

## VISCOELASTICITY OF HOLOTHURIAN BODY WALL

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### SUMMARY

1. Stress-relaxation tests and creep tests were performed on the body-wall dermis of two sea cucumbers, *Actinopyga echinites* (Jäger) and *Holothuria leucospilota* Brandt.

2. These viscoelastic connective tissues had mechanical properties which agreed well with those of a four-element mechanical model composed of two Maxwell elements connected in parallel.

3. The elastic stiffness of the dermis of *Actinopyga* was 1.7 MPa and that of *Holothuria* was 0.42 MPa.

4. The viscosity of the dermis showed great variation of more than two orders.

5. Chemical stimulation with artificial sea water containing 100 mM potassium increased the viscosity but not elasticity.

6. The viscosity change is suggested to be caused by the change in weak (non-covalent) bonds between macromolecules which constitute the dermis.

### INTRODUCTION

Sea cucumbers have quite thick body walls that are composed mainly of connective tissue dermis. The dermis determines the mechanical properties of the body wall, and the 'stiffness' shows great variation from species to species. One of the stiffest is *Microthele nobilis* and one of the softest is *Stichopus variegatus*. The only quantitative description of the mechanical properties of the body wall of sea cucumbers is that for *Stichopus chloronotus* (Motokawa, 1982a). Further variation of 'stiffness' of the body wall is found in individual sea cucumbers, which are able to control the tone of the body. The change is rapid and reversible, and it is controlled neurally (Jordan, 1919; Motokawa, 1981, 1982a,b, 1984). As the body-wall dermis contains little or no muscle (Stott, Hepburn, Joffe & Heffron, 1974; Motokawa, 1982b), the 'stiffness' change has been attributed to a change in the mechanical properties of the extracellular components of the dermis (Motokawa, 1981; Eylers, 1982). The mechanism of the change is yet to be understood.

The present report compares the mechanical properties of the pliant body wall of *Holothuria leucospilota* and the rigid wall of *Actinopyga echinites*. This report also describes the mechanical properties of the body wall at different states of 'stiffness'.

Viscoelasticity was simulated using a mechanical model with four elements. The mechanical properties of such material cannot be simulated by the conventional Young's modulus alone. The differences in body-wall mechanical properties between species and between the state of tone in individual animals were clearly shown by comparison of model parameters.

## MATERIALS AND METHODS

### *Dermis sample*

Two species of sea cucumbers, *Actinopyga echinites* and *Holothuria leucospilota* were used. They belong to the same family Holothuriidae, order Aspidochirotida. Specimens were collected from the lagoon of Sesoko Island. All the experiments were performed on the island at the Marine Science Centre of the University of the Ryukyus. A dermal piece of dorsal interambulacrum was used, because this part contains no muscles (Motokawa, 1982*b*). The dermis was cut out and trimmed to a column whose long axis was parallel to the long axis of the animal. The dermis was stretched in the direction of the long axis in mechanical tests.

### *Stress-relaxation test*

A stress-relaxation test was performed to describe the mechanical properties of the dermis. One end of a dermis sample was attached to a holder on a platform of an experimental trough with cyanoacrylate glue. The trough was mounted on a steel plate, on which semiconductor strain gauges were attached. The small deflection of the strain gauge, which was proportional to the tension in the dermis, was converted into an electric signal through a bridge amplifier and recorded on a pen recorder. The other end of the dermis was glued to a holder which was connected to a beam. The size of the sample was measured to the nearest 0.1 mm using callipers. It was usually 2 mm thick, 2 mm wide and 6 mm long. The length of the sample between the sample holders was 2 mm. The sample never slipped out from the sample holders during the experiments. The beam could be displaced by a desired amount with a speed-controllable motor and that displacement could be held till the end of the experiment. Either artificial sea water (ASW) or artificial sea water containing 100 mM potassium (KASW) was introduced to the trough, and the preparation was rested for 1 h in the trough before the mechanical test.

The preparation was stretched with a strain rate of  $1.5 \text{ s}^{-1}$  to a strain of 0.5, and that strain was maintained. The strain was measured with a linear variable differential transformer which was attached to the beam. Because the stress-strain curve of the present sea cucumbers had a 'toe region', the strain of 0.5 was chosen, as it was in the linear region of stress-strain curves (Fig. 1). The strain of 0.5 may be larger than that employed in many stress-relaxation experiments, but is well within the working range of the material. The extended length of *Holothuria leucospilota* is more than three times the contracted length.

The stress-relaxation tests assume that the stretch is instantaneous. In the present study, the time needed to stretch the sample to the final strain was 0.33 s, which was one-fifth of the relaxation time of a smaller dashpot of *Holothuria* and was one-third

sixth of that of *Actinopyga* (see Results). Therefore the strain rate of  $1.5 \text{ s}^{-1}$  is fast enough. Such a fast stretch, however, may result in mechanical stimulation. Stott *et al.* (1974) reported that mechanical stimuli stiffened the body wall of a sea cucumber. In preliminary experiments, I employed the strain rate of  $1.5 \times 10^{-2} \text{ s}^{-1}$ . The results were not significantly different from those with a strain rate of  $1.5 \text{ s}^{-1}$ . A small (about 10%) decrease in the elastic stiffness was observed in the slower-strain-rate experiments. This difference will be explained by the stress relaxation during the stretch. A single stress-relaxation test was finished within 15 min, because many dermis completely relaxed in this period, and because mechanical properties could change spontaneously during a longer experimental period.

#### *Creep test*

A creep test was performed to show the effect of chemical stimulation upon the viscosity of a single dermis sample. A more slender sample (approx.  $0.5 \times 0.5 \times 7 \text{ mm}$ ) than that used in the stress-relaxation test was used so as to reduce the diffusion time of the chemical. One end of a sample was attached to a holder on a platform of an experimental trough. The other end was attached to a holder which was connected to a lever. The dermis was rested in ASW for 10 min, and a weight was applied to the other side of the lever. The resulting elongation of the sample was measured with a linear variable differential transformer and recorded on a pen recorder. KASW was introduced after the dermis had attained the constant rate of elongation.

#### *Mechanical model*

A four-element mechanical model (Fig. 4, inset B) was used to simulate the stress-relaxation behaviour of the dermis. The model consists of two Maxwell elements connected in parallel, each of which is composed of a spring and a dashpot connected in series (Fig. 4, inset A). Springs represent elasticity and dashpots viscosity. The stress  $\sigma(t)$  of the model at time  $t$  of stress relaxation is described as follows for a unit strain:

$$\sigma(t) = E_1 e^{-t/\tau_1} + E_2 e^{-t/\tau_2}$$

where  $E_1$  and  $E_2$  are the elastic moduli, and  $\tau_1$  and  $\tau_2$  ( $\tau_1 \leq \tau_2$ ) are the relaxation times of respective Maxwell elements (Goto, Hirai & Hanai, 1962).

$$\eta_1 = E_1 \tau_1, \eta_2 = E_2 \tau_2$$

where  $\eta_1$  and  $\eta_2$  are viscosities of respective Maxwell elements. The parameters of a model were determined as follows. Twelve points were selected from a stress-relaxation curve of a dermis. Among them eight points were selected with the same interval between time 0 and the time when the stress became 37% ( $1/e$ ) of the initial stress, and four points were selected with the same interval between the latter time and the time when the stress became 9% ( $1/4e$ ) of the initial stress (see Fig. 4). The method of least squares was used to determine the model parameters with the best fit. The square of the differences in stresses between the dermis and the model at corresponding time was calculated for twelve points and the values were summed. The model parameters were changed so as to make the sum less and the model with the

least sum was chosen as the model for that dermis. These procedures were done with the aid of an eight-bit microcomputer (PC 8801, NEC).

The analysis of the data obtained by creep tests was performed using another four element mechanical model, composed of a Maxwell element and a Voigt element connected in series (Fig. 6, inset). The Voigt element is composed of a spring and a dashpot connected in parallel. This model more readily described the creep behaviour than did the two-Maxwell element model. These two models are in fact theoretically equivalent and the parameters of this model can be described by those of the model used in stress-relaxation tests (Goto *et al.* 1962):

$$E_4 = E_1 + E_2$$

$$\eta_4 = \eta_1 + \eta_2$$

$$E_3 = E_1 E_2 (\eta_1 + \eta_2)^2 (E_1 + E_2) / (\eta_1 E_2 - \eta_2 E_1)^2$$

$$\eta_3 = \eta_1 \eta_2 (E_1 + E_2)^2 (\eta_1 + \eta_2) / (\eta_1 E_2 - \eta_2 E_1)^2$$

$$\tau_3 = \eta_3 / E_3$$

where  $E_4$  and  $\eta_4$  are the elastic modulus and the viscosity of the Maxwell element respectively, and  $E_3$ ,  $\eta_3$  and  $\tau_3$  are the elastic modulus, the viscosity and the retardation time of the Voigt element respectively (Fig. 6, inset). In creep tests the dermis was first elongated in ASW and then the medium was changed to KASW. The rate of elongation decreased in KASW (see Results). The change in the rate of elongation will be influenced by the change in all the four elements of the model. The effect of  $E_4$  is, however, instantaneous and that of a Voigt element (i.e.  $E_3$  and  $\eta_3$ ) is only transient. In the time of  $3 \times \tau_3$ , the effect of the Voigt element decreases to less than 10% of the effect at the moment of change. Therefore if we observe the change caused by KASW for a sufficiently long time (at least  $3 \times \tau_3 +$  diffusion time of the chemical + reaction time of the tissue), the elongation rate at that time is determined by  $\eta_4$  alone: the elongation rate is inversely proportional to  $\eta_4$ .  $\tau_3$  was 40 s for *Actinopyga* dermis and 9 s for *Holothuria* when they were calculated from the mean values in KASW given in Table 2. Therefore the decrease in elongation rate more than about 3 min after the application of KASW will correspond to the increase in the viscosity  $\eta_4$ . In this report the inverse of the elongation rate was plotted to show the change in  $\eta_4$ . The plotted values were normalized by the value just before the application of KASW.

The averages of model parameters are given in geometric means, because standard deviations were roughly proportional to arithmetic means (Snedecor & Cochran, 1967).

#### *Experimental solutions*

Composition of artificial sea water (ASW) was as follows (in mM): NaCl, 433.7; KCl, 10.0; CaCl<sub>2</sub>, 10.1; MgCl<sub>2</sub>, 52.5; NaHCO<sub>3</sub>, 2.5 (pH 8.1–8.2). In artificial sea water containing a high concentration of potassium (KASW), the potassium concentration was increased to 100 mM, decreasing sodium concentration to maintain the osmotic concentration constant. The temperature of the experimental solutions was 19.5–20.5 °C in the stress-relaxation experiments and 24.5–25.5 °C in the creep experiments.

## RESULTS

*Stress-strain curve*

Fig. 1 shows typical stress-strain curves of the dermis of *Actinopyga* and *Holothuria*. The curves have a toe region where the curve becomes concave. The stress-strain relation becomes linear when the strain exceeds 0.1–0.3. The slope of the linear region was defined as the elastic stiffness and was four times greater for *Actinopyga* than for *Holothuria* (Table 1).

*Stress-relaxation curve*

When stretched to 0.5 strain, the dermis developed a stress, which gradually decreased when the sample was held at that strain. The rate of relaxation greatly varied from sample to sample (Fig. 2). Some relaxed rapidly to zero stress whereas

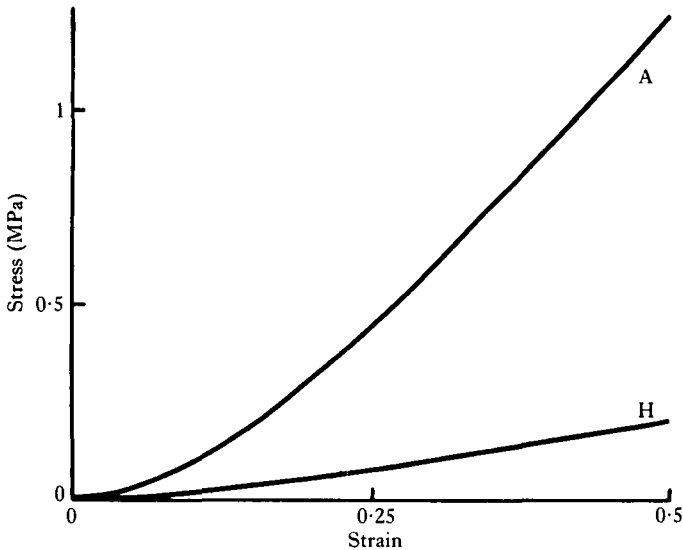


Fig. 1. Stress-strain curves of the dermis of *Actinopyga* (A) and of *Holothuria* (H) in artificial sea water.

Table 1. *Elastic stiffness and 'relaxation time' of sea cucumber dermis*

Solution	<i>Actinopyga</i>		<i>Holothuria</i>	
	Elastic stiffness (MPa)	'Relaxation time' (s)	Elastic stiffness (MPa)	'Relaxation time' (s)
ASW	1.67 (0.21, $N = 16$ )	80.3 (0.54, $N = 19$ )	0.42 (0.15, $N = 9$ )	15.3 (0.65, $N = 9$ )
KASW	2.08 (0.20, $N = 15$ )	295* (0.33, $N = 15$ )	0.59 (0.17, $N = 10$ )	34.9 (0.36, $N = 10$ )

Geometric mean (s.d. of logarithmically transformed values,  $N =$  number of experiments).

\* Significantly different from the mean in ASW by  $t$ -test ( $P = 0.001$ ).

ASW, artificial sea water; KASW, artificial sea water with a high potassium concentration.

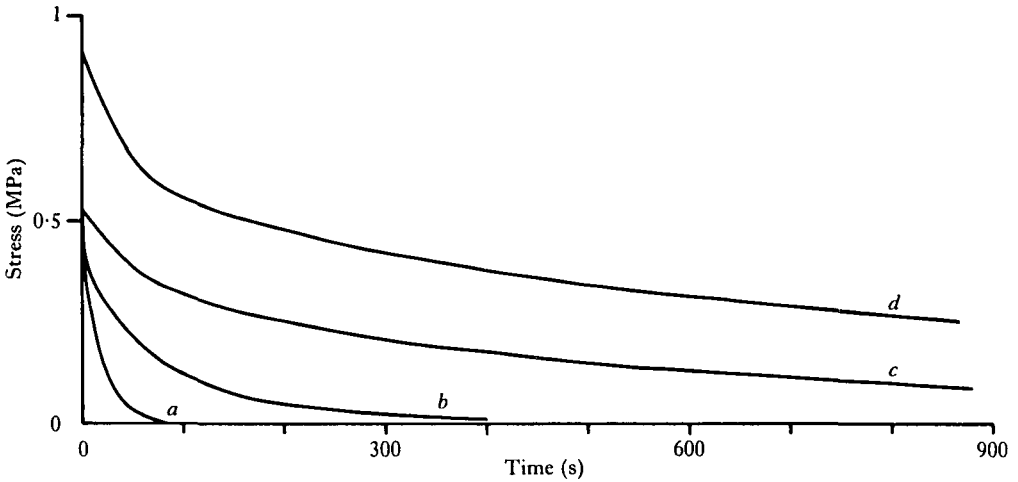


Fig. 2. Stress-relaxation curves of *Actinopyga* in artificial sea water. The dermis was stretched to 0.5 strain at time zero, and it was held at that length. Notice that the rate of relaxation varies greatly from dermis to dermis. Parameters of the model which corresponds to each curve are given in Table 2.

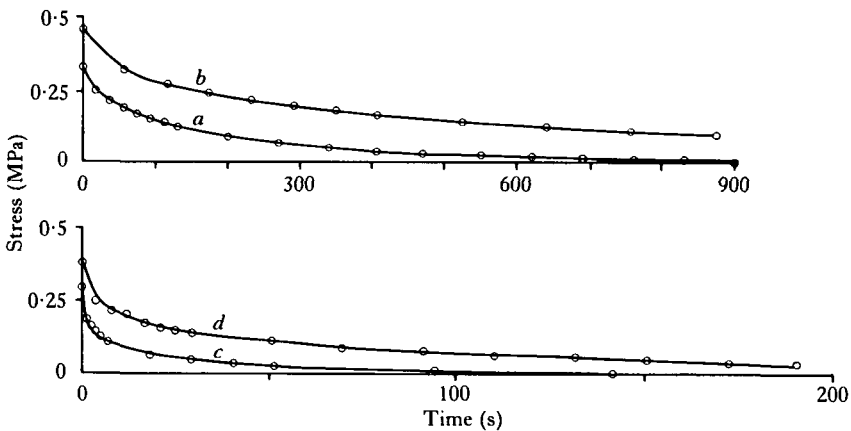


Fig. 3. Stress-relaxation curves of *Actinopyga* (*a*, in ASW; *b*, in KASW) and those of *Holothuria* (*c*, in ASW; *d*, in KASW) at 0.5 strain. These curves have median 'relaxation time' in respective experimental groups. The hollow circles are the stress of the dermis and continuous lines are the stress-relaxation curves of models. The parameters of models are given in Table 2.

others maintained various stresses at the end of experiments. The time when the stress falls to  $e^{-1}$  of the initial stress was taken as the measure of the rate of relaxation and called the 'relaxation time'. *Actinopyga* had a five- to nine-fold longer 'relaxation time' than *Holothuria* (Table 1, Fig. 3). In both species KASW increased the 'relaxation time' (Table 1, Fig. 3). The variation of 'relaxation time' in ASW was far greater than that of elastic stiffness (compare the standard deviations in Table 1). The former varied by two orders in both species. The elastic stiffness seemed to be greater as the 'relaxation time' increased. The former, however, increased by 0.15–0.18th power of the latter ( $r = 0.5$ ,  $N = 9-16$ ), thus the increase of elastic stiffness was small. There was no statistically significant difference between the means of elastic stiffness in ASW and in KASW.

Mechanical model

Because the stress of the dermis finally relaxed to zero (Figs 2, 3), the mechanical properties of a dermis can be described by a mechanical model which is composed of large numbers of Maxwell elements in parallel. It was found that a model consisting

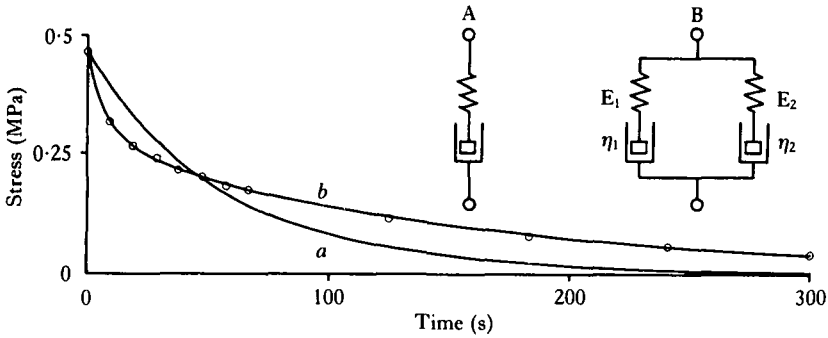


Fig. 4. A stress-relaxation curve of a two-element model and that of a four-element model at 0.5 strain. Twelve points (hollow circles) were selected from a stress-relaxation curve of a dermis of *Actinopyga* in ASW. The parameters of mechanical models were selected so as to minimize the sum of the squares of the difference in stresses between the dermis and the model at these points. A simple Maxwell model shown in inset A generated the best fit stress-relaxation curve *a*. A four-element model shown in inset B generated the best fit curve *b*. The stress-relaxation curve of the four-element model agrees well with that of the dermis. The model parameters of curve *b* are given in Table 2.

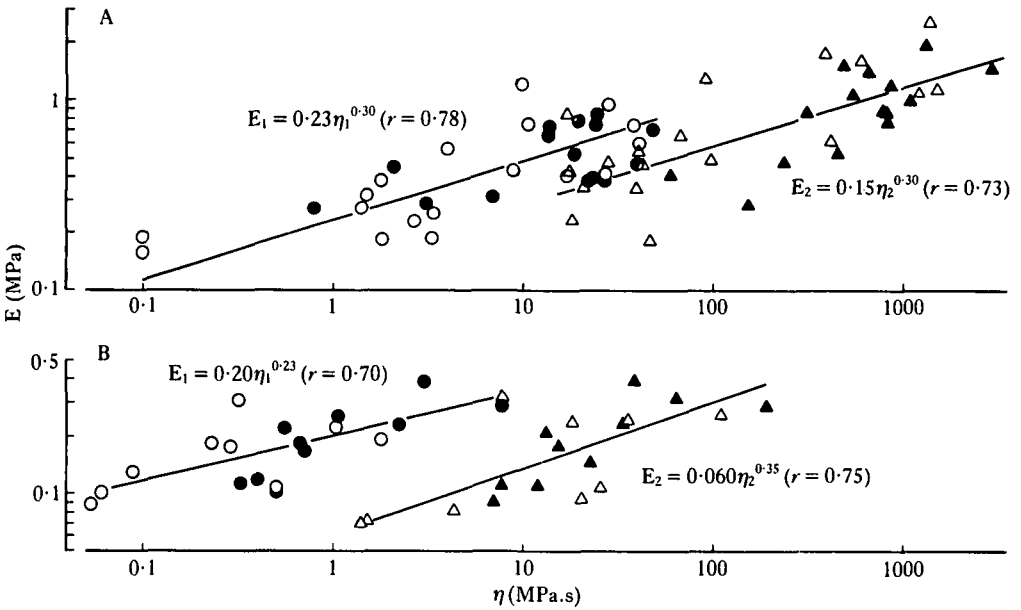


Fig. 5. Correlation between model parameters of *Actinopyga* (A) and *Holothuria* (B). Circles represent the correlation between  $E_1$  and  $\eta_1$ . Triangles represent the correlation between  $E_2$  and  $\eta_2$ . Open symbols are for the data in ASW and filled symbols are for those in KASW.

of a single Maxwell element was inadequate to describe the stress-relaxation behaviour of the dermis, whereas a four-element model containing two Maxwell elements in parallel was adequate for the two species in ASW and also in KASW (Figs 3, 4).

The model consists of two Maxwell elements. One element had lower viscosity: it was 2–7% that of the higher one. The elastic moduli of the two elements were about the same. These relations held for the models of two species in ASW and also in KASW. The absolute values of parameters, however, showed marked difference between species and between experimental solutions. The parameters of some models are given in Table 2. The relations between  $E_1$  and  $\eta_1$  and between  $E_2$  and  $\eta_2$  are shown in Fig. 5. As would be expected from the large variation in 'relaxation time' and relatively small variation in elastic stiffness, the viscosities ( $\eta_1$ ,  $\eta_2$ ) had larger variations than those of the elastic moduli ( $E_1$ ,  $E_2$ ). The range of viscosities in ASW was more than two orders whereas that of elastic moduli was about one order. Both  $E_1$  vs  $\eta_1$  and  $E_2$  vs  $\eta_2$  had positive correlations. Elastic modulus increased by 0.2–0.3th power of the viscosity. KASW increased viscosities. The distribution patterns of  $\eta_1$  and  $\eta_2$  of *Actinopyga* and  $\eta_1$  of *Holothuria* in KASW were significantly different from

Table 2. Parameters of models of sea cucumber dermis

Model number*	Elastic modulus		Viscosity	
	$E_1$ (0.1 MPa)	$E_2$ (0.1 MPa)	$\eta_1$ (0.1 MPa.s)	$\eta_2$ (10 MPa.s)
<i>Actinopyga</i> in ASW				
1 (2-a)	1.58	8.95	1.40	1.74
2 (4-b)	3.90	5.39	18.3	4.10
3 (2-b)	2.34	6.65	26.8	6.80
4† (3-a)	1.91	4.92	32.7	9.79
5 (2-c)	4.15	6.22	274	41.7
6 (2-d)	7.38	11.1	384	120
average‡	3.61 (0.32)	6.62 (0.31)	41.9 (0.77)	10.2 (0.70)
<i>Actinopyga</i> in KASW				
7	7.34	14.2	239	63.9
8† (3-b)	3.78	5.44	224	44.8
9	7.26	10.0	139	107
average‡	4.93 (0.17)	8.78 (0.23)	129 <sup>1</sup> (0.50)	53.4 <sup>2</sup> (0.41)
<i>Holothuria</i> in ASW				
10	0.85	0.70	0.53	0.14
11† (3-c)	3.04	2.80	3.14	0.77
12	1.76	2.34	2.29	1.81
average‡	1.51 (0.18)	1.36 (0.26)	2.59 (0.53)	1.11 (0.64)
<i>Holothuria</i> in KASW				
13	1.83	1.75	6.57	1.56
14† (3-d)	3.73	3.78	19.1	3.88
15	2.67	2.89	77.7	19.0
average‡	1.87 (0.19)	1.85 (0.21)	9.02 <sup>1</sup> (0.45)	2.35 (0.44)

\* The models whose stress-relaxation curves are shown in the figures are indicated in the brackets following the model number: a numeral corresponds to a figure number and a letter corresponds to that of each curve. The models with longer 'relaxation time' are placed nearer to the bottom in each group.

† The model with median 'relaxation time' in each experimental group.

‡ Geometric mean (s.d. of logarithmically transformed values);  $N = 18$  for *Actinopyga* in ASW, 15 for *Actinopyga* in KASW, 9 for *Holothuria* in ASW, and 10 for *Holothuria* in KASW.

<sup>1,2</sup> The mean in KASW is significantly different from that in ASW by  $t$ -test at the level of 5% and 1% respectively.



those in ASW by Mann-Whitney U test ( $P = 0.05$ ). Elastic moduli seemed to increase a little in KASW but the change was statistically insignificant.

The relaxation times of *Holothuria* were much shorter than those of *Actinopyga*. In ASW the averages of  $\tau_1$  and  $\tau_2$  of *Holothuria* were 1.7 s and 82 s respectively, whereas  $\tau_1$  and  $\tau_2$  of *Actinopyga* were 12 s and 160 s respectively. Because the viscosity increase was far larger than the elasticity increase in KASW, the relaxation time increased in KASW. The averages of  $\tau_1$  and  $\tau_2$  of *Holothuria* in KASW were 4.8 s and 140 s respectively, and  $\tau_1$  and  $\tau_2$  of *Actinopyga* were 26 s and 590 s respectively.  $\tau_1$  of *Holothuria* and  $\tau_2$  of *Actinopyga* in KASW were statistically different from those in ASW by *t*-test at the level of 5 and 0.1 % respectively.

### Creep test

Fig. 6 shows the extension curve of a dermis of *Holothuria* in ASW. At the application of a load, the dermis elongated instantaneously, and then elongated at a rapidly decreasing rate. Within 1 min the rate of elongation became constant. The dermis continued to elongate with the elongation rate, which increased little by little, to rupture. The creep behaviour of *Actinopyga* dermis was similar to that of *Holothuria*. This creep behaviour can be interpreted by the four-element mechanical model shown in Fig. 6 (inset). The instantaneous elongation is the elongation of the spring whose elastic modulus is  $E_4$ . The phase of rapidly decreasing rate of elongation corresponds to the retarded elongation of the Voigt element. The following phase of constant flow is the flow of the dashpot whose viscosity is  $\eta_4$ . As the dermis elongated the cross sectional area decreased and thus the stress in the dermis increased. For this reason the flow rate gradually increased as the dermis elongated.

KASW decreased the elongation rate reversibly, and thus increased  $\eta_4$  reversibly (Fig. 7). In an extreme case,  $\eta_4$  increased to more than 100 times that before application of KASW (Fig. 7B). The average values of  $\eta_4$  are given in Table 3.

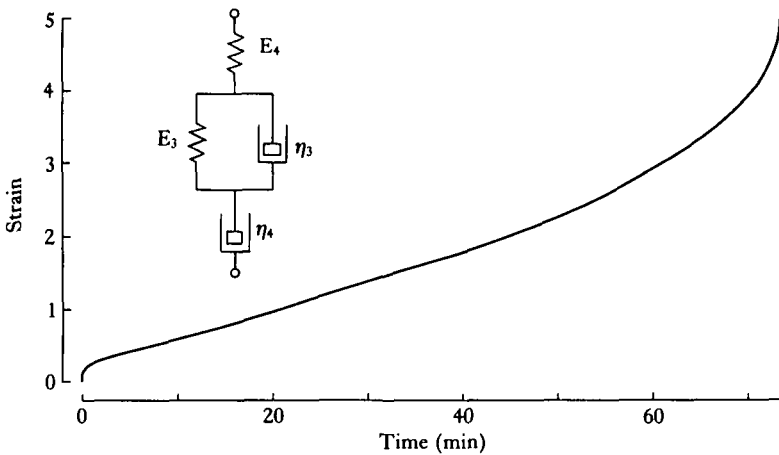


Fig. 6. The elongation curve (creep curve) of the dermis of *Holothuria* under a constant load. A load was applied at time zero. The stress at time zero was 56 KPa. The dermis continued to elongate and ruptured at the end of the curve. A four-element mechanical model, which can simulate the creep behaviour of the dermis, is shown in the inset.

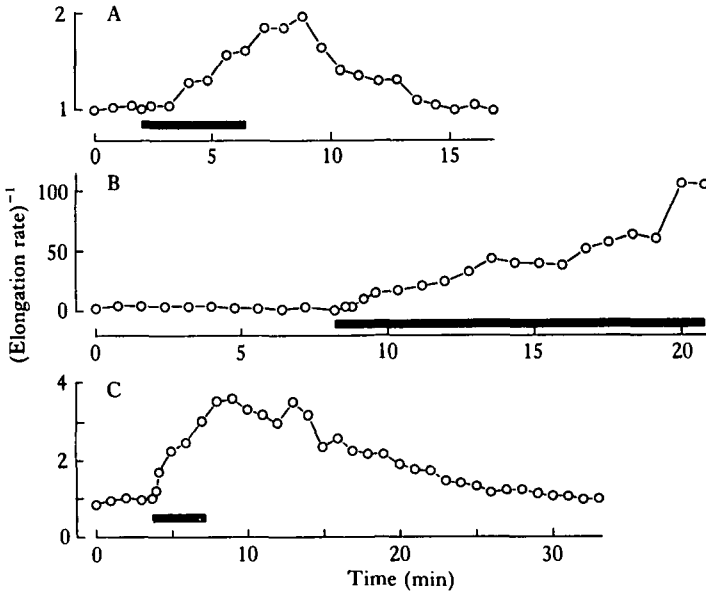


Fig. 7. Effect of KASW on the viscosity  $\eta_4$  of *Actinopyga* dermis (A, B) and of *Holothuria* dermis (C). The ordinate is the inverse of elongation rate which is normalized by the value at the application of KASW. This parameter is a measure of  $\eta_4$ . The bars indicate the periods of application of KASW.

Table 3. Viscosity  $\eta_4$  (in MPa.s) of the dermis of sea cucumber body walls

Species	Average	Minimum	Maximum
<i>Stichopus chloronotus</i>	0.047	0.012	1.7
<i>Stichopus japonicus</i>	1.1	0.076	35
<i>Holothuria leucospilota</i>	11	1.6	200
<i>Actinopyga echinites</i>	100	17	2900

The averages are the geometric means of the viscosity measured in ASW. The minima and maxima are those of the data in which measurements in ASW and in KASW are pooled. Minima are found in ASW samples. Maxima are found in KASW samples except that of *S. japonicus*. The data of *S. chloronotus* and *S. japonicus* are obtained from Motokawa (1982a) and (1984) respectively. The data of *H. leucospilota* and *A. echinites* are calculated from the data obtained from stress-relaxation experiments.

#### DISCUSSION

The body wall of *Actinopyga* is far stiffer than that of *Holothuria*, which is in the same family. *A. echinites* is found exposed on the shore where waves break, whereas *H. leucospilota* is found in a calm moat of lagoons. The difference in the mechanical properties may represent adaptation to the environment. *A. echinites* changes the body shape little, whereas *H. leucospilota* is usually found partially hidden under coral rocks: it extends the front part of the body out from the rock, but when disturbed, it rapidly retracts. The animal sometimes shortens to less than one-third of the extended length. The short relaxation time, together with the thinner body wall compared with *A. echinites*, will make possible this large and rapid deformation, because the short relaxation time implies that the component molecules of the body wall can easily slip to rearrange in a short time.

There will be other factors which influence the mechanical properties of body walls. *Stichopus chloronotus* has both lower elastic stiffness (Motokawa, 1982a) and viscosity (Table 3). Yet it is found exposed in the same location as *A. echinites*. *S. chloronotus* deforms the body, but less rapidly and by a smaller amount than does *H. leucospilota*. Critical factors which have influenced the evolution of the mechanical properties of the body wall may be different in each species of sea cucumber. The sea cucumber body wall is a composite material in which collagen fibres are embedded in a proteoglycan matrix (Tanikawa, 1955). The mechanical properties of composite materials are determined by the arrangement, the relative amount and the chemistry of the components (Vincent, 1982). Both morphological and chemical studies are needed to understand the basis of the difference in mechanical properties of the dermis of these sea cucumbers.

There was a change in mechanical properties of the dermis of sea cucumbers in response to KASW. The main change was an increase in viscosity. The change in elasticity was small. The viscosity increase was clearly shown in creep tests. In stress-relaxation tests, however, the extreme scatter of the viscosity values, which were distributed over more than two orders in ASW, made the effect of KASW not so clear. The wide distribution of the viscosity values may be a reflection of the ability of the dermis to change the viscosity. In ASW the dermis may be either in a relaxed state, in an intermediate state or in a catch state. The full catch state will have 100 times larger viscosity than that of the fully relaxed state. KASW may stimulate the relaxed dermis to increase the viscosity. If this idea is correct, KASW will increase the viscosity by 100 times when applied to a fully relaxed dermis. The 100-fold increase in viscosity was actually observed in some creep tests (Fig. 7B). The variations of viscosity by two orders are also found in other sea cucumbers (Table 3). Therefore it is quite likely that the sea cucumbers change the viscosity of the dermis by as much as 100 times and thus control the body tone.

Several examples of a reversible change in mechanical properties of extracellular materials have been reported. The blood-sucking bug *Rhodnius* softens the abdominal cuticle when the bug sucks blood (Reynolds, 1975). The uterine cervix of mammals softens at parturition (Hollingsworth, 1981). Echinoderms employ the 'stiffness-adjustable' connective tissue for controlling the tone of the body. The tone control by connective tissues has been called connective tissue catch (Rüegg, 1971). Spines of sea urchins (Takahashi, 1967; Motokawa, 1983) and of starfish (Motokawa, 1982c) are held at the spine joint with catch connective tissues. The flexibility of the cirri of crinoids (Wilkie, 1983) and the tensile strength of the arms of ophiuroids (Wilkie, 1978) are controlled by the catch connective tissues. How sea cucumbers use the catch connective tissue of the body wall was discussed by Motokawa (1981).

The mechanical properties of holothurian dermis could be simulated by a four-element mechanical model. This does not imply, however, that the dermis has four structures which correspond to either springs or dashpots. Many materials of quite different chemical compositions and structures can be simulated well by four-element models (Goto *et al.* 1962). The mechanical properties of such materials are described more precisely by models with more elements, or exactly by relaxation spectra. The merit of using simple models is that they visualize the mechanical properties with only a few parameters. This will be a help in understanding the molecular mechanism of

the change in the mechanical properties of the holothurian dermis. The dermis of sea cucumbers is a composite material which is composed of macromolecules such as collagens and proteoglycans. The stress-relaxation of macromolecules has several mechanisms which have their own relaxation times (Goto *et al.* 1962). The relaxation times  $\tau_1$  and  $\tau_2$  of the present study were of the order of 1–100 s. The relaxation time in this range will be caused by the translational movement of the macromolecules. The viscosity is determined by the resistance to this translation, or in other words, by the strength and the number of the non-covalent bonds between the molecules. The dermis is a polymer blend in which various polymer molecules interact with one another, and thus have various relaxation times.  $\tau_1$  and  $\tau_2$  will be the results of summation of these various relaxation times.

KASW increased the viscosity of the dermis. This means that the interactions between macromolecules become stronger. The interactions which are candidates for the change are those between proteoglycan chains and those between proteoglycans and collagens. As these molecules are polyelectrolytes, the interacting forces may be expected to be influenced by the ionic environment (Nagasawa, 1978). The viscosity of the dermis of a sea cucumber has been shown to be influenced by concentrations of some ions in the bathing medium (Eylers, 1982). The effect of ionic environment on the mechanical properties of catch connective tissues was studied in detail in the catch apparatus of the sea-urchin spine joint (Hidaka, 1983). This tissue changes mechanical properties under nervous control (Maeda, 1978). Hidaka's conclusion is that the change in mechanical properties of the catch apparatus is caused by the viscosity change. The most likely mechanism of the viscosity change is that a change in the ionic environment (either  $H^+$  or  $Ca^{2+}$  concentration) in the tissue occurs, thus changing the interactions of the polyelectrolytes. The intervertebral ligament of a brittlestar, which is another catch connective tissue, is also sensitive to the ionic environment (Wilkie, 1978). The rapid change in mechanical properties of catch connective tissues of echinoderms seems to be controlled through a change in the ionic environment in the connective tissues. Enzymatic digestion of proteoglycans, however, has been proposed for the softening mechanism of a sea cucumber body wall (Junqueira *et al.* 1980). The molecular mechanism of connective tissue catch in sea cucumber body wall is yet to be understood.

Although some ions may work directly on the interactions of macromolecules, KASW is likely to work indirectly *via* stimulating nerves. There are some observations which suggest nervous control of the mechanical properties of the holothurian body wall. Photic stimulation increases the viscosity of the dermis by stimulating 'photoreceptors' in the epidermis (T. Motokawa, in preparation). Acetylcholine changes the viscosity of the dermis (Motokawa, 1981, 1984). Possible neurosecretory cell processes are found abundantly in the dermis (Motokawa, 1982*b*). As menthol anaesthesia suppressed the effect of KASW (unpublished), KASW is most probably stimulating the nerves which are controlling the mechanical properties of the dermis.

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