomized by constriction. Its processes are similar to those of reported synaptids (Apoda) with the anchor-shaped ossicles, but there are some differences in details between *P. fusca* and these synaptids. First, mechanical stimulation effectively induced autotomy in *P. fusca*, but not in *Synapta maculata* (Domantay, 1931). Second, autotomy occurred at a single site in *P. fusca*, but at plural sites simultaneously in *S. maculata* (Domantay, 1931) and successively in *Leptosynapta inhaerens* (Pearse,

1909). Third, in *L. inhaerens* (Pearse, 1909) and *S. maculata* (Domantay, 1931), the posterior body piece produced by autotomy did not further divide in response to stimulation. In *P. fusca* the posterior piece indeed divided seldomly just after separation, but the autotomy frequency increased with time and recovered to 75% in two hr.

The isolated posterior piece of the above synaptids died (Pearse, 1909; Domantay, 1931). On the other hand, the anterior of *Leptosynapta crassipatina* lived and could regenerate the lost portion, even if it was comprised only of the oral disk (Smith, 1971). The culture of *P. fusca* showed that the isolated posterior also dies. The anterior lived for the culture period of a month, but no sign of regeneration was detected under the condition of no food supply. Crawling and wriggling movements such as observed during autotomy in *P. fusca* have been reported in holothurian fission (Chadwick, 1890 & Monticelli, 1896 after Emson & Wilkie, 1980; Crozier, 1917; O'Loughlin, 1991).

In the present studies a hypothesis was examined that an increase in the tensility of LBWM provoked by stimulation triggers autotomy, but evidence supporting it was not obtained. Autotomy induction after loss of muscular tensility such as observed in *P. fusca* was reported in *Thyone briareus* by Smith & Greenberg (1973); in the specimen whose coelomic fluid pressure had been dissipated by an incision through the body wall, breakage of the pharyngeal retractor muscle (PRM), a process characteristic of evisceration, was provoked. Furthermore in *Eupentacta quinquesemita*, mechanical stimulation of the body wall and isolated PRMs elicited muscular contraction but not autotomy (Byrne, 1986). However in *P. fusca* there are data suggesting that an increase in the tensility shortens the stimulation time required for initiating autotomy. In addition, small flags with a LBWM evoked autotomy more frequently when previously both ends of the flags were pinned than when one end was pinned (unpublished observations). Thus it is inferred that somehow an increase in the LBWM tensility may enhance the autotomy-inducing effect of tapping.

The nervous control of holothurian autotomy has been not fully understood (Emson & Wilkie, 1980;

refer also to Prosser & Mackie, 1980). The experimental results in *P. fusca* indicated that autotomy suppression was introduced in the body piece or the flag region posterior to a autotomy site, and that the suppression was based on interruption of the breakage process in the region where otherwise the occurrence of autotomy is expected, neither on unproductiveness of an autotomy-inducing signal at the stimulation site nor on nonconduction of the signal along the radial nerve. The suppression covered a period of even two hr. Such a long suppression period implies that a substance released or activated in the region to autotomize works for suppression rather than a neural pathway; for example, with an excitation-inhibition system.

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