Ultrastructure of the corneal stroma: a comparative study

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ABSTRACT Using a high intensity synchrotron x-ray source, we have recorded diffraction over a range of angles from the corneas of a wide variety of species. The results show that the interfibrillar Bragg spacing varies from 39 nm to 67 nm, the fibril diameter varies from 24 nm to 43 nm, but in the species studied intermolecular Bragg spacing is constant (1.58 ± 0.03 nm). Using these data, a number of other structural parameters were calculated including the interfibrillar volume, V, and the surface-to-surface fibril separation, s. Large variations were found, particularly between aquatic and terrestrial animals. We found that the parameter which appears to be most constant *throughout* the species was the volume fraction, that is, the proportion of the tissue occupied by the hydrated fibrils. Ignoring the volume of the stroma occupied by cells, the tissue fibril volume fraction was (28 ± 3)% for both aquatic and land animals.

The observation of a constant volume fraction led us to propose a simple model in which collagen molecules and interfibrillar glycosaminoglycans occur in a fixed ratio in all the species—thus species with narrow fibrils have fewer interfibrillar glycosaminoglycans and the fibrils are thus more closely spaced, and vice versa. This model agrees with many of the experimental data on corneal composition and on the physical properties of the tissue reported in the literature.

INTRODUCTION

The corneal stroma is remarkable among all collagen-rich tissues in that it transmits about 99% of the incident light unscattered. This transparency is due to the uniform size of the constituent collagen fibrils and to the degree of ordering in their packing (Maurice, 1957; Hart and Farrell, 1969). The factors which limit the lateral growth of the collagen fibrils and maintain their spatial arrangement are not known. The fibrils in most species studied contain predominantly type I collagen with smaller amounts of types V and possibly type III (Davson et al., 1979; Newsome et al., 1982; Fitch et al., 1984; Marshall et al., 1991*a*, *b*). It has been proposed that collagen fibril diameters in cornea may be controlled by the incorporation of minor collagens (such as type V) into the collagen fibrils (Birk et al., 1986). The interfibrillar space contains type VI collagen filaments (Zimmermann et al., 1986; Linsenmayer et al., 1986) and is filled with glycosaminoglycans (GAGs), which exert a swelling pressure sufficient to inflate the collagen matrix (Hedbys, 1961). The GAGs are attached to protein cores to form two types of proteoglycan: keratan sulphate (lumican) and dermatan sulphate (decorin). Balazs (1965) speculated that the interfibrillar volume containing the sulphated GAGs is structured. Using catonic dyes to specifically stain GAGs, it has been demonstrated that there is indeed regularity in the association between some of the proteoglycans and the collagen fibrils (Scott and Haigh, 1985; Meek et al., 1986), and it has been proposed that fibril organization is maintained by this regular association (Scott, 1991).

When corneas swell, light scattering increases. This effect is thought to arise from a non-uniform distribution of water (Benedek, 1971) and disruption to the collagen packing. Using x-ray diffraction, we have recently shown that when bovine corneas contain approximately 66% or more water, the fibrils themselves swell very little, and most of the additional water goes into the interfibrillar spaces. Above physiological hydration (approximately 76%), a proportion of the additional water goes into regions devoid of collagen fibrils (Meek et al., 1990). The distribution of water within the corneal stroma, therefore, is of some importance in determining the relative proportions of the total tissue volume occupied by collagen fibrils and interfibrillar GAGs, respectively, and hence in understanding the basis of many of the optical and structural properties of the tissue.

The low angle x-ray diffraction pattern from the cornea can provide information about the mean center-to-center spacing of the collagen fibrils (which we call the interfibrillar separation, i, and about fibril diameters, D, as a function of tissue hydration (Goodfellow et al., 1978; Sayers et al., 1982). From wide angle x-ray diffraction we can determine the mean center-to-center separation a of the collagen molecules within the fibrils (Meek et al., 1990; Fullwood et al., 1992; Malik et al., 1992). We have previously shown that interfibrillar separations vary between species at a constant hydration near to 76%, particularly between aquatic and land animals (Gyi et al., 1988). We also noted that some fish and aquatic mammals have smaller fibril diameters than land mammals (Gyi and Meek, 1987; Gyi et al., 1988; Gyi, 1988). The difference between fibril diameters of fish and land mammals has also been reported, using a low temperature embedding procedure in electron microscopy (Craig et al., 1986; Craig and Parry, 1989).

In this paper we compare the interfibrillar separation, fibril diameter, and intermolecular separation for a wide variety of species from x-ray diffraction data obtained using a high intensity synchrotron source. The results lead to a simple model in which all tissues are shown to

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have an approximately constant volume fraction occupied by the hydrated collagen fibrils at physiological hydration.

MATERIALS AND METHODS

Where possible, corneas were taken from animals that had died either naturally, in road accidents, in the course of farming, in zoos, or in private menageries.

Eyeballs were removed as soon as possible post mortem and stored at −40°C. The maximum time between death and freezing was 3 d although for certain species, i.e., rat, trout, rabbit, and cow it was possible to remove the corneas immediately after death. Corneas were excised from frozen eyeballs, wrapped tightly in clingfilm, and stored at −40°C. (We have found that freezing has no detectable effect on the x-ray diffraction patterns from the corneal stroma).

X-ray diffraction patterns were recorded at the high intensity synchrotron radiation source at Daresbury, UK.

Low angle diffraction

Low angle diffraction patterns were recorded at Station 8.2 with a wavelength of 0.154 nm and beam dimensions 4 mm × 0.5 mm. The beam was directed through the center of each cornea along its optical axis. The specimen-to-film distance was about 3 m and the camera was calibrated using the 67 nm meridional spacing of rat tail tendon. The first order of the equatorial pattern was obtained with an exposure of about 20 s; the first subsidiary maximum required 10 mins and the second subsidiary maximum required 20 min. Because of beam-time restrictions at the synchrotron, it was not possible to obtain the second subsidiary maximum from most specimens. Diffraction patterns were recorded on Caeverken AB film (Caeverken, Strangnass, Sweden).

High angle diffraction

High angle patterns were recorded at Station 7.2b. The same experimental procedure as at Station 8.2 was followed, but in this case the wavelength of the x-rays was 0.1488 nm, the beam was collimated to a diameter of 0.5 mm, exposure times were 2–5 min, and the specimen-to-film distance was 12 cm. The camera was calibrated using the 0.305 nm lattice reflection of calcite.

For all experiments, the corneas were mounted in airtight cells to minimize dehydration. They were weighed before and after exposure to obtain a mean wet weight, then placed in a desiccator over silica gel and allowed to dry to a constant weight. The hydration was calculated as:

$$H = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100\%.$$

ANALYSIS OF DATA

X-ray diffraction patterns consist of contributions from a great many scatterers. Consequently, the analysis yields the mean values of structural parameters averaged over a large number of fibrils or molecules. In each individual cornea there is a certain amount of variation in fibril spacings and fibril diameters, which causes the diffraction rings to spread out (Gyi et al., 1988). All measurements, therefore, refer to the mean value of a quantity, either from an individual cornea, or from a number of corneas from the same species.

Interfibrillar spacing

The Bragg spacing, p, from the packing of the collagen fibrils within the corneal stroma, was obtained from the first-order diffraction ring in the low angle equatorial pattern. The fibrils were assumed to be arranged in a two-dimensional liquid-like array such that their center-to-center spacing, i, is given by $i = 1.12p$[1] (Worthington and Inouye, 1985). This type of packing is well supported by both electron microscopy (Cox et al., 1970) and x-ray diffraction (Worthington and Inouye, 1985).

Since the interfibrillar spacing is known to vary with hydration (Goodfellow et al., 1978; Sayers et al., 1982), corneas were compared only over a narrow range of hydrations (H = 74% to 78%). Data from corneas subsequently found to be outside this hydration range were therefore rejected. This range covers the natural variation found within and between most species. The estimated experimental error was the precision with which the mean diameter of a typical diffraction ring could be measured, converted to real space. Where several corneas from a particular species were examined, it was possible to calculate the standard deviation of the measurements.

Fibril diameter

The subsidiary maxima in the low angle equatorial pattern have been shown to arise from the uniform cylinder transform of the individual collagen fibrils with essentially no contribution from the interference function due to fibril packing (Worthington and Inouye, 1985). The first two of these maxima would therefore be expected to occur at:

$$R = 5.14/\pi D \quad \text{and} \quad R = 8.42/\pi D \quad \text{respectively,}$$

where R is the reciprocal space coordinate of the reflection, D is the fibril diameter, and the numerical factors derive from Bessel functions (Vainshtein, 1966). The positions of these maxima in the diffraction pattern may therefore be used to find R and hence calculate D. Unfortunately, in most cases it was only possible to expose the specimens long enough to obtain the first of these subsidiary maxima. In order to assess the effect of calculating D using one rather than two orders of the cylinder transform, measurements were made from a few individual diffraction patterns where *both* subsidiary maxima were present (Table 1). In all cases, the two measurements were the same within the range of the experimental errors, which were taken as the precision to which the individual diffraction rings could be measured, converted to real space.

Intermolecular spacings

Measurement of the first-order diffraction ring from the packing of the collagen molecules allows the intermolecular Bragg spacing, a, to be obtained (Meek et al., 1991).

[1] The factor 1.12 changes only slightly if one assumes that fibrils are arranged in either a hexagonal lattice (1.15) or a pseudo-hexagonal lattice (1.11).

TABLE 1 **Diameter measurements using the first and second subsidiary maxima in individual low angle x-ray patterns**

Animal	Fibril diameter calculated from first subsidiary maximum	Fibril diameter calculated from second subsidiary maximum
	nm	*nm*
Human	30.8 ± 0.8	30.7 ± 0.9
Monkey	30.7 ± 0.8	31.4 ± 1.0
Rabbit	38.0 ± 1.3	37.2 ± 1.4
Coypu	29.5 ± 0.5	29.0 ± 0.6
Coypu	29.2 ± 0.7	29.1 ± 0.9
Fox	38.8 ± 1.1	37.6 ± 1.2
Fox	37.9 ± 0.9	36.5 ± 1.1
Cat	39.9 ± 1.3	38.5 ± 1.5
Cat	38.8 ± 1.1	38.5 ± 1.5

The Bragg spacing is increased by a factor of 1.11 to give the mean intermolecular center-to-center spacing. The factor of 1.11 assumes that the collagen molecules are packed into a "pseudo-hexagonal" lattice, which is the most common arrangement for a noncrystalline assembly of rod-like molecules (Maroudas et al., 1991). On a molecular level, the corneal collagen can be considered as a lattice with unit cell "volume" (per unit length), $v = 1.11a^2$. An estimate of the number of molecular unit cells within a fibril cross-section, N, is given by N = fibril volume/molecular unit cell volume. Because $D \gg a$, this is a reasonable approximation of the number of molecules in a fibril cross-section.

The estimated experimental error was the precision with which the mean diameter of a typical diffraction ring could be measured, converted to real space.

RESULTS AND DISCUSSION

Interfibrillar Bragg spacings, p, fibril diameters, D, and intermolecular Bragg spacings, a, were calculated from diffraction patterns as described above and the results are presented in Table 2. Although fibril diameters vary from D = 24 nm to 43 nm and interfibrillar Bragg spacings from p = 39 nm to 67 nm, no clear pattern is evident, except that land animals have larger diameters and spacings than aquatic animals at the same hydration. Intermolecular Bragg spacings, a, on the other hand, are remarkably constant at $a = (1.58 \pm 0.03)$ nm.

Using the data presented in Table 2, it is possible to calculate a number of other important parameters for the corneal stromas of different species. Fig. 1 shows a diagram of a group of four fibrils within the corneal stroma. There is no crystalline lattice of fibrils in the stroma and hence, strictly speaking, there is no crystallographic unit cell. However, in order to define some parameters, it helps if we imagine such a unit cell associated with fibrils of diameter D separated by a center-to-center distance, i. The volume (per unit length) of the cell, U, is given by $U = 1.12p^2$ since $i = 1.12p$ (see Methods). This cell can be regarded as a typical "average" for the corneal stroma, which describes the volume of interfibrillar space associated with each collagen fibril. Using this "unit cell" together with data presented in Table 2, it is possible to calculate a number of other structural parameters in the cornea.

It has been proposed that, for a range of land mammals, the GAG chains on proteoglycans link together adjacent collagen fibrils and are thus implicated in the control of the interfibrillar separation (Scott, 1991). The proposal was strengthened by the experimental observation that proteoglycan filaments stained with cupromeronic blue appear, in the electron microscope, to be of similar length in a number of species and also appear to link adjacent fibrils (Scott, 1991). We have therefore estimated the *surface-to-surface* fibril separation, s (Table 3). From these calculations we find that the mean value of s is (30.1 ± 3.4) nm for the land mammals and birds, and (24.3 ± 2.4) nm for the marine mammals and fish. These values do not seem to correlate with the sizes of the proteoglycan filaments observed in cow and trout, respectively (Gyi and Meek, 1987).

Scott and Bosworth (1990) have suggested that, for a number of mammals, the interfibrillar volume, V_i, is constant. We have therefore estimated V_i for some of the animals in Table 2 and these estimates are also presented in Table 3. Once again the animals appear to fall into two distinct groups. In land mammals and birds, $V_i = (2971 \pm 423)$ nm^2, and in aquatic mammals and fish, $V_i = (1698 \pm 246)$ nm^2.

Examination of the data in Table 2 suggests that there is some correlation between animals with smaller fibril diameters and those with closer fibril packing, and yet neither the interfibrillar volume V_i, nor the fibril surface separation, s, were independent of the "unit cell" volume, U, for all species. We therefore decided to investigate the correlation between the interfibrillar volume, V_i

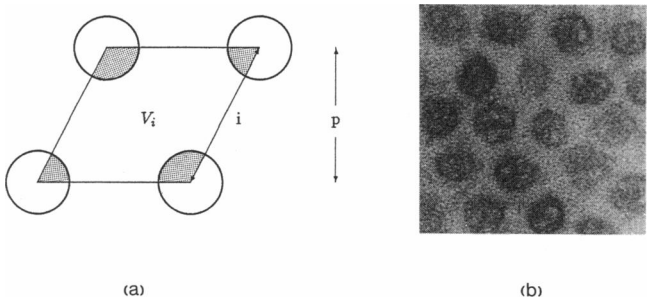

FIGURE 1 (*a*) Diagrammatic representation of four collagen fibrils (in cross-section) packed in such a way that the volume per unit length of a unit cell, U is related to the Bragg spacing, p, and the center-to-center spacing, i, by $U = ip$, where $i = 1.12p$. The volume, U, comprises two fractions, the fibrillar volume, V_f (shown shaded) and the interfibrillar volume, V_i. (*b*) Electron micrograph of collagen fibrils in the corneal stroma of a rabbit. Magnification × 160,000 (courtesy of I. M. Rawe).

TABLE 2 **The interfibrillar Bragg spacing (p), fibril diameter (D), and intermolecular Bragg spacing (a) for a number of different vertebrate species**

Name	Scientific name	Interfibrillar Bragg spacing, p	Fibril diameter, D	Intermolecular Bragg spacing, $a \pm 0.10$
		nm	*nm*	*nm*
PISCES				
Teleostei				
Herring	*Clupea spp.*	38.9 ± 2.1 (1)*	—	—
Salmon	*Salmo salar*	47.6 ± *1.0* (2)	26.7 ± *0.8* (2)	1.62 ± *0.08* (2)
Trout	*Salmo trutta*	45.4 ± 3.0 (5)*	24.7 (—)‡	1.56 ± *0.03* (8)
AMPHIBIA				
Anura				
Frog	*Rana temporaria*	55.5 ± 7.1 (1)	33.2 ± 1.5 (1)	1.54 ± *0.04* (4),
AVES				
Ciconiiformes				
Abdim's stork	*Ciconia abdimii*	60.7 ± 4.5 (1)	34.7 ± 1.8 (1)	—
Anseriformes				
Swan	*Cygnus olar*	65.0 ± 6.0 (2)*	39.5 ± 2.0 (1)	1.51 ± *0.04* (2),
Black swan	*Cygnus atratus*	59.0 ± 4.5 (2)*	—	—
Strigiformes				
Barn owl	*Tyto alba*	58.9 ± 4.5 (2)*	32.4 ± *1.3* (2)	1.57 ± *0.01* (2),
Short-eared owl	*Asio flammeus*	63.9 ± 2.2 (1)	36.1 ± *2.3* (1)	1.57 (1)
Psittaciformes				
Buffan's macaw	*Ara buffani*	62.0 ± *3.3* (2)	35.2 ± *1.1* (2)	—
Slender-billed cockatoo	*Kakatoe tenuirostris*	65.8 ± *1.9* (2)	34.5 ± *2.3* (2)	—
MAMMALIA				
Marsupialia				
Opossum	*Didelphis virginiana*	53.0 ± 3.0 (1)*	29.9 ± 0.7 (1)	—
Wallaby	*Macropus rufogrinseus*	57.7 ± 4.5 (2)	37.4 ± *3.1* (2)	1.56 ± *0.03* (3)
Insectivora				
Hedgehog	*Erinaceus europeaus*	52.9 ± 7.1 (2)	29.5 ± 3.0 (1)	—
Primates				
Human	*Homo sapiens*	55.3 ± 4.0 (4)*	30.8 (—)‡	1.63 (10)
Monkey	*Macaca fascicularis*	60.6 ± *1.7* (6)	32.6 ± *1.6* (3)	1.58 ± *0.03* (12)
Lagomorpha				
Rabbit	*Oryctologus cuniculus*	58.8 ± 4.5 (10)*	38.8 (—)‡	1.58 ± *0.06* (6)
Hare	*Lepus timidus scotius*	57.9 ± 4.5 (4)*	38.6 ± *0.5* (2)	—
Rodentia				
Squirrel	*Scuirus carolinensus*	62.8 ± *5.3* (3)	42.4 ± *1.4* (4)	1.59 (2),
Mouse	*Mus musculus*	64.6 ± *6.5* (3)	39.7 ± *1.8* (3)	—
Rat	*Rattus norvegicus*	61.5 ± 6.0 (3)*	36.7 ± *1.5* (3)	—
Mara	*Dolichotis patagonium*	53.6 ± 3.0 (2)*	—	—
Guinea-pig	*Cavia porcellus*	66.0 ± *2.8* (6)	39.5 ± *0.8* (3)	1.58 ± *0.04* (6),
Coypu	*Myocastor coypu*	52.3 ± *4.5* (4)	30.2 ± *1.0* (4)	—
Cetacea				
Fin whale	*Balaenoptera physalus*	42.2 ± 2.0 (2)*	24.0 ± 0.5 (1)	1.61 (1),
Dolphin	*Lagenorhynchus acutus*	45.3 ± 3.0 (1)*	25.6 ± 1.0 (—)‡	1.57 ± *0.04* (2)
Carnivora				
Foxhound	*Canis familiaris*	61.7 ± 5.1 (3)*	35.7 ± *2.8* (3)	1.60 ± *0.06* (3)
Fox	*Vulpes vulpes*	62.2 ± 4.1 (3)*	38.5 ± *1.1* (3)	1.57 ± *0.05* (8)
Brown bear	*Ursus arctos*	54.4 ± 3.0 (4)*	31.2 ± *0.4* (4)	—
Badger	*Meles meles*	60.3 ± 5.1 (6)*	31.9 ± *0.5* (3)	1.59 ± *0.07* (3)
Cat	*Felis catus*	62.3 ± *2.0* (4)	40.2 ± *0.3* (4)	—
Tiger	*Panthera tigris*	—	40.2 ± 1.0 (1)	1.55 ± *0.01* (2)
Polecat	*Mustela erminea*	—	—	1.59 ± *0.01* (2),
Pinnipedia				
Seal	*Halichoerus grypus*	56.3 ± 4.0 (1)*	29.7 ± *0.6* (2)	1.56 ± *0.00* (2)
Perissodactyla				
Zebra	*Equus burchelli grevii*	57.0 ± *5.7* (2)	34.4 ± *0.5* (2)	—
Artiodactyla				
Pig	*Sus scrofa*	58.6 ± 4.5 (4)*	36.9 ± *3.2* (2)	1.57 ± *0.01* (2)
Wild boar	*Sus scrofa*	59.7 ± 4.5 (2)*	37.8 ± *1.6* (2)	—
Camel	*Camelus bactrianus*	67.0 ± 6.0 (1)	43.0 ± 1.0 (1)	—

TABLE 2 (continued)

Name	Scientific name	Interfibrillar Bragg spacing, p	Fibril diameter, D	Intermolecular Bragg spacing, $a \pm 0.10$
		nm	*nm*	*nm*
MAMMALIA *continued*				
Artiodactyla				
Muntjac deer	*Muntiacus reevesi*	58.7 ± *1.9* (3)	38.5 ± *1.4* (2)	1.60 ± *0.01* (2)
Chinese water deer	*Hydropotes inermis*	58.2 ± 4.5 (3)*	38.3 ± *0.08* (3)	—
Cow	*Bos taurus*	56.8 ± 4.5 (7)*	38.2 (—)‡	1.60 (31)
Goat	*Capra hircus*	57.9 ± 4.5 (1)*	—	—
Sheep	*Ovis aires*	60.4 ± 5.0 (6)*	37.0 ± *1.8* (4)	1.59 ± *0.01* (2)
Peccary	*Tayassu tajacu*	—	—	1.61 ± *0.02* (2)

The experimental errors are estimates of the precision of measurement of the diffraction rings, (*normal print*), converted to real space. In some cases it was possible to calculate standard deviation (*italic print*). *Refers to data interpreted from Gyi et al. (1988). Numbers in parenthesis refer to the number of specimens used. ‡Refers to data taken from Gyi (1988), where the number of specimens and experimental errors were not quoted. , refers to data taken from specimens which were dried and rehydrated.

and the "unit cell" volume, U. We first calculated the volume fraction of the tissue occupied by GAGs (V_i/U) and the results are shown in Table 3. There is a remarkable consistency in this value for *all* the animals studied ($V_i/U = (75 \pm 4)\%$). Fig. 2 shows the result of plotting V_i against U; the relationship has a linear correlation coefficient of 0.97 and the gradient gives the mean volume fraction as (72 ± 3)%. The volume fraction occupied by the fibrils, V_f/U, is therefore (28 ± 3)%. If we ignore the stromal volume occupied by keratocytes, 2–3% (Maurice, 1969), these volume fractions represent the total tissue volumes occupied by the extrafibrillar GAGs, etc., and by the hydrated collagen fibrils, respectively.

Since most animals appear to have the same intermolecular Bragg spacing (Table 2), it was possible to estimate the average number of molecules in a fibril cross-section on the assumption that, as with most collagens, molecules are packed in a pseudo-hexagonal array (see Methods). These estimates are also presented in Table 3. It is clear that, because of the similarity of the intermolecular spacing between the species, differences in fibril diameter arise from different numbers of molecules per fibril rather than from different spacings of the same number of molecules.

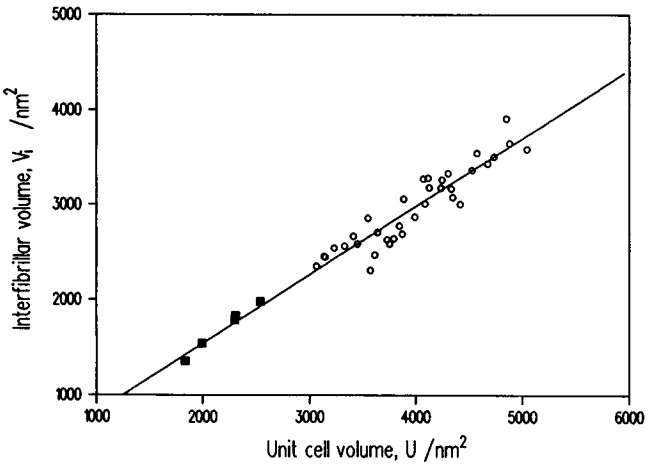

FIGURE 2 Interfibrillar volume, V_i, plotted against "unit cell" volume, U, using data calculated from Table 2. The aquatic animals are represented by ■ and the terrestrial animals by ○. The linear correlation coefficient is 0.97 and the intercept on the vertical axis is 100 ± 119.

The results presented in Table 2 lead to one further prediction. Since the volume fraction occupied by the fibrils, V_f/U, appears to be approximately constant, and since V_f is proportional to N, the number of molecules per fibril cross-section, it follows that N/U should be constant, in other words, there should be a constant number of molecules per unit tissue volume. This relationship is plotted in Fig. 3. Furthermore, since U is proportional to V_i, N/V_i must also be constant, that is, there must be a linear relationship between the number of molecules per fibril and the volume between the fibrils. The linear dependency between N and U (or V_i) leads to a simple model, which applies to the mature corneas of all the species studied undergoing normal turnover of collagen and proteoglycans.

The model supposes that fibrillar collagen molecules and interfibrillar GAGs (which constitute most of the interfibrillar volume) are synthesized in a fixed numerical ratio. Thus, the greater the number of molecules within a collagen fibril, the larger the number of GAGs in the interfibrillar volume and hence the farther apart the fibrils are spaced. Fibrils with small diameters (such as those in fish) thus pack together more closely than fibrils with larger diameters (such as those in some land mammals). The spacing between the fibrils is therefore a direct consequence of the ultimate diameter that the fibrils can reach. In all species, however, the relative proportions of the volume occupied by the hydrated collagen fibrils and the interfibrillar GAGs is constant.

The model described above has a number of physical and chemical consequences that can be examined. First,

TABLE 3 **Calculated structural parameters for a number of different vertebrate species**

Common name	Scientific name	Fibril surface separation s	Interfibrillar volume V_i	V_i/U	No. molecules per fibril cross section, N
		nm	nm^2		
PISCES					
Teleostei					
Salmon	*Salmo salar*	26.6	1978	0.78	198
Trout	*Salmo trutta*	26.1	1829	0.79	169
AMPHIBIA					
Anura					
Frog	*Rana temporaria*	29.0	2584	0.75	306
AVES					
Ciconiiformes					
Abdim's stork	*Ciconia abdimii*	33.3	3181	0.77	334
Anseriformes					
Swan	*Cygnus atratus*	33.3	3507	0.74	433
Strigiformes					
Barn owl	*Tyto alba*	33.6	3061	0.79	291
Short-eared owl	*Asio flammeus*	34.5	3550	0.78	362
Psittaciformes					
Buffon's macaw	*Ara ambigua*	34.2	3332	0.77	344
Slender-billed cockatoo	*Cacatua tenuirostris*	39.2	3914	0.81	330
MAMMALIA					
Marsupialia					
Opossum	*Didelphis virginiana*	29.5	2444	0.78	248
Wallaby	*Macropus rufogriseus*	27.2	2630	0.71	388
Insectivora					
Hedgehog	*Erinaceus europaeus*	29.7	2450	0.78	242
Primates					
Human	*Homo sapiens*	31.0	2668	0.78	263
Monkey	*Macaca fascicularis*	35.3	3282	0.80	295
Lagomorpha					
Rabbit	*Oryctologus cuniculus*	27.1	2690	0.69	418
Hare	*Lepus timidus scotius*	26.2	2584	0.69	414
Rodentia					
Squirrel	*Scurius carolinensis*	29.7	3005	0.68	499
Mouse	*Mus musculus*	32.7	3436	0.74	437
Rat	*Rattus norvegicus*	32.2	3178	0.75	374
Guinea-pig	*Cavia porcellus*	34.4	3653	0.75	433
Coypu	*Myocastor coypu*	28.4	2347	0.77	253
Cetacea					
Fin whale	*Balaenoptera physalus*	23.3	1543	0.77	160
Dolphin	*Lagenorhynchus acutus*	25.1	1784	0.78	182
Carnivora					
Foxhound	*Canis familiaris*	33.4	3263	0.77	354
Fox	*Vulpes vulpes*	31.2	3169	0.73	411
Brown bear	*Ursus arctos*	29.8	2562	0.77	270
Badger	*Meles meles*	35.6	3273	0.80	282
Cat	*Felis catus*	29.6	3078	0.71	448
Tiger	*Panthera tigris*	23.1	2306	0.65	448
Pinnipedia					
Seal	*Halichoerus grypus*	33.4	2857	0.80	245
Perissodactyla					
Zebra	*Equus grevyi*	29.4	2709	0.74	328
Artiodactyla					
Pig	*Sus scrofa*	28.7	2777	0.72	378
Wild boar	*Sus scrofa*	29.1	2870	0.72	397
Camel	*Camelus bactrianus*	32.2	3590	0.71	513
Muntjac deer	*Muntiacus reevesi*	32.7	3366	0.74	411
Chinese water deer	*Hydropotes inermis*	26.9	2642	0.70	407
Cow	*Bos taurus*	25.4	2467	0.68	405
Sheep	*Ovis aries*	30.6	3011	0.74	380

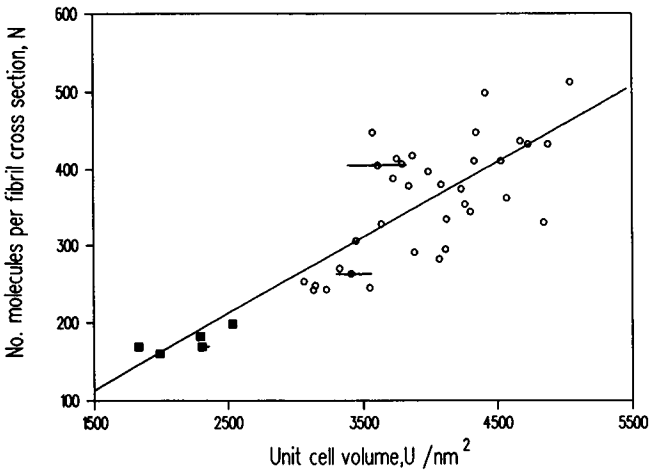

FIGURE 3 Number of molecules per fibril cross-section, N, plotted against "unit cell" volume, U, using data calculated from Table 2. The aquatic animals are represented by ■ and the terrestrial animals by ○. The horizontal bars represent the variation in U, over the natural range of hydration for trout, human, cow. The linear correlation coefficient is 0.82 and the intercept on the vertical axis is -35 ± 42.

the hydration of corneas from different species would be expected to be approximately constant. Scott and Bosworth (1990) have measured the water content of corneas from a number of land mammals and found them all to have (79 ± 1)% water by weight. However, their method of determining hydrations differs from ours in that their corneas were freeze dried rather than air dried. We have found the water content of trout corneas to be (78 ± 2)% (five specimens, air dried) or (81 ± 2)% (same specimens, oven dried, 4 d at 100°C). The variation between species seems to be less than the natural variation between corneas of the same species, which, for cow, is about ±2%. The change in the unit cell volume, U, with hydration is known for trout, man, and cow (Gyi, 1988) and the effect on this volume of a ±2% variation in the tissue hydration can thus be calculated. This effect is depicted for these three animals by the horizontal bars in Fig. 3. There is no change in the fibril volume over this hydration range (Meek et al., 1990). Natural variations in hydration therefore only cause small changes in the volume fraction and do not affect our conclusion that the volume fractions in most species are probably the same.

The constant ratios V_i/U and V_f/U suggest that the glycosaminoglycan content (mass of sulphated GAGs/mass of dry tissue) and the collagen content (mass of collagen/mass of dry tissue) should be the same for all species. Gyi (1988) has shown that the glycosaminoglycan content is (5.1 ± 1.4)% of the tissue dry weight for a number of species, including fish. Scott and Bosworth (1991) have shown for a number of land mammals, that the collagen content is constant. Finally the constant volume fractions V_i/U and V_f/U predict, according to Gladstone and Dale's law of mixtures, that the refractive indices would not vary across the species. From a review of measured refractive indices from 26 species (including fish and whales) (Sivak, 1988) the mean corneal refractive index is found to be (1.372 ± 0.009).

The model discussed above requires a constant collagen/sulphated GAGs ratio, but the fibrillar collagen content can be composed of more than one collagen type and the GAG content by more than one type of GAG. Fibril diameters in the cornea may be controlled by the proportion of type V collagen within the predominantly type I collagen fibrils. We are currently examining the content of type V collagen of a variety of species to see if there is any correlation between the type V content and the ultimate diameter that fibrils in each species can attain (and hence their ultimate spacing). Similarly, Scott and Bosworth (1990) have shown, for a number of land mammals, that the polyanionic charge per unit volume is constant even though the ratio of keratan sulphate to dermatan sulphate shows remarkable variation. Gyi (1988) has shown that this is also the case when comparing trout and bovine corneas. It is thought that keratan sulphate "stands in" for dermatan sulphate in animals whose oxygen supply to the cornea is restricted (Scott and Haigh, 1988).

At present we cannot explain why fibril diameters should vary between species. There may be some optical reason, for example, relating to the wavelength transmission characteristics of the cornea, or it may simply be a mechanical effect. However, the observed differences seem to relate to the size of neither the animal nor the cornea, but rather to environmental factors. The clear separation between the aquatic and land species and the implications of these variations within the paradigm for transparency will be addressed when further experimental evidence has been collected.

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