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INDUCTION OF EVISCERATION IN THE HOLOTHURIAN *EUPENTACTA QUINQUESEMITA* AND EVIDENCE FOR THE EXISTENCE OF AN ENDOGENOUS EVISCERATION FACTOR

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SUMMARY

The stimuli provoking evisceration of *Eupentacta quinquesemita* (Selenka) and autotomy of isolated pharyngeal retractor muscle (PRM) tendons were investigated. Tendon autotomy is a two-part response involving PRM contraction and breakdown of tendon connective tissue. An evisceration factor (EF) was detected in coelomic fluid expelled during evisceration. EF was isolated in tissue extracts and the haemal system and peritoneum were sources of EF activity. Autotomy and evisceration were induced by electrical stimulation, K^+ and EF, and the effect of these agents was inhibited by anaesthetics. The acetylcholine antagonist tubocurarine chloride elicited evisceration, suggesting that evisceration may involve inhibition of cholinergic transmission. Evisceration and autotomy appear to be neurally controlled and the presence of an endogenous EF suggests neurosecretory or hormonal activity. Cells involved in evisceration may be located at a distance from the autotomy tissues and effect connective tissue breakdown through the medium of the coelomic fluid. Hypothetical sequences of events and possible roles for EF are presented.

INTRODUCTION

Evisceration is a behaviour characteristic of many holothurian echinoderms and results in autotomy of the internal organs (Emson & Wilkie, 1980). There are numerous behavioural studies of evisceration where the response was elicited by injection of chemicals into the coelom or by electrical stimulation (Pearse, 1909; Bertolini, 1932; Domantay, 1931; Kille, 1935; Dawbin, 1949; Bai, 1971; Jespersen & Lützen, 1971; Tracey, 1972; Smith & Greenberg, 1973; Byrne, 1985*a*). Echinoderm autotomy results from rapid breakdown of connective tissue structures (Wilkie, 1978; Holland & Grimmer, 1981; Byrne, 1982, 1985*b*) and is part of the phenomenon of variable tensility of echinoderm connective tissues (reviewed by Motokawa, 1984*a* and Wilkie, 1984). The changes associated with variable tensility have attracted attention because they occur in an extracellular tissue and are considered by many workers to be neurally controlled (Jordan, 1919; Serra-von Buddenbrock, 1963;

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Wilkie, 1978, 1983, 1984; Holland & Grimmer, 1981; Hilgers & Spletchna, 1982; Motokawa, 1981, 1982*a*, 1984*a,b,c*; Hidaka & Takahashi, 1983; Wilkie, Emson & Mladenov, 1984). Echinoderm autotomy differs from that of other invertebrates, where autotomy is associated with muscle tissue responses and for which neuromuscular mechanisms have been established (McVean, 1974, 1975).

Evisceration by the dendrochirote holothurian *Eupentacta quinquesemita* results from the rapid softening of three autotomy structures: (1) the tendon (P-L tendon) connecting the pharyngeal retractor muscle (PRM) to the longitudinal body wall muscle (LBWM), (2) the intestine-cloacal junction and (3) the introvert (Byrne, 1985*a*). These structures are predominantly composed of connective tissue (Byrne, 1982, 1985*b*). Investigation of the mechanical properties of the tendon and introvert revealed that they do not have a pre-existing mechanical weakness to account for their failure during evisceration (Byrne, 1985*c*). The mechanical properties of these tissues are sensitive to changes in ionic concentrations and it was suggested that the most likely mechanism of autotomy is through an alteration of ionic interactions within the connective tissue (Byrne, 1985*c*).

Although current research suggests that variable tensility of echinoderm connective tissues is neurally controlled (Motokawa, 1984*a*; Wilkie, 1984), the morphological and physiological bases for neural control have not been established. I investigated several evisceration-inducing agents with *E. quinquesemita* for evidence of neural involvement in the connective tissue change. Ionic and pH mechanisms have been proposed in numerous studies of variable tensility (Wilkie, 1979, 1983; Holland & Grimmer, 1981; Smith, Wainwright, Baker & Cayer, 1981; Eylers, 1982; Hidaka, 1983; Byrne, 1985*c*) and so a broad range of ionic solutions was tested for their capacity to induce evisceration of *E. quinquesemita*. In high concentrations, K^+ decreases the viscosity of the introvert (Byrne, 1985*c*), whereas low concentrations of the ion increase the viscosity of the echinoid spine catch apparatus and the holothurian body wall (Takahashi, 1967; Motokawa, 1981, 1982*a*, 1984*b*). It was suggested that at high concentrations, K^+ destabilizes the introvert by its direct effect on ionic interactions within the connective tissue (Byrne, 1985*c*), and that at low concentrations, K^+ stiffens the connective tissue by stimulating neurones controlling variable tensility (Motokawa, 1982*a*). In this investigation, a broad range of K^+ concentrations was tested to differentiate between the direct and indirect effects of the ion on the autotomy response of *E. quinquesemita*. The K^+ concentration and pH of normal and eviscerated coelomic fluid were measured to see if they changed during evisceration. The echinoderm nervous system contains cholinergic neurones and is sensitive to acetylcholine (ACh) antagonists (Pentreath & Cottrell, 1968; Florey, Cahill & Rathmayer, 1975; Shelkovnikov, Starshinova & Zeimal, 1977), and the effect of ACh antagonists on the evisceration response was tested. I also compared the stimulus-evisceration response of *E. quinquesemita* with that of other holothurians.

An evisceration-inducing factor (EF) was detected in the coelomic fluid expelled by eviscerating *Sclerodactyla briareus* and factors that affect the mechanical properties of echinoderm connective tissues have been isolated from several echinoderms

(Smith & Greenberg, 1973; Motokawa, 1982*a*,*b*). These factors were suggested to be involved with neural control of variable tensility. I tested the coelomic fluid and tissues of *E. quinquesemita* for the presence of autotomy-inducing factors and to elucidate their role in the control of autotomy.

MATERIALS AND METHODS

Specimens of *Eupentacta quinquesemita* were collected subtidally near Victoria, B.C. and held in a recirculating sea water system for at least 24 h before use in experiments. Holothurians to be dissected were relaxed in 6·7 % $MgCl_2$ or 0·1 % propylene phenoxetol (PPOX) in sea water for 3–5 h. Some specimens were cut along an interambulacrum, pinned in a dissecting tray and placed in 1:1 6·7 % $MgCl_2$-sea water for 1–2 h, followed by a wash in sea water for 3–6 h. Isolated pharyngeal retractor muscles (PRMs) were dissected as described in Byrne (1985*c*), and PRM autotomy was used as an assay to investigate the presence of autotomy-inducing activity in test solutions. PRM autotomy is a two-part response involving PRM contraction and rapid softening of the P-L tendon, resulting in separation of the PRM and LBWM. The PRMs were placed in a small dish and kept in sea water until tested.

Mechanical, chemical and electrical stimuli were applied to intact and dissected holothurians, and to isolated PRMs. These stimuli were tested for their capacity to induce evisceration and P-L tendon autotomy. A Grass stimulator and stimulus isolation unit were used to deliver pulsed electrical stimuli (40–100 V, 20 Hz frequency, 25 ms duration, 1–3 min stimulation time) to the holothurians by two methods. In the first method, a stimulus isolation fork was used to stimulate the epidermal surface. The second method involved intracoelomic stimulation, where one electrode, a stainless steel insect pin, was inserted into the coelom through the anus, and the second electrode, an AgCl wire, was laid over the body wall. The influence of anaesthetics ($MgCl_2$ and PPOX) and the ACh antagonists, tubocurarine chloride and atropine, on the electrical stimulation response was investigated. The antagonists were tested at 10^{-2}–10^{-4} mol l^{-1} concentrations, levels suggested from studies of molluscan ACh receptors (Kehoe, 1972). They were dissolved in artificial sea water (ASW) and 0·5 ml samples were injected into the coelom through the body wall with a tuberculin syringe.

Chemical stimuli were tested by injecting 0·5 ml of several experimental solutions into the coelom of intact specimens and by pipetting the solutions directly onto isolated PRMs. The test solutions included ASW (in mmol l^{-1}) NaCl, 423·0; KCl, 9·0; $CaCl_2$, 9·27; $MgCl_2$, 22·94; $MgSO_4$, 25·5; $NaHCO_3$, 2·15); Ca^{2+}-free sea water (CaFSW) (NaCl, 436·71; KCl, 9·0; $MgCl_2$, 22·94; $MgSO_4$, 25·5, $NaHCO_3$, 2·14); MgFSW (NaCl, 481·53; Na_2SO_4, 50·96; KCl, 9·0; $CaCl_2$, 9·27; $NaHCO_3$, 2·14), and CaMgFSW (NaCl, 461·85; KCl, 10·73; Na_2SO_4, 7·04; $NaHCO_3$, 2·14) made according to M.B.L. Formulae (Cavanaugh, 1975), and isosmotic (0·30 mol l^{-1}) $CaCl_2$. The effect of K^+ ions was also investigated and the K^+ concentration was elevated by two methods: (1) by adding isosmotic KCl (0·45 mol l^{-1}) to 0·45 mol l^{-1}

NaCl (KNaASW) and, (2) by substituting 0·45 mol l^{-1} KCl for an appropriate amount of NaCl in ASW containing the normal complement of other sea water ions (KASW). The K^+ response specificity was examined by testing isosmotic (0·45 mol l^{-1}) solutions of other monovalent cations, Rb^+ (RbCl), Cs^+ (CsCl), Na^+ (NaCl) and Li^+ (LiCl). The mean response times of the holothurians to different K^+ concentrations were compared by means of Student's *t*-tests to determine if they differed significantly. The K^+ concentration of coelomic fluid from intact specimens and eviscerates was measured with an atomic absorption spectrophotometer and the pH measured with a combination microelectrode. Neurotransmitter (10^{-4} mol l^{-1}) injection (listed in Table 4) was also tested.

The volume of coelomic fluid per *E. quinquesemita* is very small (0·0–0·5 ml, $N = 20$). This volume cannot be measured without dissecting the holothurians; therefore, it was not possible to determine the final concentrations of the test solutions in the injection experiments. The concentrations listed in Tables 2–4 are those of the injected solutions not the final experimental concentrations. This problem was avoided in the isolated PRM experiments where the solutions were pipetted directly onto the preparations. For these tests, the concentrations listed in Table 3 are the actual experimental concentrations.

Coelomic fluid and tissue extracts were assayed for the presence of evisceration- and autotomy-inducing factors. Coelomic fluid was collected from intact specimens by inserting scissors into the anus, making an incision, and collecting the drops of coelomic fluid released from the cut. Eviscerated coelomic fluid was collected as it was expelled during evisceration. Extracts were made of the following *E. quinquesemita* tissues, dissected from intact specimens: (1) gut/haemal system, (2) body wall/peritoneum, (3) body wall alone and (4) peritoneum peeled away from the body wall (includes body wall muscles and radial nerve cords). The haemal system of *E. quinquesemita* is difficult to isolate from gut tissue, and so the larger haemal systems of the holothurians *Cucumaria miniata* and *Parastichopus californicus* were used to make haemal system extracts. This also provided a cross species test for EF. The coelomic fluid of these species was also tested.

The EF extraction procedure was based on Smith & Greenberg (1973). Tissues dissected from 5–12 specimens were boiled in a small amount of autologous coelomic fluid or ASW for 3–5 min in a water bath, and then homogenized with a motor-driven pestle. A blender was used to homogenize body wall tissue. The homogenate was reboiled and centrifuged at 27 000×***g*** for 30 min at 4°C. The supernatant was tested immediately or frozen and lyophilized. The extracts were assayed by injection into intact specimens or on isolated PRMs.

RESULTS

Mechanical stimulation

Simple mechanical stimuli (tugging at tentacles or pinching the body wall with forceps) did not elicit evisceration, but when pressure was put on the whole coelom (squeezing specimen transversely with forceps), evisceration was provoked within

0·5–30 min. ($\bar{X}_{10} = 5{\cdot}1$ min; S.E. = 1·21). Mechanical stimulation of the body wall or PRMs of dissected specimens elicited muscle contraction but not autotomy.

Electrical stimulation

Electrical stimulation of the epidermis with the stimulation fork induced muscle contraction, but not evisceration. Stimuli that failed to elicit evisceration by external application were effective with the intracoelomic method (Table 1). Electrical stimulation of 30–100 V induced body wall muscle contraction followed by evisceration, and the response time decreased to 0·5 min at the highest voltages tested. Twenty-volt stimulation was never successful. Sham experiments where insect pins were inserted into the coelom without an electrical pulse did not elicit evisceration. Anaesthetized specimens (6·7% $MgCl_2$ or 0·1% PPOX) did not respond to intracoelomic electrical stimulation ($N = 14$). The specimens appeared normal on recovery from anaesthesia and were restimulated. All 14 responded with muscle contraction and nine of them eviscerated.

Specimens treated with the ACh antagonists, tubocurarine chloride (TC) and atropine (A) eviscerated soon after electrical stimulation (Table 2). The response times in these tests did not differ significantly from those of untreated controls (Table 1). Two of seven specimens injected with 10^{-3}mol l^{-1} TC protracted their tentacles followed by full or partial retraction, and touching their tentacles resulted in momentary retraction. Injection of 10^{-2} mol l^{-1} TC rapidly induced evisceration. Atropine induced tentacle protraction in nine ($N = 11$) specimens, followed by full or partial retraction (Table 2). The holothurians were unable to keep their tentacles fully retracted, in spite of repeated tactile stimulation. Atropine did not induce evisceration.

Dissected specimens were also presented with electrical stimuli (60 V). The nerve cords could not be dissected free of associated tissue and so the radial nerve cord–LBWM and oral nerve ring–introvert regions were stimulated directly. This resulted in muscle contraction, but not autotomy ($N = 10$). Direct stimulation

Table 1. *The effects of intracoelomic electrical stimulation on intact specimens and direct electrical stimulation on the PRMs*

Stimulation method	Voltage	*N*	Response (% eviscerated or autotomized)	Response time [min (S.E.)]
Intracoelomic	80–100	8	88	0·5 (0)
	60	7	71	1·7 (0·2)
	50	4	75	1·6 (0·5)
	40	4	75	1·5 (0·25)
	30	4	50	1·0 (0)
	20	4	0	
Direct–intact PRMs	60	15	87	1·8 (0·4)
Direct–isolated PRMs	60	7	71	1·6 (0·3)

of the PRMs induced muscle contraction followed by tendon autotomy (Table 1). Anaesthetized ($MgCl_2$) PRMs did not respond to electrical stimulation ($N = 5$), and upon recovery did not regain the ability to autotomize, perhaps due to tissue damage resulting from direct stimulation.

Table 2. *The effect of acetylcholine antagonists on the response to intracoelomic electrical stimulation (60 V)*

Solution	N	Response to injection	Response to electrical stimulation (% eviscerated)	Response time* [min (S.E.)]
10^{-4} mol l^{-1} TC	2	no response	100	1·5 (0)
10^{-3} mol l^{-1} TC	7	2 protracted tentacles	71	0·85 (0·23)
10^{-2} mol l^{-1} TC	9	7 eviscerated	0	
10^{-4} mol l^{-1} A	3	3 protracted tentacles	67	1·5 (0·04)
10^{-3} mol l^{-1} A	3	3 protracted tentacles	67	0·05 (0)
10^{-2} mol l^{-1} A	5	3 protracted tentacles	100	0·94 (0·36)

TC, tubocurarine chloride; A, atropine.
* Response time to electrical stimulation.

Table 3. *The effect of cation solutions injected into intact specimens or pipetted directly onto isolated PRMs*

Stimulation method	Solution	N	% Eviscerated or autotomized	Response time [min (S.E.)]
Injection	ASW	7	0	
	0·45 mol l^{-1} KCl	10	100	2·4 (0·5)
	0·45 mol l^{-1} RbCl	8	88	2·3 (1·0)
	0·45 mol l^{-1} CsCl	5	100	0·5 (0·1)
	0·45 mol l^{-1} NaCl	5	0	
	0·45 mol l^{-1} LiCl	5	0	
	0·2 mol l^{-1} KCl (KNaASW)	12	100	3·9 (1·0)
	0·1 mol l^{-1} KCl (KNaASW)	11	55	6·5 (4·4)
	0·05 mol l^{-1} KCl (KNaASW)	16	75	4·3 (1·5)
	0·01 mol l^{-1} KCl (KNaASW)	11	73	4·4 (0·8)
	0·005–0·075 mol l^{-1} KCl (KNaASW)	10	0	
	0·05–0·1 mol l^{-1} KCl (KASW)	14	57	12·6 (2·0)
	$MgCl_2$ anaes.-KCl*	9	0	
	PPOX anaes.-KCl*	9	0	
Direct–isolated PRMs	ASW	5	0	
	0·3 mol l^{-1} KCl (KNaASW)	5	100	1·0 (0·2)
	0·2 mol l^{-1} KCl (KNaASW)	5	100	2·5 (0·9)
	0·1 mol l^{-1} KCl (KNaASW)	5	100	4·3 (1·1)
	0·05 mol l^{-1} KCl (KNaASW)	5	60	6·7 (1·7)
	0·01 mol l^{-1} KCl (KNaASW)	7	57	5·8 (1·5)
	0·45 mol l^{-1} NaCl	5	0	

* Anaesthetized specimens injected with 0·45 mol l^{-1} KCl.

Table 4. *The effect of neurotransmitter injection into intact specimens*

Neurotransmitter*	*N*	Response
Acetylcholine	4	Immediate muscle contraction
Epinephrine	4	Relaxed†
Dopamine	2	Relaxed†
Gamma amino butyric acid	2	Relaxed†
Norepinephrine	2	No response
5-hydroxytryptamine	2	No response

* 10^{-4} mol l^{-1}; † tentacles protracted.

Chemical stimulation

Evisceration rapidly followed injection of isosmotic (0·45 mol l^{-1}) KCl, RbCl and CsCl in virtually all tests, but the response was not elicited by NaCl or LiCl (Table 3). Concentrations of 0·01–0·3 mol l^{-1} KCl in KNaASW also provoked evisceration of intact specimens and autotomy of isolated PRMs (Table 3). The response to injection of 0·01–0·2 mol l^{-1} KCl was slower than that to isosmotic KCl and this difference was significant ($P < 0·05$). Isolated PRMs also took longer to autotomize at low concentrations of K^+ (0·01–0·1 mol l^{-1}) compared with 0·3 mol l^{-1} tests ($P < 0·01$). KNaASW containing K^+ concentrations below 0·01 mol l^{-1} were not effective. The concentration of K^+ in coelomic fluid is 0·012 mol l^{-1} (see below). ASW contains the normal complement of seawater cations, including 0·01 mol l^{-1} KCl, and this solution was not effective. The influence of other seawater cations on the K^+ response was tested in KASW. KASW containing 0·05–0·1 mol l^{-1} KCl induced evisceration, but the response time increased significantly ($P < 0·01$) compared with KNaASW tests (Table 3). Injection of ASW lacking divalent cations (CaFSW, $N = 7$; MgFSW, $N = 7$; CaMgFSW, $N = 8$) or injection of excess Ca^{2+} (0·30 mol l^{-1} $CaCl_2$, $N = 7$) did not elicit evisceration.

The effect of $MgCl_2$ and PPOX on the K^+ response was tested by injection of 0·45 mol l^{-1} KCl into 18 anaesthetized specimens (Table 3). No response was observed. Ten of the specimens were dissected while anaesthetized and their P-L tendons were found intact. The remaining eight eviscerated upon recovery in sea water. Injection of some common neurotransmitters did not elicit evisceration, although muscle responses were observed (Table 4).

Evisceration factor

To test for the presence of an evisceration factor (EF), coelomic fluid collected from intact and eviscerating *Eupentacta quinquesemita* was injected into intact holothurians. Injection of coelomic fluid from eviscerates provoked evisceration in four of nine specimens (Table 5). Those that did not eviscerate formed a mid-body constriction, apparently due to PRM contraction. Injection of control fluid from intact specimens and of coelomic fluid from *Parastichopus californicus* and *Cucumaria miniata* did not induce these responses (Table 5).

The presence of evisceration-inducing activity in coelomic fluid from eviscerates, but not from intact holothurians, suggests that EF is released into the coelom from an

internal source at evisceration. To investigate the source of EF, tissue extracts were tested by injection into intact specimens and on isolated PRMs. Injection of extracts made from all the *E. quinquesemita* tissues tested, and the extracts of *P. californicus* and *C. miniata* haemal systems provoked evisceration (Table 5).

Extract injection may stimulate release of endogenous EF by recipient holothurians. This potential artifact was avoided in isolated PRM tests where the ambient medium was experimentally controlled. All extracts, except those made from the body wall (stripped of peritoneum), induced PRM contraction and tendon autotomy (Table 5). The muscle contracting and tendon autotomizing action of EF was not induced in PRMs anaesthetized with $MgCl_2$ ($N = 5$).

Spectrophotometry and pH of coelomic fluid

The response to K^+ injection mimics evisceration, suggesting that coelomic K^+ may be elevated during evisceration. To test this, the K^+ concentration of coelomic fluid from intact specimens and eviscerates was measured by atomic absorption spectrophotometry. No change was detected in coelomic K^+ before or after evisceration, and the K^+ concentration was $0{\cdot}012\,mol\,l^{-1}$ ($0{\cdot}452\,mg\,ml^{-1}$; S.E. =

Table 5. *The effect of EF extracts injected into intact specimens or pipetted directly onto isolated PRMs*

Stimulation method	Extract	*N*	Response (% eviscerated or autotomized)	Response time [min (S.E.)]
Injection	*Eupentacta quinquesemita* extracts			
	coelomic fluid*	4	0	
	eviscerated coelomic fluid*	9	44	8·8 (2·0)
	gut/haemal system	9	89	3·1 (1·1)
	body wall/peritoneum	8	100	7·0 (5·6)
	body wall	8	100	2·0 (0·8)
	peritoneal tissue	8	100	4·1 (1·0)
	Parastichopus californicus extracts			
	coelomic fluid*	5	0	
	coelomic fluid†	5	0	
	haemal system	8	100	6·6 (1·2)
	Cucumaria miniata extracts			
	coelomic fluid†	8	13	3·0
	haemal system	9	100	7·1 (3·0)
Direct–isolated PRMs	*Eupentacta quinquesemita* extracts			
	gut/haemal system	9	89	1·3 (0·1)
	body wall	7	0	
	peritoneal tissue	10	80	2·2 (0·4)
	Parastichopus californicus extracts			
	coelomic fluid†	5	0	

* Tested directly without boiling.
† Coelomic fluid boiled and treated as other extracts.

$0{\cdot}02\,mg\,ml^{-1}$, $N = 8$), slightly elevated over local sea water, $0{\cdot}01 mol\,l^{-1}$ ($0{\cdot}410\,mg\,ml^{-1}$). The pH of coelomic fluid also did not change during evisceration and was 6·98 (S.E. = 0·08, $N = 8$).

DISCUSSION

Evisceration of *Eupentacta quinquesemita* elicited by mechanical and electrical stimulation may be associated with EF activity. Mechanical stimulation provoked evisceration if pressure was put on the coelom as a whole, perhaps due to EF release from damaged internal organs. The intracoelomic method induced evisceration of *E. quinquesemita,* whereas direct epidermal stimulation did not. This suggests that current flow through the coelom and across the dermis may be required for electrical induction of evisceration in *E. quinquesemita,* perhaps stimulating EF release into the coelom. However, direct epidermal stimulation induces evisceration in *Sclerodactyla briareus* (Smith & Greenberg, 1973). Anaesthetics inhibited electrical and K^+-induced evisceration of intact *E. quinquesemita* and prevented autotomy of isolated PRMs, similar to results obtained with *S. briareus* (Smith & Greenberg, 1973).

Evisceration was provoked by K^+, Rb^+ and Cs^+, but not by the other monovalent cationa, Na^+ and Li^+, suggesting that ionic induction of autotomy may involve some common characteristic of these ions. The inability of Na^+ and Li^+ to substitute for K^+ may be associated with their small size: K^+, Rb^+ and Cs^+ are all larger than these ions (Masterton & Slowinski, 1973). Similarly, the mechanical properties of the autotomy tissues of *E. quinquesemita* are affected by K^+ and Rb^+, but not by Na^+ (Byrne, 1985*c*). There may be a competitive relationship between K^+ and divalent cations in K^+-induced evisceration. Induction of evisceration and autotomy by KNaASW containing physiological concentrations of K^+ ($0{\cdot}01 mol\,l^{-1}$) may be due to the absence of the divalent cations, found in sea water and coelomic fluid. The K^+ response was delayed in KASW containing normal concentrations of divalent cations, and ASW (containing $0{\cdot}01 mol\,l^{-1}$ KCl) did not elicit evisceration. High concentrations of K^+ ($0{\cdot}15$–$0{\cdot}45 mol\,l^{-1}$) reduced introvert viscosity, whereas low concentrations of the ion ($0{\cdot}01$–$0{\cdot}075\,mol\,l^{-1}$ KCl) have no discernible effect (Byrne, 1985*c*). In this investigation, $0{\cdot}01\,mol\,l^{-1}$ KCl induced autotomy and evisceration. The autotomizing influence of K^+, especially in high concentrations, may be through a direct effect of K^+ on ionic interactions within the connective tissue (Byrne, 1985*c*). The induction of autotomy by low concentrations of K^+ suggests that the ion may also exert an indirect influence, through stimulating neurones or EF-secreting cells involved with connective tissue autotomy. The peritoneum and radial nerves were removed from the introvert preparations used in the mechanical tests. Perhaps low concentrations of K^+ did not affect the viscosity of the introvert because cells controlling autotomy were removed. Injection of divalent-cation-free sea water did not elicit autotomy, whereas these solutions reduce introvert viscosity (Byrne, 1985*c*). These results, together with the variable influences of K^+ in low and high concentrations, demonstrate the importance of differentiating

between the potential mechanism of autotomy (ionic) and the control of the response (neural).

The ACh antagonists did not block the response to electrical stimulation, and in high concentrations tubocurarine chloride induced evisceration. If the ACh receptors of *E. quinquesemita* are sensitive to these antagonists, then the results suggest that: (1) evisceration may be induced by blocking these receptors, and (2) although the echinoderm nervous system contains cholinergic neurones (Pentreath & Cottrell, 1968), a different neurotransmitter may be involved in the response, perhaps under inhibitory influence by ACh. The inability of drugged specimens to retract their tentacles is presumably a result of blocking ACh transmission which in echinoderms functions in muscle contraction (Hill, 1983). In vertebrates, tubocurarine chloride inhibits nicotinic receptors and atropine inhibits muscarinic receptors (Prosser, 1973). The similar influence of these antagonists in this study might be taken to suggest that the ACh receptors of *E. quinquesemita* are not specific muscarinic or nicotinic types. However, the presence of muscarinic and nicotinic receptors has been demonstrated for the PRM of *Cucumaria japonica* through the use of a broad range of pharmacological agents (Shelkovnikov *et al.* 1977).

The evisceration-inducing activity of coelomic fluid expelled during evisceration and in tissue extracts, suggests the presence of an EF, as found for *S. briareus* (Smith & Greenberg, 1973). The extract test results suggest that the haemal system and peritoneum may be potential sources of EF. All extracts, except those made from body wall alone, induced tendon autotomy, suggesting that the EF activity in body wall/peritoneal extracts resides in the peritoneum. Considering that the haemal system is overlain by peritoneum, the haemal peritoneum – rather than the extracellular haemal fluid – may be the source of EF in haemal system extracts. The haemal system of *S. briareus* also has a high EF activity (Smith & Greenberg, 1973). It would be interesting to test haemal fluid alone, but acquiring sufficient quantities for assay is difficult. EF is presumably a cellular product, and peritoneal cells, cells within haemal vessels, or neurones associated with these tissues are potential sources. Injection of eviscerated coelomic fluid did not elicit the entire evisceration response in all specimens; perhaps the concentration of EF was too low. Smith & Greenberg (1973) found that low concentrations of EF induce PRM contraction that was not followed by tendon autotomy.

EF was extracted from *Cucumaria miniata*, a species that rarely eviscerates (personal observation) and from *Parastichopus californicus*, a species that eviscerates if handled roughly (Swan, 1961). As suggested by Smith & Greenberg (1973), EF may be a widespread substance in holothurians, having roles other than its involvement with evisceration. Therefore, the term 'evisceration factor' may be a misnomer, but without knowing the chemical composition of EF it is difficult to speculate what these other roles may be.

The holothurian tissues retained their EF activity through the boiling-extraction procedure, demonstrating that EF is heat stable and is probably a small molecule, as suggested for *S. briareus* EF and for coelomic fluid factors derived from other echinoderms (Smith & Greenberg, 1973; Motokowa, 1982*a*,*b*). Gel filtration of

S. briareus EF indicates that its molecular weight is approximately 150 Da and it was suggested to be an unidentified neurotransmitter (Smith & Greenberg, 1973). Preliminary separation of *E. quinquesemita* EF also suggests that it is a small molecule (M. Byrne, unpublished results). Common transmitters were tested on *E. quinquesemita* but did not elicit evisceration. Two factors were extracted from the coelomic fluid of *Stichopus chloronotus*, one that stiffens and one that softens the holothurian body wall and the echinoid spine central ligament (Motokawa, 1982*a*). It was suggested that they effect change by stimulating stiffening and softening nerve pathways (Motokawa, 1982*a*). Although these factors altered the mechanical properties of connective tissues *in vitro*, their physiological role in variable tensility has not been established. It is not clear how coelomic factors influence the tensility of connective tissues not in close proximity to coelomic fluid *in vivo*, such as the echinoid spine central ligament.

EF induced the two-part PRM autotomy response, muscle contraction and tendon autotomy, suggesting that for *E. quinquesemita* either, (1) EF is a single factor that induced both responses, (2) that it induced one response which lead to the other, or (3) that EF has two components, one affecting muscle and another affecting connective tissue. Factors isolated from the coelomic fluid of *S. chloronotus* affect connective tissue and have no discernible effect on muscle tissue (Motokawa, 1982*a*). Both parts of the PRM autotomy response to EF were blocked by $MgCl_2$. Although PRM contraction is involved with autotomy, the mechanism of autotomy depends on the breakdown of P-L tendon connective tissue. As demonstrated by the response to ACh injection, autotomy does not always follow strong muscle contraction.

The autotomy tissues of *E. quinquesemita* consist of connective tissue and a muscle tissue component (Byrne, 1982, 1985*b*), whereas the arm autotomy ligaments of ophiuroids and crinoids are composed entirely of connective tissue (Wilkie, 1978; Holland & Grimmer, 1981). Axon-like processes containing large dense vesicles (LDV) appear characteristic of echinoderm connective tissues and have been proposed to be neurosecretory neurones involved in the control of variable tensility (Wilkie, 1979, 1984; Holland & Grimmer, 1981; Smith *et al.* 1981; Motokawa, 1982*c*; Wilkie *et al.* 1984). It has also been suggested that the LDVs contain agents that effect connective tissue change (Wilkie, 1979; Holland & Grimmer, 1981). LDV-filled processes are present in the autotomy tissues of *E. quinquesemita* in association with muscle and connective tissue (Byrne, 1982, 1985*b*). The LDVs appear to remain intact during autotomy, suggesting that they may not be the source of agents that effect connective tissue change. However, the LDVs may be involved in variable tensility of the autotomy tissues in a manner not evident by structural examination. LDV-exocytotic profiles observed in autotomized crinoid ligaments were taken as evidence to suggest that LDV contents play a role in effecting autotomy (Holland & Grimmer, 1981). However, it is not clear whether these profiles are a cause or a result of autotomy. If the LDV-filled processes of ophiuroids and crinoids are neurosecretory and effect autotomy, then the LDVs may contain an EF-like substance. Alternatively, they may contain substances such as chelating agents or acidic substances (rather than a neurosecretory product), agents known to affect

connective tissue tensility (Wilkie, 1978; Holland & Grimmer, 1981; Hidaka, 1983; Byrne, 1985*c*).

The presence of a coelomic EF suggests that cells involved in autotomy may be located at a distance from the tissues and effect change through the medium of the coelomic fluid. This indirect mode of action and the possible neural origin for EF, suggests neurosecretory-like activity (Maddrell & Nordmann, 1979). During evisceration, P-L tendon autotomy is coincident with the presence of EF in the coelom and ultrastructural examination revealed that softening of the introvert occurs from the internal surface outwards (Byrne, 1985*b*), perhaps associated with infiltration of EF from the coelom. In holothurians, the autotomy tissues are partially or completely surrounded by coelomic fluid and so there is potential for hormonal or neurosecretory activity using the coelomic fluid as a transport medium. The autotomy tissues of ophiuroids and crinoids are not associated with a large coelom and so autotomy in these groups may depend on locally distributed cells. It would be interesting to monitor the coelomic vessels of ophiuroid and crinoid arms during autotomy for the presence of autotomy-inducing factors.

EF may be secreted from activated cells, thereby effecting autotomy, or it may be a result of evisceration, a product released from damaged tissues. The extract test results suggest that EF is not an artifactual substance, but without knowing its chemical composition, we can only speculate about its mode of action. Hypothetical

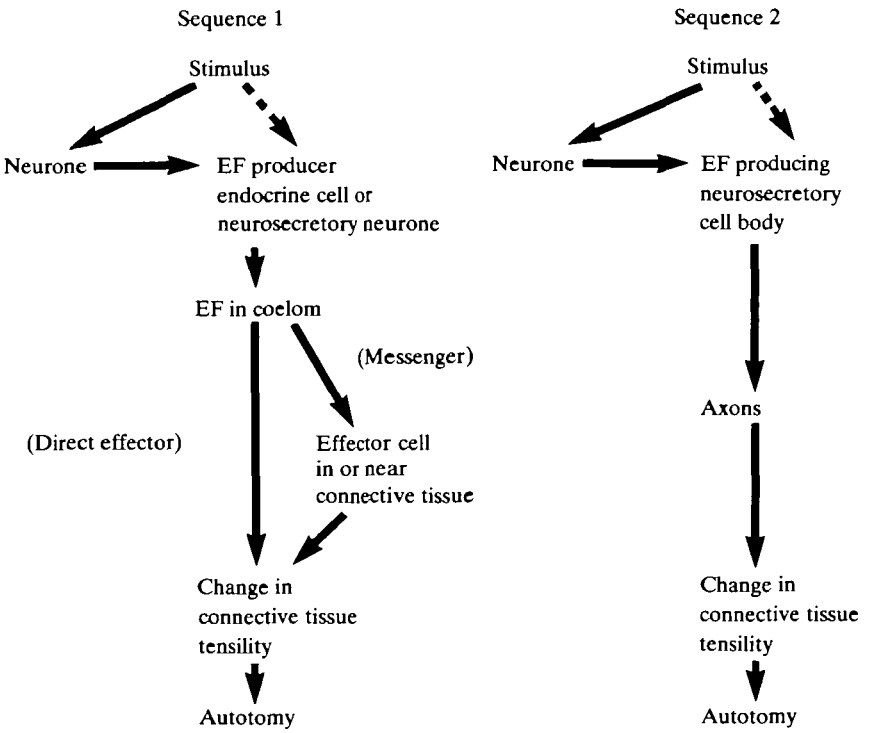

Fig. 1. Hypothetical sequences of events leading to autotomy, see text.

sequences of events leading to autotomy are shown in Fig. 1. Sequence 1 depicts the events that may lead to holothurian evisceration. The evisceration stimulus induces activity in neurones or directly on EF-producing cells causing EF release into the coelom. Once in the coelom, EF may act as a direct effector and alter connective tissue ionic interactions, or it may act as a messenger substance which activates effector cells. Sequence 2 is proposed for ophiuroid and crinoid arm autotomy (Wilkie, 1979; Holland & Grimmer, 1981), where EF is produced by neurosecretory neurones that send axons into the connective tissue where it effects tensility change directly.

The mechanism of connective tissue change appears to be an ionic one (Byrne, 1985*c*) and the influence of K^+, electrical stimulation and anaesthetic antagonism indicates that the process is neurally controlled. The presence of an EF suggests neurosecretory or hormonal involvement. Holothurian evisceration appears to be a good system for further investigation of variable tensility of echinoderm connective tissues, and the logical first step would be the characterization of EF.

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