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THE MECHANICAL PROPERTIES OF THE AUTOTOMY TISSUES OF THE HOLOTHURIAN *EUPENTACTA QUINQUESEMITA* AND THE EFFECTS OF CERTAIN PHYSICO-CHEMICAL AGENTS

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SUMMARY

Evisceration in the holothurian *Eupentacta quinquesemita* (Selenka) results from a rapid softening of autotomy structures comprised of connective tissue. The mechanical properties of two autotomy tissues, the introvert and the retractor muscle tendon, were tested to investigate their function in the non-evisceration state and their behaviour during autotomy. The results show that these structures do not have a pre-existing mechanical weakness to account for their rapid failure during evisceration. The autotomy response was mimicked *in vitro* by increasing K^+ concentration. The introvert exhibited viscous behaviour and the absence of Ca^{2+} and Mg^{2+} decreased introvert viscosity, whereas excess Ca^{2+}, and low and high pH, increased viscosity. These agents may influence the mechanical properties of the autotomy structures by directly affecting connective tissue ionic interactions and may induce proteoglycan conformational changes. K^+ may also exert an indirect effect through responses of cells controlling connective tissue tensility. The most likely mechanism of autotomy is through an alteration of connective tissue ionic interactions.

INTRODUCTION

The phenomenon of variable tensility in echinoderm connective tissue is associated with two types of change. Echinoderm catch ligaments exhibit reversible stiffening/softening tensility changes, whereas autotomy ligaments undergo an irreversible sudden reduction in tensility leading to loss of body parts. The echinoid spine catch apparatus is the classic example of an echinoderm catch ligament. When in catch, the connective tissue ring surrounding the spine base holds the spine in place so firmly that it is impossible to move the spine without tearing the apparatus (von Uexküll, 1900; Takahashi, 1967). Similar changes in tensility have been described for holothuroid, asteroid and crinoid connective tissues (Lindemann, 1900; Jordan, 1914; von Uexküll, 1926; Serra-von Buddenbrock, 1963; Meyer, 1971; Stott, Hepburn, Joffe & Heffron, 1974; Freinkel & Hepburn, 1975; Eylers, 1976*a*,*b*; Wilkie,

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1983). The mechanical properties of echinoderm catch ligaments have been examined in numerous studies and it appears that variable tensility is associated with the viscous behaviour of the connective tissue matrix and is effected by a change in the connective tissue environment (Takahashi, 1967; Eylers, 1976*b*, 1982; Biglow, 1981; Motokawa, 1981, 1982, 1983, 1984*a,b*; Wilkie, 1983, 1984; Hidaka, 1983; Hidaka & Takahashi, 1983).

The ability to autotomize body parts is characteristic of echinoderms (Emson & Wilkie, 1980), but the mechanical properties of autotomy connective tissues have received relatively little attention. Two autotomy structures that have been studied are the holothurian retractor muscle tendon and the ophiuroid intervertebral ligament (Smith & Greenberg, 1973; Wilkie, 1978).

Evisceration in the dendrochirote holothurian *Eupentacta quinquesemita* is associated with sudden softening of three autotomy connective tissues: (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, the anterior extensible portion of the body wall (Byrne, 1982). Autotomy of the P-L tendon usually occurs within 30 s and that of the introvert takes approximately 3 min but took up to 5 min in some specimens (Byrne, 1983). During evisceration the introvert changes from a firm opaque structure to one that is soft and translucent. It becomes distended as it is filled with coelomic fluid and autotomized organs propelled anteriorly by contraction of the body wall muscles. Autotomy results from changes within the connective tissue and internal hydrostatic pressure plays a role in the eventual detachment of the introvert. In this study, the mechanical properties of the P-L tendon and the introvert were examined to correlate their mechanical properties *in vitro* with their function in the non-evisceration state and with their behaviour during autotomy. The introvert is comprised predominantly of connective tissue and creep tests were used to quantify its mechanical properties. Muscle fibres dispersed in the introvert connective tissue occupy 1–4 % of the introvert cross-sectional area and do not appear to influence introvert autotomy (Byrne, 1983). The connective tissue of the P-L tendon is intimately associated with PRM muscle bundles making it impossible to isolate the tendon (Byrne, 1982) and so entire PRM preparations were used for tests. The PRMs were extended under a constant load to examine the mechanical properties of the tendon and PRM, especially for the position of failure.

Variable tensility of echinoderm connective tissues can be mimicked *in vitro* by altering the pH and cation composition of test solutions (Wilkie, 1978, 1983, 1984; Biglow, 1981; Smith, Wainwright, Baker & Cayer, 1981; Eylers, 1982; Motokawa, 1982, 1983, 1984*a,b*; Hidaka, 1983) and similar experiments were used to investigate the mode of action of physico-chemical agents used in other studies on the autotomy structures of *E. quinquesemita*.

MATERIALS AND METHODS

Tissue samples

Specimens of *Eupentacta quinquesemita* were collected subtidally near Victoria, B.C. and near the Friday Harbor Laboratories, Washington, and acclimated in an ambient sea water system for at least 24 h before use in experiments. They were

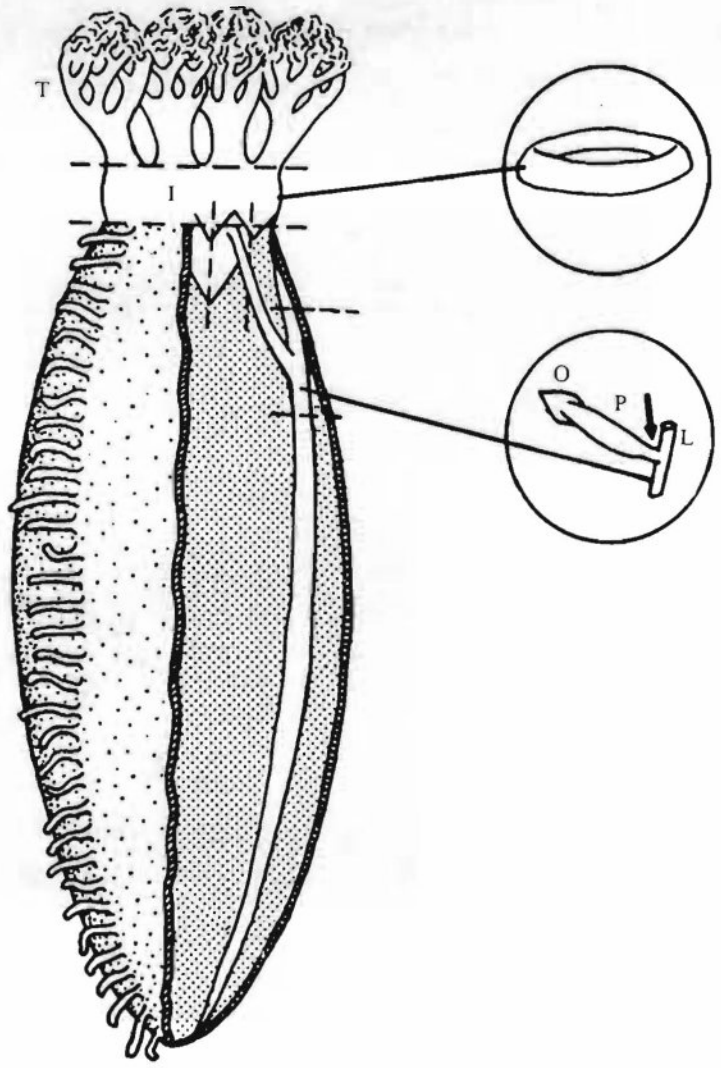

Fig. 1. Diagram showing the dissection and isolation of the introvert (I) and the pharyngeal retractor muscle (P) of *Eupentacta quinquesemita* (Selenka). Incisions indicated by the broken lines. O, ossicle; L, longitudinal body wall muscle; T, tentacle; arrow, P-L tendon.

relaxed in 6·7 % $MgCl_2$ or 0·1 % propylene phenoxetol (PPOX) in sea water for 3–5 h before dissection. The introvert was dissected as follows (Fig. 1): it was cut along its posterior margin where it joins the body wall, the tentacles were cut off at their bases and the body wall muscle tissue was removed. For ease of handling, circumferential rings were used for all tests. The PRMs were isolated from relaxed specimens by dissection around their junction with the LBWM and at their antcrior insertion into the ossicle (Fig. 1). Surgical silk thread was tied at either end of the preparations and thread loops were made to facilitate connections of the PRMs to the testing apparatus (see below). Before testing, the preparations were washed in sea water for 3–5 h.

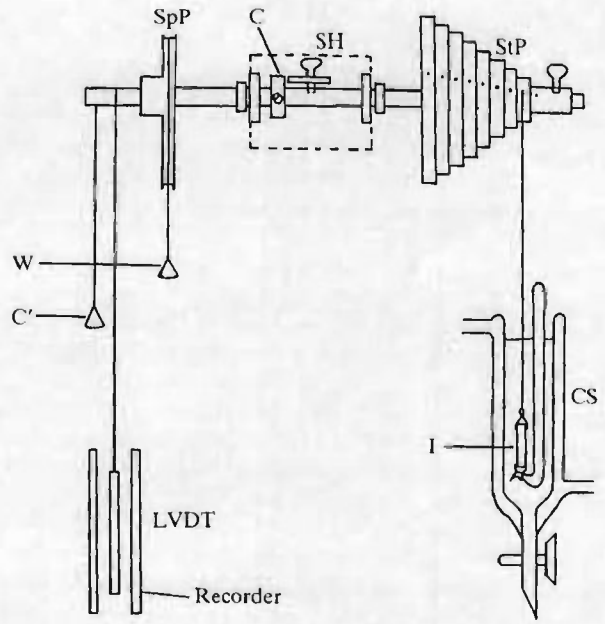

Fig. 2. The constant stress creep-testing machine, modified from Vogel & Papanicolaou (1983). The introvert (I) is in a bathing solution and is attached to the 'step' of the step pulley (StP) with a diameter closest to the original length of the specimen. C, counterweight for the step pully; C', counterweight for the LVDT core rod; CS, cooling sleeve; LVDT, linear variable differential transformer, measures specimen elongation and is connected to a chart recorder; SH, shaft holder; SpP, spiral pulley; W, applied weight, when multiplied by the moment arm of the spiral pulley, gives the force on the specimen.

Introvert

A constant stress creep machine (Fig. 2) was used to quantify the mechanical properties of the introvert. Stress (σ) is $= F/A$, where F = force and A = cross-sectional area of the specimen. It was assumed that the volume of the introvert remained constant during tests. The rationale behind the creep machine's design and construction is described in Vogel & Papanicolaou (1983). The machine has two essential features: a circular step pulley with steps of different radii and a spiral pulley, both of which are on the same shaft. The specimen is attached to that step of the step pulley with a diameter nearest to the initial length of the specimen. The spiral pulley is designed so that the product of specimen length and the pulley lever arm is constant, i.e. the lever arm length decreases directly in proportion to specimen elongation, keeping stress constant. The stress on the specimen is given by: $\sigma = F'Ro'/RSo$, where F' = applied weight, Ro' = initial lever arm length, R = radius of the step pulley chosen and So = initial cross-sectional area of the specimen. Shaft rotation was measured by a linear variable differential transformer (LVDT) and provided a measure of specimen length, which was required to calculate strain. The introvert proved to be a highly extensible tissue, therefore natural or true strain; $e = \ln(L/Lo)$ was used, where L = actual length and Lo = original length of the tissue (Wainwright, Biggs, Currey & Goslinc, 1976). The introvert rings were held between two heart

clips, one connected to a clamped glass hook and the other to the step pulley by silk thread (Fig. 2). During creep tests the tissue and test solutions were placed in a cooling sleeve and kept at 12 °C by ambient sea water running through the outer jacket.

The slope of the creep curve was used to calculate the creep rate; $\dot{e} = de/dt$. Constant stress divided by the creep rate provides a measure of viscosity, $N = \sigma/\dot{e}$ (Wainwright *et al.* 1976). For tests where the creep curve was not a straight line, the slope of the best fit line was calculated by least squares linear regression and the correlation coefficients are listed in Table 2. Compliance (D), an index of extensibility, was also used to express creep data (Table 1) and is given by: $D(t) = e(t)/\sigma$, where e(t) is the strain at time t and σ is the constant stress. Time t = 300 s was chosen because the duration of some tests was not longer than 300 s. The stress used for all creep tests was $\sigma = 3 \times 10^4$ N m^{-2}.

Pharyngeal retractor muscle

Isolated PRMs were placed on a lever system (Fig. 3) and extended with a constant load, that is, stress increased as specimen cross-sectional area decreased. The LBWM end was connected to a glass hook clamped to a micromanipulator and the ossicle end was attached to a jeweller's chain connected to the lever. A weight was attached to the other side of the lever and an LVDT was used to measure specimen elongation. The duration of each test and the position of tissue failure were recorded.

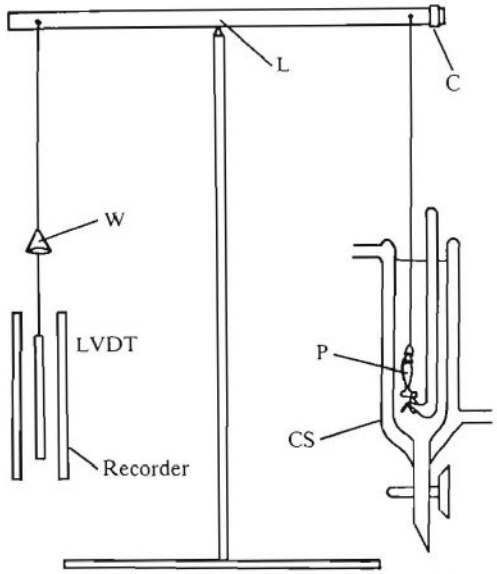

Fig. 3. Lever system, the PRM (P) is in a bathing solution kept cool by ambient sea water running through the cooling sleeve (CS). The LBWM end is attached to a clamped glass hook and the ossicle end to the lever (L). A weight (W) is attached to the other side of the lever and specimen elongation is measured by a LVDT. C, counterweight for LVDT core rod.

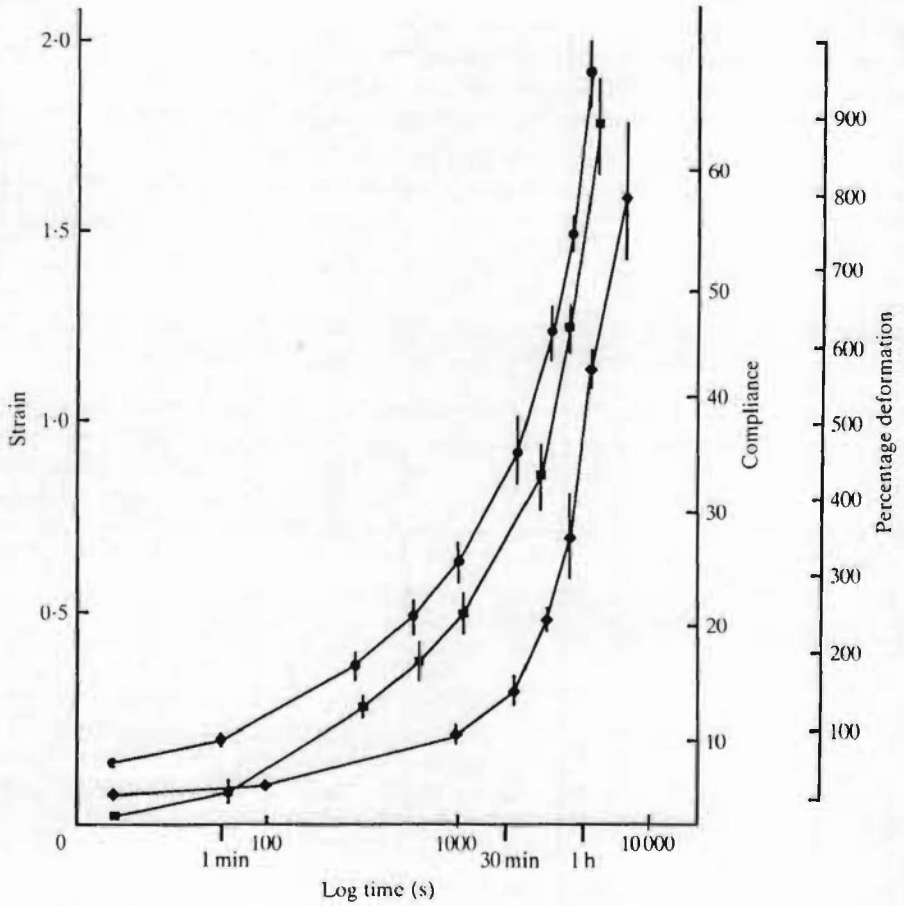

Fig. 4. Creep curve (—●—), compliance (—■—) and percentage deformation (—◆—) of introvert tissue tested in ASW. $N = 9$ at t_o, $N = 6$ at $t = 3500$ s, $N = 4$ at $t = 6000$ s. Means ± S.E.M.

Experimental solutions

Creep tests were done while the introvert was bathed in artificial sea water (ASW), Ca^{2+}-free sea water (CaFSW), MgFSW, or CaMgFSW, made according to M.B.L. Formulae (Cavanaugh, 1956). The salinity of local sea water varies between 28 and 32 ‰. Solutions containing various concentrations of K^+ were made by adding isosmotic KCl (0·45 mol l^{-1}) to 0·45 mol l^{-1} NaCl (KNaASW), i.e. ASW containing the cations K^+ and Na^+ only. Isosmotic KCl and solutions of other monovalent cations, Rb^+ (0·45 mol l^{-1} RbCl) and Na^+ (0·45 mol l^{-1} NaCl), were used to examine the K^+ response specificity. Isosmotic $CaCl_2$ (0·30 mol l^{-1}) was also tested. To test the effect of anaesthetics on the K^+ response, tissues were tested without recovery from anaesthesia. Buffer solutions, pH 2–12, were used to test the effect of pH on introvert viscosity. For pH 4–10, Tris buffers were made with appropriate amounts of Tris-HCl and Trisma base (Sigma). For pH 2, McIlvaine's buffer was used (Pearse, 1968) and 0·1 mol l^{-1} NaOH was used for pH 12. The effect of experimental

solutions on the PRM was tested while the tissue was attached to the lever maintained in position by a light load (F/A at $t_o = 0{\cdot}98 \times 10^4$ N m^{-2}).

RESULTS

Introvert

The introvert was creep-tested in ASW to examine the mechanical properties of the tissue in a solution approximating to physiological conditions. The combined creep curve for nine specimens shows that the tissue deformed at a slow and constant rate until failure (Fig. 4). When strain is plotted against log time, the creep curve is exponential due to the constant creep rate. The creep curve between 600 and 4000 s was used to calculate viscosity. This was to avoid the influence of initial slack in the tissue and after 4000 s the sample size decreased (Fig. 4). Introvert compliance increased as the creep test progressed (Fig. 4) and deformations at failure were up to 900 % relative to the original length of the tissue (Fig. 4). The introvert is very viscous and deformations beyond approximately the first 10 min were irreversible. Specimens in creep tests arrested after 10 min did not return to their original dimensions before eventually degenerating. During the first 5–10 min the introvert appeared relatively stiff when manipulated and examined directly. The introvert cross-sectional area appeared to decrease uniformly during creep tests. Narrowing or necking of the tissue was not observed even as the introvert approached failure. Introvert viscosity in ASW tests was of the order of 10^8 N m^{-2} s (Table 2).

Pharyngeal retractor muscle

Eight PRMs were placed on the lever and two were loaded with $0{\cdot}98 \times 10^4$ N m^{-2} and six with $4{\cdot}9 \times 10^4$ N m^{-2}. Strains at failure were similar for all tests despite the different initial stresses (Fig. 5). Six PRMs broke at or near the ossicle but two of the PRMs at the higher load broke at the tendon.

Table 1. *Introvert compliance D(t) at t = 300 s, D(t) = e(t)/σ*

Solution	D(t)*	S.E.	*N*
ASW	1·2	0·09	9
CaFSW	2·7	0·46	9
MgFSW	1·5	0·18	5
CaMgFSW	4·3	0·29	2
0·45 mol l^{-1} KCl	2·5	0·29	3
0·25 mol l^{-1} KCl	1·8	0·36	2
0·15 mol l^{-1} KCl	1·2	0·06	5
0·075 mol l^{-1} KCl	1·2	0·15	4
0·45 mol l^{-1} NaCl	1·0	0·20	2
$MgCl_2$–KCl†	1·9	–	1‡
PPOX–KCl‡	0·7	0·05	4

* All values $\times 10^{-5}$ N^{-1} m^2.
† Anaesthetized tissue tested in 0·45 mol l^{-1} KCl without recovery in sea water.
‡ Only one of three specimens lasted 300 s before failure.
PPOX, propylene phenoxetol.

Table 2. *Introvert viscosity* (η) *in test solutions*, $\eta = \sigma/\dot{e}$

Solution	η*	N	r^2
ASW	10·0	9	1·0
CaFSW	6·0	9	1·0
MgFSW	5·0	5	1·0
CaMgFSW	1·25	2	1·0
0·45 mol l^{-1} KCl	2·08	3	0·89
0·45 mol l^{-1} RbCl	1·07	2	0·97
0·25 mol l^{-1} KCl	5·6	2	0·96
0·15 mol l^{-1} KCl	7·5	5	0·9
0·075 mol l^{-1} KCl	10·0	4	0·97
0·45 mol l^{-1} NaCl	12·0	2	0·87
$MgCl_2$–KCl†	0·9	3	1·0
PPOX–KCl†	10·0	4	1·0

*All values $\times 10^7$ N m^{-2} s.
†Anaesthetized tissue tested in 0·45 mol l^{-1} KCl without recovery in sea water.
r^2, coefficient of determination. The regressions were calculated for the creep curves, see Figs 4, 6 and 8 for standard errors.
PPOX, propylene phenoxetol.

Effect of experimental solutions

Introvert

The softening of the introvert observed during evisceration was mimicked *in vitro* by increasing the K^+ concentration in the bathing solution. Excess K^+ ions induced an increase in compliance and decrease in viscosity (Fig. 6; Tables 1, 2). Isosmotic KCl had the greatest effect with rapid failure at low strain values (Table 3). The

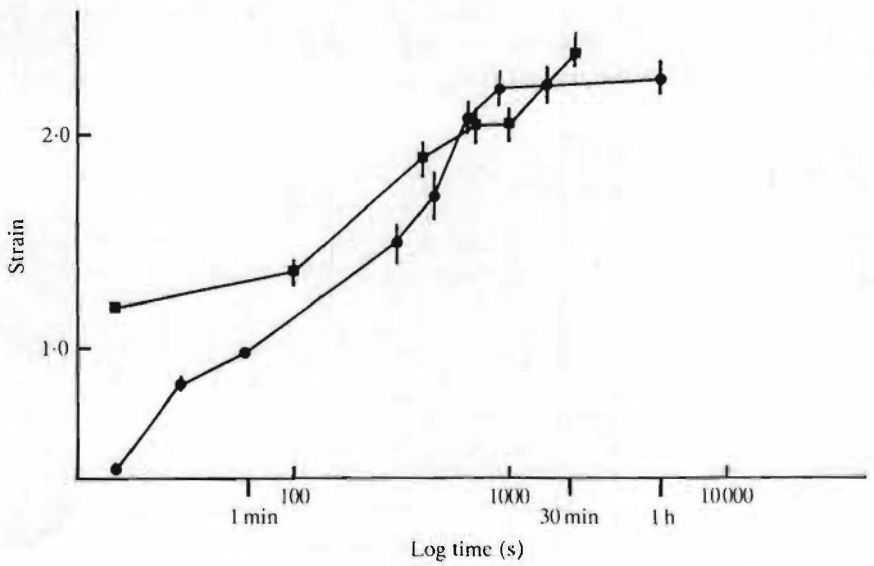

Fig. 5. Elongation of the PRMs extended with a constant load while bathed in ASW. Strains resulting from the two initial stresses, (—●—) $\sigma = 0{\cdot}98 \times 10^4$ N m^{-2} ($r^2 = 0{\cdot}92$) and (—■—) $\sigma = 4{\cdot}9 \times 10^4$ N m^{-2} ($r^2 = 0{\cdot}97$), were similar. Means ± S.E.M.

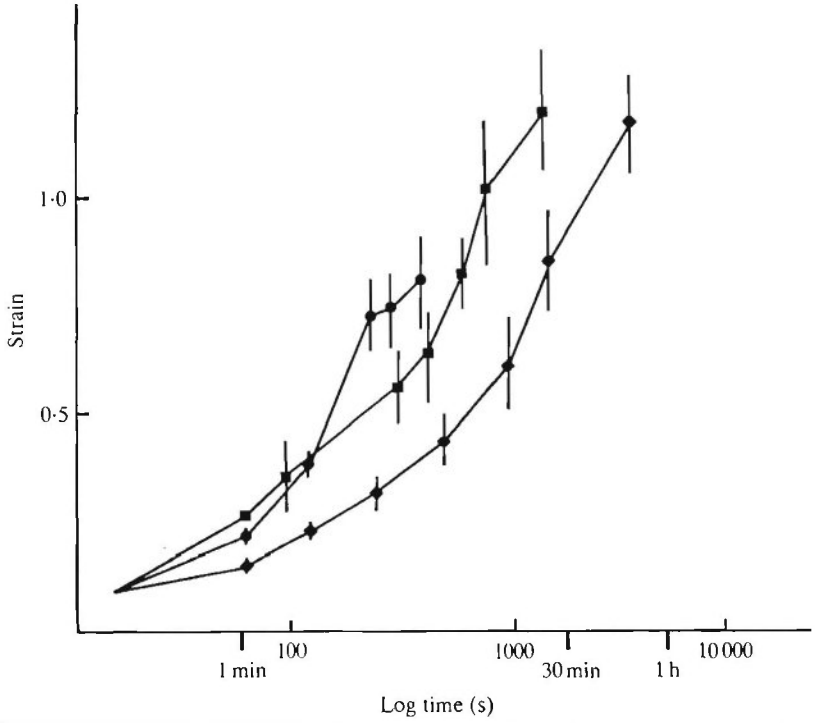

Fig. 6. The influence of K^+ on introvert creep behaviour. 0·45 mol l^{-1} KCl (—●—) induced a rapid creep rate and failure ($N = 3$). 0·25 mol l^{-1} KCl (—■—) ($N = 2$). 0·075 mol l^{-1} KCl (—◆—) tests resembled controls ($N = 4$). Means ± S.E.M.

influence of the ion decreased as the concentration was lowered and 0·15 mol l^{-1} KCl was the lowest concentration tested that affected introvert viscosity. For tissues tested in 0·075 mol l^{-1} KCl, the creep curves were similar to controls. The concentration of K^+ in the coelomic fluid of *Eupentacta quinquesemita* is 0·012 mol l^{-1} (Byrne, 1983). Isosmotic RbCl produced results similar to those with KCl and 0·45 mol l^{-1} NaCl tests were similar to controls (Tables 2, 3). The decrease in compliance induced by 0·45 mol l^{-1} KCl was arrested when the bathing solution was replaced with isosmotic $CaCl_2$ (Fig. 7). Excess Ca^{2+} stiffened the introvert and the tissue did not fail in tests.

Anaesthetized tissues tested without prior recovery exhibited variable responses to K^+. $MgCl_2$ did not interfere with K^+-induced softening, but PPOX inhibited the response and may have had a stiffening influence. PPOX-anaesthetized tissues had viscosity values similar to controls and the creep tests had a similar duration, although the breaking strains were lower than those of controls (Tables 2, 3). Introvert preparations that were anaesthetized in $MgCl_2$ or PPOX, and then washed in sea water for 3–5 h before testing, had similar responses to those tested in excess K^+.

The initial stiff period apparent in control tissue when handled directly was not present in tissues treated with divalent-cation-free sea water. Introvert preparations

Table 3. *Breaking strain and failure times for introvert creep tests*

Solution	Breaking strain (S.E.)	Time (s) (S.E.)	*N*
ASW	1·52 (0·25)	4200 (800)	9
CaFSW	1·93 (0·08)	2455 (470)	9
MgFSW	1·33 (0·18)	1520 (345)	5
CaMgFSW	1·46 (0·11)	350 (70)	2
0·45 mol l^{-1} NaCl	1·53 (0·08)	6000 (1180)	2
0·45 mol l^{-1} KCl	0·83 (0·08)	350 (54)	3
0·45 mol l^{-1} RbCl	1·00 (0·03)	390 (30)	2
0·25 mol l^{-1} KCl	1·19 (0·13)	1250 (250)	2
0·15 mol l^{-1} KCl	1·10 (0·20)	1720 (186)	5
0·075 mol l^{-1} KCl	1·40 (0·17)	3400 (1300)	4
$MgCl_2$–KCl*	0·63 (0·14)	130 (78)	3
PPOX–KCl*	1·00 (0·19)	3125 (315)	4

* Anaesthetized tissue tested in 0·45 mol l^{-1} KCl without recovery in sea water.
PPOX, propylene phenoxetol.

soaked for 2 h and tested in CaFSW, MgFSW or CaMgFSW deformed at a faster rate than ASW controls, with decreased viscosity and increased compliance (Fig. 8; Tables 1, 2). The lack of Ca^{2+} made the tissue more compliant and deformations at failure were higher than those of ASW controls (Table 3). The lack of Mg^{2+} hastened failure and at strains similar to controls (Table 3). The lack of both ions elicited the greatest increase in compliance and decrease in viscosity with a rapid failure at strains similar to controls (Fig. 8; Tables 1–3). In contrast, excess Ca^{2+} (isosmotic $CaCl_2$) induced stiffening and the tissue did not fail in tests.

Manipulation of pH affects introvert viscosity. The viscosity value was lowest at pH 7·0, similar to that of ASW tests, and increased sharply at either side of this pH (Fig. 9). The pH of *E. quinquesemita* coelomic fluid is 7·0 (Byrne, 1983).

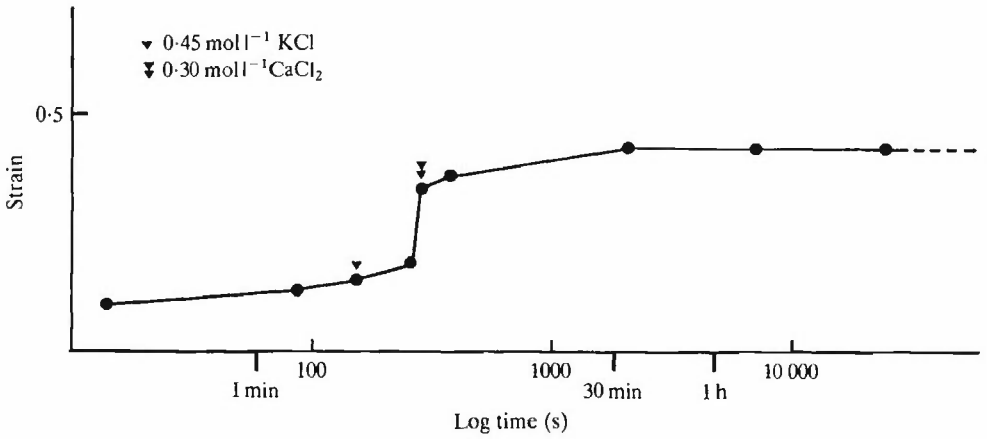

Fig. 7. Antagonistic interaction of K^+ and Ca^{2+} on introvert creep behaviour. The increased compliance induced by 0·45 mol l^{-1} KCl is arrested if the bathing solution is replaced with 0·30 mol l^{-1} $CaCl_2$. The tissue remained stiff in $CaCl_2$ for the duration of the test and did not fail.

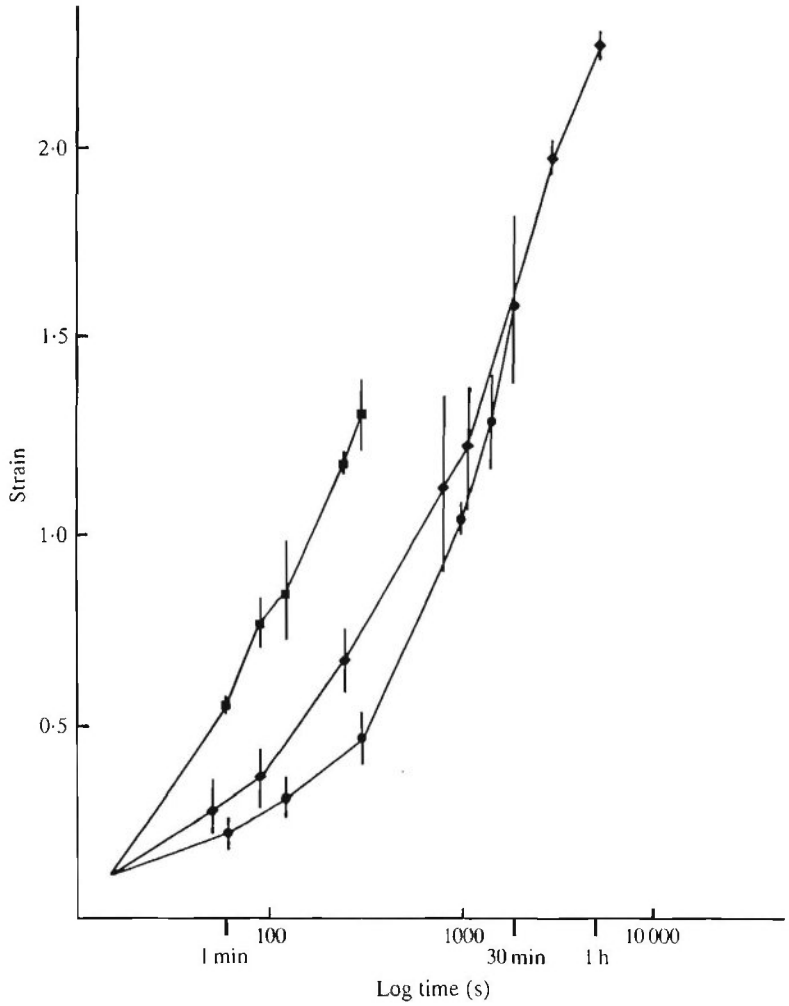

Fig. 8. Creep curve of introvert tissue bathed in cation-free ASW. CaFSW (—◆—) induced a decrease in viscosity, and deformations at failure were greater than controls ($N = 9$). MgFSW (—●—) also decreased introvert viscosity and hastened failure at strains similar to ASW tests ($N = 5$). The lack of both ions (—■—) elicited the greatest increase in compliance and rapid failure ($N = 2$). Means ± S.E.M.

Pharyngeal retractor muscle

Muscle preparations tested in ASW and 0·45 mol l^{-1} NaCl did not fail in tests (Table 4). KCl and RbCl were most effective in eliciting tendon autotomy. These solutions induced muscle contraction, tendon softening and a rapid separation of the PRM and LBWM (Table 4). $MgCl_2$ did not block these responses but PPOX inhibited K^+-induced autotomy. Tendon failure occurred with other solutions, but the response times were considerably longer (Table 4) and the results cannot be reliably compared with the rapid tendon autotomy *in vivo* during evisceration. Excess Ca^{2+} induced muscle contraction resulting in a narrow neck at the P-L tendon and

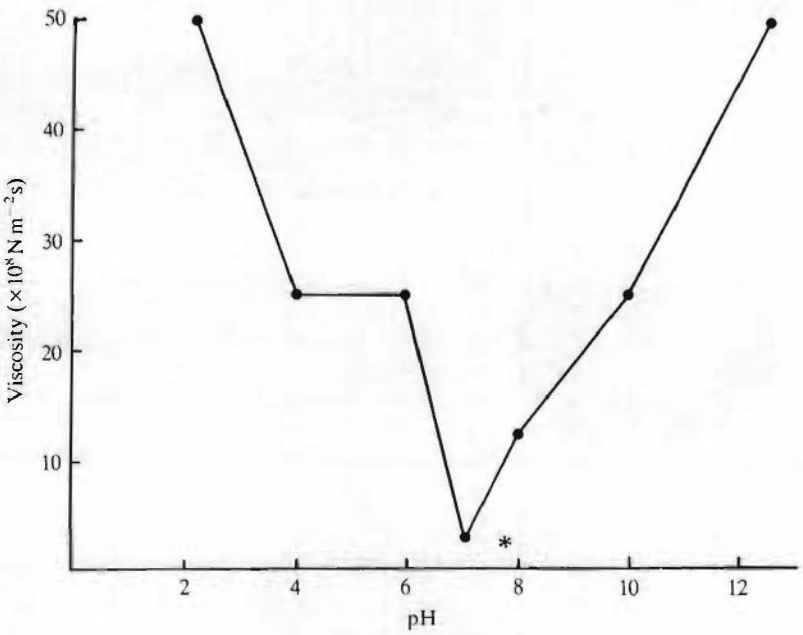

Fig. 9. The influence of pH on introvert viscosity. The asterisk marks introvert viscosity in ASW (pH 7·8) tests.

concentration of stress forces at the neck may have caused eventual tendon rupture. Muscle contraction induced by KCl and RbCl may also have resulted in stress concentration, but the rapid response of the P-L tendon suggests that these agents also affected the connective tissue. CaFSW induced muscle relaxation and uniform cross-sectional area along the PRMs and they did not fail in CaFSW tests. Most of the PRMs tested in MgFSW broke at the tendon.

Table 4. *Response of PRM preparations to test solutions*

Solution	*N*	P-L tendon failure, *N*	Response time (s) (S.E.)
ASW	5	0	
0·45 mol l^{-1} NaCl	5	0	
0·45 mol l^{-1} KCl	10	10	102 (12)
0·45 mol l^{-1} RbCl	9	9	90 (130)
0.30 mol l^{-1} $CaCl_2$	5	5	4806 (570)
CaFSW	5	0	
MgFSW	9	7	4800 (1038)
$MgCl_2$–KCl*	5	5	126 (60)
PPOX–KCl*	7	1	90

* Anaesthetized tissue tested in 0·45 mol l^{-1} KCl without recovery in sea water.
PPOX, propylene phenoxetol.

DISCUSSION

The compliant properties of the introvert correlate with the extensibility it exhibits during suspension feeding in association with tentacle protraction and retraction and with the characteristic infolding of the introvert that occurs when the tentacular crown is withdrawn. In comparison, the body wall posterior to the introvert was considerably less extensible in mechanical tests (M. Byrne, unpublished results). The introvert is comprised of a dense connective tissue layer containing collagen fibrils and matrix and a predominant layer of loose connective tissue containing matrix and occasional small unstriated fibrils (Byrne, 1983). Morphologically and histochemically the introvert appears to be comprised predominantly of glycosaminoglycan (GAG) (Byrne, 1983, 1985) which probably accounts for its viscous properties *in vitro* and its extensible behaviour *in vivo*. Other echinoderm connective tissues have been shown biochemically to possess a high GAG and proteoglycan content (Junqueira *et al.* 1980; Minafra *et al.* 1980; Bailey, Gathercole, Dlugosz & Voyle, 1982).

The creep curves in ASW were similar for all introvert preparations although they differed in duration, which suggests that some specimens were stiffer than others. The proportion of loose and dense connective tissue in the introvert varies between specimens (Byrne, 1983) and may be a source of creep test variability. The introvert may also have catch properties as described for the holothurian body wall (Motokawa, 1984*b*) and so the test tissues may have been in various states of catch. The introvert creep rate appears to be less variable than that of the body wall (Motokawa, 1984*b*). Introvert creep curves are similar to those obtained for other echinoderm connective tissues (Takahashi, 1967; Eylers, 1976*a*, 1982; Wilkie, 1978; Motokawa, 1981; Smith *et al.* 1981) and for sea anemone mesogloea (Alexander, 1962; Koehl, 1977). As for other echinoderm connective tissues the introvert exhibited viscoelastic properties. Assuming that introvert collagen fibrils are similar to vertebrate tendon collagen, relatively inextensible and of high tensile strength (Harkness, 1961), the large deformations obtained during creep tests indicate that the collagen fibrils are discontinuous, as suggested for holothurian dermis and mesogloea (Gosline, 1971; Motokawa, 1981).

Initially during creep tests, the collagen fibrils may act as tensile elements with the matrix transferring the load from fibre to fibre. As deformation continues, the collagen fibrils must slip past each other perhaps associated with a decrease in fibre-matrix adhesion. This may be associated with an alteration in the GAG-collagen electrostatic interactions (see below) and a decrease in matrix viscosity. The interfibrillar slippage results in a decrease in stiffness and increase in compliance. During the final stages of the creep tests, matrix proteoglycan molecules may be pulled upon directly, with possible breakage of intermolecular ionic bonds and subsequent tissue failure. Initial reinforcement by fibrillar elements followed by viscous behaviour dominated by matrix proteoglycans is similar to that described for sea anemone mesogloea and insect cuticle (Gosline, 1971; Reynolds, 1975).

Introvert compliance and its viscous matrix are central to its function *in vivo* where introvert extension and inversion are generated by the tentacle muscles, the PRMs and the body wall musculature. During tentacle protraction and retraction, introvert collagen fibrils may slip past each other, but it is unlikely that the tissue is extended

to the point where the collagen fibrils no longer overlap. In the non-evisceration state the introvert does not approach failure and so it is unlikely that matrix proteoglycans are ever pulled upon directly, as is suggested to occur in the latter portion of the creep tests.

During evisceration, the introvert serves as a specialized autotomizing structure. The sudden softening characteristic of the introvert during autotomy was not observed in control tests in ASW, suggesting that the tissue does not have a pre-existing mechanical weakness to account for its failure during evisceration and that autotomy involves a physiological change in the tissue. Ultrastructural examination of autotomizing introvert revealed that changes in the connective tissue matrix caused interfibrillar slippage resulting in complete fibril disarray and that the subsequent viscid flow was followed by autotomy (Byrne, 1985).

The P-L tendon serves as a connection between the PRM and LBWM and as an autotomizing structure. The tendon is comprised of collagenous connective tissue associated with PRM muscle fibres and it functions in conjunction with the PRM (Byrne, 1982). Consequently, the test results largely reflect PRM properties. The tendon was found to be as strong or stronger than associated muscle tissue and it thus forms a strong connection between the PRM and LBWM. PRM failure usually occurred at or near the anterior insertion into the ossicle but not at the tendon. Similar results were obtained for the P-L tendon of *Sclerodactyla briareus,* which also autotomizes during evisceration (Smith & Greenberg, 1973).

Introvert and P-L tendon autotomy during evisceration was mimicked *in vitro* by elevated K^+, especially with isosmotic KCl. Introvert compliance also increased in the absence of divalent cations. The results of ion experiments may be a function of the connective tissue chemistry. Connective tissue polysaccharides in solution undergo reversible conformational changes induced by altering ion concentrations (Cael, Winter & Arnott, 1978) and their polyanionic nature creates potential for GAG-GAG and collagen-GAG electrostatic interactions (Obrink, 1975; Comper & Laurent, 1978; Lindahl & Höök, 1978). Experimental alteration of ion concentrations may have affected introvert viscosity by changing these interactions or by inducing proteoglycan conformational change. Manipulation of the ionic milieu has been used with similar results for other echinoderm connective tissues (Wilkie, 1978; Biglow, 1981; Smith *et al.* 1981; Eylers, 1982; Hidaka, 1983). Viscosity changes induced by altering K^+ and Ca^{2+} concentrations were obtained in solutions of holothurian dermis where cellular inclusions were completely disrupted (Biglow, 1981). The effect of K^+ may involve the masking of GAG anionic sites, thereby reducing collagen-GAG and GAG-GAG ionic interactions, as suggested for other echinoderm connective tissues (Wilkie, 1978; Biglow, 1981; Eylers, 1982). K^+ and Rb^+ reduced introvert viscosity and induced P-L tendon autotomy, but Na^+ did not, perhaps because K^+ and Rb^+ have similar ionic characteristics, both are larger than Na^+ (Masterton & Slowinski, 1973).

Excess Ca^{2+} stiffened the introvert, perhaps by acting as a divalent cross-linker; that is, as an ionic bridge facilitating ionic interactions. Conversely, the lack of Ca^{2+} would have a softening effect. Treatment with CaFSW decreased introvert viscosity, as has been found for other echinoderm connective tissues (Biglow, 1981; Smith *et al.* 1981; Eylers, 1982; Hidaka, 1983). MgFSW also decreased introvert viscosity and

hastened failure compared with ASW controls. This solution had a similar effect in other studies (Wilkie, 1978, 1983; Smith *et al.* 1981). The lack of both Mg^{2+} and Ca^{2+} had the greatest influence on the introvert with results similar to those from isosmotic KCl tests. Both Mg^{2+} and Ca^{2+} appear to play a stabilizing role in the connective tissue, as suggested in other investigations (Wilkie, 1978, 1983; Smith *et al.* 1981), although excess Mg^{2+} lowered the viscous resistance of the echinoid catch apparatus (Hidaka, 1983). The response of the introvert to altering ion concentrations suggests that the mechanism of autotomy may involve a change in the ionic environment *in vivo*.

Besides the potential effect of K^+ on ionic interactions, there is evidence that the action of K^+ may be indirect through cellular mediation, especially in studies where low concentrations of K^+ were tested (Table 5). Wilkie (1983) found that the K^+ response that makes crinoid cirral ligaments more pliant is Ca^{2+}-dependent and is inhibited by Mg^{2+}. Concentrations of 0·075–0.1 mol l^{-1} KCl in KNaASW had no discernible effect on the introvert, perhaps due to the absence of other cations. In studies where K^+ was tested with an appropriate decrease in Na^+ and where other sea water cations were balanced, K^+ was found to have a stiffening or a relaxing effect depending on the tissues tested (Table 5). Inconsistent responses were observed for the ophiuroid oral plate ligament which stiffens or softens in response to isosmotic KCl (Wilkie, 1984). The influence of K^+, especially at low concentrations, may not be due to a direct physico-chemical effect and at high concentrations the ion potentially exerts direct and indirect effects. The inconsistent effect of K^+ suggests that the K^+ response may be tissue-specific, perhaps involving cells controlling variable tensility.

Introvert mechanical properties were influenced by pH. Viscosity was lowest at pH 7·0 and increased sharply with increasing pH (8–12) and decreasing pH (pH 6–2). The ophiuroid intervertebral ligament and the echinoid catch apparatus were also

Table 5. *The effect of K^+ on the mechanical properties of echinoderm connective tissues*

Class and species	Tissue	$[K^+]$ (mol l^{-1})	Response	Reference
Crinoidea				
Antedon bifida (Pennant)	cirral ligament*	0·015–0·05	softening	Wilkie (1983)
Echinoidea				
Anthocidaris crassispina (A. Aggassiz)	catch apparatus*	0·1	hardening	Takahashi (1967)
Diadema setosum Leske	spine central ligament*	0·1	hardening	Motokawa (1983)
Holothuroidea				
Sclerodactyla briareus (Lesueur)	P-L tendon†	0·1	softening	Smith & Greenberg (1973)
Stichopus chloronotus Brandt	dermis*	0·05–0·1	hardening	Motokawa (1981)
Eupentacta quinquesemita (Sclenka)	introvert‡	0·075	no effect	present study

*KASW, †KCl, ‡KNaASW.

influenced by pH, but the viscosities and tensile strength of these tissues were lowest at pH 10 and 5 respectively (Wilkie, 1978; Hidaka, 1983). These different experimental results may reflect differences in tissue physiology, perhaps associated with matrix composition. They may also be influenced by the different test solutions employed. Wilkie (1978) also used a series of buffer solutions while Hidaka (1983) used buffered ASW where the presence of other sea water cations may have influenced the results. Altering pH may influence the mechanical properties of echinoderm connective tissues by changing the net surface charge of the matrix and thereby affect connective tissue ionic interactions, as suggested by Hidaka (1983). Although *in vitro* tests demonstrated that the introvert stiffens at low and high pH, the sensitivity of the tissue to pH change suggests that the mechanism of autotomy may involve an alteration of tissue pH.

The results of ion and pH experiments have been used to suggest that the mechanism of variable tensility involves ion or pH changes that alter electrostatic interactions within the connective tissue, thereby causing tensility change (Wilkie, 1979; Motokawa, 1981, 1982, 1983; Eylers, 1982; Hidaka, 1983). Large ionic or pH changes are unlikely *in vivo* and the results of experiments described here and elsewhere involving ion concentrations and pH values above or below physiological levels may be *in vitro* artifacts reflecting proteoglycan conformational changes. Although ion and pH change are potential mechanisms, their physiological role in variable tensility of echinoderm connective tissues has yet to be established.

Anaesthetic antagonism of the K^+ response has been taken as evidence to suggest that variable tensility is neurally controlled (Wilkie, 1978, 1983). The influence of anaesthetics on the introvert and P-L tendon was variable. $MgCl_2$ did not block K^+-induced softening, perhaps because Mg^{2+} is a muscle relaxant and it may have a limited effect on connective tissue, especially in competition with a high concentration of K^+ ions. $MgCl_2$ partially blocked the response of the ophiuroid intervertebral ligament to excess K^+ (Wilkie, 1978). Propylene phenoxetol inhibited the K^+ response as shown in other studies (Wilkie, 1978, 1983), but its mode of action has not been established and it may have exerted a direct stabilizing influence on the connective tissue.

In general, the mechanical properties of echinoderm connective tissues that exhibit variable tensility appear to be associated with changes in the matrix, not with collagen or muscle events. At present, the most likely mechanism of variable tensility is through an alteration of connective tissue ionic interactions, but how this is brought about is not known. There is evidence for neural control of evisceration autotomy and for the presence of an endogenous evisceration factor in *Eupentacta quinquesemita* and this evidence will be presented in a following report (M. Byrne, in preparation).

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