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FINE STRUCTURE AND MECHANICAL PROPERTIES OF THE CATCH APPARATUS OF THE SEA-URCHIN SPINE, A COLLAGENOUS CONNECTIVE TISSUE WITH MUSCLE-LIKE HOLDING CAPACITY

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SUMMARY

The catch apparatus (CA) of the sea-urchin spine has been known to have a muscle-like holding property, though it is composed mainly of extracellular collagen fibres. An electron microscopic study has been made on the CA of the sea urchin, *Anthocidaris crassispina*, with special reference to its content of muscle cells and to structural changes of the collagen components on elongation of the CA. The stretch resistance of the CA in a highly extensible state and in a very inextensible state was also measured. Although very thin smooth muscle cells were found scattered among the collagen fibres in the CA, the difference in the passive tension was greater than the estimated stress which could be generated by the muscle cells in the CA by three orders of magnitude. The collagen fibrils remained undeformed but slid along one another during the length change of the CA. The present results suggest that the cohesive force between the collagen fibrils rather than the contractile activity of the muscle cells plays a significant role in determining the mechanical properties of the CA.

INTRODUCTION

It has been suggested that, in certain echinoderms, movement and maintenance of posture depend to a considerable extent on the passive mechanical properties of the collagenous connective tissue (catch apparatus or ligament of the echinoid spine, Takahashi, 1966, 1967*a*; Smith, Wainwright, Baker & Cayer, 1981; crinoid ligament, Meyer, 1971; holothuroid body wall, Stott, Hepburn, Joffe & Heffron, 1974; asteroid body wall, Eylers, 1976; ophiuroid ligament, Wilkie, 1978*a*,*b*; echinoid tube-foot wall, Florey & Cahill, 1977). These collagenous tissues are supposed to be able to change their passive mechanical properties reversibly, whereas connective tissues such as tendons and ligaments are generally regarded as passive, inert materials.

This peculiar function of echinoderm connective tissue was first studied systematically in the catch apparatus (CA) of the sea-urchin spine (Takahashi, 1967*a*,*b*). The CA is a fibrous cylinder which surrounds the joint at the base of the spine (Fig. 1).

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Key words: Sea urchin, connective tissue, mechanical properties.

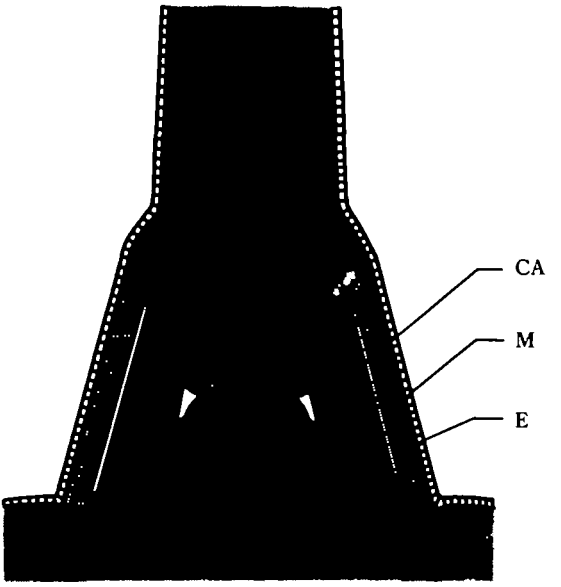


Fig. 1. A diagrammatic scheme of the joint connecting the spine to the test. CA, catch apparatus. M, spine muscle. E, epithelium.

The CA is in turn surrounded by the spine muscle which runs longitudinally from the base of the spine to the test. A sea urchin can bend its spine freely in any direction around the joint, but it can also hold the spine tightly to the test, resisting the bending force for some time. While spine movement is brought about by contraction of the spine muscle, it has been known that the ability to hold the spine stiffly in a given posture is due to a temporary increase in the stretch resistance of the CA. Although the CA was long believed to be a tonic muscle on account of its holding function (von Uexküll, 1900), it has been shown that the CA is mainly composed of extracellular collagen fibres, with only a small volume of cellular elements (Takahashi, 1967*a*; Smith *et al.* 1981). The isolated preparation of the CA behaves like a viscous body under a light load and responds to various kinds of stimuli with changes in its extensibility (Takahashi, 1967*b*); for example, acetylcholine (ACh) and high concentrations of potassium ions make the CA highly inextensible and adrenaline (Adr) increases the rate of isotonic extension, but the CA does not respond by contraction to the stimuli known to produce contraction in normal muscular tissues. The inextensible state of the CA is similar to the passive stretch-resistant state of the molluscan catch muscle, and this phenomenon was called 'connective tissue catch' by Rüegg (1971).

However, the mechanism by which the mechanical properties of the CA are altered is not understood. To understand this mechanism it is necessary to know which components determine the mechanical properties of the CA. Though Kawaguti & Kamishima (1965) reported that there were no muscle cells in the CA of *Anthocidaris crassispina* on the basis of their electron microscopic study, some results of the physiological experiments carried out with the CA suggested the presence of contractile elements (Takahashi, 1967*b*). A recent study of the CA of the sea urchin

Echinometra lucunter, has revealed very thin muscle fibres which occupy 2–3 % of the cross-sectional area of the CA (Smith *et al.* 1981).

In the present study, we made an electron microscopic study of the CA of the sea urchin, *Anthocidaris crassispina*, with special reference to its content of muscle cells and structural changes of the collagen components which might occur on elongation of the CA. We also made quantitative measurements of the changes in the mechanical properties of the CA. It was concluded that the observed large changes in the stretch resistance of the CA cannot be accounted for by the small number of muscle cells found in the CA. It is suggested that the mechanical properties of the CA are determined by the resistive force against the slippage of collagen fibrils over each other.

MATERIALS AND METHODS

Specimens of *Anthocidaris crassispina*, measuring 4–6 cm in test diameter, were collected near the Misaki Marine Biological Station, University of Tokyo. They were used immediately or after being maintained in an aquarium of artificial sea water at 17–20 °C.

For electron microscopy, an interambulacral primary spine with a piece of the test was cut out. The epithelium and the spine muscle were carefully removed with forceps to expose the CA. A longitudinal strip of the CA, about 1 mm wide, was prepared on either side of the spine joint by cutting the rest of it. The specimens were first fixed in 3 % glutaraldehyde in 0·1 M-s-collidine buffer (pH 7·4) with 2 % NaCl for 2 h at room temperature. They were rinsed in the same buffer containing 3 % NaCl, and were post-fixed in 1 % OsO_4 in the same buffer with 2 % NaCl for 2 h on ice. Dehydration was performed in a graded series of ethanol. The strip of the CA was cut off from the test and from the base of the spine during dehydration, and was embedded in Epon. Thin sections were cut with glass knives on a Porter-Blum MT2-B ultramicrotome, stained with 5 % uranyl acetate in 50 % ethanol, and were examined with a Hitachi HS-9 electron microscope.

To examine the changes in the structure of collagen components on elongation of the CA, strips of the CA were stretched slowly under a small load to a predetermined length in sea water or in 10^{-5} M-Adr in sea water. The CA becomes highly extensible in 10^{-5} M-Adr and can be stretched easily up to three times or more. The length of the strip was measured with a dissecting microscope provided with an ocular micrometer, and was expressed relative to the standard length, which was defined as the length the CA would assume when the spine stands perpendicularly to the test. The stretched CA was fixed as described above.

The dimensions of the collagen components of both stretched and unstretched CA were measured on the electron micrograph prints. The magnification of the electron microscope was calibrated with polystyrene latex particles of 0·234 μm diameter as the standard and was found to be almost constant during this experiment. The periodicity of the cross-striation of the collagen fibrils was measured on the longitudinal sections of the CA. All the measurements were performed with the collagen fibrils on which at least 20 periods can be followed, so that the error produced by tilting of the sectioning plane from the true long axis of the collagen fibrils should be negligible.

The diameters of the collagen fibrils were measured on transverse sections of the CA in which the collagen fibrils displayed circular profiles. The diameters of the collagen fibrils contained within an arbitrarily selected square of 2 μm^2 in each of five electron micrograph prints were measured for each specimen.

The cross-sectional areas of the muscle cells found in the CA were measured by tracing the profiles of the muscle cells on a tracing section paper and counting the number of 1 mm^2 squares contained in each profile. To obtain the areal density of the muscle cells in the CA, cross-sectional areas of muscle cells contained in an area of at least 240 μm^2 were measured for each specimen and such a measurement was repeated on four specimens.

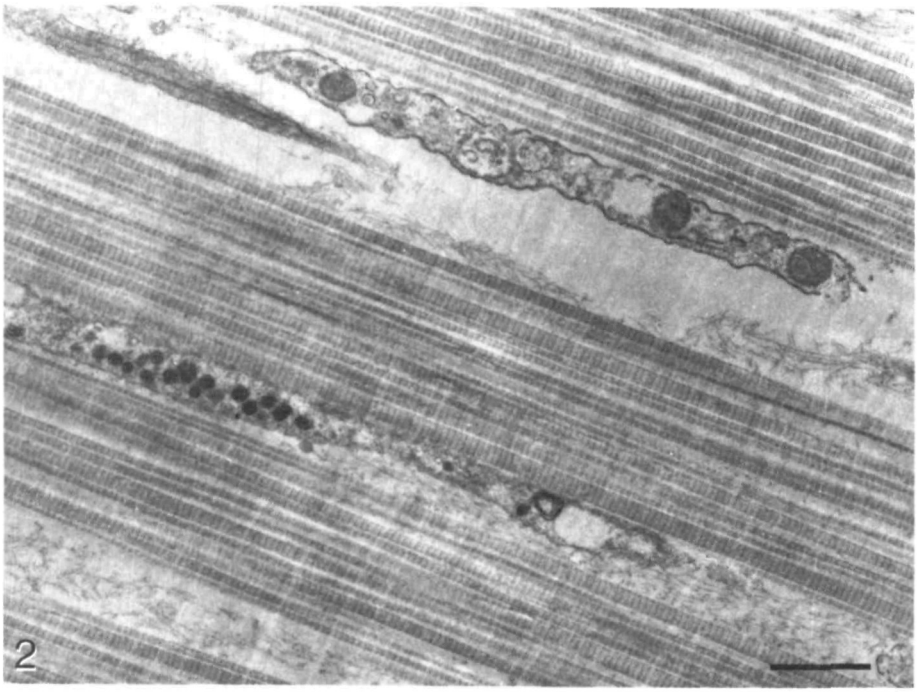
To measure the changes in the passive mechanical properties of the CA, CAs in a highly stretch resistant state and those in an extremely extensible state were subjected to tensile tests. For tensile testing, a strip of the CA 0·5 mm wide was prepared. The thickness and the length of the strip were measured under a dissecting microscope with an ocular micrometer to calculate the volume of the specimen. Then the specimen was attached to the testing apparatus and was equilibrated in artificial sea water for 15 min. During this time a slight stretch was given to the specimen until a small tension (5–10 g) was generated in the specimen to remove the slack in the specimen. Then the initial length of the specimen was measured, since the length of the specimen usually changed during these procedures. The initial length of the specimen ranged 2–3 mm in this experiment. The initial cross-sectional area was obtained by dividing the volume of the specimen by the initial length. After 15 min of equilibration, 0·5 ml of 5×10^{-3} M-ACh or Adr in deionized water was added to the medium (25 ml) to make the final concentration 10^{-4} M. Preliminary experiments have shown that the effect of ACh is temporary; the stretch resistance of the CA becomes maximal in 1 or 2 min after application of ACh and then falls (Hidaka, 1979). When treated with Adr, the CA showed a marked fall in stretch resistance, that levelled off after about 5 min. Specimens were treated with 10^{-4} M-ACh for 1 min or with 10^{-4} M-Adr for 5 min before tensile testing. The specimens were stretched at three different speeds, 7, 74 and 740 $\mu m\,s^{-1}$ up to rupture and the resulting stress-strain curves were recorded on a pen-recorder. The tensile testing apparatus used in this experiment is described elsewhere (Hidaka, 1983). In the case of the slowest stretch (7 $\mu m\,s^{-1}$), the stretch was applied 30 s earlier than usual so that the specimens should rupture at almost the same time after the application of ACh or Adr as those stretched at faster speeds.

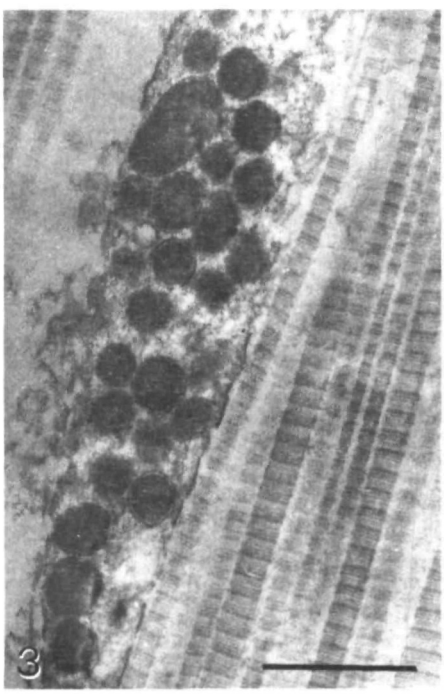
The solutions were continuously stirred by a magnetic stirrer during the experiment and the temperature of the solution was held at 20 °C. The artificial sea water contained (in mM) NaCl, 433·7; KCl, 10·0; $CaCl_2$, 10·1; $MgCl_2$, 52·5; buffered to pH 8·0 at 20 °C with Tris-HCl (ionic strength, $\mu = 0{\cdot}01$).

RESULTS

Electron microscopic observations

The CA consists mainly of collagen fibres which run longitudinally from the test to the shaft of the spine as described previously (Kawaguti & Kamishima, 1965; Takahashi, 1966, 1967*a*; Smith *et al.* 1981). The collagen fibres are composed





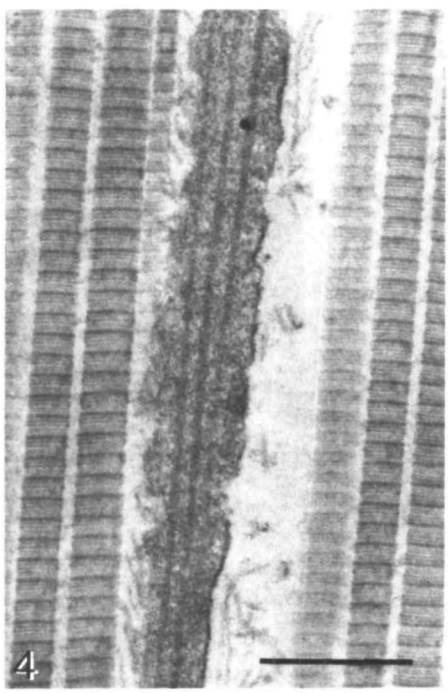
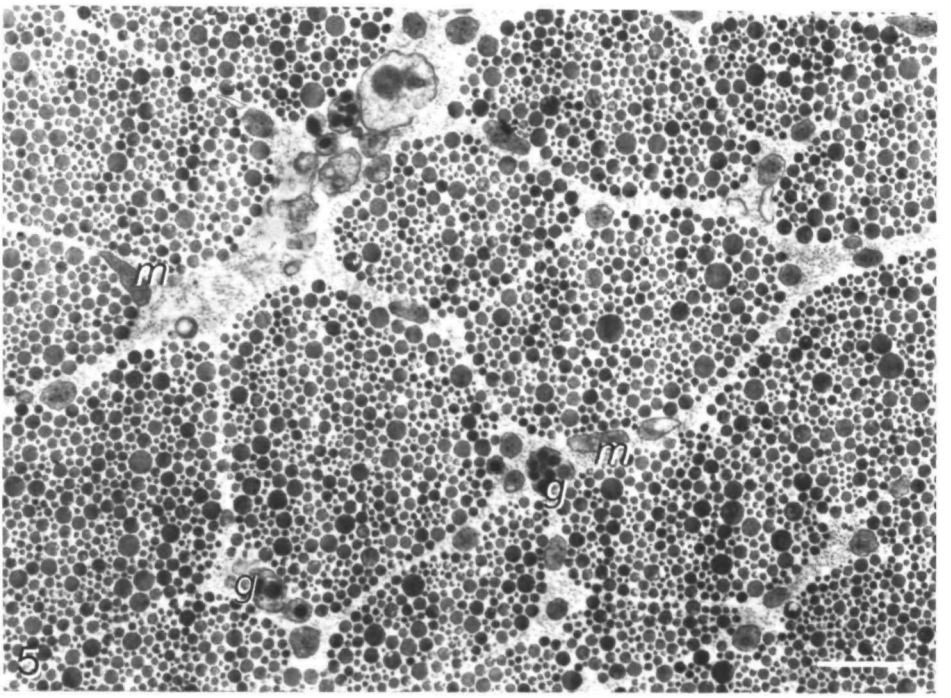


Fig. 2. A longitudinal section of the CA. Collagen fibrils showing characteristic cross-striation are arranged in parallel to form collagen fibres. Scale bar, 1 μm.
Fig. 3. A longitudinal section of the CA showing a cellular process which contains many electron-dense granules. Scale bar, 0·5 μm.
Fig. 4. A longitudinal section of the CA showing a muscle cell with thick myofilaments. Scale bar, 0·5 μm.



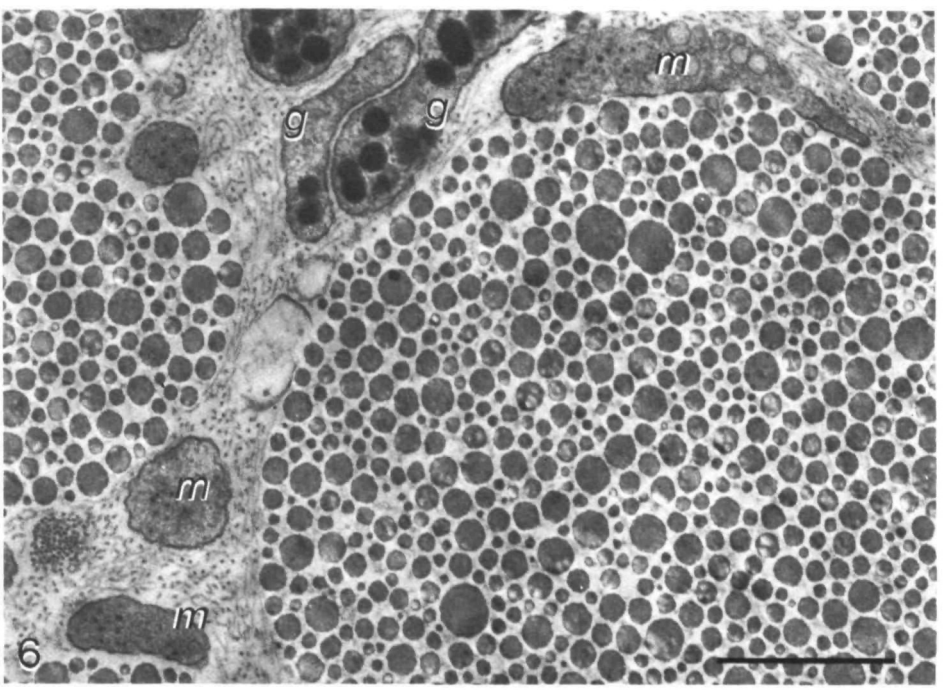


Fig. 5. A transverse section of the CA. Collagen fibres have more or less rounded profiles. Granule-containing cells (*g*) and thin muscle cells (*m*) are scattered among the collagen fibres. Scale bar, 1 μm.
Fig. 6. A transverse section of the CA showing the granule-containing cells (*g*) and the muscle cells (*m*) at higher magnification. Note thin filaments, 'microfibrils' in the space among the collagen fibres. Scale bar, 1 μm.

many collagen fibrils which run parallel to one another (Figs 2–4). The collagen fibrils display characteristic cross-striation of about 70 nm periodicity. This value is slightly larger than those reported for the collagen of mammals and of echinoderms in electron microscopic studies (about 64 nm) (Baccetti, 1967; Meyer, 1971; Matsumura, 1974; Smith *et al.* 1981), although it is similar to those reported for the wet collagen of mammalian tendon on the basis of X-ray diffraction study, i.e. 68 nm (Bear, 1944). In transverse sections, the collagen fibrils show circular profiles and assemble in more or less rounded clusters to form collagen fibres (Figs 5, 6). The collagen fibres are mostly 1–5 μm in diameter. They are surrounded by a loose meshwork of thin filaments of about 10 nm diameter (Fig. 6). These filaments are hereafter referred to as '*microfibrils*'. Thus, each collagen fibre is separated from the neighbouring ones by a sheath of microfibrils. Sometimes two fibres which are very close to each other with no distinct gap or microfibrils between them were observed. This suggests that the collagen fibres in the CA occasionally anastomose with each other, as is the case with the mammalian tendon (Viidik, 1973). The CA is divided into radially arranged sheets by many clefts (Takahashi, 1967*a*). In such a sheet, the collagen fibres were densely packed and occupied about 80 % of the total cross-sectional area. In the clefts were clustered many cellular elements. A small number of cellular elements were also found scattered among the collagen fibres.

At least two kinds of cellular elements were found in the CA: granular cells and muscle cells. The granular cells appeared as long cellular processes which run parallel with the collagen fibres in the longitudinal section (Fig. 3). They contained many electron-dense granules whose diameters ranged between 120–220 nm. These granular cells frequently contained small clear vesicles of various sizes. In transverse sections, the granular cells were found scattered among the collagen fibres (Fig. 5). They were usually surrounded by microfibrils and showed no apparent contact with the collagen fibres nor with the muscle cells (Fig. 6). Many granular cells were found to be clustered in the space between the sheets of collagen fibres. In addition to the granular cells, cellular processes containing clear vesicles of 50–100 nm diameter were sometimes observed. But these cells were rare as compared with the granular cells and appeared to be confined to the spaces between the sheets of collagen fibres.

Smooth muscle cells were found in the CA, though their contribution to the total cross-sectional area was very small (Figs 4, 5, 6). The areal density of the muscle cells was about 3 %. The muscle cells were very thin and their cross-sectional areas were mostly less than 0·2 μm^2 except for the expanded portion which contained a nucleus. The muscle cells contained thick myofilaments whose diameters were 10–40 nm. One or two mitochondria were occasionally found in a small expanded portion of the muscle cell.

Though precise arrangement of the muscle cells and the collagen fibres in the CA could not be known, it is likely that the muscle cells are attached to the collagen fibres at both ends. Some muscle cells appeared to be closely attached to the collagen fibres leaving only a small gap between them. In such a case, the microfibrils were not present in the gap between them but surrounded the muscle cells and the collagen fibre together (Fig. 6). It was often observed that the membrane of the muscle cell undulated to follow the contour of the collagen fibre. There were other muscle cells

which were located independently in the interfibre space surrounded by many microfibrils. It is likely that some muscle cells were cut at their attachment sites while others were cut at an intermediate region.

Morphological changes in the collagen components on elongation of the CA were examined to determine whether the collagen components themselves deform or slide along each other when the CA changes its length. The collagen fibrils appeared unchanged in the longitudinal section of the CA stretched to about three times the standard length in 10^{-5} M-Adr in sea water (Fig. 7). The periodicity of the cross-striation of the collagen fibrils was constantly about 70 nm regardless of the length of the CA at fixation (Fig. 10). The profiles of the collagen fibrils in the transverse section of the CA stretched to about three times the standard length also appeared to be unchanged (Figs 8, 9). The diameter of the collagen fibrils varied widely even in the same collagen fibre, but the distribution of fibril diameters was almost the same for the unstretched CA and the stretched CA (Fig. 11). The diameters of the collagen fibrils were mostly in the range 20–260 nm and averaged 90–110 nm in both stretched and unstretched CAs. Thus both the axial period and the average diameter of the collagen fibrils remained unchanged even when the CA was stretched up to three times the standard length. This indicates that the collagen fibrils themselves are essentially undeformed during the length change of the CA.

However, the collagen fibres in the stretched CA underwent considerable deformation (Figs 8, 9). The collagen fibres which assumed more or less rounded profiles in transverse sections of the unstretched CA became irregular in shape. It was often difficult to identify individual collagen fibres in the stretched specimen, since the gap between the collagen fibres almost disappeared and the boundary of each collagen fibre became obscure. Furthermore many empty spaces occurred in the collagen fibres. When the microfibril sheath was observed, these empty spaces were always located inside the sheath. This suggests that some of the collagen fibrils have slipped within the fibre during elongation of the CA, resulting in the empty space. If this is true, the collagen fibres cannot be independent structural units which can move along one another, but the fibres must be continuous from the test to the base of the spine or anastomose with each other to form a network of collagen fibres. The above observations strongly suggest that sliding of the collagen fibrils is the main process that accounts for the length change of the CA.

One or two muscle cells which had become much thinner were usually found in the empty space close to the microfibrils (Fig. 9). The microfibrils in the stretched CA aligned with the stress axis, indicating that they are connected to each other or to other components (Fig. 7).

Mechanical measurements

Typical stress-strain curves of the CA treated with ACh and Adr are shown in Fig. 12. The specimen was treated with 10^{-4} M-ACh for 30 s (Fig. 12A) or with 10^{-4} M-Adr for 4·5 min (Fig. 12B) before the stretch. The rate of stretch in both cases was $7\,\mu m\,s^{-1}$. The CA treated with ACh resisted the stretch with a large tension. The stress-strain curve started with an initial rounded region, which was followed by a more or less linear region. Then the curve bent off towards the strain axis and the maximum stress was attained at a strain of 9–17 %. Further stretches caused a gradu

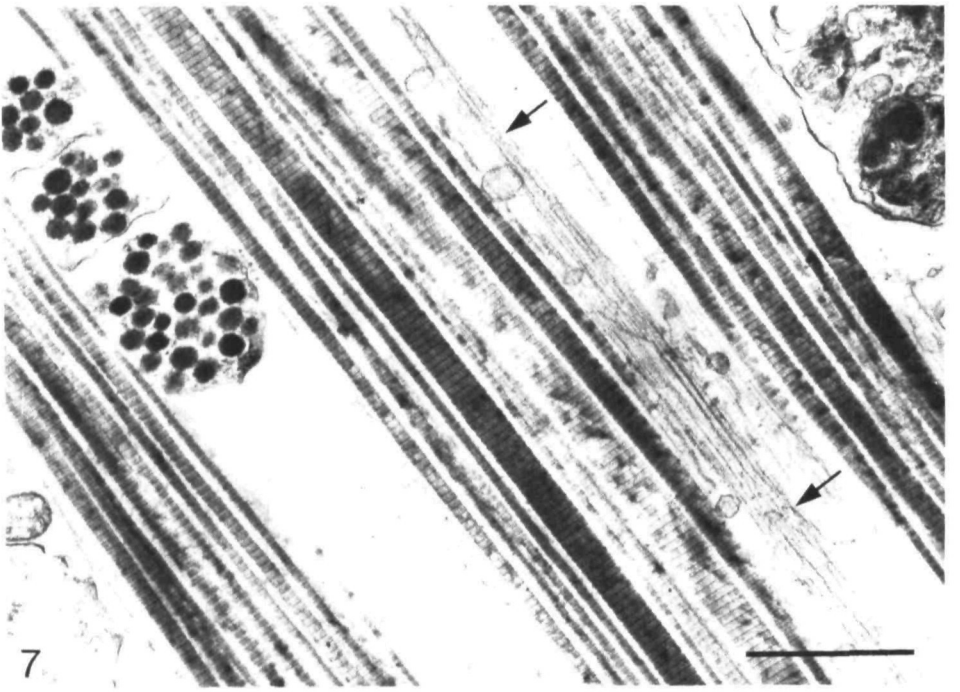
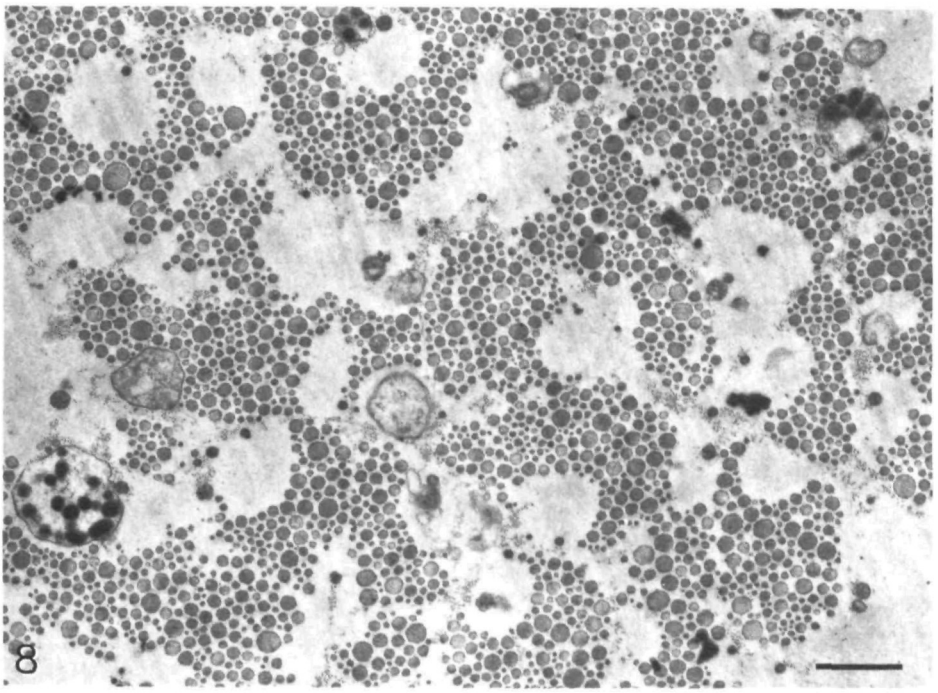


Fig. 7. A longitudinal section of the CA stretched to about three times the standard length in 10^{-5} M-Adr. The collagen fibrils appear unchanged but the collagen fibres appear to be less densely packed than those in the unstretched specimens. Microfibrils (arrows) have aligned parallel in the longitudinal direction. Scale bar, 1 μm.



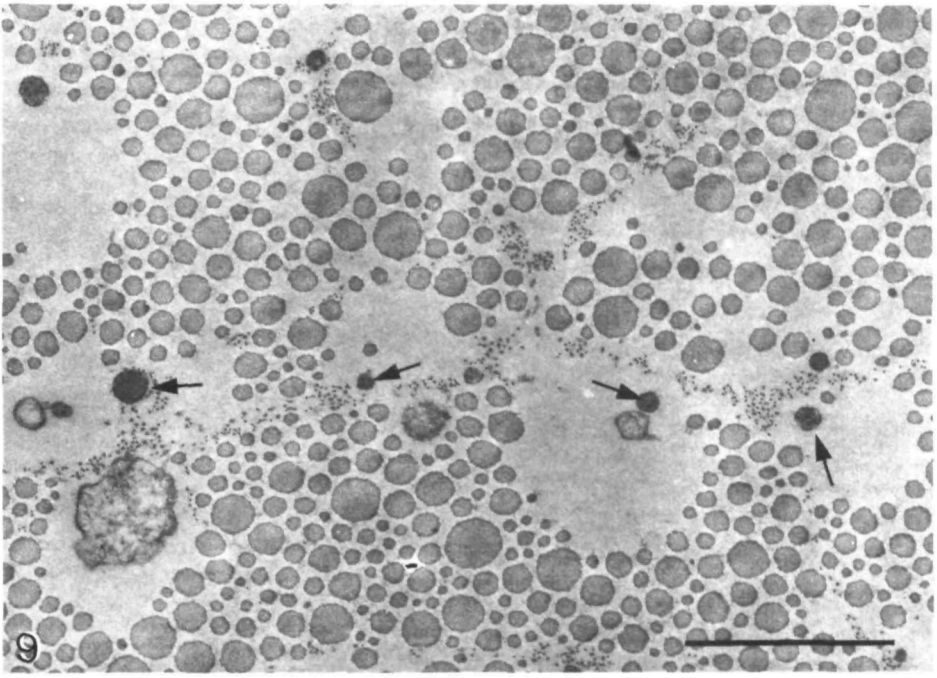


Fig. 8. A transverse section of the CA stretched to about three times the standard length in 10^{-5} M-Adr. The collagen fibres underwent considerable deformation and empty spaces occurred in them. Scale bar, 1 μm.

Fig. 9. A transverse section of the same specimen as shown in Fig. 8 at higher magnification. Very thin muscle cells (arrows) are observed in the empty space close to the microfibrils. Scale bar, 1 μm.

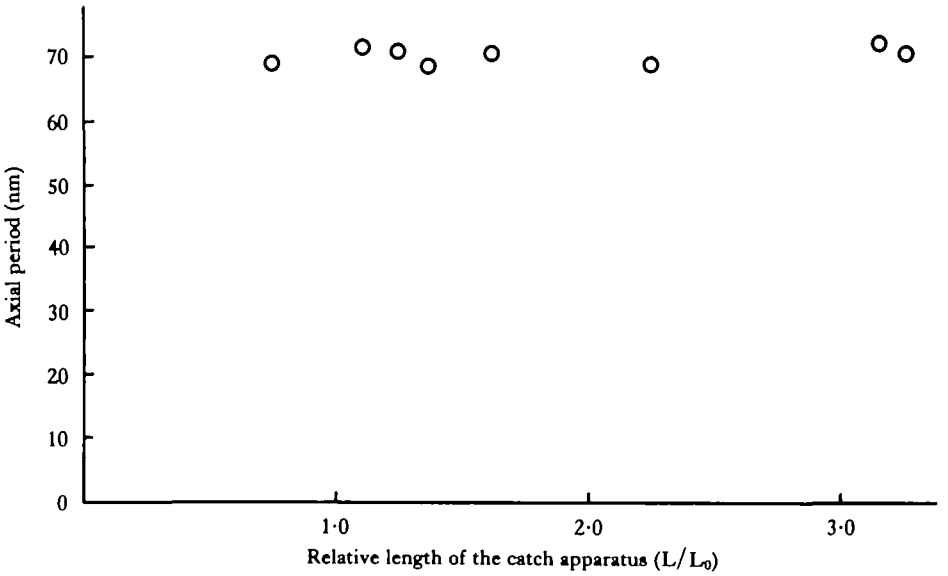


Fig. 10. Axial period of the collagen fibrils in the CA fixed at various degrees of extension.

decrease in tension and the tension finally fell to zero, indicating that the specimen was completely broken. When the CA treated with Adr was stretched at 7 μm s^{-1}, the specimen developed only a slight tension which was more or less maintained during the period of stretch. Sometimes no detectable tension was developed. This indicates that the CA treated with Adr barely resists the stretch at this speed.

To describe the stretch resistance of the CA quantitatively, the maximum slope of the stress-strain curve (apparent stiffness) and the maximum tension per unit initial cross-sectional area of the specimen (tensile strength) were measured. Fig. 13 shows the apparent stiffness and the tensile strength of the CA treated with 10^{-4} M-ACh and with 10^{-4} M-Adr measured at three different speeds. Both the apparent stiffness and the tensile strength were little affected by the rate of extension in the CA treated with ACh, at least in the range 7–740 μm s^{-1}. The apparent stiffness and the tensile strength were relatively high, ranging between 2·3–4·2 $\times$ 10^8 N m^{-2} and 1·8–3·8 $\times$ 10^7 N m^{-2}, respectively. On the contrary, the CA treated with Adr displayed stress-strain relations which were greatly dependent on the rate of extension. The apparent stiffness and the tensile strength were very high and were nearly equal to those of the CA treated with ACh when the specimens were stretched at 740 μm s^{-1}. However, they decreased sharply as the rate of extension was lowered. When stretched at 7 μm s^{-1}, the specimen developed only a small tension, about 9×10^4 N m^{-2}.

The above observations prove the visco-elastic nature of the CA. Since the apparent stiffness and the tensile strength measured at a relatively high speed were almost the same for the CA treated with both ACh and Adr, it is clear that the elastic components of the CA did not appreciably change. The changes in the mechanical properties of the CA must be due to changes in the viscous components of the CA.

To examine the possibility that a sustained contraction of the muscle cells produces

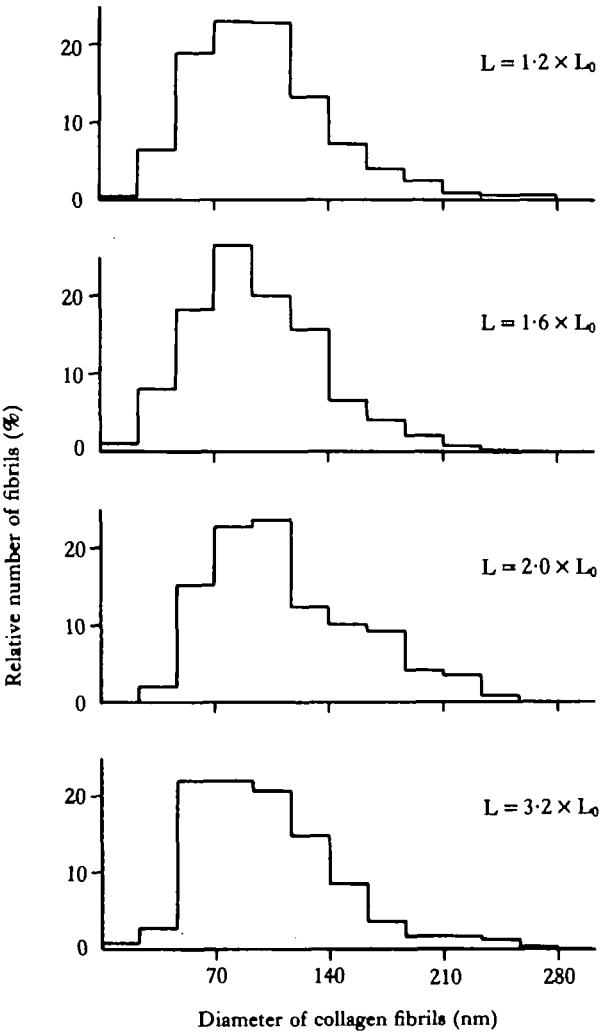


Fig. 11. Distribution of collagen fibril diameters in the CA fixed at various degrees of extension.

the apparent increase in the viscosity of the CA, actual changes in the tensile strength were compared with the estimated stress which could be generated by the muscle cells in the CA. The area occupied by the muscle cells was only about 3 %. The largest tension ever reported for muscular tissues is that of the anterior byssus retractor muscle of *Mytilus*: $1{\cdot}2$–$1{\cdot}3 \times 10^6\,N\,m^{-2}$ (Jewell, 1959; Twarog, 1967). Even if we assume that the muscle cells in the CA can generate as large a tension as that developed by the anterior byssus retractor muscle, the maximum stress in the CA amounts to only $4 \times 10^4\,N\,m^{-2}$. When the specimen was stretched at $7\,\mu m\,s^{-1}$, the passive tension generated in the CA treated with ACh was larger than that generated in the CA treated with Adr by $2 \times 10^7\,N\,m^{-2}$. This difference in the passive tension is about three orders of magnitude greater than the estimated stress which could be generated by the muscle cells in the CA. This excludes the possibility that the changes in the mechanical properties of the CA can be accounted for by the contractile activity of the muscle cel

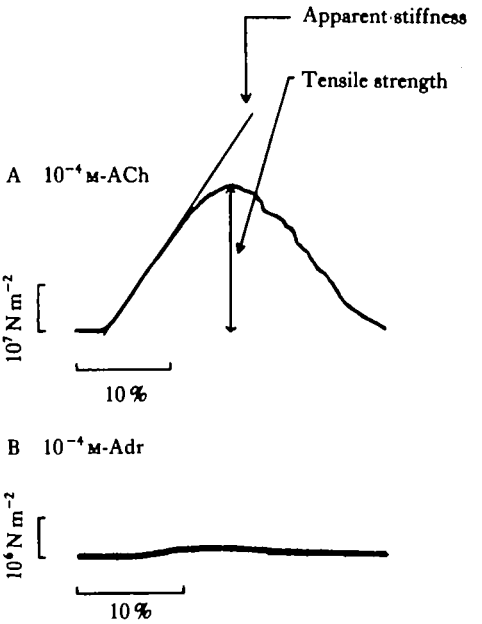


Fig. 12. Stress-strain curves of the CA. Specimens were treated with 10^{-4} M-ACh for 30 s (A) and with 10^{-4} M-Adr for 4·5 min (B) before stretch. Horizontal bars represent 10 % strain. Rate of extension, 7 μm s^{-1}.

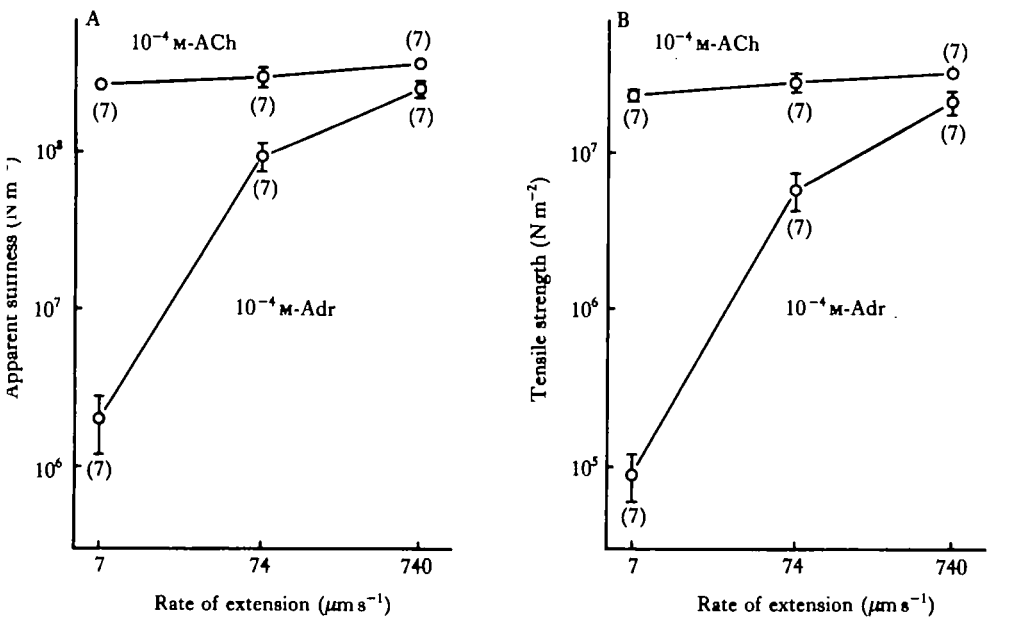


Fig. 13. Effect of ACh and Adr on the apparent stiffness (A) and the tensile strength (B) of the CA. The number of measurements is shown in the parentheses. Means±S.E.M.

in the CA. Consequently the mechanical properties of the CA seem to depend on components other than the muscle cells.

DISCUSSION

The present electron microscopic examination of the CA has shown that the CA is mainly composed of extracellular collagen fibres as described previously (Kawaguti & Kamishima, 1965; Takahashi, 1966, 1967*a*). However, a small number of isolated smooth muscle cells were found in the CA contrary to the previous observation by Kawaguti & Kamishima (1965), but in accord with the findings of Smith *et al.* (1981). These muscle cells were very thin and the areal density of these muscle cells was only about 3 %. Based on the areal density of the muscle cells in the CA, the maximum stress in the CA which could be generated by the muscle cells was estimated to be about 4×10^4 N m^{-2}. This value was calculated by assuming that the muscle cells in the CA were as powerful as those in the most powerful muscles known. Yet, this value is three orders of magnitude smaller than the observed difference in the passive tension between the CA treated with ACh and those treated with Adr. So, it is clear that the muscle cells alone cannot account for the changes in the stretch resistance of the CA. The changes in the mechanical properties of the CA must be due to some changes in the collagen fibre system.

The above discussion is based on the assumption that the muscle cells extend the whole length of the CA from the test to the base of the spine. Although the muscle cells run parallel with the collagen fibres, it is possible that the muscle cells are relatively short and serve as cross-links between the collagen fibres. The present observation suggests that the muscle cells are attached to the collagen fibres at both ends. If this is the case, it is possible that some muscle cells that do not appear in the same transverse section of the CA exert forces on the same collagen fibre and that the tension per unit cross-sectional area of the CA is greater than a simple sum of the tensions generated by the individual muscle cells which appear in the same transverse section of the CA. In such an arrangement of muscle cells, an imposed change in the length of the CA will produce a proportionately greater change in the length of the muscle cells. A preliminary measurement has revealed that the muscle cells in the stretched CA became 2–3 times thinner than expected from the actual change in the length of the CA. This suggests that such a mechanical advantage, if present, must be small.

When the CA treated with Adr was stretched at a relatively slow speed (7 μm s^{-1}), only a small tension (about 9×10^4 N m^{-2}) was developed. This indicates that the CA in an extensible state can be stretched by a small tension such as that generated by the muscle cells in the CA. Probably the muscle cells serve to restore the extended CA to its original length. This is consistent with the previous observation that the CA shortened when it was kept slack for a period (Takahashi, 1967*b*), and is in accord with the conclusion of Smith *et al.* (1981) based on their electron microscopic observations of the CA of *Echinometra lucunter*.

The present electron microscopic examination of the CA fixed at various degrees of extension has shown that the collagen fibrils remain essentially undeformed during the elongation of the CA. On the other hand, the collagen fibres underwent considerabl

deformation when the CA was stretched. Circular empty spaces frequently occurred in the collagen fibre of the stretched CA. These observations strongly suggest that the length change of the CA can be accounted for by the slippage of collagen fibrils relative to one another (Fig. 14). Smith *et al.* (1981) have also suggested that the collagen fibrils slide in the CA of *Echinometra lucunter*, because the CA does not kink when it shortens in normal function on the side to which the spine is being pointed. Consequently interactions between the collagen fibrils and the interfibrillar matrix and those between the interfibrillar matrix molecules must play a significant role in determining the mechanical properties of the CA.

This is quite contrary to the case with the mammalian tendon, a similar parallel-fibred collagenous tissue. The tendon is known to be highly inextensible with a modulus of elasticity of about 8×10^8 N m^{-2} (Rigby, Hirai, Spikes & Eyring, 1959). It has been reported that the periodicity of the collagen fibrils can be enlarged proportionately to the strain when the tendon is stretched up to about 10 % strain (Cowan, North & Randall, 1955). This indicates that the elongation of the individual collagen fibrils can account for the strain of the tendon and that the high modulus of the tendon might be due to the relative inextensibility of the collagen fibrils.

Gosline (1971), however, has shown that, in the body-wall connective tissue of the sea anemone, the spacing of the X-ray diffraction patterns of the collagen are the same for the unstretched mesogloea and the mesogloea stretched to twice the resting length. Harkness (1968) has also suggested that movement of the collagen fibrils relative to one another might account for the large change in the circumference of the uterine cervix of the rat during pregnancy. It seems that, in highly extensible tissues, the collagen fibrils are relatively short and can move past one another. The characteristic feature of the CA seems to be its capacity to change the ease with which the collagen fibrils can move relatively to one another.

The responsiveness of the CA to neurotransmitters, such as ACh and Adr, and to excess potassium ions suggests that the mechanical properties of the CA are under

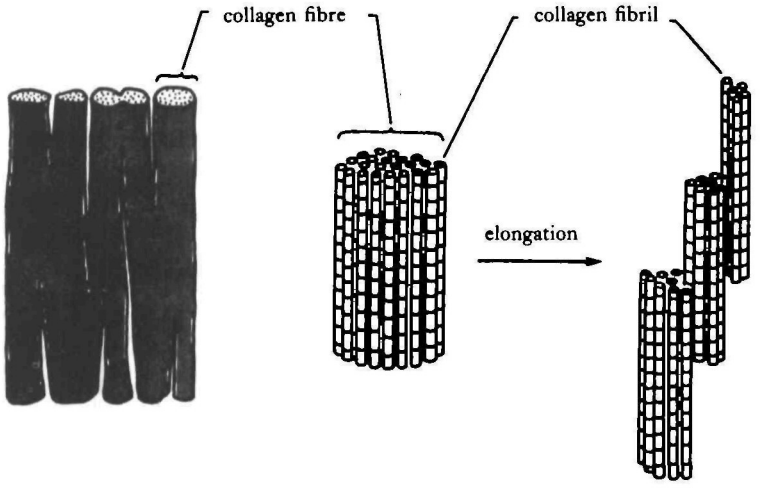


Fig. 14. A model of the collagen fibre system in the CA. Collagen fibres anastomose with each other to form a network. Collagen fibrils are relatively short and slide along one another when such a network is stretched at both ends.

nervous control (Takahashi, 1967*b*; Hidaka, 1979). In fact, electrical stimulation the radial nerve reduces the 'stiffness' of the CA of *A. crassispina* (Maeda, 1978). Though the mechanical properties of the CA appear to be under nervous control, the structural basis of this cellular control is not clear. Cellular processes filled with granules of 120–220 nm diameter were abundant throughout the CA. These granules are also found in the CA of *Echinometra lucunter* (Smith *et al.* 1981) and are similar to those found in the tube-foot wall of echinoids, especially within the connective tissue layer and at the collageno-muscular junctions (Coleman, 1969; Florey & Cahill, 1977). Similar granules are also found in the juxtaligamental cells in ophiuroids (Wilkie, 1979). Both of these tissues are supposed to be able to change their mechanical properties. It has been supposed that the connective tissue of tube-foot wall may be plasticized during the movement of the tube foot (Florey & Cahill, 1977). Wilkie (1978*a*) has reported that the mechanism of arm autotomy in ophiuroids depended on a sudden decrease in the tensile strength of the intervertebral ligament and other intersegmental connective tissue structures. It has been suggested that the granules in the juxtaligamental cells may be involved in the rapid loss of tensile strength of the intervertebral ligament during arm autotomy (Wilkie, 1979). Although similar granules were found also in the hyponeural tissue of *Echinus* (Cobb & Laverack, 1966) and of *Asterias* (Hehn, 1970), it is tempting to assume that these granule-containing cells are characteristic of echinoderm connective tissues and are involved in the control mechanism for altering the mechanical properties of these tissues.

The microfibrils were abundant around the collagen fibres and also near the cellular elements. These are similar to the slender fibrils found in *Echinometra lucunter* (Smith *et al.* 1981). The function of the microfibrils is not clear at present. However, it is likely that they play some mechanical role, since the microfibrils in the stretched CA aligned with the stress axis indicating that they are connected to each other or to some other components. Hanak & Böck (1971) have suggested that the 'microfibrils' of 10–12 nm diameter provide the junction between the muscle cells and the collagenous tendon fibres in mammalian muscular tissues. The attachment between the muscle cell and the microfibrils could not be found in this study. Some microfibrils, however, appeared to bridge between collagen fibrils of different fibres and between the muscle cells and the collagen fibrils. Since the muscle cells in the stretched CA were more closely attached to the microfibril sheath rather than to the collagen fibres, it is likely that the microfibrils have some role in anchoring the muscle cells to the collagen fibres.

There is an increasing amount of evidence which supports the idea that certain echinoderms can change the mechanical properties of their collagenous connective tissues rapidly and reversibly. Meyer (1971) stated that the collagenous ligaments in crinoids appeared to be capable of maintaining a high degree of stiffness over a longer period of time than a normal muscle can remain contracted. He suggested that the crinoid ligaments functioned in a manner similar to the holding mechanism of the CA of the sea-urchin spine. It is known that a sea cucumber can undergo an enormous change in size when handled and that there is a marked increase in body wall rigidity on shrinking. The isolated preparation of holothurian dermis also responds to mechanical stimulation by an increase in stiffness (von Uexküll, 1926). Stott *et al.* (1974) have measured this change in stiffness semi-quantitatively. Motokawa (198

82) has reported the stiffness change of the isolated preparation of the holothurian dermis caused by chemical and electrical stimulation. Stott *et al.* (1974) claimed that they found no muscle in a light microscopic examination of the holothurian dermis nor any muscle-like biochemical activity in the extract of dermis. It has been suggested that the collagenous connective tissue which surrounds the ossicles in the body wall of the starfish may be responsible in part for the varying rigidity of the ray (Eylers, 1976). However, direct measurement of changes in mechanical properties have previously been made only with the CA (Takahashi, 1967*b*; Smith *et al.* 1981), the holothurian dermis (Stott *et al.* 1974; Motokawa, 1981, 1982), and the intervertebral ligament of ophiuroids (Wilkie, 1978*a*,*b*).

In the present study, we measured the change in the mechanical properties of the CA and showed that the change in the stretch resistance of the CA cannot be accounted for by the contractile activity of the muscle cells found in the CA. Since the collagen fibrils slide past one another on elongation of the CA, the stretch resistance of the CA must be due to cohesive forces between the collagen fibrils. The present study points out the importance of interactions between the collagen fibrils and the interfibrillar matrix and between the interfibrillar matrix molecules in determining the mechanical properties of the CA. To elucidate the mechanism of changes in the mechanical properties of the CA, it is necessary to study the nature of the interactions and to examine the factors that modify these interactions. Some results of such a study have been described (Hidaka, 1979) and will be described in the following paper (Hidaka, 1983).

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