and the interactions between the collagen and the matrix constituents, all of which appear to vary with age and also with anatomical location. It cannot be doubted that connective tissue is a biological composite of considerable complexity but the challenge posed in determining its structure and function at the molecular level has proved to be of such great interest scientifically and medically that it has become the focus of a substantially increased research effort in the past decade.

The molecular and fibrillar structure of collagen and its relationship to the mechanical properties of connective tissue cover such a vast area of research that it is clearly impossible to do justice to it in a short review such as this. Consequently, a decision has been made to discuss only those more recent results which are pertinent to current thinking, though past observations of note are mentioned in context. For a complete background to the contents of this paper the reader is referred to those more substantial reviews on specific aspects of collagen structure and function that may be found in the literature (see, for example, refs. 1–6).

## 2. Molecular structure

The three $\alpha$-chains which constitute the collagen molecule each contain a substantial portion of their amino acid sequences in the form (glycine-X-Y)$_n$, where X and Y can be any amino acid but are frequently proline and hydroxyproline, respectively. Each $\alpha$-chain has a left-handed structure similar to that found in polyglycine II [7] and polyproline II [8]. In vivo three $\alpha$-chains aggregate in parallel to form a right-handed, triple-stranded, coiled-coil molecule whose general conformation has been formulated by Ramachandran and Kartha [9] and by Rich and Crick [7,10]. Specifically, the detailed high-angle X-ray diffraction patterns of collagen obtained by Fraser et al. [11,12] showed that the molecules have 10 repeating units in three turns, a unit rise of 0.2894 nm and a pitch length of 0.9647 nm (fig. 1). One of these features – the meridional reflection at a spacing of about 0.29 nm – is generally considered to be a diagnostic feature of a collagenous structure. For a type I collagen molecule two of the three $\alpha$-chains are identical ($\alpha_1$(I)) and the third ($\alpha_2$) is homologous but chemically distinct. The chain structure of the molecule may thus be written in the form ($\alpha_1(I)_2\alpha_2$). In both the $\alpha_1$ and $\alpha_2$ chains the triplet region extends for 1014 residues in toto (i.e., 338 triplets) and is terminated at either end by short non-triplet-containing sequences called telopeptides. These telopeptides have particular importance since each of them (with the exception of that at the C-terminal end of the $\alpha_2$ chain) contains a hydroxylysine residue involved in the formation of stabilising intermolecular covalent cross-links.

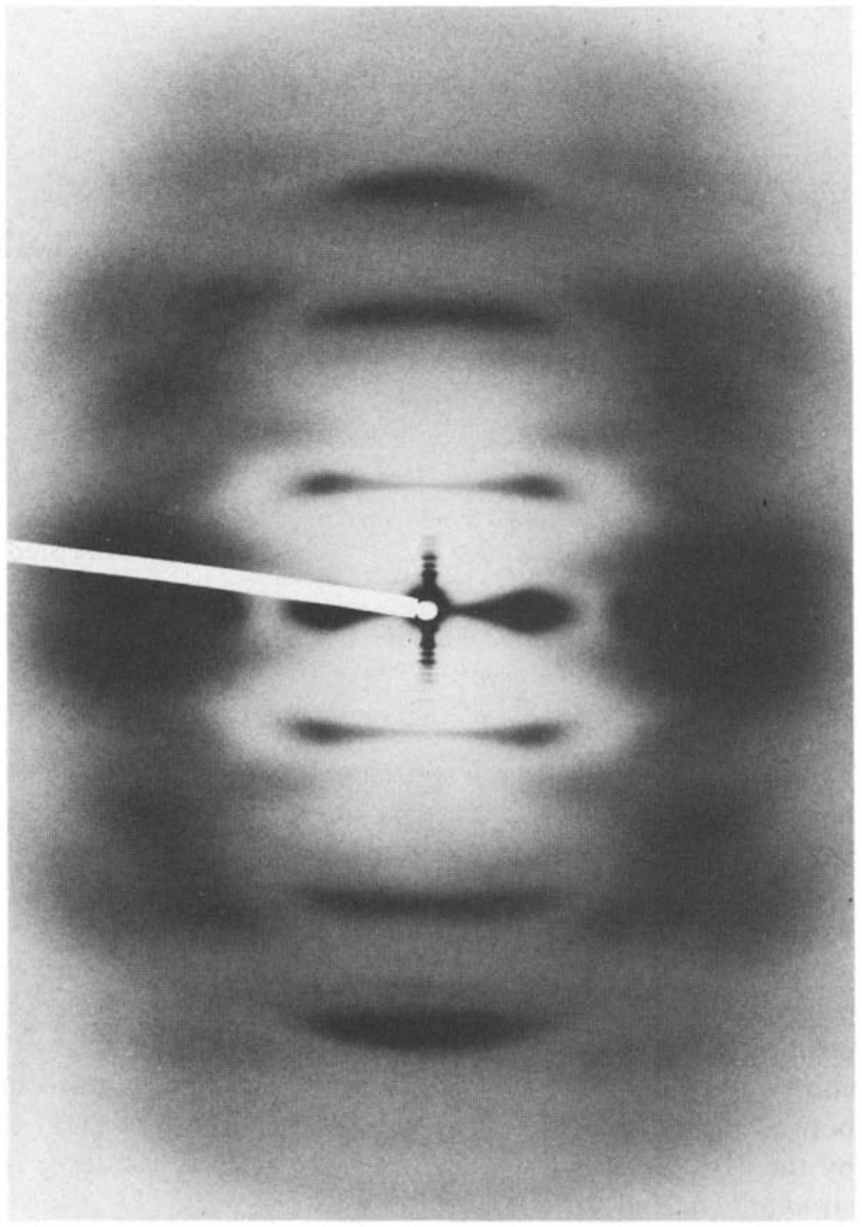

Fig. 1. High-angle X-ray diffraction pattern from a kangaroo tail-tendon which has been stretched by 10% and held at a relative humidity of 75%. The fibre axis is vertical and a characteristic meridional reflection at a spacing of about 0.29 nm can be seen. Reprinted from Fraser et al. [11] with permission of the authors and Academic Press.

The models proposed for collagen [7,9,10] dif-

fered significantly in the topology of interchain hydrogen bonding and considerable effort and ingenuity were involved in the debates on the relative merits of what became known as the one hydrogen bond per triplet structure and the two hydrogen bond per triplet structure. The issue was finally decided by two important pieces of work. The first of these was the sequential polypeptide studies undertaken by Traub and co-workers [13,14] who synthesised a variety of polytripeptides and polyhexapeptides based on the collagen triplet substructure. The sequences differed in their ability to form one or two interchain hydrogen bonds per triplet. Thus, by a judicious choice of the sequence, Traub was able to show that X-ray diffraction patterns similar to those from collagen in vivo could be obtained from those sequential polypeptides that permit the formation of only a single hydrogen bond per triplet, i.e., that between the peptide NH group of a glycyl residue and the peptide carbonyl group of an X residue in another chain. The conformation is thus the one embodied in the Rich-Crick II model. In 1979 this conclusion was confirmed directly and extended further in an elegant study undertaken by Fraser et al. [11]. In this study they collected high-resolution high-quality X-ray data and used them, in conjunction with a linked-atom

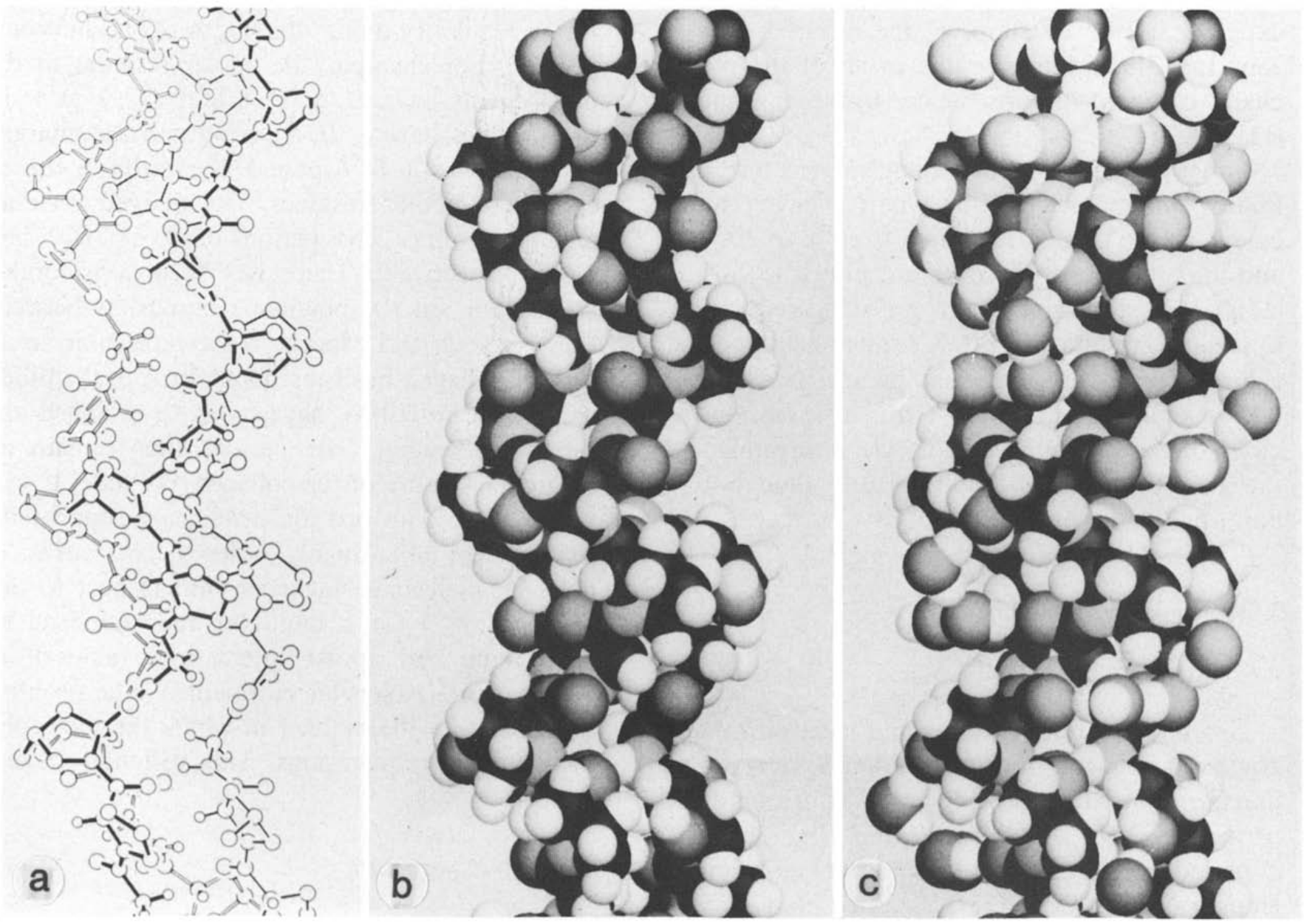


Fig. 2. (a) Model of the molecular structure of collagen refined using a linked-atom least-squares procedure against quantitative X-ray diffraction data [11]. In this model the $\alpha$-chains adopt a left-handed helical structure but wind round the axis of the molecule in a regular right-handed manner. (b) Space-filling model of the structure shown in panel a. (c) Space-filling model of the collagen molecule including water molecules which bridge the glycyl NH to the C = O of a prolyl residue in another chain and the OH group of a hydroxyprolyl residue in the same chain. Reprinted from Fraser et al. [6] with permission of the authors and Academic Press.

least-squares analysis, to refine a model for the collagen molecule (fig. 2). The only stereochemically acceptable conformation proved to be almost identical to the Rich-Crick II model. Also the pitch length of the individual $\alpha$-chains was refined to a value of 8.68 nm. This model provided the possibility of investigating stereochemically feasible positions for water molecules in the structure which may act as hydrogen-bond linkers between main-chain sites. In fact, for a single water molecule it was not possible to find any site which was capable of spanning two main-chain carbonyl groups. However, a pair of water molecules could be positioned which would link the carbonyl groups of the glycyl and Y residues of adjacent chains. Furthermore, the orientation of the doubly bonded water molecule was compatible with the experimental observations of the infrared dichroism [15]. This scheme results in all of the main-chain carbonyl groups being hydrogen-bonded [11].

Other aspects of the collagen structure are known; the diameter of the type I collagen molecule is about 1.4 nm, its length is close to 300 nm and the probable order of its $\alpha$-chains is $\alpha_2$–$\alpha_1$–$\alpha_1$ [16], where successive chains are staggered axially by a single residue [16,17]. It seems likely that the chain ordering, however, may be specified largely by the amino- and carboxy-terminal extension regions of the procollagen molecule rather than by the triplet region of the structure, though this point has yet to be confirmed.

## 3. Molecular aggregation

### *3.1. Axial structure*

X-ray diffraction patterns and electron micrographs of collagen fibrils in tendon have revealed that the molecular assembly has a significant axial periodicity ($D$) of about 67 nm (fig. 3a); the $D$-period in skin and cornea is a little less (65 nm). Hodge and Petruska [18] suggested that similar observations could be explained if the molecules were axially staggered in the fibril by a distance $D$ (or a multiple). The length of the collagen molecules including the telopeptides ($\sim$ 300 nm), however, is not an integral multiple of the $D$-period and it follows that the fibril structure will have alternating regions of dense molecular packing (the overlap regions of length $0.47D$) and less dense molecular packing (the gap regions of length $0.53D$) (fig. 3b). A theoretical analysis [19] showed that this model was strongly supported by the nature of the amino acid sequence of the collagen $\alpha$-chains, i.e., if two collagen molecules are moved past one another and the number of potential apolar and ionic interactions are scored then clear maxima in the interaction curves are apparent at relative axial staggers of $nD$, where $n = 0$, 1, 2, 3 and 4 (fig. 3c).

Further, Fourier analyses of the amino acid sequences of both the $\alpha_1$(I) and $\alpha_2$ chains of collagen has shown that certain amino acids or amino acid groupings occur with a high degree of linear regularity along the length of the molecule [19–22]. For example, the apolar residues in the $\alpha_1$(I) chains have $D/5$, $D/6$ and $D/11$ periods, the prolines have a $D/5$ period and the charged residues have a $D/6$ period. Similarly, in the $\alpha_2$ chain the apolar residues, the charged residues and the prolines have periods of $D/11$, $D/6$ and $D/5$, respectively. There has been considerable speculation on the possible relationship between these periods and aspects of the molecular structure of collagen but subsequent structural studies, as already described, have failed to establish any direct connection. The periods thus remain an enigmatic feature of the collagen molecule.

All these data provide convincing support for the concept of a highly ordered axial array of collagen molecules staggered with respect to one another by $D$ (or a multiple) and stabilised by both ionic and apolar interactions (as well as covalent intermolecular cross-links). The problem remains as to the manner in which the molecules pack in three dimensions. This will now be addressed.

### *3.2. Lateral structure*

Rat-tail tendon, stretched and treated with phosphotungstic acid, has a detailed X-ray diffraction pattern which reveals many sharp Bragg reflections lying on a number of well-defined rowlines (fig. 4). These data imply that the collagen

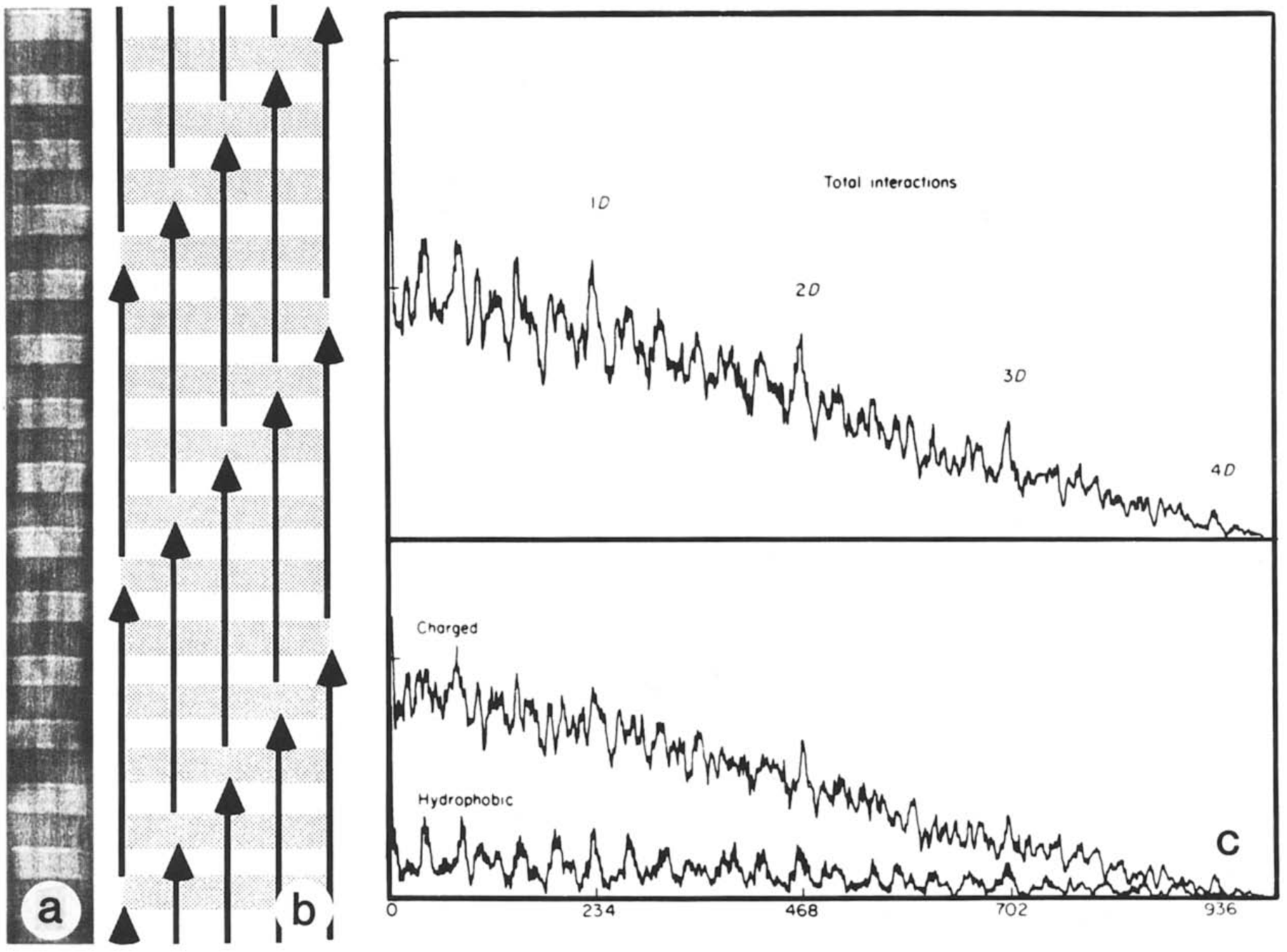


Fig. 3. (a) Electron micrograph of a negatively stained, whole-mount preparation of a collagen fibril from Cuvierian tubules (*Holothuria forskali*). The polar fibril shows a characteristic banding pattern with a period ($D$) of length approx. 67 nm; each asymmetric unit consists of a light-staining and a dark-staining band known as the 'overlap' and 'gap' regions, respectively. (b) This pattern arises from the regular axial staggering of collagen molecules (of length $4.47D$) by an integral number of $D$-periods. (c) The number of possible ionic and apolar interactions between collagen molecules calculated as a function of relative axial stagger reveals maxima at multiples of $D$. (c) Reprinted from Hulmes et al. [19] with permission of Academic Press. These data, both experimental and theoretical, provide convincing evidence of the likely one-dimensional arrangement of the molecules in the collagen fibrils.

molecules (or parts thereof) have a high degree of both axial and lateral order. Indeed, careful measurements of the positions of the reflections and a least-squares analysis allow the parameters defining the triclinic unit cell to be determined with a high degree of precision ($a$, $b$ and $c$ axes, 3.997, 2.695 and 67.797 nm, respectively; $\alpha$, $\beta$ and $\gamma$, 89.24, 94.59 and 105.58°, respectively; $\theta_a$, $\theta_b$ and $\theta_c$, 92.79, 89.99 and 1.82°, respectively; $\phi_a$, $\phi_b$ and $\phi_c$ are −15.60, 90.00 and 155.72°, respectively; see ref. 12 for details). Of particular note is the observation that the $c$-axis is not parallel to the axis of the collagen fibril. The quasi-hexagonal packing of the collagen molecules, as arises from the shape and size of the unit cell determined [12,23] is such that the lateral separation of planes of molecules is different in the three principal directions. This can be determined in part by the observed splitting of the 1.3 nm equatorial/near-equatorial maximum (fig. 4). The X-ray data in the turn layer line region of the helix pattern clearly show that the direction of the molecules contributing to that region is inclined relative to the crystal lattice by about 4.2° (i.e., $\theta = 4.19°$,

$\phi = 245.46°$). The implication is a profound one for if the molecules were perfectly straight over their entire lengths then their direction in the lattice would be closely specified by a line joining the lattice coordinates (0, 0, 0) to (0, −2, 1) (see fig. 5). This, however, is physically impossible since it would result in portions of different molecules occupying the same region in space. This apparent anomaly may be explained, however, by postulating that the molecular segments giving rise to the Bragg diffraction maxima have a limited axial extent (less than the *D*-period) and that they are joined together by molecular segments exhibiting sufficient disorder that they do not give rise to discrete high-angle X-ray diffraction maxima. This

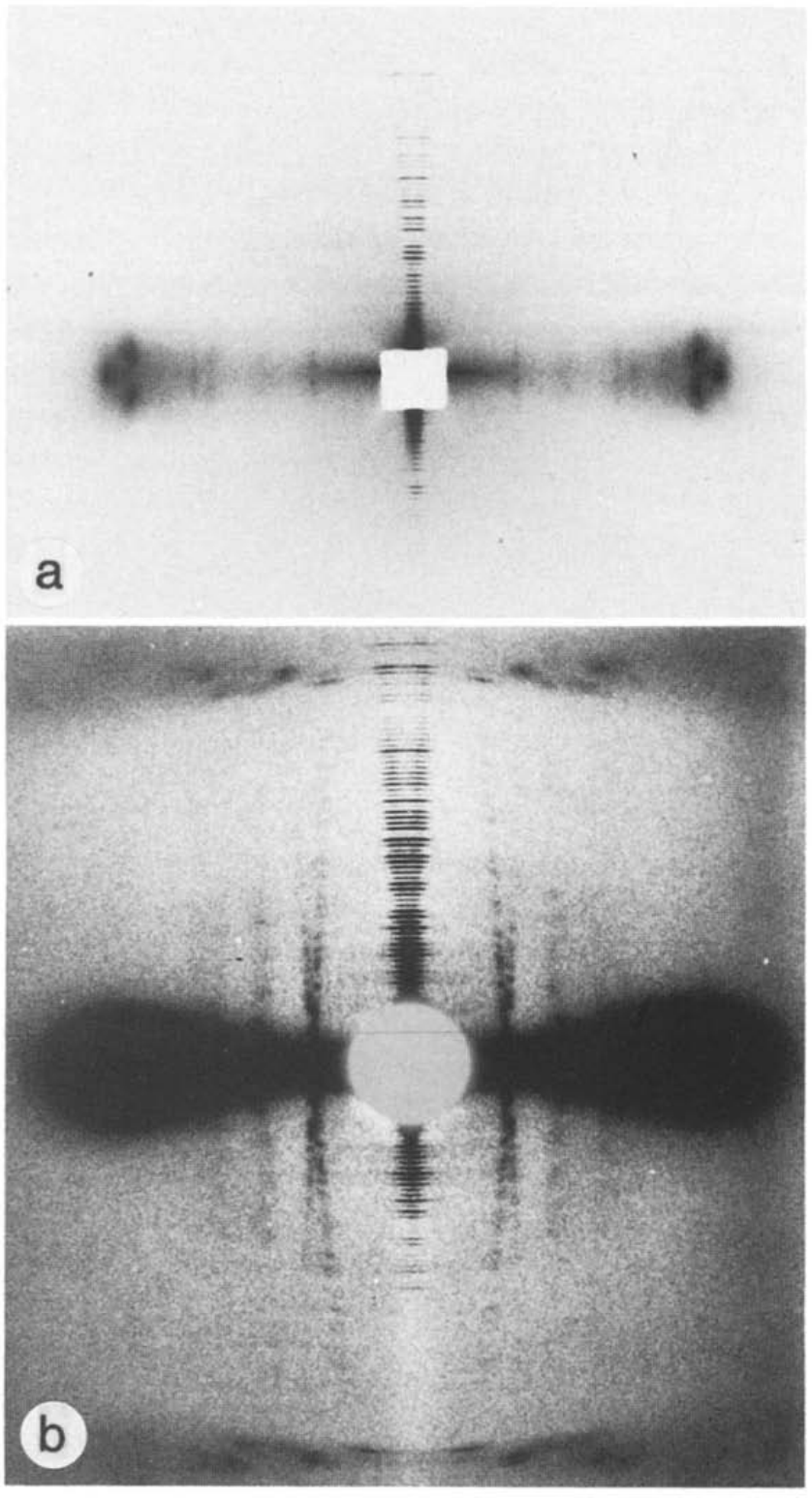


Fig. 4. X-ray diffraction patterns of stretched rat-tail tendon stained with phosphotungstic acid. (a) Low-angle pattern showing the composition of the 1.3 nm equatorial/near-equatorial region. (b) High-angle pattern showing the row lines intersecting the band of intensity relating to the molecular pitch length. (b) Reprinted from Fraser et al. [12] with permission of Academic Press.

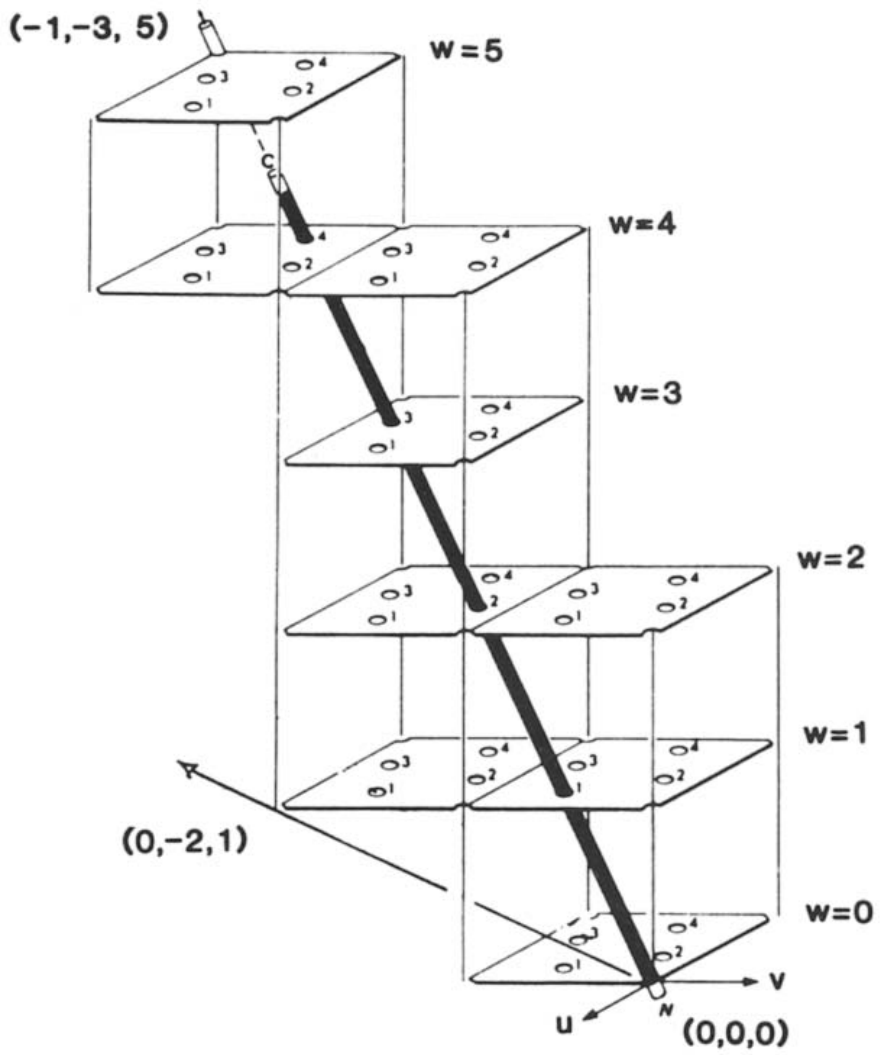


Fig 5. In this figure (not drawn to scale) the N-terminal end of the triple-helical region of a collagen molecule has been arbitrarily placed at the origin (0, 0, 0) of the lattice coordinates ($u$, $v$, $w$). X-ray diffraction data indicate that a collagen molecule with a straight axis must pass through positions 0, 1, 2, 3 and 4 in the planes $w = 0$, 1, 2, 3 and 4, respectively. There are, however, four possible ways in which these positions may be allocated that will result in sheets of molecules being produced with constant intermolecular staggers. Detailed analysis of the Bragg reflections lying within the band of intensity originating from the molecular pitch length has shown that those parts of the collagen molecules that are highly ordered lie in a direction close to (0, −2, 1). This observation precludes the possibility that collagen molecules are straight, since it would necessitate that they occupy overlapping positions in space. It follows that molecular segments, rather than intact molecules, lie in the direction (0, −2, 1). Redrawn and modified from the original [12] with permission of the authors.

suggestion receives direct support from an estimate of the measurement of the half-widths of the X-ray maxima, which indicate an axial coherence of only about 31 nm, i.e., 0.46$D$ [12]. The model thus emerges of the overlap region of the fibrils being composed of highly ordered straight segments of tilted collagen molecules linked to one another by relatively disordered molecular segments that constitute the gap regions. Regarding the disordered segments it is necessary only to postulate that these segments are mobile and subject to positional variation from one cell to another. It is not difficult to rationalise this point since the gap region (relative to the overlap region) has a much higher water content, a lower packing density, a lower content of hydroxyproline residues, a lower content of (glycine-imino-imino) triplets and a lower content of aromatic residues [24]. Each of these factors taken individually implies an increase in molecular mobility of the gap region relative to the overlap region and, when taken as a group, provide strong support for this postulate.

In addition to the discrete Bragg reflections previously discussed, there is considerable diffuse scatter in the X-ray pattern of stretched tendon. Diffuse scatter generally indicates regions of disordered or partially disordered structure and in this case may be associated with the structure of the gap regions of the collagen fibrils. Two particular features of the distribution of diffuse scatter are of importance [24]. Firstly, the scatter is fanned about the equator to the same extent as the Bragg reflections, thus indicating that the angle of inclination of the molecules in the gap region is similar to that in the highly ordered overlap region. Secondly, the diffuse scatter has an equatorial maximum at a spacing of about 1.3 nm (i.e., the same spacing as the triplet of key Bragg reflections). This indicates that the molecules remain clumped together in the gap region thus leaving a water-filled hole where the missing molecular segment would have been located.

The Fourier transform of the tilted molecular segments comprising the overlap region of the fibril indicates that this part of the structure makes no significant contribution to the low-angle X-ray diffraction pattern. Consequently, it was suggested by Fraser et al. [24] that this part of the pattern may be attributable to one (or more) of the following possibilities: (i) the water-filled 'hole' in the gap region, (ii) the telopeptides or (iii) the regular disposition of certain amino acids or amino acid groupings with periods of $D/5$ or $D/6$. The degree of disorder in the gap region of the fibril would, as previously indicated, reduce its contribution to the diffraction pattern to an insignificant level in the high-angle region. Fig. 6 illustrates the calculated and observed positions of the low-angle diffraction maxima for a variety of models based on the first two of the possibilities listed above. The calculated diffraction maxima for a 'hole' aligned parallel to the direction of the molecular segments comprising the overlap region is in poor agreement with the observed data. However, if the hole is realigned appropriately (see fig. 6c, d and legend) then agreement with the observed data is much improved. Similarly, inclusion of the telopeptides in the model (fig. 6f) results in the best qualitative fit yet achieved between the experimental and the calculated data in the low-angle region.

The work described thus far shows that the X-ray data are explicable in terms of arrays of appropriately ordered collagen molecules. There is no requirement that any sub-fibrillar structure greater in extent that an individual molecule should exist. That is not to say, however, that a microfibrillar structure defined by some specific pattern of cross-links and interactions is not formed. A variety of models, based on an original proposal by Smith [25], have received widespread attention over the last 10–15 years and certain of these do seem capable of explaining a number of electron microscope observations indicative of substructures larger in diameter than a single molecule (see ref. 26 for a summary of these data). In particular, a compressed microfibril model [27,28] is capable of accounting for both the low-angle and the high-angle X-ray diffraction patterns [24] provided that (i) the overlap region of the fibrils still contains straight molecular segments tilted with respect to the axis and (ii) the gap region consists of a disordered but twisted array of molecular segments that is suitably orientated (fig. 6). It is important to note that no viable model exists which will explain the X-ray pattern in terms of

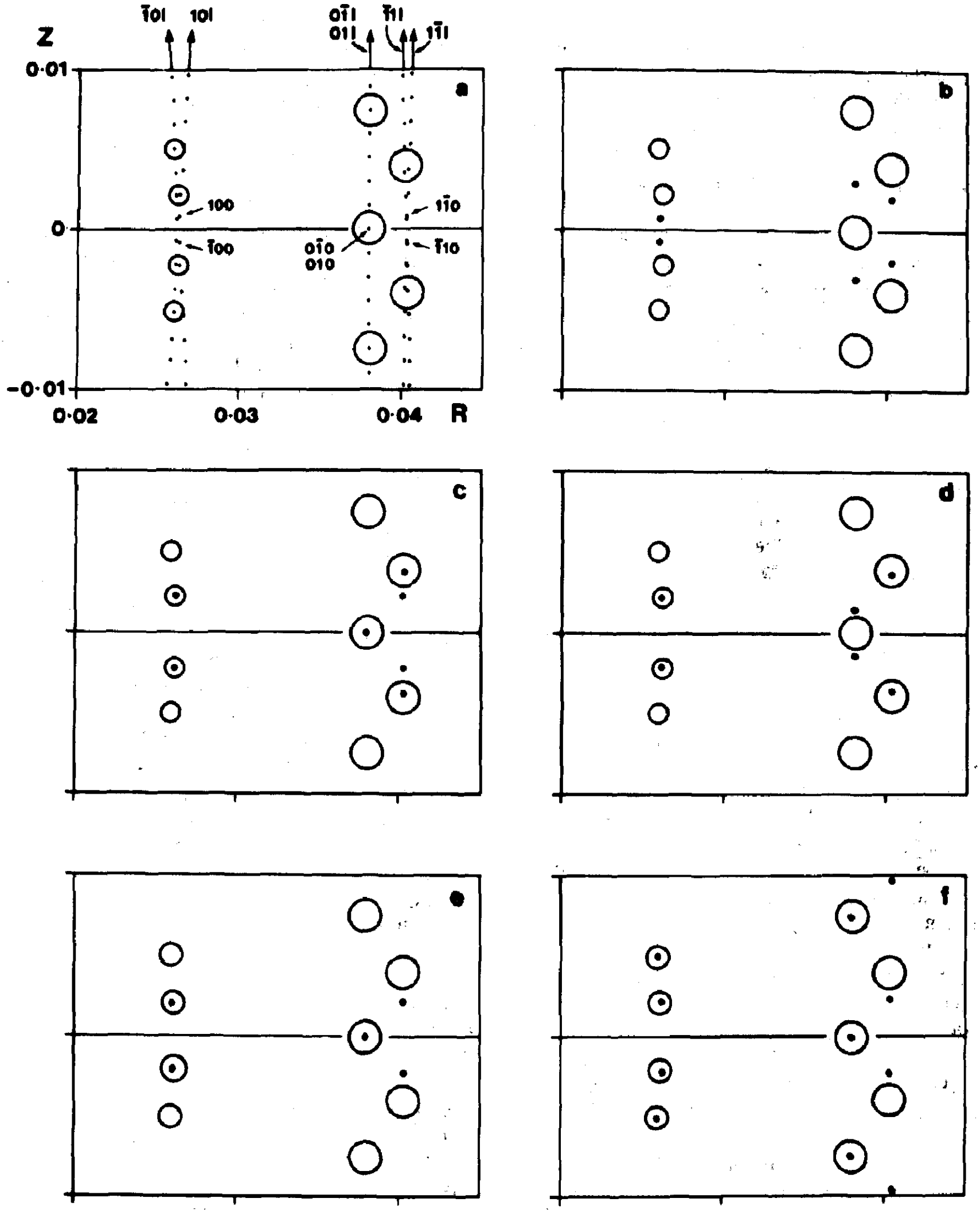


Fig. 6. (a) Possible positions for the Bragg reflections originating from the crystalline segments of type I collagen fibrils are indicated by small dots. The positions of the observed reflections, which are broadened considerably as a result of finite crystal size and disorientation present in the specimen, are marked by circles. (b) The maxima predicted by the $m = 0$, $n = 0$ helix layer plane of the molecular segments in the overlap region (heavy dots). (c and d) The maxima predicted by the layer plane for a gap extending from $(-0.4, -1.14, 0.47)$ to $(-1, -1, 1)$ and $(-0.2, -0.54, 0.47)$ to $(1, -2, 1)$ respectively. (e) The maxima predicted by the layer plane generated by the gap region of a compressed microfibril with axis running from $(0, 0, 0)$ to $(1, -1, 1)$. (f) The maxima predicted for a specific telopeptide arrangement. Reprinted from Fraser et al. [13] with permission of the authors and Academic Press.

either straight molecules or straight microfibrils, i.e., a *D*-periodic crimp is an inescapable conclusion from the X-ray data.

In passing it should be noted that the structure described here is almost certainly not unique to type I collagen-containing tissues since Eikenberry et al. [29] have shown that a related X-ray pattern with discrete Bragg reflections is obtainable from lamprey notochord, a type II collagenous connective tissue. The X-ray data are more difficult to analyse than those from rat-tail tendon, since the natural width of the reflections is much higher in notochord (where the fibrils are small) than in tendon (where the fibrils are much larger).

The model as described has the capability of explaining observations that were incompatible with the original quasi-hexagonal model [23]. For example, a *D*-periodic molecular crimp allows the formation of both large *and* small diameter collagen fibrils whereas a model containing straight inclined molecules of considerable length is unable to account for the smallest diameter collagen fibrils seen by electron microscopy (i.e., their projected lengths are greater than the observed fibril diameters). Also the molecular crimp allows the smaller *D*-period in skin and cornea (compared to that in tendon) to be explained by a relative shearing of the surfaces that define the boundaries of the overlap region of the fibril [12,24]. The molecular packing is not significantly affected.

## 4. Fibril growth and development

The morphologies of collagen fibrils have been studied by electron microscopy for a diverse selection of connective tissues at stages of development ranging from early foetal through maturity to senescence. A basic pattern of development of the fibrils is common to most tissues and may be summarized as follows:

(a) The first collagen appears as widely dispersed bundles of limited extent comprising small constant-diameter fibrils (fig. 7a).

(b) The lateral extent of the bundles then increases without a concomitant increase in diameter of the constituent collagen fibrils. It follows that the number of fibrils is increasing and, in all probability, that the lengths of the fibrils are also increasing.

(c) At the next stage of development the fibrils in neighbouring bundles often have different (constant) diameters.

(d) The fibril diameter distribution broadens and each bundle is composed of populations of fibrils of discrete sizes (fig. 7b). This stage of development occurs prenatally in precocial animals and postnatally in altricial animals.

(e) As the animal matures the collagen fibril diameter distribution becomes either unimodal or bimodal depending on the tissue function (fig. 8) [4,5,30,31].

(f) At senescence the collagen fibrils are often irregular in section and may display a markedly changed diameter distribution [4].

In contrast to the extensive data now available on the distribution of collagen fibril diameters, there is virtually no hard information on the lengths of the collagen fibrils in any tissue at any age. The reasons for this gap in our knowledge are easy to state: (i) individual collagen fibrils cannot be followed in the optical microscope as their diameters are smaller than the resolving power of the instrument, (ii) the small (natural) disorientation of collagen fibrils even in a highly orientated connective tissue such as tendon results in the axes of the fibrils leaving the plane of a longitudinal section prepared for study in the electron microscope, (iii) whole-mount specimens of collagen fibrils ex vivo cannot be prepared for any tissue. It appears, therefore, that fibril lengths may be measurable only by high-voltage electron microscopy, by microdissection techniques in conjunction with scanning electron microscopy or by other less direct methods. Of these, the latter has recently yielded mean fibril lengths in young (5 day) and mature (4 month) rat-tail tendon of about 125 and 1700 $\mu$m, respectively (Craig, Conway and Parry, unpublished data). These values are about 3–10-times greater than the so-called critical lengths (section 5) required for the tissue to be capable of sustaining the stress levels that occur in vivo.

The physical study of collagen fibril size has not been restricted to electron microscopy. X-ray diffraction studies of collagen fibrils from corneal

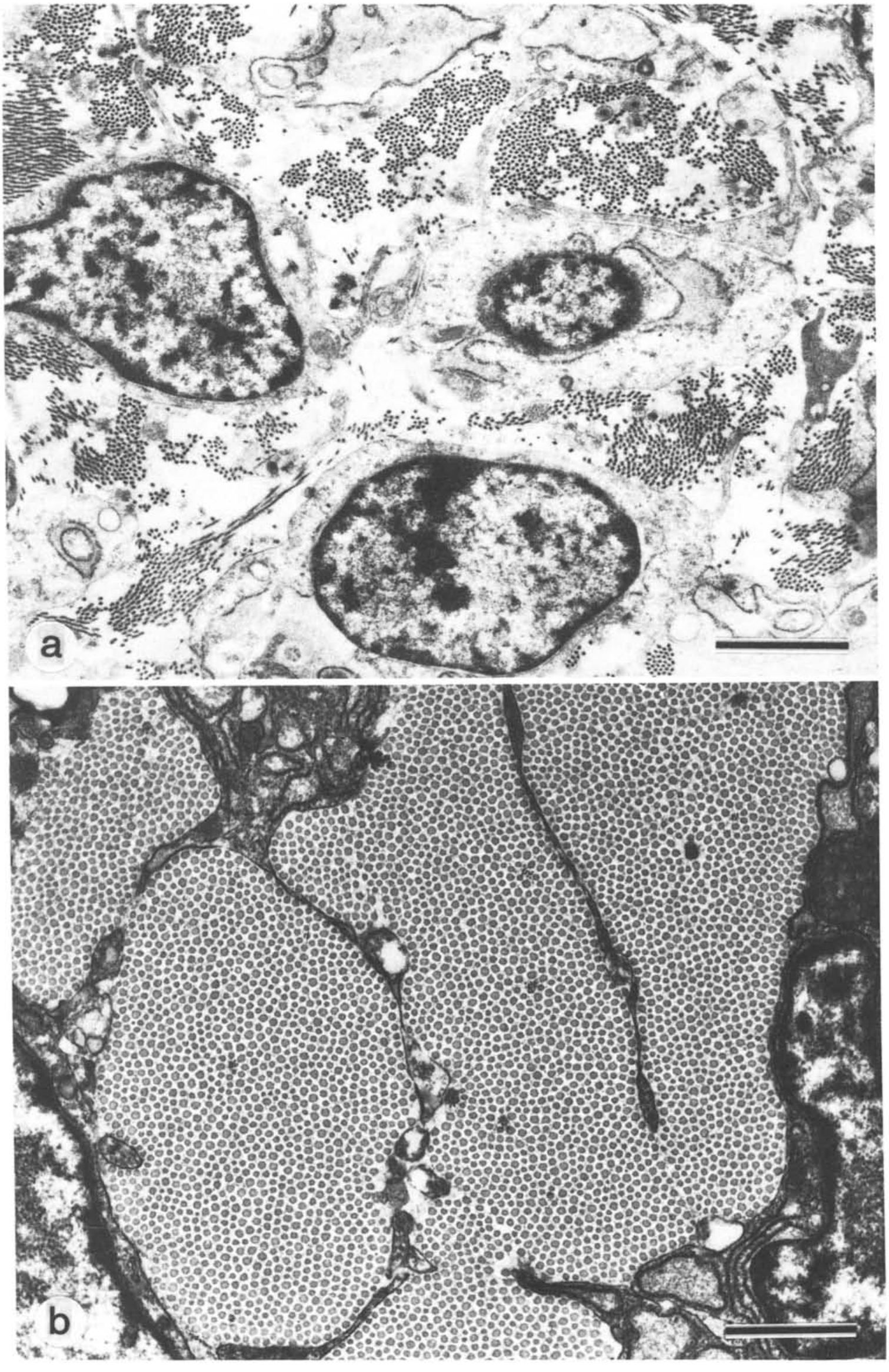


Fig. 7. (a) Transverse section of collagen fibrils from 60 day foetal sheep tendon. The fibrils have a sharp, unimodal distribution of diameters and are loosely grouped in bundles. At this early stage of development the tissue has a high cellular content. Bar = 2 $\mu$m. (b) Transverse section of collagen fibrils from 18 day foetal chick metatarsal tendon. The distribution of fibril diameters has begun to broaden, the collagen fibres (i.e., fibril bundles) are clearly delineated and the cellular content of the tissue is beginning to decrease relative to that in the earliest stages of development. Bar = 1 $\mu$m.

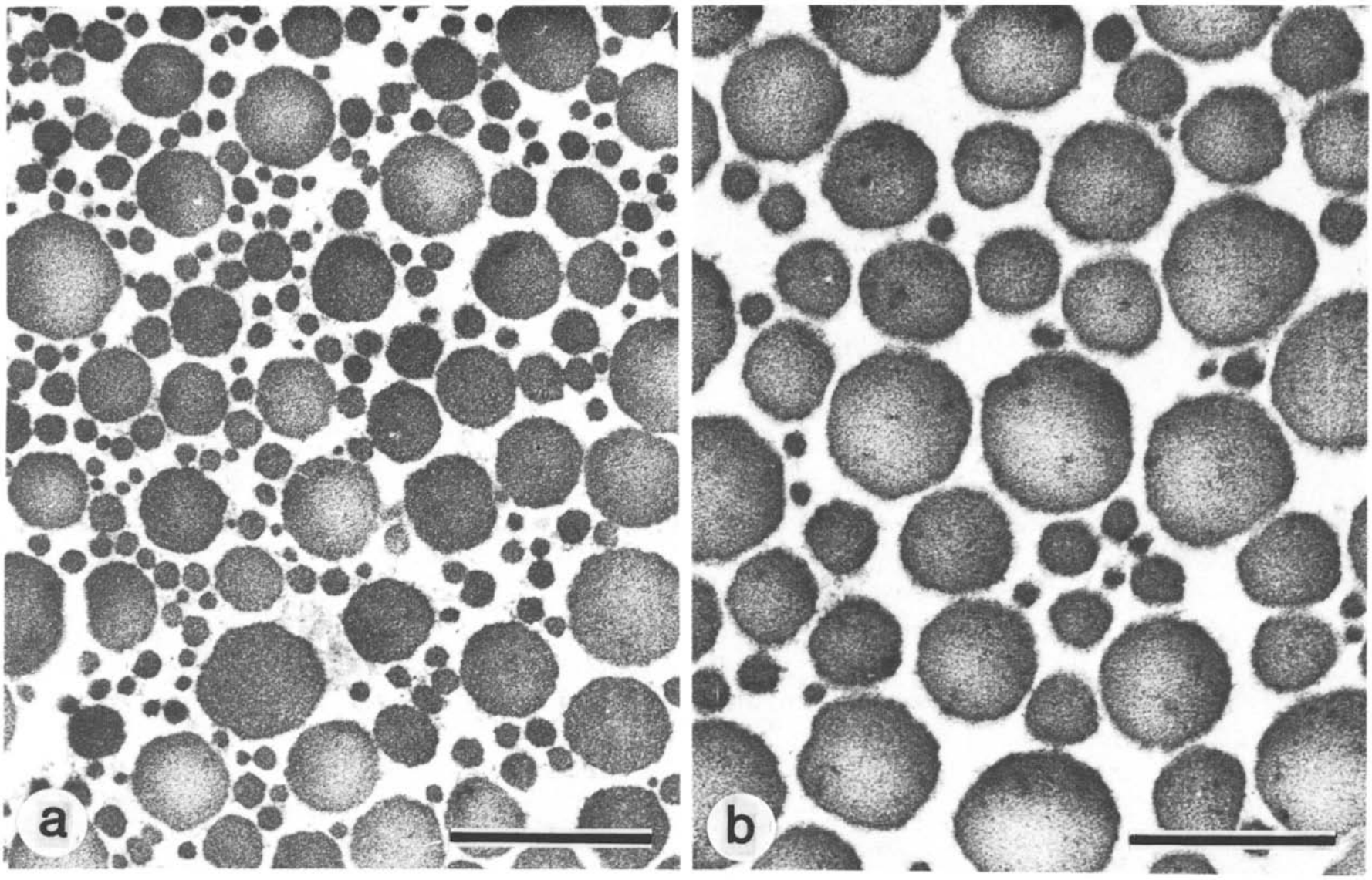


Fig. 8. Transverse section of the collagen fibrils from (a) the suspensory ligament of a 5-year-old horse showing a bimodal distribution of diameters and (b) the common digital extensor tendon of the same horse showing a unimodal distribution of diameters. Bars = 0.5 μm. Each of the pairs of histograms (below) contains a number distribution (left) and a mass distribution (right) plotted as a function of diameter. The graduation marks on the diameter axis are 50 nm apart. Reprinted from Parry and Craig [5] with permission of CRC Press.

stroma [32–34] and from foetal or immature tissues [34,35] which contain fibrils of constant size have also proved possible since low-angle X-ray maxima and minima are interpretable in terms of fibril diameter. Furthermore, the values so determined refer to those from hydrated specimens. Hence, it is possible to assess quantitatively the degree of specimen dehydration suffered by specimens during electron microscope preparative procedures (see, for example, refs. 35 and 36). Unfortunately X-ray diffraction techniques are not able to reveal fibril size distributions in mature tissues containing a variety of fibril diameters.

## 5. Mechanical properties

What are the structural and functional features of the collagenous components of connective tis-

sue, i.e., the collagen molecules, the fibrils and the fibres, that allow them to bestow appropriate mechanical properties to tissues of such widely varying function? Clearly, it is not yet possible to answer this question in detail but many of the problems have been addressed and some answers are now beginning to emerge. This section will consider a selection of those but it most be noted that the contribution of the non-collagenous components of connective tissue to its mechanical properties is not considered here, even though their role is crucial.

(a) Although collagen fibrils have little strength in either flexion or torsion (especially the smaller diameter fibrils) they exhibit high tensile strength. This property is largely attributable to the presence of intermolecular covalent cross-links. In the earlier stages of fibril development such cross-links are said to be 'reducible' and link axially those molecules staggered by a distance of $4D$ in the collagen fibril. As the tissue matures the cross-links become 'stable' and they then link collagen molecules both axially *and* laterally [2]. Note that an important step prior to cross-link formation is the specific alignment of the collagen molecules in the fibril through the maximisation of apolar and ionic interactions [19].

(b) The collagen fibril diameter distribution plays a major part in determining the mechanical properties of a connective tissue. In particular, it has been suggested by Parry and Craig [4,5,30] that the creep-inhibition property of a tissue, i.e., its ability to resist plastic deformation, is directly related to the percentage of small-diameter fibrils present whereas the ability of the tissue to withstand high stress levels is related to the percentage of large-diameter fibrils in the tissue. This hypothesis, based on extensive electron microscope data on the form of the collagen fibril diameter distribution in a variety of different tissues over a wide range of ages, has been rationalised as follows. Firstly, the interface between the fibrils and the matrix is, for a given mass of collagen, greater when the fibrils are small than when they are large. Therefore, the area over which the shear stress exists at the fibril/matrix interface is much enhanced and permanent creep is less likely to be a significant problem in the tissue as a whole. Secondly, when the tissue contains large-diameter fibrils a greater percentage of lateral covalent cross-links will be made than in a tissue containing a high percentage of small-diameter fibrils. The reason is simply stated: lateral cross-links cannot be made in toto round the periphery of a fibril, and since the ratio of internal volume to periphery increases with fibril diameter it follows that the tensile strength of a fibril is predicted to increase with diameter, i.e., larger diameter fibrils are predicted to have a particularly important role in sustaining the tensile loading of the tissue.

(c) Provided that the length of a collagen fibril is greater than some particular value – the critical length ($l_c$) – then that fibril is able to act as a high-tensile element, i.e., it will not be pulled out of the tissue when stress is applied. An expression for the critical length [37] can be written as follows:

$$l_c = \{\text{radius of a collagen fibril} \times \text{the breaking stress of that fibril}\} \times \{\text{shear stress exerted on that fibril by the matrix}\}^{-1}.$$

This indicates that $l_c$ increases with fibril diameter and hence with tensile attributes; it also emphasises that when a connective tissue is stressed great importance is associated with the shearing which occurs at the fibril/matrix interface. In turn, the magnitude of the shear stress depends on the interactions between the collagen fibrils and the hydrated glycosaminoglycans constituting the matrix. It is generally accepted that the shear stress plays a major part in determining the elastic and viscous properties of connective tissue. Recent estimates of fibril length have been made for immature and mature rat-tail tendon and in each case the values determined were greater than $l_c$ by a (safety) factor of between three and ten. There is, however, no requirement that any collagen fibril should extent over a length significantly greater than $l_c$ though the tissue loses nothing by so doing.

(d) A factor which affects the ability of a tissue to sustain an applied load is the collagen content per unit mass of tissue. In general those tissues with greater tensile strengths are those with the highest percentage collagen contents. While in

principle higher collagen contents could be achieved by increasing the diameters of the fibrils (and reducing their number), there are several practical reasons why this solution is not the complete answer (e.g., larger diameter fibrils have decreased flexibility, a decreased ability to resist crack propagation and decreased creep-inhibition properties). Certainly, collagen fibrils are larger in tissues like tendon (mean diameter ~200 nm) than they are in skin (~100 nm) or cartilage (~50 nm) and this can be correlated to tensile loading [4,5,30]. However, an increased collagen content can also be achieved, to some extent, by an appropriate form for the collagen fibril diameter distribution. For example, in a bimodal or skewed distribution the smaller diameter fibrils can fill the voids left between the larger ones. As noted later, this will also result in enhanced creep-resistance properties.

(e) The fibril-matrix nature of connective tissues has an important advantage over a single-component system in that it has a greatly enhanced ability to withstand crack propagation. The fibrils, being arranged in parallel, may suffer individual damage or destruction without marked alteration in the properties of the tissue as a whole. In contrast, the propagation of a crack through a single large component would ultimately result in its destruction and in permanent incapacitation of the tissue.

(f) The orientation of collagen fibrils varies markedly from one tissue to another. For example, fibrils are orientated in a direction parallel to the long axis of tendon whereas in skin and cornea the fibrils occur in orientated layers. In other connective tissues, such as vitreous humour, the fibrils appear randomly orientated. In all cases the fibril orientation function is closely related to the direction(s) in which the tissue is generally stressed. It has been pointed out by Wainwright et al. [38] that fibrils whose axes do not lie close to the direction of applied stress will add weight to the tissue without providing compensating increased tensile strength, i.e., the strength/weight ratio will decrease to the detriment of the animal concerned. The effect of fibril orientation can be quantitated through the term $\eta$, known as the efficiency of reinforcement. This has been defined by Krenchel [37] as

$$\eta = \sum a_n \cos^4\phi$$

where $a_n$ is the fraction of fibrils lying in some particular direction and $\phi$ the angle between the fibril groupings and the loading axis. Numerically, $\eta$ is 0.2 for fibrils randomly orientated in three dimensions and stressed in any direction, 0.375 for fibrils lying in a plane and stressed in a direction lying within that plane, and 1.0 for fibrils orientated parallel to the direction of stress. The Young modulus of the composite ($E_c$) is then related to the Young moduli of the fibrils ($E_f$) and the matrix ($E_m$) by the expression

$$E_c = \eta E_f V_f + E_m(1 - V_f)$$

where $V_f$ is the volume fraction occupied by the collagen fibrils.

(g) The collagen fibre, which is a highly interwoven rope of collagen fibrils, shows two features of particular relevance to the mechanical properties of connective tissue. Firstly, electron microscopy has revealed that the collagen fibrils are not confined to a single fibre but may move freely from one to another forming an integral part of each in turn. This mechanism thus acts to link or cement fibres together to form even larger structural units, which ultimately form the intact tendon, cornea, etc. The second feature, which is shown by fibres in tissues subjected to rapid increases in stress levels, is a macroscopic crimp structure with a period of about 1–100 $\mu$m and a crimp angle in the range 5–25° [39]. It is worth restating at this juncture that individual collagen fibrils also exhibit a *D*-periodic crimp. Both this and the macroscopic crimp may act together as a compliance mechanism to prevent permanent damage to a tissue on application of stress. This feature is most clearly visualised in the low-stress region of a stress-strain curve and represents the so-called toe region.

In this section an attempt has been made to summarise a few of the important mechanical attributes that collagen bestows upon a connective tissue. However, all components of this biological composite necessarily contribute to its mechanical properties and while it is clear that collagen is a

major factor in specifying tensile characteristics it is unrealistic to consider its role in isolation. A coordinated approach, aimed at understanding the interactions between components, is now becoming increasingly important.

## 6. Summary

In the last decade impressive progress has been made in elucidating the conformation of the collagen molecule and on its mode of packing in vivo. To a large extent, this particular aspect of the more extensive problem posed by the structure and function of connective tissue is complete, though further refinement of details is likely. Increasingly, attention is being paid to the mechanism of collagen fibrillogenesis, to fibril growth and development and to the role and organisation of collagen fibrils in providing a tissue with the appropriate mechanical properties for it to be able to function optimally. Thus, even though research is moving towards lower resolution studies of connective tissue structure the complexity of the problem is even greater than before since we are now involved in trying to understand how the mix of different chemical entities constituting a connective tissue – collagen, glycosaminoglycans, minerals, elastic fibres, water and cells – interact with and influence one another during the physical and chemical changes which occur naturally in vivo. We can remain optimistic, however, that the next 10 years will see further progress and resolution of some our current problems. We may also hope that these efforts will play an increasingly important part in the study and treatment of connective tissue disorders.

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