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CONNECTIVE TISSUE MECHANICS OF *METRIDIUM SENILE*

I. STRUCTURAL AND COMPOSITIONAL ASPECTS

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The body-wall connective tissue (mesogloea) of the sea anemone *Metridium senile* constitutes the highly extensible, tensile container for the hydrostatic skeleton of this animal (Wainwright, 1970). Light-microscopic (Chapman, 1953*a*) and electron-microscopic (Grimstone, Horne, Pantin & Robson, 1958) studies of anemone mesogloea show this tissue to be densely fibrillar, with the fibres forming right-handed and left-handed helices around the columnar body wall. The fibrous component has been identified as a collagen on the basis of periodic banding in electron micrographs (Grimstone *et al.* 1958; Piez & Gross, 1959; Batham, 1960) and of amino acid analyses (Piez & Gross, 1959).

Chapman (1953*b*) observed that the mechanical properties of the body wall of sea anemones could be attributed to the mesogloea alone and did not depend on the muscle fibres. Alexander (1962) carried out creep tests (extension under constant stress) on *Metridium* mesogloea over long periods of time. He found that under very small loads the material elongated for about 12–15 h, reaching more than three times its initial length, and then maintained constant length. When the load was removed the material showed nearly complete recovery over a similar time period. Alexander's observations provide valuable insight into the relationship between the mechanical properties and the function of the tissue in the animal, but they shed little light on the molecular mechanism that accounts for these properties. A macromolecular model which can account for these mechanical properties must stay within the limits of the observed structure and composition of the tissue and explain the remarkable extensibility and apparent slow elasticity. This paper attempts to set down a structural and compositional framework within which such a macromolecular model can be constructed.

The mechanical properties of a composite material of fibres in a matrix, such as mesogloea, are affected by the degree of preferred orientation of the high-strength, fibrillar component (in this case collagen), by the volume percentage of the components in the composite, and by the mechanical properties of the components themselves (Kelly, 1967). Each of these aspects has been considered to some extent in this study. I observed fibrillar arrangement with polarized light microscopy and the fine structure with electron microscopy. Preliminary quantitative analyses were carried out to determine the volume fraction of collagen present in the composite and to investigate

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the nature of the matrix. A series of experiments dealing with the nature of the mesogloeal collagen was carried out to determine the extent of mechanical similarity between mesogloeal and vertebrate collagens.

MATERIALS AND METHODS

Large *Metridium senile* from the California coast were maintained in refrigerated aquaria (12–14 °C) filled with artificial sea water ('Instant Ocean', Aquarium Systems, Inc., Wickliffe, Ohio). Animals damaged during collecting usually died within 2 weeks, but healthy animals were maintained for up to 2 months without problem. The periodic contractions and expansions characteristic of normal behavioural patterns of *Metridium* (Batham & Pantin, 1950) persisted for about 1 month. After this time the periodic changes in shape became less frequent. In all cases only healthy animals were used.

Mesogloea was isolated by first relaxing the animal for at least 6 h at 0 °C in artificial sea water to which isosmotic magnesium sulphate (20 % $MgSO_4.7H_2O$ in distilled water) had been added. The tentacles, oral disk, mesenteries and pedal disk were dissected away leaving a flat sheet of body wall. Parietal muscle fibres running longitudinally on the inner surface of the body wall were dissected away as completely as possible. This process yielded a material which will be referred to hereafter as mesogloea.

Mesogloea for polarized light microscopy was relaxed in $MgSO_4$–sea water and then fixed in 1 % formaldehyde in sea water. Sections were cut on a thermoelectric freezing-stage microtome (Komatsu Solidate Co., Ltd., Tokyo, Japan) and observed at low magnifications with a compound polarizing microscope (Leitz, Ortholux-pol). The intrinsic birefringence of collagen fibres was determined by measuring the retardation of single fibres in tissue sections equilibrated with media of varying refractive index. The media used were as follows: acetone, $n = 1{\cdot}356$; chloroform, $n = 1{\cdot}443$; benzene. $n = 1{\cdot}498$; 1-bromonaphthalene, $n = 1{\cdot}656$; and mixtures of benzene and 1-bromonaphthalene for values between $n = 1{\cdot}498$ and $n = 1{\cdot}656$. The refractive index of these solutions was measured with an Abbé refractometer; retardation was measured with a Berek compensator.

Mesogloea prepared for electron microscopy was fixed for 1 h in 6 % glutaraldehyde in sea water, washed in sea water, post-fixed in 1 % osmium tetroxide in sea water for 1 h, and passed through an alcohol series all at 0 °C. The tissue was then put into propylene oxide at room temperature and embedded in Epon. Sections were cut on a Poter–Blum MT-1 ultramicrotome, stained with 1 % uranyl acetate (Watson, 1958) and lead tartrate (Millonig, 1961) and observed on a Hitachi HS-7 electron microscope at 50 kV.

Mesogloea prepared for X-ray diffraction analysis was washed with running distilled water for several hours to remove salts and then air-dried. Stretched samples were elongated in sea water before washing with distilled water. The diffraction patterns were taken with Ni-filtered Cu Kα radiation ($\lambda = 1{\cdot}54$ Å) on Kodak no-screen medical X-ray film in a flat-plate camera.

Dry-weight measurements were made after holding mesogloea overnight at 110 °C. Salt-free dry weights were measured on mesogloea washed for several hours in running distilled water. Mesogloea that was to be autoclaved was washed with distilled

water, air-dried, powdered in a ball mill, and weighed. The autoclave-soluble material was extracted in distilled water at 18 psi (124 °C) for 12–15 h.

Hexosamine determinations were carried out on material hydrolysed in 6N-HCl in sealed tubes at 110 °C for 12 h. Chromatographic separation and the Elson–Morgan reaction were carried out according to the procedure of Boas (1953). Hexuronic acids were determined with the carbazole reaction as described by Dische (1947). Neutral hexoses were determined with the anthrone reaction according to Roe (1955).

RESULTS

Microscopy

The results of polarized-light microscopy on mesogloea are shown in Plates 1 and 2. Light areas in these pictures are indications of a birefringent material; in this case collagen. Collagen, however, is not birefringent if the observer is looking down the fibre axis. Thus, only radial and circumferential fibres are seen in a transverse section through the animal, only radial and vertical fibres are seen in a longitudinal section, and so on. Pl. 1, fig. 1A shows a horizontal section through the anemone body wall. The inner third of the mesogloea is seen as a broad, intensely birefringent band. The birefringence in this area is due to a high concentration of circumferential and radial fibres. In horizontal sections observed with a first-order red compensator the birefringence is positive in the circumferential direction, probably because the number of circumferential fibres present is much greater than the number of radial fibres. Small pieces of mesentery can be seen extending from the inner side of this layer. The outer two-thirds of the mesogloea appears as a broad band containing radially orientated fibres, but the density (number/unit area) of these fibres appears to be considerably lower. A thin layer of circumferential fibres is located along the outer surface of the mesogloea just below a number of projections which extend into the ectodermal cell layer. The cells appear dark between crossed polaroids because they do not contain any collagen or other strongly birefringent material.

Pl. 1, fig. 1B shows a longitudinal section through the body wall. The inner layer is seen to contain a number of discrete bundles of radial fibres that extend about one-quarter of the thickness of the material. Circumferential fibres are not seen in this layer, as mentioned earlier, because they are cut in cross-section. The outer portion of the mesogloea shows a layered appearance. Observation of these bright layers with a first-order red compensator suggests that the layers are concentrations of longitudinally orientated fibres. The extreme outer edge again shows projections which extend into the ectodermal cell layer.

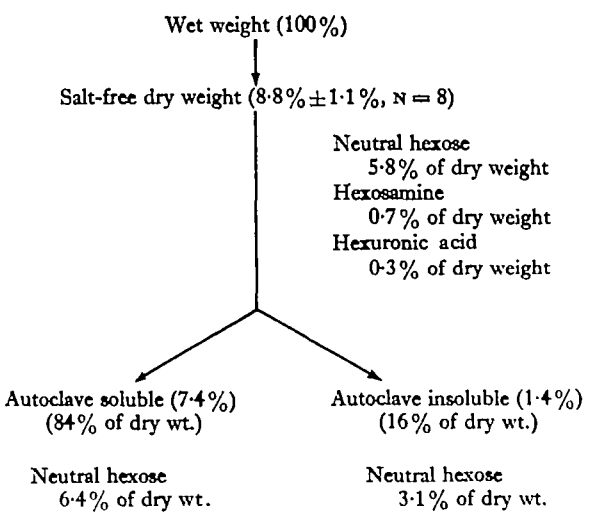
Pl. 2, fig. 2B and C are tangential sections cut at different levels in the mesogloea. Pl. 2, fig. 2B passes through the broad band of diffuse radial and vertical fibres seen in Pl. 1, fig. 1A and B. The crossed-fibrillar arrangement of the collagen fibres is quite apparent. The edge of this section, seen at the right of the picture, was cut parallel to the vertical axis of the animal. As these micrographs are from 'relaxed' animals, it should be possible to measure the fibre angles (the angle between the fibre and the vertical axis of the animal) for unstressed mesogloea. Unfortunately, the relaxing procedure is not completely effective, and the animals tend to contract when they are dissected and fixed. Thus the angles measured in these micrographs range from

32° to 50° and can only be taken as approximations to the unstressed fibre angle. The mean fibre angle (± twice the standard deviation, $N = 25$) from Pl. 2, fig. 2B is $37 \pm 7°$. Mean fibre angles of $43 \pm 4°$ and $45 \pm 4°$ were obtained from photomicrographs of two other sections from the same piece of mesogloea. In a small area of a single micrograph the range of values is quite small, but values from different areas in a single micrograph or from different micrographs cover a much broader range. Pl. 2, fig. 2C shows the transition between the outer, crossed-fibrillar region and the dense circumferential and radial fibres of the inner layer. Spaces between the circumferential fibres are probably occupied by the radial fibre bundles seen in Pl. 1, fig. 1B.

Pl. 2, fig. 2A is a low-power electron micrograph showing the densely fibrillar nature of mesogloea. The collagen fibres appear to be arranged in a number of tracts or bundles with the bundles running in apparently random directions. The collagen fibres appear to be the only structures, apart from occasional cells, in this micrograph. In places where the collagen fibres have been cut in cross-section it can be seen that the individual fibres are completely surrounded by space that is assumed to be filled with non-staining matrix. The fibres apparently do not branch. In similar preparations at higher magnifications it is possible to detect traces of the 210–220 Å axial banding pattern as well as the hollow appearance of the fibres observed by Grimstone *et al.* (1958) and Piez & Gross (1959).

Table 1. *Quantitative analyses of* Metridium *mesogloea*

(Salt-free dry weights were obtained from tissue washed in running tap water. Autoclave soluble material was extracted in water at 18 psi (124° C) for 12 h.)



Quantitative analyses

Table 1 shows the results of quantitative analyses run on *Metridium* mesogloea. The salt-free dry weight makes up approximately 9% of the wet tissue. Analyses of dry mesogloea show that about 6% of the dry weight is neutral hexose with only traces of hexosamine and hexuronic acid. After autoclaving, 84% of the dry weight goes into solution leaving 16% as an autoclave-insoluble fraction. Collagen charac-

ristically becomes soluble with autoclaving while other proteins are precipitated. Thus, the soluble fraction can be used as a rough estimate of the amount of collagen present. A large fraction of the neutral hexose is found in the soluble material. The insoluble fraction must contain non-collagen protein and polysaccharides as well as any traces of collagen that do not dissolve on autoclaving.

Mesogloeal collagen structure

The results of X-ray diffraction studies on mesogloeal collagen and rat-tail tendon collagen are shown in Table 2. The 2·86 Å meridional spacing, the 11·5 Å equatorial spacing and the 9·5 Å layer line (indicated by diagonally placed reflections) characteristic of all collagens are seen for both mesogloeal and rat-tail tendon collagen. The diffuse 4·5 Å equatorial reflection appears to be more intense in mesogloeal collagen patterns.

Table 2. *Characteristic X-ray diffraction spacings of* Metridium *mesogloeal collagen and rat-tail tendon collagen*

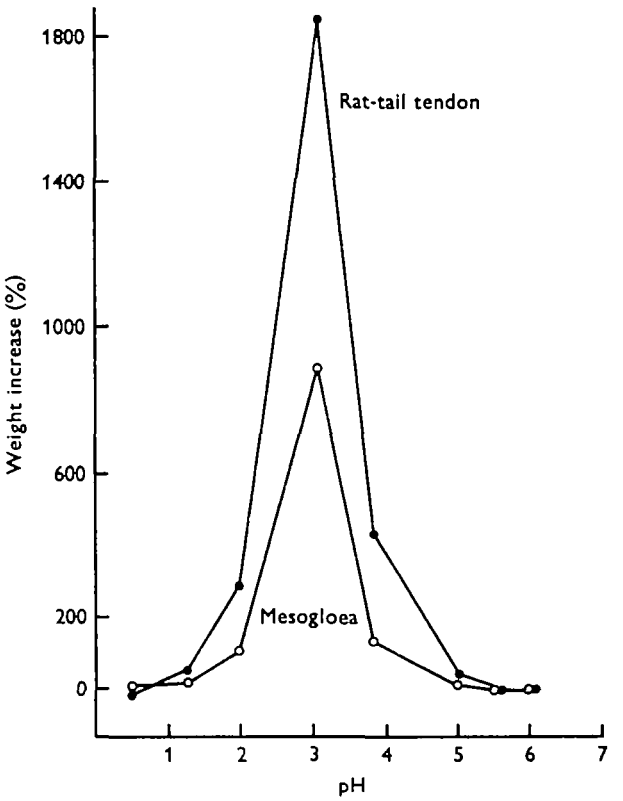
Reflexion	Mesogloeal collagen (Å)	Rat-tail tendon collagen (Å)
Equatorial (diffuse)	4·56	4·5
Equatorial	11·5	11·2
Meridional	2·87	2·86
Layer line	9·5	9·4

The X-ray diffraction patterns of mesogloea stretched to twice resting length is made up of relatively short arcs, indicating a high degree of preferred orientation of the collagen fibres. The degree of preferred orientation of stretched mesogloea compares quite closely with that of rat-tail tendon collagen. The X-ray diffraction patterns of unstretched mesogloea is made up of diffuse haloes or complete circles, indicating a very random orientation of the collagen fibres. The spacings are the same for both stretched and unstretched mesogloea. Apparently, the collagen fibres align with the stress axis when mesogloea is deformed.

Pieces of rat-tail tendon and of mesogloea were washed in running tap water, blotted and weighed, placed in dishes of distilled water of known pH (adjusted with HCl) and left overnight at 0 °C. Text-fig. 1 shows the percentage weight change as a function of the pH of the medium at the end of the test period. Both rat-tail tendon and mesogloea show a maximum weight increase at pH 3·1. The swelling in rat-tail tendon is greater presumably because rat-tail tendon has a higher collagen content than mesogloea. Besides swelling, rat-tail tendon collagen, normally opaque, becomes translucent, almost transparent, over the range pH 2–4. Mesogloea showed a similar change in transparency.

In a similar experiment weighed pieces of rat-tail tendon and mesogloea were placed in various concentrations of urea and allowed to stand overnight at 0 °C. Text-fig. 2 shows the percentage weight change as a function of urea concentration. The curves for both materials are very similar. Both can be extrapolated to zero weight change at slightly less than 2 M urea, and both tend to level off at higher urea concentrations. Rat-tail tendon showed a greater change at every point, again, because rat-tail tendon has a higher collagen content.

The intrinsic birefringence of mesogloeal collagen and rat-tail tendon collagen a[illegible] compared in Text-fig. 3. A minimum in retardation at refractive index (n) = 1·55 is observed for both types of collagen. This indicates that the refractive index of the collagen crystallite in a direction perpendicular to the optic axis (the optic axis is parallel to the long axis of a collagen fibre) is the same in both mesogloeal and rat-tail tendon collagen. The increase in retardation at values of refractive index above and below n = 1·55 is an indication of form birefringence that can be ascribed to the parallel arrangement of collagen fibres in a medium of differing refractive index.

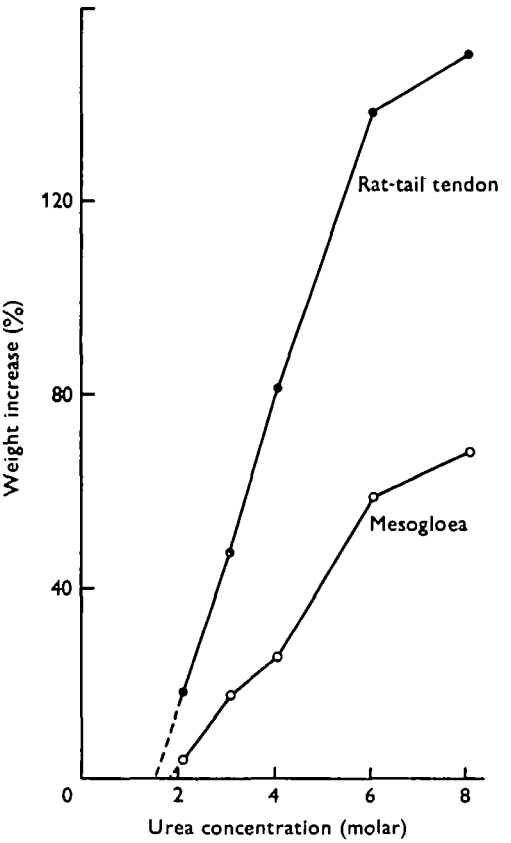


Text-fig. 1. The osmotic swelling of mesogloeal collagen. The percentage weight change of mesogloea and rat-tail tendon was measured in distilled water over a range of pH.

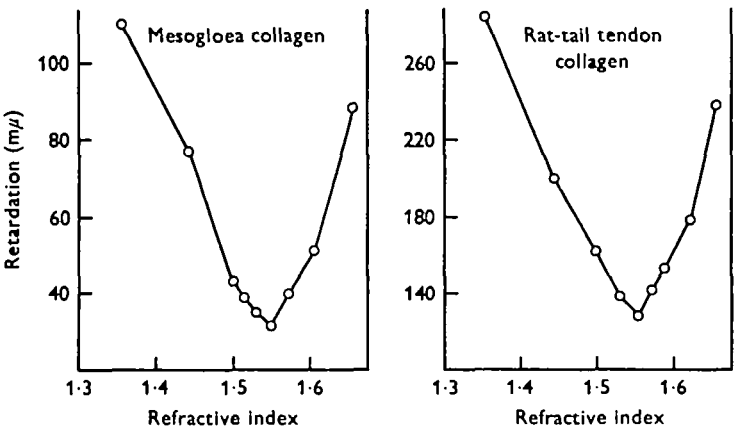
DISCUSSION

The polarized-light micrographs of mesogloea from large *Metridium senile* clearly demonstrate the structural complexity of this material. The mesogloea appears to be made up of two distinct layers: an outer layer which comprises about two-thirds of the thickness of the material and is characterized by a crossed-fibrillar arrangement of collagen fibres running helically in the plane of the body wall, and an inner layer making up the other third which is characterized by densely packed radial and circumferential fibres.

The crossed-fibrillar arrangement in the outer layer is similar to the structure found by Chapman (1953*a*) in *Calliactis*. The X-ray diffraction patterns I have taken of stretched and unstretched mesogloea clearly indicate that the collagen fibres align



Text-fig. 2. Lyotropic swelling of mesogloeal collagen in urea.



Text-fig. 3. Intrinsic birefringence of mesogloeal collagen. The retardation of collagen fibres in mesogloea and in rat-tail tendon was measured in organic media of differing refractive index.

with the stress axis when the material is stretched. Crossed-fibrillar structures in which the fibres align when the structure is deformed provide a reasonable arrangement for a highly extensible material (Picken, Pryor & Swann, 1947). The variability in the mean fibre angle measured in different sections from a single piece of mesogloea suggests that the stress conditions in the sample are not uniform. Indeed, during dissection the animals do tend to contract, and Batham & Pantin (1951) report that the mesogloea of *Metridium* is thrown into horizontal folds during such contractions, where the degree of folding is related to the magnitude of the contraction. The formation of such folds would put some parts of the body wall under tension, others under compression, and leave some parts unaffected. As the contractions in the material used in this study were relatively minor, the degee of folding should be small. These folds could account for the variations observed between sections and within a single section, and thus make accurate determinations of the unstressed fibre angle difficult. A value of 40° to 45° for the unstressed fibre angle is probably as reasonable a figure as can be obtained. This is similar to the 45° fibre angle observed in *Calliactis* by Chapman (1953*a*). The extensibility of a fibre lattice with an unstressed fibre angle of 45° would be about 40% assuming the fibres are inextensible and they are joined where they cross. The extensibility of mesogloea is much greater than 40% (Alexander, 1962) indicating that the fibres in the mesogloea lattice are either extensible or are not joined together.

The inner, densely fibrillar layer is most interesting. In a composite material the distribution and orientation of the high-strength, fibrillar component plays a major role in determining the properties of the material. This high concentration of radial and circumferential fibres must surely have some mechanical significance in mesogloea. Perhaps it provides a rigid attachment layer for the parietal muscles that lie perpendicular to these fibres on the inner surface of the mesogloea, or perhaps it is a mechanism that provides mechanical anisotropy to the material as a whole.

Electron micrographs of mesogloea show more clearly the relationship between the collagen and the matrix. The mesogloea of *Metridium* can be described, on the basis of the electron-microscopic evidence, as a two-phase system of fibres in some non-staining matrix. The crossed-fibrillar nature of mesogloea has been observed in a number of sections, although it is not very apparent in the section in Pl. 2, fig. 2A. Grimstone *et al.* (1958) also report the presence of a crossed-fibrillar arrangement in electron micrographs of *Metridium* mesogloea. It is very difficult to estimate the volume percentage of the matrix in mesogloea from electron micrographs. The embedding procedure for electron microscopy involves dehydrating the material in alcohol, and the resulting removal of water and replacement with alcohol is likely to cause considerable shrinkage. As the collagen fibres are probably not as highly hydrated as the matrix, the shrinkage of these fibres should be less than that of the matrix. Thus, the relative proportions that are observed in electron micrographs may not accurately reflect the volume percentage of the two components in the native mesogloea. Even without taking this differential shrinkage into account, it is obvious that the matrix makes up a considerable portion of the volume of the material.

Quantitative analyses of mesogloea disclosed neutral hexoses in large amounts and hexosamines and hexuronic acids in trace amounts. Gross, Dumsha & Glazer (1958) also found large amounts of neutral hexose (8·9% with an orcinal reaction) as well

as 0·8 % neutral pentose. Piez & Gross (1959) report finding 0·6 % hexosamine, but make no mention of hexuronic acids. The trace appearance of hexuronic acid in this study is probably due to the interfering reaction of neutral hexose with carbazole as pointed out by Dische (1947). The lack of hexuronic acids is in agreement with the findings of Katzman & Jeanloz (1970), who report finding no acidic or sulphated polysaccharides in gelatin from *Metridium dianthus*.

The 85 % of the dry weight that becomes soluble with autoclaving is a reasonable figure for the collagen content of mesogloea. A major portion of the neutral hexose and hexosamine was found in the autoclave-soluble fraction, suggesting that it may have been bound in some way to the collagen. The insoluble fraction, which makes up 16 % of the dry weight or 1·4 % of the wet weight, must contain the matrix protein and the remaining polysaccharide.

Mesogloea appears to be a very highly hydrated tissue. The salt-free dry weight is 9 %. Thus in a 100 g sample of mesogloea, assuming that there is approximately 5 g of salt, there will be about 86 g of water. This water must be partitioned in some manner between the collagen and the matrix. The 7·4 g which makes up the autoclave-soluble fraction contains a substantial amount of neutral hexose which must be classed as matrix. After adjusting for this, one obtains a value of about 6·7 g of collagen and 2 g of matrix per 100 g of wet tissue. NMR studies of collagen at low temperatures have indicated that the water of hydration of collagen is around 0·54 g of water per gram of collagen or about 2·6 water molecules per amino acid residue (Dehl, 1970). Using these figures as an indication of the amount of water associated with the collagen in mesogloea, one finds that of the 86 g of water about 3·7 g is associated with the collagen leaving the remaining 82·3 g associated with the matrix. Thus, 2 g of matrix protein and polysaccharide is associated with roughly 82 g of water giving a 2·4 % polymer solution. It is of interest that numerous polymer solutions (both natural and synthetic) form solid gels at concentrations of 3 % or below. Agar, for example, forms stable gels at concentrations as low as $\frac{1}{2}$ %, and gives very rigid gels at a concentration of 2 %. Gelatin forms stable gels at concentrations around 1 %.

The series of experiments on the structure of mesogloeal collagen was carried out in order to investigate the mechanical properties of the mesogloeal collagen. Each of the experiments, X-ray diffraction, osmotic and lyotropic swelling, and determination of the intrinsic birefringence, investigate aspects of the three-dimensional structure of the collagen molecule or the crystalline, fibrillar nature of these molecules. The comparison with a vertebrate collagen in each of these experiments is made because the mechanical properties of vertebrate collagens, which are well documented (Elden, 1968), are known to depend on the structure of the collagen molecule and the aggregation of these molecules into fibres. The tropocollagen monomer is a long (3000 Å), narrow (14 Å), rod-like molecule (molecular weight = 300000; Boedtker & Doty, 1956) made of a triple helix of three polypeptide chains (see Bailey, 1968). These monomers aggregate in nearly crystalline parallel arrays to form fibres. The present experiments on collagen structure attempt to demonstrate that mesogloeal collagen is structurally similar to vertebrate collagen. Once this structural similarity is established, it is reasonable to assume mechanical similarity as well. Information about the mechanical properties of mesogloeal collagen will be very important when attempts are made to model the mechanical behaviour of mesogloea as a whole (Gosline, 1971).

Wide-angle X-ray diffraction is capable of detecting 'crystalline' periodicities on the inter-atomic scale. The spacings observed for mesogloeal collagen appear to be identical to those observed for rat-tail tendon collagen. The 11·5 Å equatorial spacing is a measure of the distance between adjacent collagen molecules, the 2·86 Å meridional spacing is an indication of the spacing of individual atoms along the helically arranged polypeptide chains, and the 9·5 Å spacing is characteristic of the helical structure and is an indication of the spacing between turns of the helix (Bear, 1952; Tanford, 1961; Glimcher & Krane, 1968). The diffuse 4·5 Å equatorial reflexion is one to which most organic materials contribute (Marks, Bear & Blake, 1949). This reflexion is more marked in diffraction patterns of mesogloea because of the non-collagen protein and polysaccharide present in the matrix. Clearly, the structure of mesogloeal collagen as seen by wide-angle X-ray diffraction is very similar to that of vertebrate collagen.

Osmotic swelling, or swelling in salt-free solutions at acid pH, is apparently due to the titration of charged groups on the surface of collagen molecules reducing the interactions between these molecules. The weight increase or swelling is due to the uptake of water into spaces created by the loosening of the aggregate of molecules (Veis, 1964). Text-fig. 1 shows that mesogloeal collagen and rat-tail tendon collagen swell in much the same manner, indicating that the interactions which cause the molecules to aggregate into fibres are similar in both kinds of collagen.

The swelling observed in various concentrations of urea or lyotropic swelling (Text-fig. 2) is believed to be due to the breaking of hydrogen and hydrophobic bonds causing irreversible changes in the triple helical structure of the collagen molecule (Veis, 1964). Mesogloeal collagen and rat-tail tendon collagen react similarly in urea, indicating a similarity in the type of bonding that stabilizes the helical structure of both collagens.

Intrinsic birefringence is a property of crystalline materials. The crystalline nature of collagen arises from the parallel arrangement of tropocollagen molecules to firm fibres. Birefringence is defined as $B = n_e - n_0$, where n_e is the refractive index in a direction perpendicular to the optic axis and n_0 is the refractive index parallel to the optic axis. The refractive index at which retardation is minimum (see Text-fig. 3) is the value n_e for collagen. Both mesogloeal and rat-tail tendon collagen have a value of $n_e = 1.55$. Assuming that n_0 is the same for both collagens, the birefringence of each is also the same. The retardation of a wave-front passing through a birefringent material depends on the spatial arrangement of the atoms in the material and on the nature and orientation of the chemical bonds which tie the atoms together (Bunn, 1961). In other words, birefringence is dependent on the molecular structure of the crystalline aggregates. Both mesogloeal and rat-tail tendon collagen have the same intrinsic birefringence, indicating further similarity of the two collagens.

Having established a basis for assuming that mesogloeal collagen is structurally similar to vertebrate collagens, I would like to propose that mesogloeal collagen be considered mechanically equivalent to vertebrate collagens. The mechanical property of interest is the relative inextensibility of collagen when stressed along the long axis of the fibre. Rat-tail tendon collagen has a Young's modulus of elasticity of about 10^{10} dynes/cm^2, can be stretched reversibly to strains of about 2 %, and breaks at strains of about 8 % (Rigby *et al.* 1959). I shall assume that these values for rat-tail tendon collagen are reasonable approximations of the properties of mesogloeal collagen.

The information now available makes it obvious that a model for the visco-elastic properties of *Metridium* mesogloea must deal with more than just the collagen alone. The collagen fibres can be assumed to be virtually inextensible, and the lattice of collagen fibres in mesogloea, with an unstressed fibre angle of about 40°, can be stretched only 30% to 40% if the fibres are joined where they cross. Yet, mesogloea itself can be extended reversibly to over three times its initial length (Alexander, 1962). Clearly, the collagen fibres are not linked directly to one another. The properties of the material as a whole must depend to a large extent on some component other than the collagen. If we are to accept the description of mesogloea as a two-phase system of collagen fibres in a matrix, this other component must be the matrix. The following paper (Gosline, 1971) describes a series of experiments carried out to investigate the role that the matrix plays in determining the mechanical properties of mesogloea and the nature of the interactions between the collagen and the matrix.

SUMMARY

The results of this study on the structure and composition of the mesogloea of the sea anemone *Metridium senile* have shown the following about this tissue.

1. Mesogloea can be described as a two-phase system of collagen fibres embedded in a matrix.

2. Polarized-light microscopy revealed two distinct layers in the mesogloea; one characterized by crossed-helices of collagen fibres with an unstressed fibre angle of 40–45°, and the other characterized by densely packed radial and circumferential collagen fibres.

3. X-ray diffraction evidence indicated that the collagen fibres align with the stress axis when the material is stretched.

4. Mesogloea is a highly hydrated material containing roughly 86% water. Salt-free, dry collagen makes up about 6·9% of the wet weight, and salt-free, dry matrix protein and polysaccharide makes up another 2%. The hydrated matrix complex makes up about 84% of the total wet weight and was found to have a concentration of about 2·4% (grams dry matrix per 100 g water).

5. The structural similarities of mesogloeal collagen and vertebrate collagens were demonstrated in a number of experiments. On the basis of the structural similarities mesogloeal collagen was assumed to be mechanically similar to vertebrate collagens.

This work is a direct result of a most pleasant and productive association with Dr S. A. Wainwright. His interest and aid in all phases of this study are gratefully acknowledged. I would also like to thank Professor T. Weis-Fogh for his critical reading of this manuscript.

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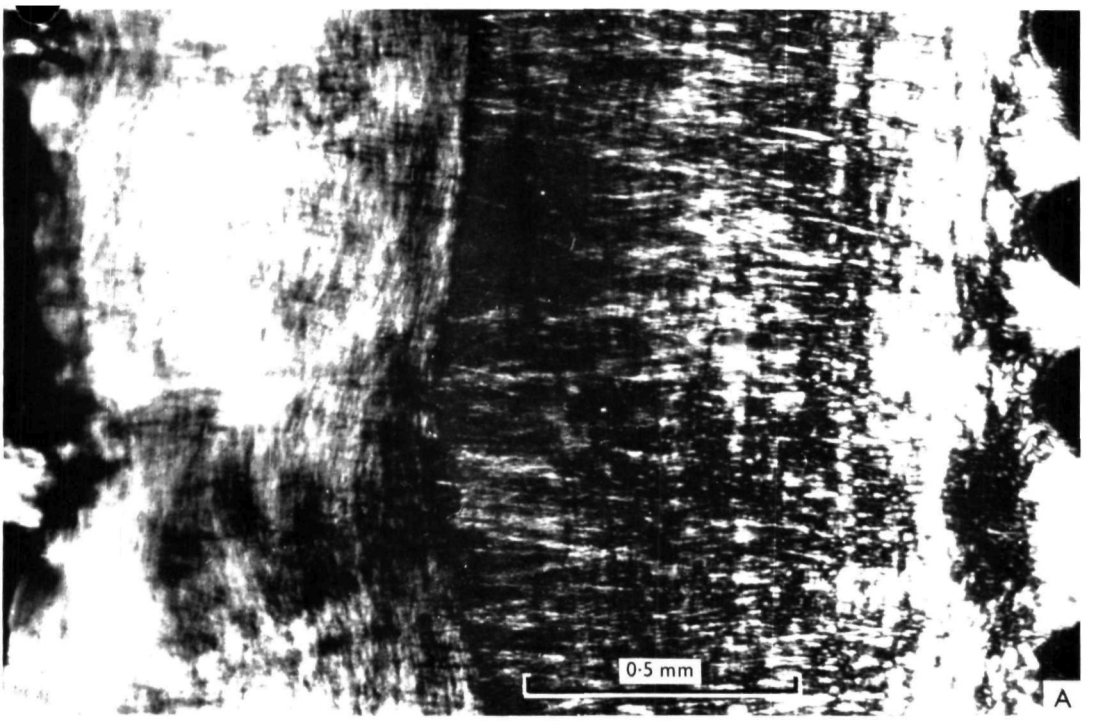
EXPLANATION OF PLATES

PLATE 1

Polarized-light micrographs of *Metridium* mesogloea. A, Horizontal section with the inner layer to the left. B, Longitudinal section with the inner layer again to the left.

PLATE 2

A, Low-power electron micrograph of *Metridium* mesogloea, showing the mesogloeal collagen fibres embedded in the matrix. B, Polarized-light micrograph of a tangential section through the outer, crossed-fibrillar layer of *Metridium* mesogloea. C, Polarized-light micrograph of a tangential section showing the transition between the outer crossed-fibrillar layer and the inner layer of densely packed circumferential and radial fibres.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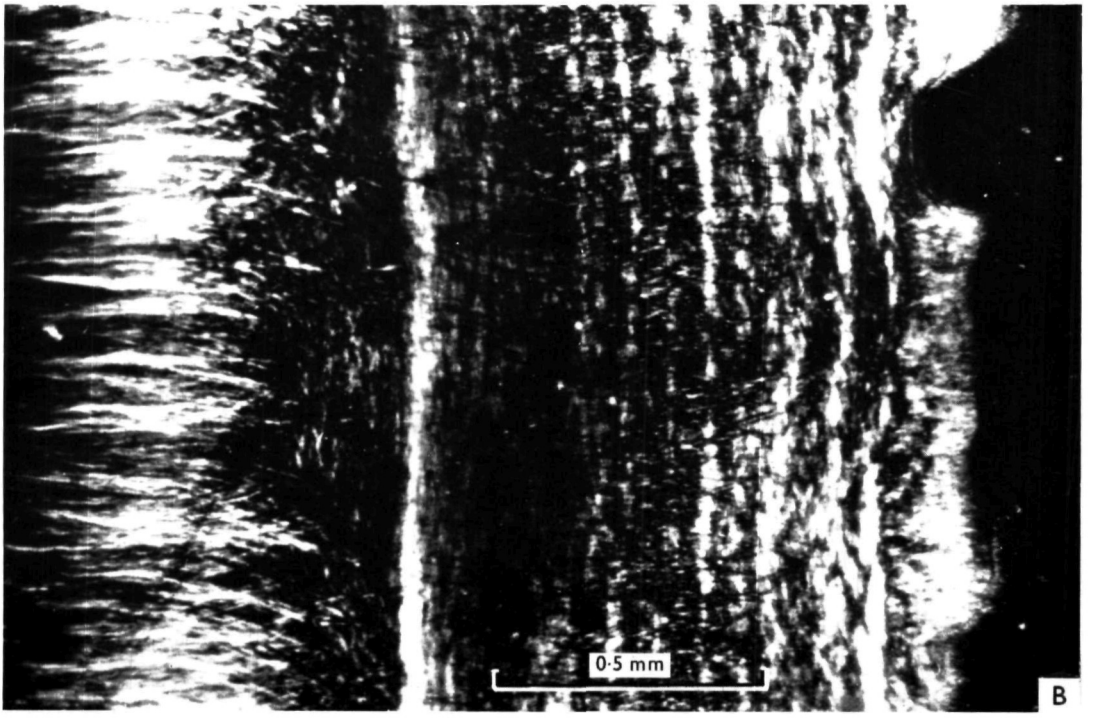
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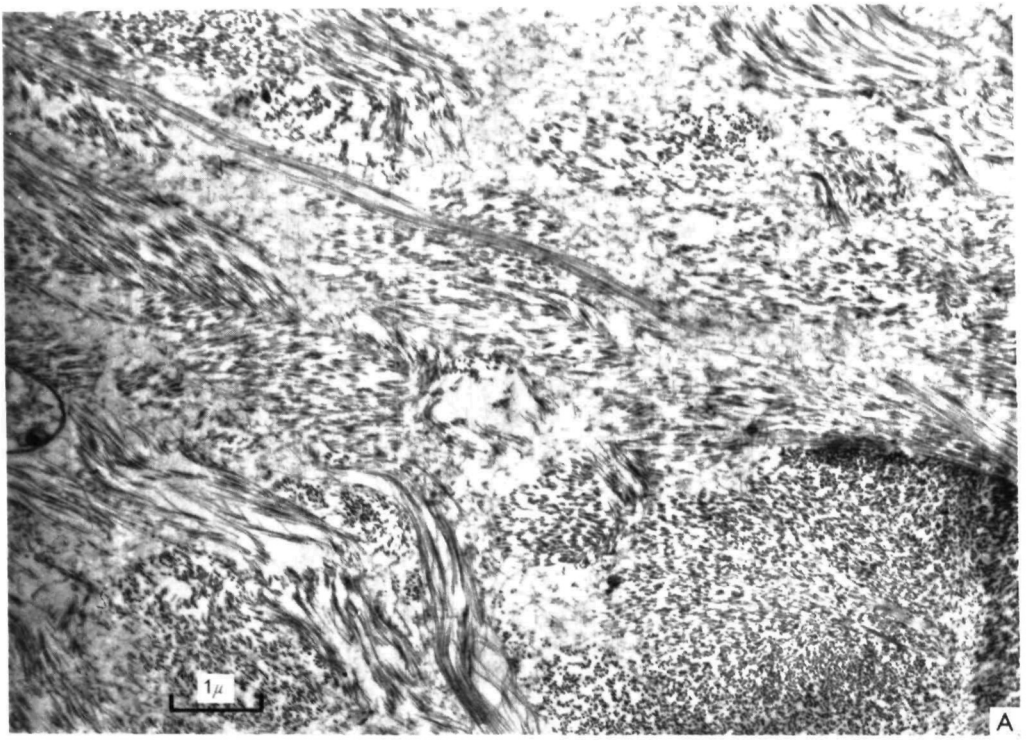
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