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MECHANICAL DIVERSITY OF CONNECTIVE TISSUE OF THE BODY WALL OF SEA ANEMONES

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SUMMARY

Techniques for analysing polymer mechanics were used to describe quantitatively the time-dependent mechanical properties of the body-wall connective tissue (mesogloea) and to indicate macromolecular mechanisms responsible for the mechanical behaviour of two species of sea anemones, *Metridium senile* and *Anthopleura xanthogrammica*.

1. The mesogloea of *M. senile* is more extensible and less resilient than that of *A. xanthogrammica* when stressed for periods comparable to the duration of flow forces the anemones encounter and the postural changes they perform.

2. Polarized light microscopy and SEM reveal that the reinforcing collagen fibres in the mesogloea are aligned parallel with the major stress axes in the body wall.

3. Mechanical tests and observations of composition and microstructure indicate that the mesogloea of *A. xanthogrammica* is less extensible than that of *M. senile* because molecular entanglements (due to more closely packed parallel collagen fibres and to a higher concentration of polymers in the interfibrillar matrix) retard the extension of *A. xanthogrammica* mesogloea.

This study illustrates how structural features on the macromolecular and microscopic levels of organization of an organism can equip that organism for the particular mechanical activities it performs and the environmental forces it encounters.

INTRODUCTION

The passive mechanical response of an organism to an imposed load depends upon both the shape of the organism and the mechanical properties of the materials composing its body (cf. Koehl, 1977*b*). The mechanical behaviour of a material in turn depends upon the molecular and microscopic structure of that material. I would therefore expect to find structural features on the molecular and microscopic levels of organization of an organism which equip that organism for the particular mechanical activities it performs and the environmental forces it encounters.

The connective tissues of the body walls of hydrostatically supported organisms are an obvious place to look for examples of molecular and microscopic features which suit tissues to the particular mechanical life-styles of organisms. There are species of

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hydrostatically supported sea anemones representing different extremes in mechanical behaviour. *Metridium senile fimbriatum* (Verrill) are calm-water anemones (Koehl, 1976, 1977*a*) that are capable of changing their body dimensions considerably (Batham & Pantin, 1950), and *Anthopleura xanthogrammica* (Brandt) are rough-water anemones that do not undergo extreme shape and size changes (Koehl, 1976, 1977*a*). I chose to investigate two questions concerning the body-wall material of these species of anemones: (1) in what ways do the mechanical properties of body-wall materials relate to the mechanical behaviour of these anemones? and (2) what macromolecular and microscopic features of the body-wall materials of these anemones are responsible for differences in their mechanical behaviour?

Structure and composition of anemone body wall

The body wall of sea anemones is a thick layer of connective tissue, mesogloea, sandwiched between single layers of ectodermal cells and endodermal epithelio-muscle cells (Stephenson, 1935). Chapman (1953*b*) observed that the passive mechanical properties of the body wall of *M. senile* and *Calliactis parasitica* were similar to that of mesogloea from which the muscle fibres had been removed.

Mesogloea is a composite material consisting of fibres contained in an amorphous matrix. Mesogloeal fibres have been judged to be collagen on the basis of ultrastructure as well as various chemical and physical properties for several species of sea anemones: *M. senile* (Chapman, 1953*a*; Grimstone *et al.* 1958; Gross, Dunsha & Glazer, 1958; Piez & Gross, 1959; Pikkarainen *et al.* 1968; Gosline, 1971*a*), *M. dianthus* (Katzman & Kang, 1972), *M. canum* (Batham, 1960), *Metridium* sp. (Kulonen & Pikkarainen, 1970), *C. parasitica* (Chapman, 1953*a*), *Aiptasia* sp. (Gosline & Lenhoff, 1968), and *Actinia equina* (Nordwig & Hayduk, 1969; Nordwig, Hieber-Rogall & Hayduk, 1970; Nordwig, Nowack & Hieber-Rogall, 1973). Elastin-like fibres have not been found in the mesogloeas of Anthozoans, including the anemone *A. equina* (Elder, 1973). The matrix surrounding the collagen fibres has been found to contain highly hydrated protein and polysaccharide polymers in two species of anemones: *M. dianthus* (Katzman & Jeanloz, 1970*a*, *b*; Katzman & Oronsky, 1971; Katzman, 1972; Katzman *et al.* 1972; Katzman & Kang, 1972), and *M. senile* (Gross *et al.* 1958; Gosline, 1971*a*, *b*). Some of the polysaccharides appear to be covalently bound to the collagen in *M. dianthus* (Katzman & Jeanloz, 1970*a*; Katzman & Oronsky, 1971). The polysaccharide chains in most collagenous connective tissues are polyelectrolytes having a high density of negative charges; because charged segments of such polysaccharide chains repel each other, the chains assume extended conformations (Schubert & Hammerman, 1968). Sea anemone mesogloea is unique in that it contains neutral rather than charged polysaccharides (Gross *et al.* 1958; Katzman & Jeanloz, 1970*b*; Gosline, 1971*a*). Gosline (1971*b*) suggests that the neutral polysaccharide chains in mesogloea take on a more nearly random-coil conformation than can charged chains, and that electrostatic binding between matrix molecules and between matrix and collagen molecules is limited by the dearth of charged polysaccharides in mesogloea.

Mechanical properties of mesogloea

Various measurements of the mechanical properties of sea anemone mesogloea indicate that they are time-dependent (Chapman, 1953*b*; Alexander, 1962; Gosline, 1971*b*; Koehl, 1977*b*) (i.e. mesogloea's response to a load depends not only on the magnitude of the force, but also on the rate, duration, and past history of force application to the mesogloea). Alexander (1962) found that *M. senile* mesogloea could be stretched uniaxially to three times its original length by a small stress (force per cross-sectional area) applied for periods of 10–20 h, and that mesogloea would nearly return to its original unstretched length over the same time period after the stress was removed. Such time-dependent large-deformation elastic behaviour is typical of networks of random-coil polymer molecules (Treolar, 1970; Ferry, 1970; Aklonis, MacKnight & Shen, 1972; Wainwright *et al.* 1976). Collagen fibres are crystalline in structure and are relatively inextensible (they can only be stretched elastically by 4% of their resting length and break at extensions of 8%) (Wainwright *et al.* 1976). Gosline (1971*b*) therefore suggested that the extensibility and elasticity of *M. senile* mesogloea is due to the random-coil polymers in the matrix rather than to the collagen fibres.

On the basis of mechanical, chemical, and structural evidence, Gosline (1971*b*) proposed that the mesogloea of *M. senile* is a pliant composite material with a discontinuous fibre system of collagen in a highly hydrated protein-polysaccharide matrix. The matrix of mesogloea is a dilute network of random-coil polymers lightly cross-linked to each other and to the collagen fibres. This viscoelastic matrix is responsible for the ability of *M. senile* mesogloea to be extended slowly by as much as 200% and to recover slowly by configurational entropy elasticity (explained in Treolar, 1970). The collagen fibres, which are not cross-linked to each other, act to reinforce the matrix. As the mesogloea is stretched, the collagen fibres tend to align with the stress axis and slide past each other and in so doing, they deform the matrix molecules between them. The closer the collagen fibres are to each other and the closer they are to being parallel with the stress axis, the greater is the rate of deformation of matrix molecules between them for a given extension rate of the mesogloea. The greater the rate of deformation of the viscoelastic matrix, the more rigid it will be.

Collagenous connective tissues vary greatly in their mechanical properties (Viidik, 1972) in ways which can often be correlated with their mode of functioning in an animal in its environment. I applied theories and techniques of polymer mechanics (discussed in Ferry (1970), Aklonis *et al.* (1972) and Wainwright *et al.* (1976) (1) to describe quantitatively the mechanical properties of the mesogloea of two species of sea anemones in relation to their diverse activities and habitats, and (2) to indicate ways in which microscopic and molecular structures of these collagenous connective tissues might vary that would result in different mechanical properties.

MATERIALS AND METHODS

Mechanical testing

Strips of body wall for mechanical testing were cut from *M. senile* and *A. xanthogrammica* which had been collected and maintained in aquaria as described by Koehl (1977*b*). To measure the passive mechanical properties of anemone body wall without the complication of contraction by the circular and longitudinal muscles of the anemone column, I trimmed away the mesenteries from a strip and then narcotized the remaining muscles by soaking the strip in a solution of 20% $MgSO_4.7H_2O$ one to one with Instant Ocean (33‰) (Pantin, 1964) or in a 0·3% solution of propylene phenoxitol in Instant Ocean. A strip was judged to be anaesthetized if it did not visibly contract when pinched with forceps and trimmed with a razor blade. Gentamicin (50 $\mu g.ml^{-1}$) was added to the $MgSO_4$ anaesthetic and to the Instant Ocean for the propylene phenoxitol anaesthetic and these solutions were then autoclaved in order to minimize bacterial decay of the body-wall strips. The strips were gripped in the various testing devices as described by Koehl (1977*b*). The length between grips, the width, and the thickness of the body wall strips were measured to the nearest 0·1 mm before each test using vernier callipers.

The compliance (D, an index of extensibility) of a material is defined as the extension produced by a given stress (σ, force per cross-sectional area) applied for a given period of time (t),

$$D(t) = \frac{\Delta L(t)/L_0}{\sigma}, \tag{1}$$

where $\Delta L(t)$ is the increase in length of the specimen at time t, and L_0 is the specimen's original length. The time-dependent compliances of strips of mesogloea were determined by conducting creep tests (constant stresses were applied to specimens and their extensions were measured through time) using the device diagrammed in Fig. 1. Extension was measured by a linear variable differential transformer (LVDT Model 1000 HR DC, Schaevitz Engineering, standard error, S.E. = ±0·25%) and recorded on a chart recorder (Gould Brush 220). Body-wall strips subjected to creep tests were bathed in one of the solutions described above and the temperature was regulated by placing the creep machine in thermostatically controlled rooms (Tyler).

Dynamic stress-strain tests were made using a non-resonance, forced-vibration instrument (Fig. 2) constructed by J. Gosline. A specimen was sinusoidally pulled by a vibrator (Ling Dynamic Systems, Type 203) at frequencies from 0·1 to 30 Hz. Extension of the specimen (±0·05 μm) was measured by a LVDT (Model 100 MHR, Schaevitz Engineering), and force (S.E. = ±2%) was measured by a BLH silicon strain gauge mounted on beam A. Signals from the LVDT and the strain gauge were amplified by carrier amplifiers (SE Laboratories, Type 4300). The amplitude and phase of the force and extension signals were then monitored by a transfer function analyser (SE Laboratories, Model SM 272DP). Specimens were immersed in a controlled-temperature bath (LAUDA K-2/RD, Brinkman Instruments) of $MgSO_4$ anaesthetic. The frequency-dependent storage modulus (E') is the elastic modulus (an index of rigidity) of a material in phase with the stress,

$$E' = E^* \cos\delta, \tag{2}$$

where E^* is the ratio of the maximum stress (σ) to the maximum extension ($\Delta L/L_0$) achieved during a cycle of stretching, and δ is the angular phase shift between stress and extension. The tangent of δ in a dynamic test is a measure of the viscous energy loss in a cycle of deformation.

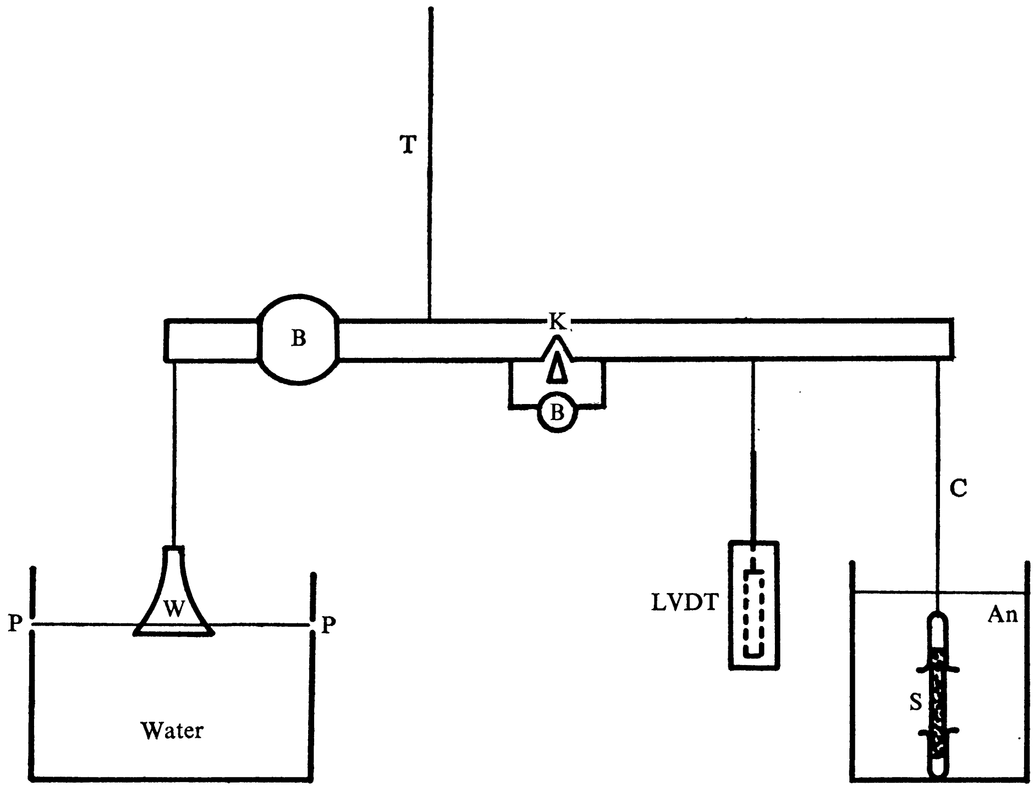

Fig. 1. The creep machine. When the thread (T) was burned, the specimen (S) was subjected to a tensile load by the weight (W). As the specimen stretched, the weight sank into water; the buoyant force of the water on the weight reduced the load on the specimen. The shape of the weight was calculated so that stress on the specimen was kept constant as the specimen extended and its cross-sectional area decreased (Andrade, 1910; Dahlquist, Hendricks & Taylor, 1950). I ran control creep tests on pieces of steel to measure extension of the creep machine itself; machine extension was then subtracted from extensions measured for mesogloea. LVDT = linear variable differential transformer; An = anaesthetic solution; B = clay weights to balance the lever; K = knife edge; L = lever; C = chain; P = overflow ports.

Structure and composition of body-wall connective tissue

Cryostat sections (Cryo-Cut Cryostat Microtome, Model 830-C) were made of fresh anaesthetized pieces of body wall from *A. xanthogrammica* and *M. senile.* These sections were immediately mounted on microscope slides in sea water and observed using a compound polarizing microscope (Wild M-21) with a first-order red compensator to indicate positive and negative birefringence.

Other anaesthetized pieces of body wall were fixed in Baker's formaldehyde-calcium fixative for marine invertebrates (Pantin, 1964), embedded in paraffin according to the procedure outlined by Grimstone & Skaer (1972), sectioned using a 820 Spencer microtome, and stained using the various techniques listed in Table 1.

Pieces of anaesthetized anemone body wall were fixed in 2% glutaraldehyde in Instant Ocean and prepared for SEM according to the procedure outlined by Mariscal (1974) for sea anemones. Some specimens were dried using a critical point drying system (Sorvall 49300) and others using a manifold freeze-dryer (Virtis Model 10-117-A). The lengths, widths, and thicknesses of the specimens were measured with

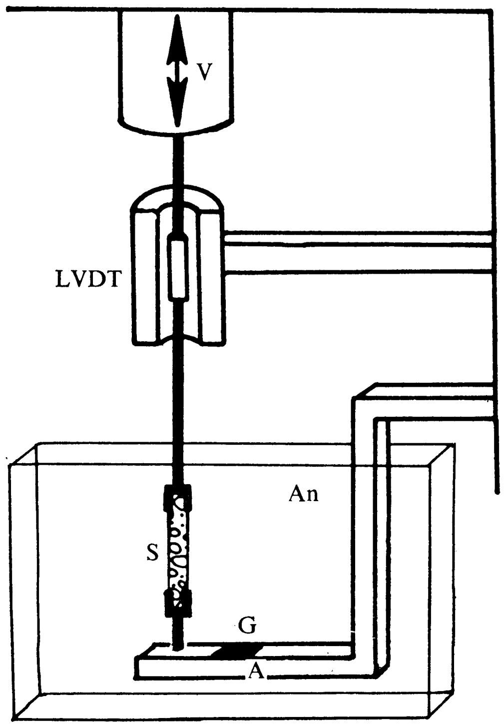

Fig. 2. The dynamic testing machine. V = vibrator; LVDT = linear variable differential transformer; S = specimen; A = force-measuring beam; G = strain gauge; An = anaesthetic in controlled-temperature bath. The double-headed arrow indicates the axis of stress application.

vernier callipers to the nearest 0·1 mm after each step in preparation to determine that tissue shrinkage was not significantly anisotropic. Dried specimens were cut along various axes using disposable histo-knives, were shadowed with gold-palladium, and observed using a JEOL JSM–SM Scanning Electron Microsope. The orientations of fibres (selected using a random number table) were measured to the nearest 1° using a protractor on scanning electron micrographs of tangential sections of body wall from *A. xanthogrammica* and *M. senile*.

Wet and salt-free dry weights, and dry weights of autoclave-soluble material were made according to the procedures outlined by Gosline (1971 *a*) for pieces of mesogloea from which the epithelium and muscle had been removed.

RESULTS AND DISCUSSION

Creep behaviour of mesogloea and its biological significance

The hydrostatic skeleton of a sea anemone is basically a tension-resisting container (the body wall) filled with compression-resisting fluid under pressure. Sea anemone mesogloea is subjected mainly to tensile stresses of three origins: (1) muscular contractions, which increase the pressure of the water in the coelenteron and, hence, subject the mesogloea to tensile stresses, (2) flow forces, which tend to bend the anemone and produce tensile stresses in the mesogloea (Koehl, 1977 *b*) and (3) ciliary pumping by the siphonoglyphs, which inflates the animal with water and thus subjects the

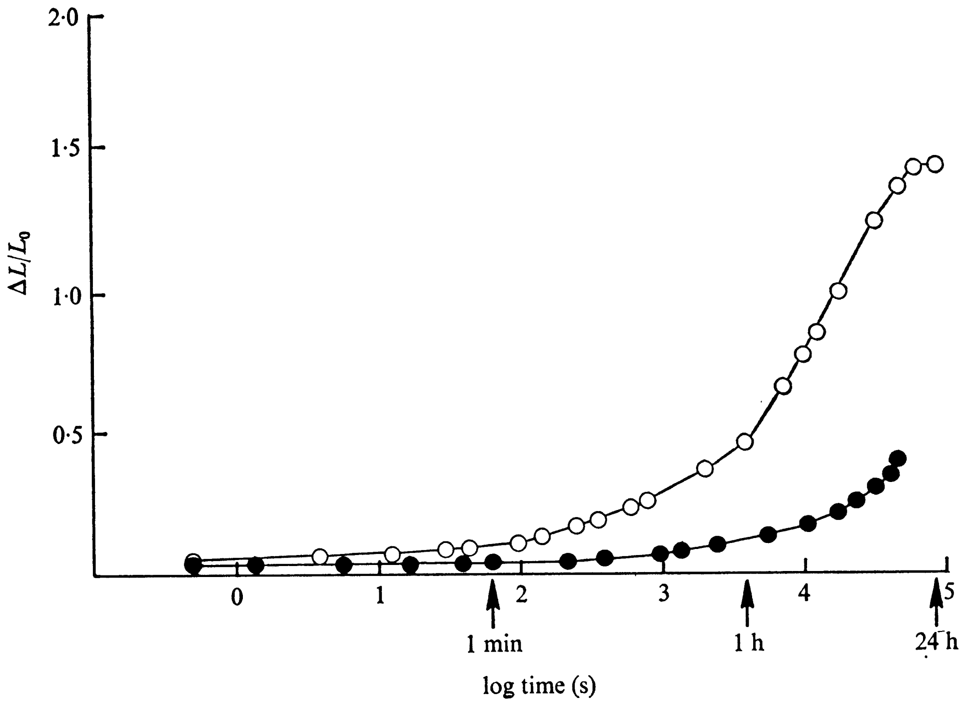

Fig. 3. Typical graphs of the extension ($\Delta L/L_0$) against log time (in seconds) for mesogloea from *M. senile* (○) and from *A. xanthogrammica* (●). These specimens were tested along their longitudinal axes at 6 °C with stresses on the order of 3000 N.m^{-2}.

mesogloea to tensile stresses. Tensile creep tests can be used to simulate these stresses in mesogloea so that the response of the mesogloea can be measured.

A typical creep curve for *M. senile* mesogloea is shown in Fig. 3. Note that *M. senile* mesogloea extends by a small amount (about 2–5 % of resting length) when stressed for short times (less than a min). If a stress is applied to *M. senile* mesogloea for an hour, it extends by about 50 %. When *M. senile* mesogloea has extended by 150 % or more after 12–24 h of being stressed, it reaches an equilibrium length beyond which it does not extend. (Three of the eight specimens of *M. senile* mesogloea did not reach an equlibrium length after 24 h (like the 'Type II' results of Alexander (1962)), but had local areas where the tissue had yielded and undergone plastic flow.

M. senile mesogloea is in essence two different materials which depend on the time scale of stress application as follows. (1) When stressed for short times, as it would be during muscular contractions involved with rapid shape changes, *M. senile* mesogloea is fairly rigid. This rigidity allows the animal to bend, straighten, sway, etc., without just ballooning out its body wall in areas where muscles are not contracting. Alexander (1962) and Gosline (1971*b*) have suggested that the rigidity of *M. senile* mesogloea during short periods of stress application allows the animal to resist waves. I tend to disagree with this interpretation because *M. senile* do not usually encounter waves (Koehl, 1976, 1977*a*), and because they would undergo local buckling if they were subjected to a wave (Koehl, 1977*b*). (2) When stressed for periods of several hours to a day, as by the tidal currents which cause bending (Koehl, 1976, 1977*a*) and by muscular contractions and ciliary pumping which produce the extreme changes in a *M.*

senile's shape and size, the mesogloea is very extensible and thus requires small forces to accomplish large movements.

A typical creep curve for 17 specimens of *A. xanthogrammica* mesogloea is shown in Fig. 3. The mesogloea of this species is fairly rigid when stressed for short times, but (unlike *M. senile* mesogloea) remains fairly rigid even after being stressed for several hours. The mean compliance ($D(t)$) of pieces of *A. xanthogrammica* mesogloea is not significantly different from that of pieces of *M. senile* mesogloea subjected to the same stress for 0·5 s ($F_{[1, 4]} = 0{\cdot}26$, $0{\cdot}50 < P < 0{\cdot}75$), but is significantly lower than that of pieces of *M. senile* mesogloea subjected to the same stress for 1 min ($F_{[1, 8]} = 7{\cdot}99$, $0{\cdot}01 < P < 0{\cdot}025$) or longer, for example, 9 h ($F_{[1, 8]} = 10{\cdot}42$, $0{\cdot}01 < P < 0{\cdot}025$) (see Figs. 9–11). This low compliance of *A. xanthogrammica* mesogloea for long times is consistent with the observation that an *A. xanthogrammica* does not undergo extreme shape and size changes and is not noticeably deformed after hours of exposure to wave action.

Short-term creep and recovery tests on *A. xanthogrammica* mesogloea using stresses and periods to simulate wave action (Koehl, 1977*a*, *b*) showed that the material stretches by about 3% of its resting length and returns to its original length before the 'next wave hits' (Fig. 4A). This complete recovery occurs even at stresses of as much as an order of magnitude greater (Fig. 4B, C) than those which are produced by non-storm waves. In contrast, *M. senile* mesogloea subjected to similar short-term creep and recovery tests does not recover completely to resting shape before the 'next wave hits' (Fig. 4D).

The time-dependent response to stress of each species' mesogloea can thus be related to the behaviour of whole anemones when subjected to environmental forces and when performing mechanical activities.

Microscopic structure of sea anemone body wall: fibre reinforcement of hydrostatic skeletons

The body wall of *A. xanthogrammica* reacts in the same way as that of *M. senile* to a variety of histological stains for collagenous connective tissues (Table 1) which suggests that its basic composition is similar to that of anemones (including *M. senile*) for which detailed chemical analyses have been performed (i.e. collagen fibres in a highly hydrated protein-polysaccharide matrix).

Both *M. senile* and *A. xanthogrammica* have definite patterns in the arrangement of fibres in their body walls. As noted by Gosline (1971*a*), polarized light microscopy reveals two distinct layers in *M. senile* body wall mesogloea: (1) an inner layer of densely packed parallel fibres oriented circumferentially, and (2) an outer layer of fibres arranged in a three-dimensional feltwork. *A. xanthogrammica* mesogloea does not appear, in polarized light, to have these two distinct layers, but rather to be composed of a feltwork of swatches of fibres running parallel to each other (Fig. 6B). It appears that more of these tracts of fibres towards the inner surface of the body wall are oriented circumferentially than are those towards the outer surface.

The observation that the fibres in the inner layer of mesogloea are more circumferentially oriented than those in the outer layer are corroborated by SEMs of *M. senile* (Fig. 5A, B) and *A. xanthogrammica* (Fig. 5C, D) body walls. The mean fibre angle

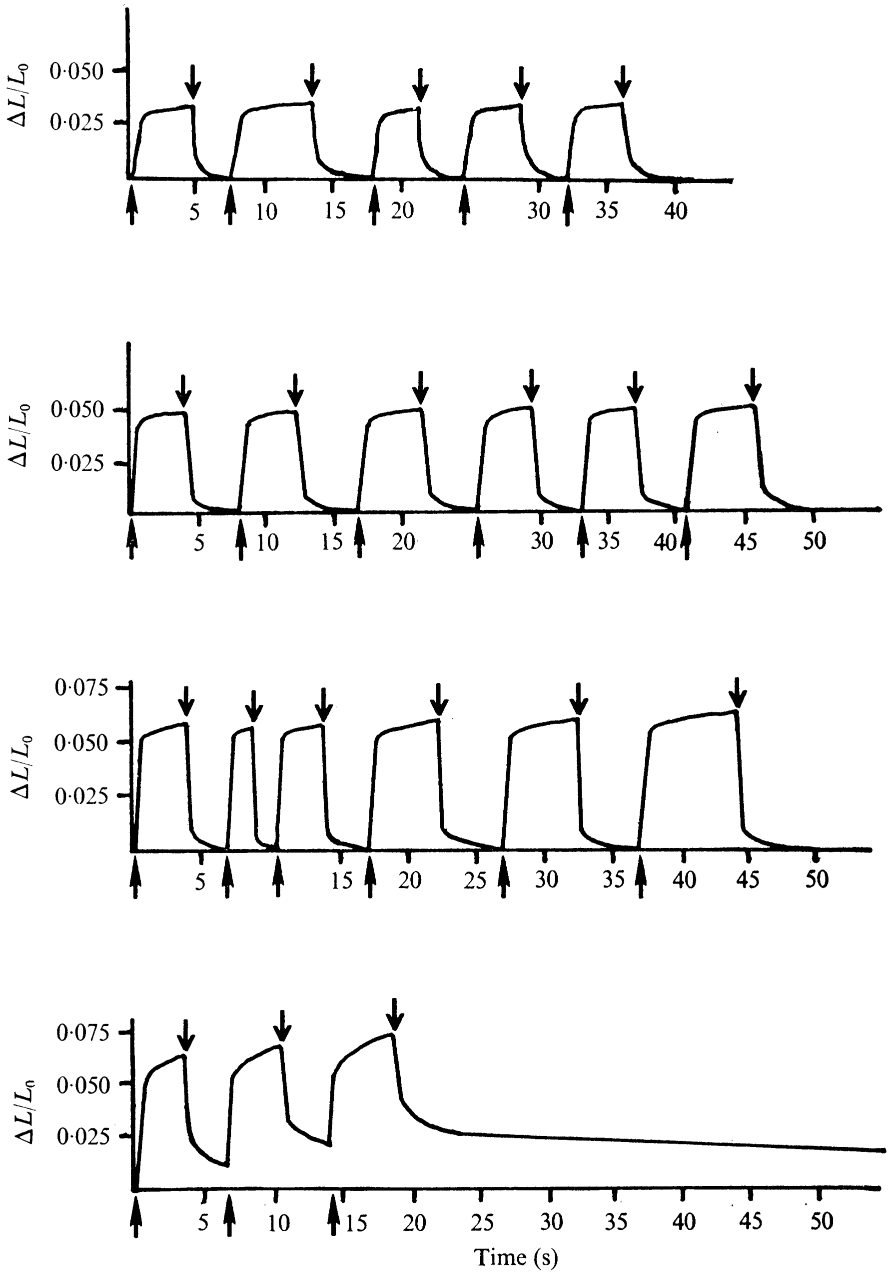

Fig. 4. Short-term creep and recovery tests run at 6 °C. Graphs of extension ($\Delta L/L_0$) against time (in s) for *A. xanthogrammica* mesogloea pulled longitudinally with a stress of (A) 1365 N.m^{-2}, (B) 5224 N.m^{-2}, and (C) 52238 N.m^{-2}, and (D) for *M. senile* mesogloea pulled longitudinally with a stress of 29946 N.m^{-2}. Solid arrows (➡) indicate when the stress was applied and open arrows (➔) indicate when the stress was removed.

(that between a fibre and the long axis of the body) of the outer layer of *M. senile* mesogloea (mean angle, $\bar{x} = 42°$) is significantly lower ($F_{[1,70]} = 259·62$, $P < 0·001$) than that of the inner layer ($\bar{x} = 82°$). Similarly, the mean fibre angle of *A. xanthogrammica* outer mesogloea ($\bar{x} = 44°$) is significantly lower ($F_{[1,70]} = 141·72$, $P < 0·001$) than that of inner mesogloea ($\bar{x} = 73°$). The fibres of the inner layer of *M. senile* mesogloea have a significantly higher ($F_{[1,71]} = 10·16$, $0·001 < P < 0·05$) mean

Table 1. *Histological staining of* M. Senile *and* A. xanthogrammica *body wall*

Method	Reference	Results
Fast green Van Gieson	Lillie (1965)	Mesogloeal fibres: reddish purple; muscle and epithelial cells: grey
HCl Biebrich scarlet-amido black variant	Lillie (1965)	Fibres: blue-black; cells: pink
Mallory's triple stain	Pantin (1964)	Fibres: deep blue; cells: purple-red
Picro-amido black	Lillie (1965)	Fibres: dark greenish blue; cells: grey

fibre angle than do those of the inner layer of *A. xanthogrammica* mesogloea (i.e. more inner fibres are closer to being circumferentially oriented in *M. senile* than are those in *A. xanthogrammica*).

This structural anisotropy observed in mesogloea of *M. senile* and *A. xanthogrammica* can be related to the mechanical anistropy of these tissues. Only collagen fibres aligned parallel to stress axes will slide past each other and shear the matrix when the tissue is stretched; if collagen fibres have preferred orientations in the tissue, the tissue will be less extensible in directions parallel to them (Gosline, 1971*b*). For short periods of stress application, both *A. xanthogrammica* and *M. senile* mesogloeas appear to be equally rigid in the longitudinal direction and the circumferential direction (Koehl, 1977*b*). However, when stressed for long times (as they would be with constant internal pressure) they are more extensible in the longitudinal than the circumferential direction (Fig. 7). This mechanical anisotropy is more pronounced in the mesogloea of *M. senile* than in that of *A. xanthogrammica*; this is not surprising in light of the observation that the anisotropy of collagen fibre arrangement is more pronounced in *M. senile*. Such circumferential reinforcement is no doubt advantageous for *M. senile* which often inflate themselves to the tall, slim shape illustrated in Koehl (1977*a*).

The circumferential stresses due to internal pressure in hydrostatically supported cylinders are twice the longitudinal stresses (Wainwright *et al.* 1976). In thick-walled hydrostatic cylinders these circumferential stresses are greatest at the inner surface (Timoshenko, 1956), which is where the circumferential fibres are located in these anemones. The longitudinal stresses in a bending anemone are greatest at the outer surface (Koehl, 1977*b*), which is where the fibres at the smallest angles to the body long axis are found in these animals. Thus, the reinforcing collagen fibres are arranged parallel to the major stress axes in different regions of the body walls of both *M. senile* and *A. xanthogrammica*.

Macromolecular mechanisms responsible for differences in mechanical behaviour of mesogloea from M. senile *and* A. xanthogrammica

Creep tests. A. xanthogrammica mesogloea is not as extensible as that of *M. senile* when subjected to tensile stresses for periods of a minute to a day. Based on the polymer mechanics illustrated in Fig. 8, I predicted that *A. xanthogrammica* was less extensible than *M. senile* mesogloea either because (1) *A. xanthogrammica* mesogloea has a longer entanglement plateau than that of *M. senile*, or because (2) *A. xanthogrammica* mesogloea is more highly cross-linked and hence reaches equilibrium length without undergoing much extension.

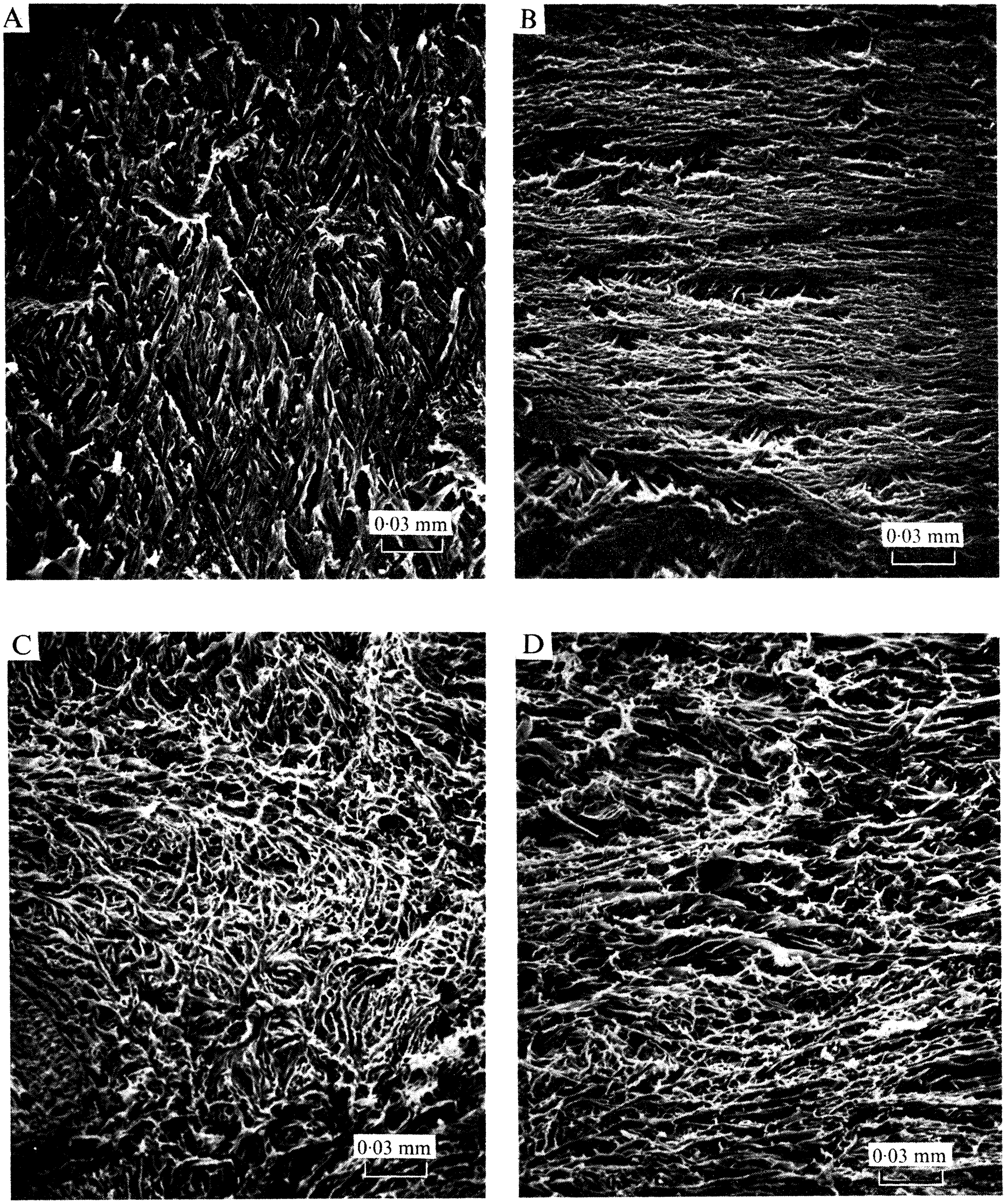

Fig. 5. SEMs of tangential sections of sea anemone body-wall mesogloea. The long axis of each photograph is parallel to the long axis of the anemone. (A) Outer mesogloea from *M. senile*. (B). Inner mesogloea from *M. senile* (notice that the inner circumferential fibres are broken away in the lower left corner of Figure B revealing the distinctly different outer feltwork layer of mesogloea). (C) Outer mesogloea from *A. xanthogrammica*. (D) Inner mesogloea from *A. xanthogrammica*.

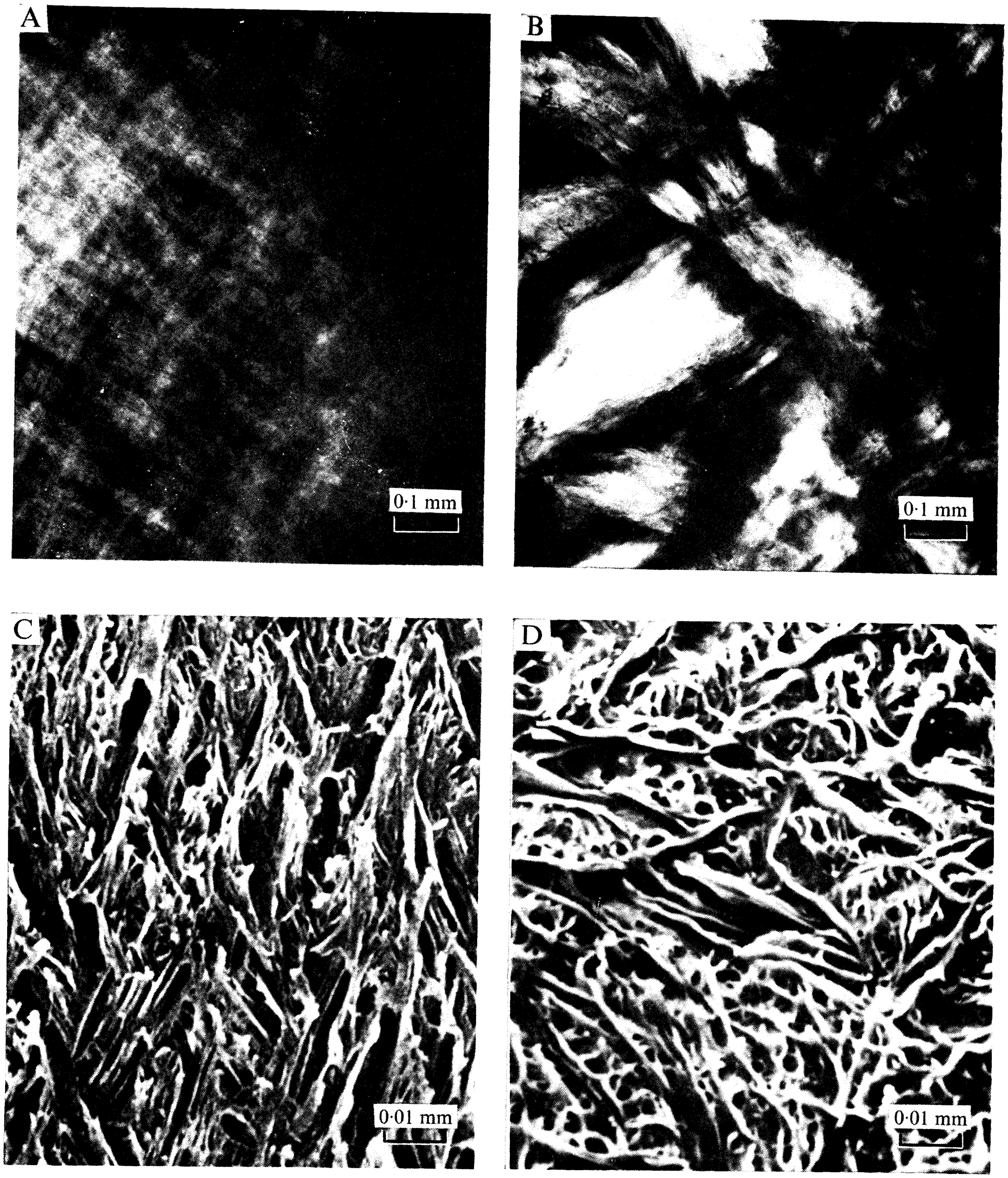

Fig. 6. (A and B) Polarized light micrographs of tangential cryostat sections 10 μm thick of fresh outer mesogloea from *M. senile* (A) and from *A. xanthogrammica* (B). (C, D) SEMs of tangential sections of outer mesogloea from *M. senile* (C) and from *A. xanthogrammica* (D).

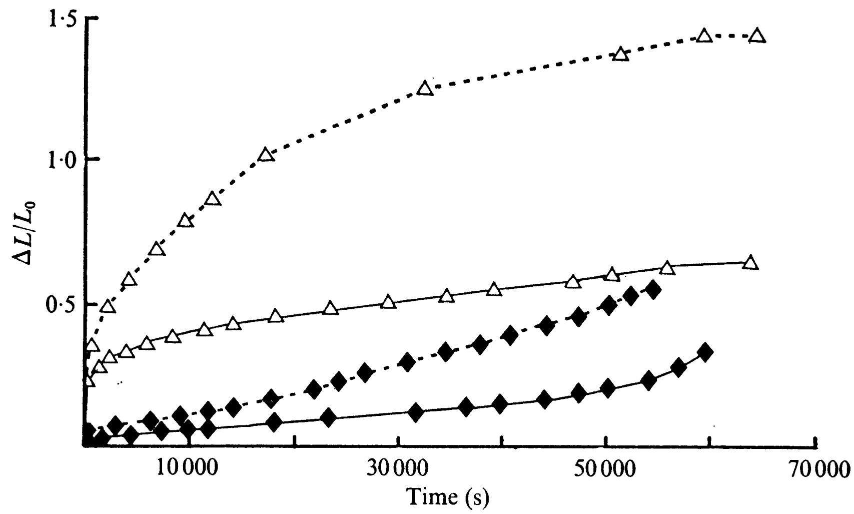

Fig. 7. Graphs of extension ($\Delta L/L_0$) against time (in s) for pieces of *M. senile* mesogloea (Δ) taken from one individual, and *A. xanthogrammica* mesogloea (◆) taken from another. Dashed lines represent creep curves of mesogloea pulled longitudinally and solid lines represent creep curves of mesogloea pulled circumferentially. The *M. senile* tests were run at 6 °C and the *A. xanthogrammica* tests at 20 °C. Stresses were on the order of 3000 N.m^{-2}.

A typical log $D(t)$ versus log t curve for *A. xanthogrammica* mesogloea at the biologically relevant temperature of 6 °C is presented in Fig. 9. *A. xanthogrammica* mesogloea behaves like a polymer solution in its entanglement platea (Fig. 8). Other creep tests at higher temperatures (analogous to running the tests for longer times) showed a rise in compliance after 12–24 h (Fig. 9). This rise probably represents the transition where entanglements in the matrix break down (perhaps by slippage of tangled molecules or by disruption of hydrogen bonds). The possibility that mesogloea of *A. xanthogrammica* (unlike that of *M. senile* (Gosline, 1971*b*)) contains mechanically continuous collagen fibres which undergo creep after long periods of stress has not been ruled out. However, if *A. xanthogrammica* mesogloea is assumed to be a composite material containing continuous fibres with elastic moduli of 10^9 N.m^{-2} (that of collagen (Wainwright *et al.* 1976)), the calculated modulus of the composite (Wainwright *et al.* 1976) is three orders of magnitude greater than its measured modulus (Koehl, 1977*b*). Thus, rearrangement of matrix molecules is a more likely explanation for the creep behaviour of *A. xanthogrammica* mesogloea than is stretching of continuous collagen fibres. Therefore, it appears that the mesogloea of *A. xanthogrammica* is less extensible than that of *M. senile* because the former remains in its entanglement plateau longer than the latter.

M. senile mesogloea reaches an equilibrium compliance after 12–24 h (Fig. 10), indicating the polymers in this mesogloea are cross-linked to each other to form a network (Gosline, 1971*b*). *M. senile* stressed at 21 °C for 24 h maintains an equilibrium compliance, indicating that the continuity of the network has not broken down and suggesting that the rise in compliance of *A. xanthogrammica* mesogloea under similar conditions is not due to tissue decomposition. Strips of mesogloea did begin to decay after 48 h of testing, however, thus *A. xanthogrammica* creep tests could not be run long enough to determine whether or not this mesogloea is also a cross-linked network.

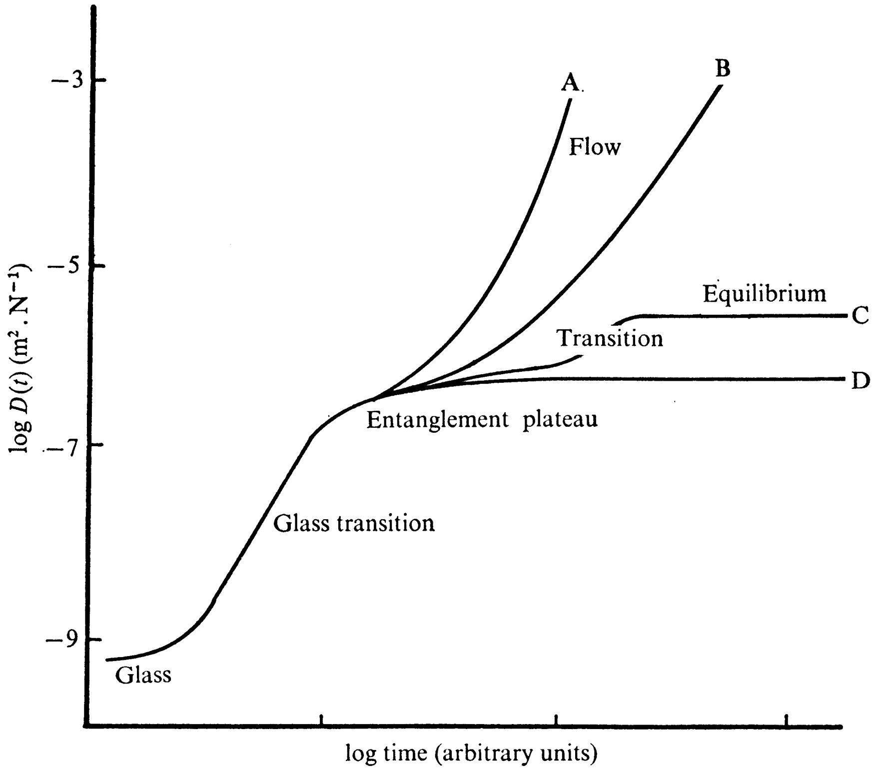

Fig. 8. Graph of the log of compliance ($D(t)$) against the log of time (in arbitrary units) for some typical random-coil polymer systems: a non-cross-linked solution of polymers of intermediate molecular weight (A) and of high molecular weight (B), and a network of polymers lightly cross-linked (C) and highly cross-linked (D). When a stress is applied to a polymeric material for a period of time shorter than that required for the rearrangement of the polymer chains in response to that stress, the compliance is low (glass). Once segments of the molecules have enough time to straighten out in response to the stress, the material extends and the compliance rises sharply (glass transition). The molecules will contine to straighten until their movements are hampered by entanglements (temporary interactions between molecules such as physical entanglements, hydrogen bonds, or electrostatic attractions, which retard the movement of molecules past each other) (entanglement plateau). If the molecular weight or concentration of polymers in solution is high, the probability of entanglements (and thus the duration of the plateau) is great. After longer periods of stress, the molecules may eventually untangle and further rearrange (transition). If the molecules are not permanently cross-linked to each other, they will continue indefinitely to slide past each other (flow). If the polymers are permanently cross-linked, an equilibrium compliance is reached when the cross-links prevent further rearrangement of the molecules. If the degree of cross-linking is great, the transition between the entanglement and equilibrium plateaux may not occur (Ferry, 1970; Wainwright *et al.* 1976).

A. xanthogrammica mesogloea displays the same sort of slow elastic recovery towards resting shape as does that of *M. senile* on cessation of prolonged stress. Because *A. xanthogrammica* mesogloea is in its entanglement plateau over the time scales involved, entanglements must be acting as the cross-links necessary for configurational entropy elasticity.

The creep of mesogloea anaesthetized in propylene phenoxitol was the same as that anaesthetized in $MgSO_4$ (Fig. 11). The similarity of these results indicates that the anaesthetics probably do not grossly alter the passive behaviour of mesogloea. The creep behaviour of unanaesthetized body wall, however, is quite different from

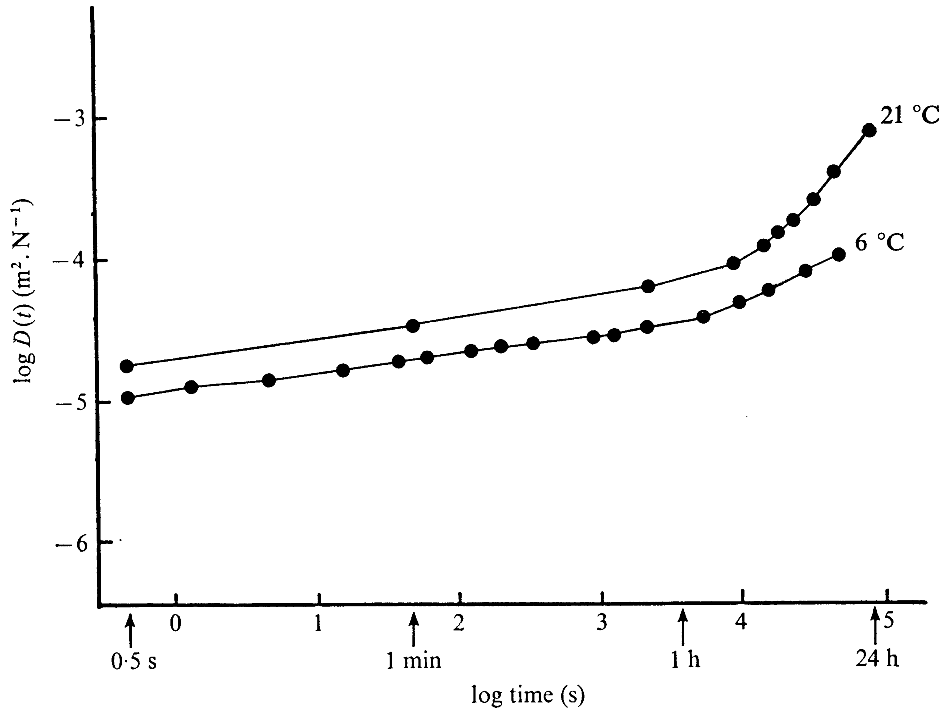

Fig. 9. Graphs of log $D(t)$ (in $m^2.N^{-1}$) against log t (in s) for *A. xanthogrammica* mesogloea stressed longitudinally at 6 °C and at 21 °C. Stresses were on the order of 3000 $N.m^{-2}$.

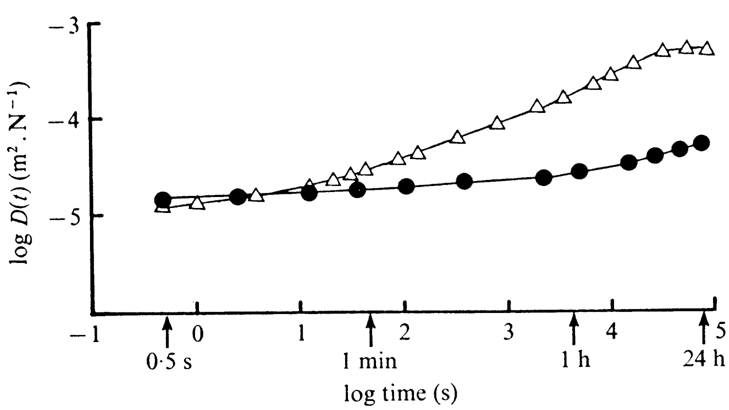

Fig. 10. Graphs of log $D(t)$ (in $m^2.N^{-1}$) against log t (in s) for *A. xanthogrammica* (●) and *M. senile* (△) mesogloeas stressed (σ = 3000 $N.m^{-2}$) at 6 °C in the longitudinal direction.

anaesthetized because the muscles sporadically contract and relax throughout the experimental period (Fig. 11). This illustrates that an anemone can prevent or alter the extension of its body wall merely by contracting its muscles – a trick that man-made polymeric composite materials are incapable of doing.

Dynamic tests. The mean logs of the frequency-dependent storage moduli (E') for both *A. xanthogrammica* and *M. senile* are plotted against the log of the inverse of frequency (roughly analogous to log time in a creep test) in Fig. 12. Over the frequency range used, the mesogloea of both species behaves like a polymer solution in its entanglement plateau with storage moduli of the order of 10^5 $N.m^{-2}$. E' is

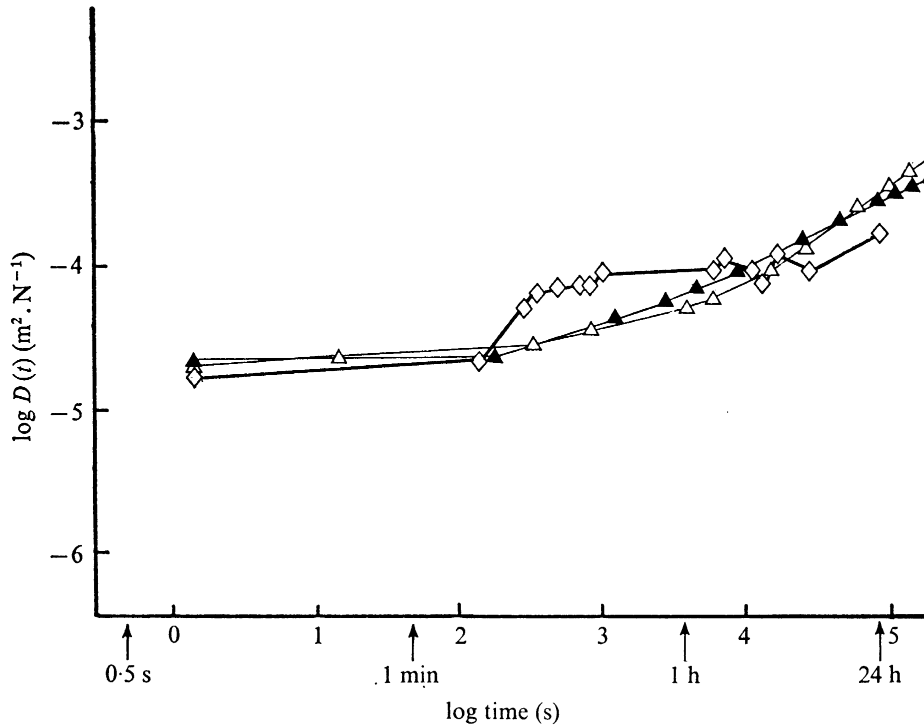

Fig. 11. Graphs of log $D(t)$ (in $m^2.N^{-1}$) against log t (in s) for *A. xanthogrammica* body wall pulled longitudinally at 20 °C by stresses on the order of 1000 $N.m^{-2}$. △, = Tissue anaesthetized in 20% $MgSO_4.7H_2O$ one to one with Instant Ocean; ▲, = tissue anaesthetized in 0·3 % propylene phenoxitol in Instant Ocean; ◇, tissue soaked in Instant Ocean containing no anaesthetic.

analogous to the inverse of $D(t)$ measured for short times in creep tests; note that $D(t)$ for *A. xanthogrammica* and *M. senile* mesogloeas at short times is 10^{-5} $m^2.N^{-1}$ (Figs. 9–11). These storage moduli are consistent with those for *M. senile* mesogloea measured by Gosline (1971*b*). With these short periods of stress application, the rigidity (E') of *A. xanthogrammica* and *M. senile* mesogloeas are not significantly different.

The E' of mesogloea pulled circumferentially in dynamic tests was not significantly different from the E' of mesogloea pulled longitudinally for both species of anemones. The range of extension rates used in the dynamic tests (0·15–75·2 % extension.s^{-1}) is roughly the same as the range of extension rates used by Koehl (1977*b*) in short-term force-extension tests of anemone mesogloea. The mean of the elastic moduli (E) measured in those force–extension tests for mesogloea pulled circumferentially is also not significantly different from the mean of the E's for mesogloea pulled longitudinally. These observations show that for short periods of stress application mesogloea from *M. senile* and from *A. xanthogrammica* is mechanically isotropic. This is consistent with the observation that circumferential and longitudinal $D(t)$'s are not different from each other at short times during creep tests for both species.

The measurements of viscous energy loss (tan δ) for *M. senile* mesogloea are consistent with those of Gosline (1971*b*) (Fig. 12). The tan δ's for *A. xanthogrammica* and *M. senile* mesogloea are not significantly different at high frequencies. However, at frequencies of 0·3 Hz and lower, *M. senile* mesogloea has a significantly higher tan δ

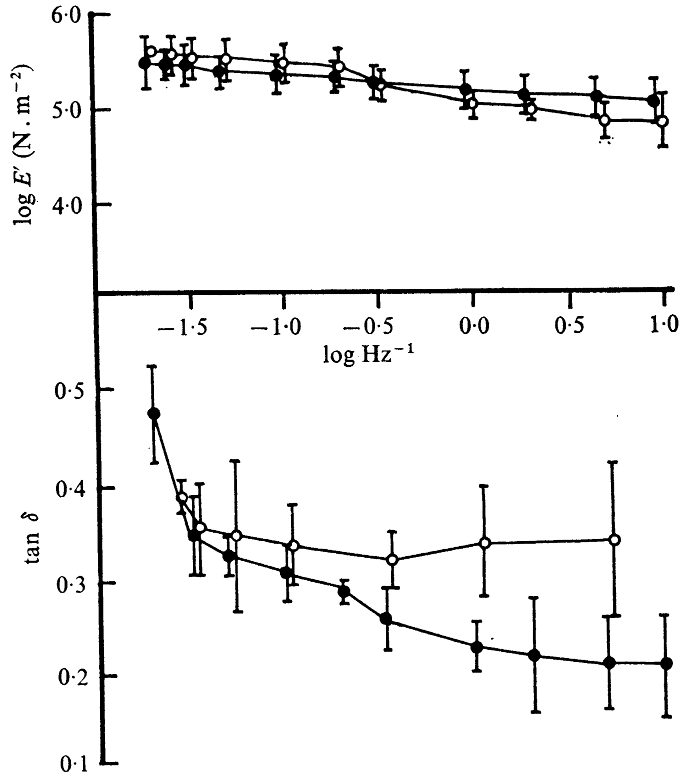

Fig. 12. Graphs of the mean logs of E' (in $N.m^{-2}$) and of the mean δ's against the log of the inverse of frequency for 14 specimens of *A. xanthogrammica* (●) and six specimens of *M. senile* (○) mesogloea tested at 7 °C with extensions of 1·5 %. Error bars indicate standard deviations.

than does *A. xanthogrammica* mesogloea (for example, $F_{[1,10]} = 10{\cdot}73$, $0{\cdot}005 < P < 0{\cdot}01$, when Hz = 0·3). What does such a difference between the tan δ's of the mesogloeas of *M. senile* and *A. xanthogrammica* mean for these anemones in nature? At a frequency of 0·2 Hz, which roughly approximates the stress cycle due to a wave, the tan δ of *A. xanthogrammica* mesogloea is significantly lower ($F_{[1,12]} = 11{\cdot}84$, $P = 0{\cdot}005$) than that of *M. senile* mesogloea (i.e. *A. xanthogrammica* mesogloea stores as potential energy a greater proportion of the energy used to stretch it than does that of *M. senile*). Since such stored energy is used in the elastic return to resting shape of the mesogloea, it is not surprising that *A. xanthogrammica* mesogloea in short-term creep and recovery tests could return to resting length before the 'next wave hit' whereas *M. senile* mesogloea could not.

Macromolecular differences responsible for mechanical differences in mesogloea. The mechanical behaviour of mesogloea from *A. xanthogrammica* and *M. senile*, when subjected to force–extension tests (Koehl, 1977*b*) and to creep and dynamic tests, can be summarized as follows. (1) When stressed for short times, mesogloea is mechanically isotropic and in *A. xanthogrammica* and *M. senile* does not differ in rigidity (E, E', or $1/D(t)$) or viscous energy loss (tan δ). (2) When stressed for longer times, the mesogloea of either species is more rigid when pulled circumferentially than longitudinally. (3) The rigidity of *M. senile* mesogloea is more time-dependent than that of *A. xanthogrammica*; when stressed for longer than 1 min *M. senile* mesogloea becomes less rigid than that of *A. xanthogrammica*. (4) When stressed at frequencies lower than 0·3 Hz, *A. xanthogrammica* mesogloea stores more energy for elastic recoil (i.e. has a lower tan δ) than does that of *M. senile*.

Thus, *A. xanthogrammica* and *M. senile* mesogloea, stressed circumferentially and longitudinally, behave in the same manner at short times when extension in response to stress is due to local straightening of polymer chains. The above differences arise because *A. xanthogrammica* mesogloea remains in the entanglement plateau longer than does that of *M. senile*, and because circumferentially stressed mesogloea remains in the entanglement plateau longer than longitudinally stressed mesogloea from the same species. This indicates that macromolecular differences that affect the length of the entanglement plateau are responsible for the observed differences in mechanical behaviour of mesogloeas.

There are several possible macromolecular differences which might increase the lengths of the entanglement plateaux of mesogloea. For example, they would be longer the higher the concentration and/or the molecular weight of protein-polysaccharide polymers in the matrix (Ferry, 1970). Entanglement plateaux would also be longer the more closely packed in parallel arrays along the stress axis are the collagen fibres in mesogloea. Such close parallel packing increases the deformation of the matrix between fibres, hence more extensive molecular rearrangements are required in the matrix for a given extension of the tissue (Gosline, 1971*b*; Wainwright, *et al.* 1976). Furthermore, the more closely packed the fibres, the greater the probability of entanglements between them and the matrix polymers. As described above, such a difference in collagen packing appears to be responsible for the differences observed in the rigidity of circumferentially versus longitudinally stressed mesogloea.

Several observations indicate that perhaps both of the macromolecular differences listed above are responsible for the observed differences in mechanical behaviour of mesogloea of *M. senile* and *A. xanthogrammica*. Estimates of the composition, by weight, indicate that there is nearly twice as much material other than water (i.e. collagen and matrix polymers) in *A. xanthogrammica* mesogloea as in that of *M. senile* (Table 2). The concentration by weight of the polymer solution in *A. xanthogrammica* mesogloea matrix is about twice that of *M. senile*. This indicates that there are probably more and/or larger molecules in *A. xanthogrammica* matrix than in that of *M. senile*. There is also almost twice as much collagen by weight in *A. xanthogrammica* mesogloea as in that of *M. senile*. If collagen fibres account for a greater proportion of the mesogloea in *A. xanthogrammica* than they do in *M. senile*, then the fibres would be more densely packed in the former than in the latter.

The suggestion that the mesogloea of *A. xanthogrammica* has more parallel collagen fibres than that of *M. senile* is corroborated by the observation that longitudinal, transverse, and tangential (Fig. 6A, B) sections of the outer mesogloea of *A. xanthogrammica* are more highly birefringent than equivalent sections of *M. senile*. The higher birefringence in the mesogloea of the former species probably indicates the presence of more collagen fibres parallel to each other in the planes of the sections. It also appears in polarized light micrographs that wider swatches of parallel fibres occur in outer mesogloea of *A. xanthogrammica* than in that of *M. senile*. The fibres visible in the light microscope and in low-magnification SEMs of *A. xanthogrammica* and *M. senile* mesogloea are actually bundles of collagen fibres. These individual

Table 2. *Composition of mesogloea from* M. senile *and* A. xanthogrammica

	*M. senile**	*A. xanthogrammica*
Measured % of total weight due to salt-free dry weight	8·8 % (S.D. = 1·0, n = 8)	14·9 % (S.D. = 1·6, n = 3)
Measured % of total weight due to autoclave-soluble fraction†	7·4 %	13·1 % (S.D. = 1·1, n = 3)
Calculated % of total weight due to collagen‡	7·0 %	12·0 %
Calculated concentration by weight of the polymer solution in the matrix§	2·0 %	4·0 %

* Values for *M. senile* were obtained by Gosline (1971 *a*).

† Collagen becomes soluble when autoclaved, whereas other proteins precipitate. The autoclave-soluble fraction of mesogloea should thus contain the collagen of the mesogloea as well as some associated polysaccharides (Gosline, 1971 *a*).

‡ By assuming that the same percentage of the autoclave-soluble fraction of *A. xanthogrammica* mesogloea is neutral hexose as Gosline (1971 *a*) reports for the autoclave-soluble fraction of *M. senile* mesogloea, I calculated using the method of Gosline (1971 *a*) an estimate of the collagen content of *A. xanthogrammica mesogloea.*

§ I calculated concentration of the matrix solution using the method of Gosline (1971 *a*).

fibres can be resolved in TEM (Grimstone *et al.* 1958; Gosline, 1971 *a*). The fibre bundles in SEMs of *A. xanthogrammica* mesogloea appear to be larger than those in SEMs of *M. senile* mesogloea (Fig. 6C, D).

My work suggests that *A. xanthogrammica* mesogloea is more rigid than that of *M. senile*, when stressed over the time scale of a minute to a day perhaps because it has a higher concentration of and/or larger polymers in its matrix, and perhaps, because it has more densely packed parallel arrays of reinforcing collagen fibres. These suggestions should be tested by TEM and by more detailed chemical analyses of the mesogloeas of both species of anemones.

CONCLUSIONS

The time-dependent response to stress of the body-wall mesogloea of two species of anemones can be related to the behaviour of the anemones when subjected to environmental forces and when performing mechanical activities. *M. senile* mesogloea is relatively rigid when stressed for short periods (as it would be during rapid muscular movements of the animals) but is very extensible when stressed for long times (as it would be by the slow tidal currents that bend these anemones over and as it would be during slow extensive postural changes).

In contrast, *A. xanthogrammica* mesogloea remains relatively rigid even after being stressed for several hours; these anemones do not undergo extreme postural changes nor do they deform noticeably when exposed to wave action for hours. *A. xanthogrammica* mesogloea stores for elastic recoil a greater percentage of the energy used to stretch it than does that of *M. senile*. The mesogloea of an *A. xanthogrammica*, when stressed by a wave, can thus elastically return to resting length before the impact of the next one.

Polarized light microscopy and SEM reveal that reinforcing collagen fibres in the body walls of the hydrostatically supported sea anemones, *M. senile and A. xantho-*

grammica, are aligned parallel with the major stress axes in different regions of the body wall.

Mechanical tests of the mesogloeas of *A. xanthogrammica* and *M. senile* indicate that a basic way in which connective tissues composed of rigid fibres in pliant matrixes may vary in their mechanical behaviour is in the length of time that molecular entanglements retard extension of the tissues. Observations of composition and microscopic structure of the mesogloeas of these two species of anemones indicate that the duration of this 'entanglement plateau' is great if (1) the fibres are closely packed and oriented parallel to the stress axis, and (2) the concentration and/or molecular weight of the polymers in the matrix is high.

This study illustrates how structural features on the macromolecular and microscopic levels of organization of an organism can equip that organism for the particular mechanical activities it performs and the environmental forces it encounters.

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