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Elastic and related fibres in the normal cornea and limbus of the domestic cat*

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INTRODUCTION

A histochemical definition of the properties of oxytalan fibres was first published by Fullmer & Lillie (1958), who found that preoxidation permits the visualisation of a specific population of connective tissue fibres in the periodontal membrane by certain staining techniques for elastic fibres. Preoxidation of these fibres also renders them susceptible to elastase digestion. Oxytalan fibres, unlike collagen, are capable of withstanding prolonged acid hydrolysis. The various oxidation procedures used for the demonstration of oxytalan fibres were later compared by Alexander, Clayton, Howes & Garner (1981).

Electron microscopy may also be used in the identification of oxytalan since ultrastructurally it is now well recognised (Carmichael & Fullmer, 1966; Cotta-Pereira, Guerra-Rodrigo & Bittencourt-Sampaio, 1976; Ross, 1973) to consist of bundles of apparently tubular fibrils 10–16 nm in diameter. During embryonic elastogenesis it appears that the first type of elastic fibre to be formed consists of bundles of this fibrillar material, oxytalan (Ross, 1973). As development proceeds, the fibrils come to lie randomly within an amorphous elastin matrix (Gawlik, 1965; Cotta-Pereira, Guerra-Rodrigo & Bittencourt-Sampaio, 1975, 1976). Ultimately in the mature elastic fibre, most of these fibrils form a collar around a central core of elastin. It appears that fibre development may arrest or slow down abruptly at any time from a late stage *in utero* to early independent life (Fullmer, Sheetz & Narkates, 1974; Alexander & Garner, 1977, 1983). This may occur at either the initial, intermediate or final stage of elastic fibre evolution (Fig. 1), giving rise to the corresponding fibre type which may persist into adult life (Alexander & Garner, 1983). The histochemical staining characteristics of these three types of fibre are given in Table 1.

Oxytalan fibres have now been demonstrated in a wide range of normal tissues (Fullmer & Lillie, 1958; Cameron, Jennings & Rannie, 1970; Fullmer *et al.* 1974; Calvo & Boya, 1983; Manning, 1974; Alexander & Garner, 1983) including human cornea. It is reported that the normal, juvenile, cornea of man contains oxytalan fibres. These fibres normally regress and ultimately disappear during late adolescence and early adulthood (Alexander & Garner, 1983), returning only in certain cases of corneal pathological conditions (Alexander & Garner, 1977; Alexander, Grierson &

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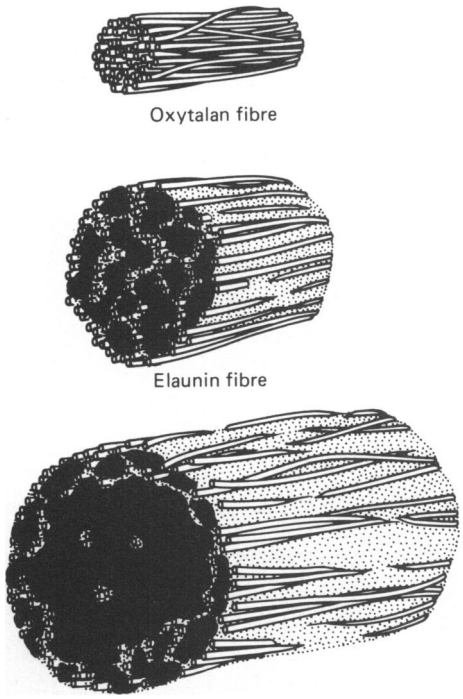


Fig. 1. Diagram of the ultrastructural relationship between oxytalan, elaunin and elastic fibres. Oxytalan fibres are comprised of microfibrils alone. In elaunin fibres these microfibrils become invested with an amorphous component (elastin) which in a mature elastic fibre forms the core of the fibre and constitutes approximately 90% of the fibre volume. (Reprinted with permission of the Editor from *Archs Ophthal., N.Y.* 1981, **99**, 1622–7, copyright 1981, American Medical Association.)

Garner, 1981). In such pathological states, further evolution of the oxytalan into elaunin or elastic fibres may occur (Alexander & Garner, 1977; Garner & Alexander, 1981).

This paper describes the presence, distribution and orientation of oxytalan fibres in the cornea and limbus of normal domestic cats from birth to maturity. It is believed to be the first such study in a non-human species.

MATERIALS AND METHODS

Anterior segment tissues were taken from the eyes of thirteen cats: newborn, 3 weeks (two animals), 6 weeks, 10 weeks, 12 weeks (three animals), 4 months, 7 months and adult (three animals). The animals were killed by barbiturate overdose immediately before removal of the tissue, which was studied by light and electron microscopy.

Light microscopy

Specimens were fixed in 10% formol saline and processed to paraffin wax before sectioning at approximately 5 μm thickness. To demonstrate elastic and precursor fibres, sections were stained by Verhoeff's iron haematoxylin and Gomori's aldehyde fuchsin techniques, both in the absence of and following oxidation, in 10% aqueous Caroat solution for 60 minutes (Alexander *et al.* 1981). Caroat (active component:

Table 1. *Histochemical staining reactions of oxytalan, elaunin and elastic fibres*

Technique	Oxytalan	Elaunin	Elastic	Colour of positive reaction
Definitive stains				
Verhoeff iron haematoxylin	−	−	+	Black
Oxidation Verhoeff	−	−	+	Black
Aldehyde fuchsin	−	+	+	Purple
Oxidised aldehyde fuchsin	+	+	+	Purple
Orcein	−	+	+	Dark brown
Oxidised orcein	+	+	+	Dark brown
Controls				
Elastase – Verhoeff's stain	−	−	A	—
Elastase – aldehyde fuchsin	−	A	A	—
Elastase – oxidised aldehyde fuchsin	S	S	A	—
Oxidation elastase – aldehyde fuchsin	A	A	A	—

−, unstained; +, stained; A, abolished; S, stained (no change).

potassium peroxymonosulphate) was obtained from Degussa Ltd, England. In selected instances, sections were exposed to elastase (Hog pancreas type I, Sigma Chemical Co.) for 6 hours at 37 °C (Fullmer, 1960) both before and after oxidation and subsequently stained with aldehyde fuchsin. Additional sections were stained by haematoxylin and eosin, Gomori trichrome, Mowry alcian blue (pH 2·5) and periodic acid–Schiff techniques.

Electron microscopy

Tissues were processed for electron microscopy by one of two different regimes.

(1) Initial, overnight, fixation was in half strength Karnovsky fixative followed by post-fixation in 1 % osmium tetroxide in cacodylate buffer, pH 7·4. After dehydration through ascending concentrations of ethanol, the tissue was block stained with 2·3 % alcoholic uranyl acetate and then passed to propylene oxide before embedding in Araldite.

(2) Initial fixation was in 2·3 % glutaraldehyde in cacodylate buffer, pH 7·4, and post-fixation in unbuffered 2 % aqueous osmium tetroxide. After block staining with 2 % aqueous uranyl acetate, the tissue was dehydrated through ascending concentrations of acetone and embedded in Epon.

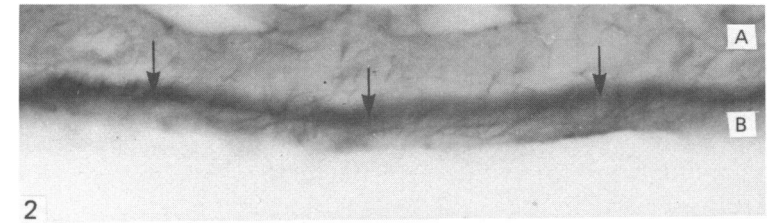
In both instances, thin sections were stained with Reynolds' lead citrate.

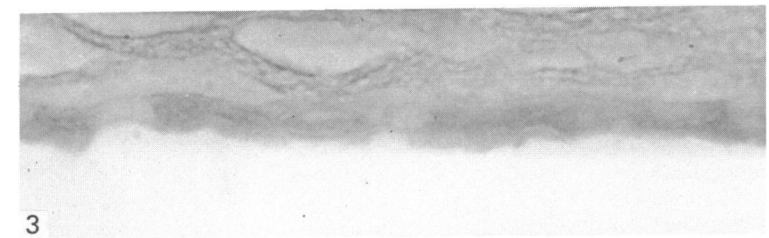
RESULTS

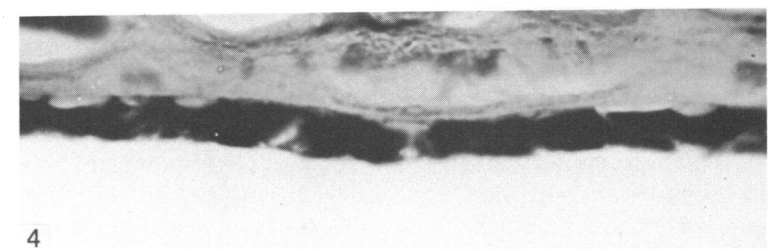
Light microscopy

Cornea

Large numbers of very fine oxytalan fibres were present throughout the substantia propria of the newborn kitten cornea. There was a gradual increase in the number and thickness of these fibres from the outer to the inner levels of the substantia propria. A similar increase was also seen from central to peripheral cornea. Oxytalan fibres were most frequent at the junction of the inner substantia propria with the posterior limiting lamina (this junction is referred to in this study as the 'junctional fibre zone'; Figs. 2–4). At this interface, fibres were largely orientated parallel to







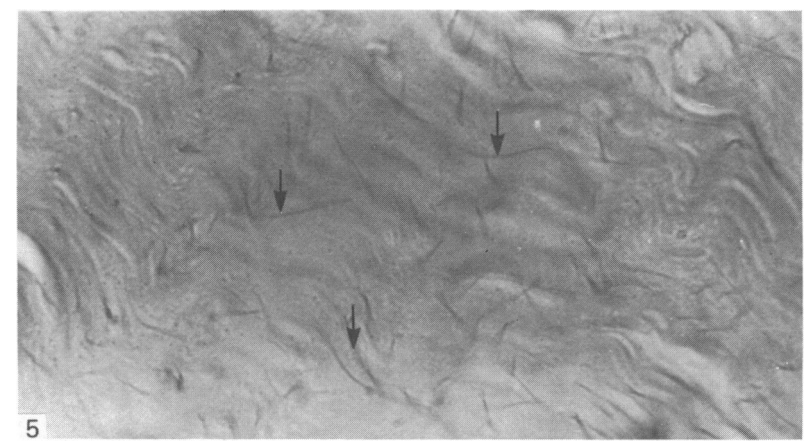


Fig. 2 (A–B). Section of newborn kitten cornea showing the junctional fibre zone (arrows) with oxytalan fibres running off into the inner substantia propria (A) and the posterior limiting lamina (B). Oxidised aldehyde fuchsin. × 1200.

Fig. 3. A similar area to that shown in Fig. 2 but stained with aldehyde fuchsin without preliminary oxidation. Elaunin and elastic fibres are not present and oxytalan fibres are unstained. × 1200.

Fig. 4. A section adjoining that shown in Fig. 3 stained by Verhoeff's iron haematoxylin confirming that no elastic fibres are present in this area. × 1200.

Fig. 5. Oxytalan fibres lying randomly within the corneal substantia propria of a seven months old kitten stained with oxidised aldehyde fuchsin. Three of the fibres are arrowed. × 1200.

the corneal surface. However, appreciable numbers ran off obliquely into both the inner substantia propria and the posterior limiting lamina itself. In places, the fibres of the peripheral junctional fibre zone, when seen in cross section, took on the appearance of small granules. A weakly defined spur of thicker and more numerous oxytalan fibres was present in the anterior peripheral part of the substantia propria, just beneath the anterior corneal epithelium (this zone is referred to in this study as the 'peripheral subepithelial spur'). The orientation of the corneal oxytalan fibres, other than in the junctional fibre zone, generally appeared to be random in sections prepared for light microscopy. In the outer peripheral part of the substantia propria of the newborn kitten, however, an increased number of fine fibres was observed running at right angles to the corneal surface. The posterior limiting lamina exhibited oxytalan fibres which tended to run obliquely outwards from just beneath the posterior epithelial monolayer into the junctional fibre zone. Rarely, fibres were observed to run parallel to the corneal surface within the posterior limiting lamina.

A gradual reduction in the number of corneal oxytalan fibres occurred with increasing age. The reduction was most rapid in the posterior limiting lamina, and, in material from the seven months old cat, no fibres could be detected at this site. Ultimately, in the adult cat only the peripheral subepithelial spur, the peripheral areas of the inner substantia propria and the junctional fibre zone contained appreciable numbers of oxytalan fibres. However, occasional fibres were identified in the middle layers of the peripheral substantia propria. The junctional fibre zone was the area within the central cornea which was the last to lose its oxytalan fibres. In the juvenile animal, the peripheral subepithelial spur was particularly prominent. However, in the mature cornea, there was some reduction in the number of fibres in the spur. Moreover, fibre maturation appeared to have taken place in this area and a small number of elaunin fibres was present. No elastic fibres were seen within the cornea at any age.

In conjunction with a decrease in the numbers of fibres as the cats matured, there was an increase in the thickness of the residual fibres. As a result, the few corneal oxytalan fibres which remained were approximately three to four times thicker than those found in the newborn kitten.

The orientation of the fibres in the corneal substantia propria (Fig. 5) showed little alteration from birth to adulthood. However, the fine, preponderantly transverse arrangement seen in the outer peripheral cornea of the newborn kitten was not observed in older animals.

Varying degrees of homogeneous, deep purple, staining were evident in the posterior limiting lamina of the cornea when oxidised aldehyde fuchsin-stained sections were examined. In the more mature specimens this staining took on a lamellar appearance which was frequently confined to the outer layers of the lamina. It was difficult to attribute this staining reaction to oxytalan since type IV collagen, which is normally present within the posterior limiting lamina (Klintworth,1977), may also stain purple with the oxidised aldehyde fuchsin technique as a result of its high glucose and galactose content (Bangle, 1954).

Corneal limbus

For the purpose of this study, the limbus approximated to the area lying immediately peripheral to the dotted line in Figure 6. In sections from the eye of the newborn kitten stained with oxidised aldehyde fuchsin, the limbus was marked by an abrupt increase in the thickness and number of oxytalan fibres. Further away from

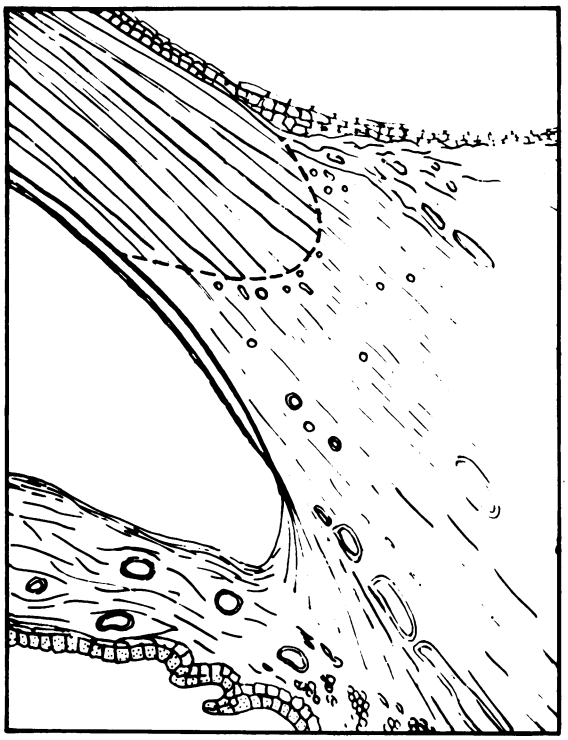


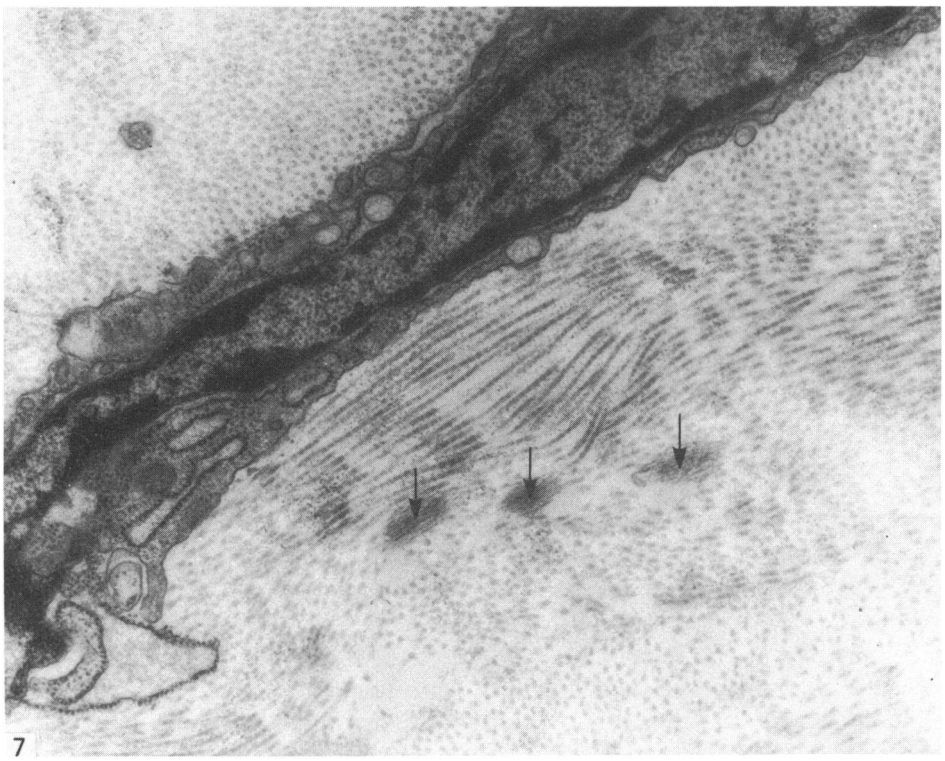
Fig. 6. Diagram of the iridocorneal angle of the cat. The dotted line represents the limits of the avascular cornea. The area immediately peripheral to this limit (to the right hand side of the dotted line) is defined as the corneal limbus for the purposes of this paper.

the limbus, a slight reduction in the number of scleral fibres was seen, giving the impression that a focus of oxytalan fibres was present around the limbal circumference. This appearance was considerably less marked, or absent, in adolescent and adult animals.

A comparison of the outer and inner parts of the limbus in the newborn kitten showed that the inner part contained considerably more oxytalan fibres. The subconjunctival connective tissue, however, where it merged with the peripheral subepithelial spur, did contain considerable numbers of fibres. Some elaunin fibres were present a short distance peripheral to the limbus in the inner scleral layers. The junctional fibre zone was continued through the limbus, associated with the remnants of the posterior limiting lamina which stretched towards the iridocorneal angle. The oxytalan present within the zone at this point appeared similar to that in the peripheral cornea and was in the form of small granules. In places these granules conjoined, to form a semi-continuous layer.

With increasing age, elastic fibre types in the inner scleral layers appeared to recede from the limbus. Ultimately in the adult animal, other than in the limbal continuation of the juctional fibre zone, the majority were situated adjacent to the reticulum trabeculare. Furthermore, some maturation of fibres was evident at this site and a significant number of elaunin fibres was present.

The outer layers of the limbus in the adolescent cat had greater numbers of oxytalan fibres than in the newborn. In the adult animal, however, a considerable reduction occurred here and ultimately only a narrow zone beneath the anterior



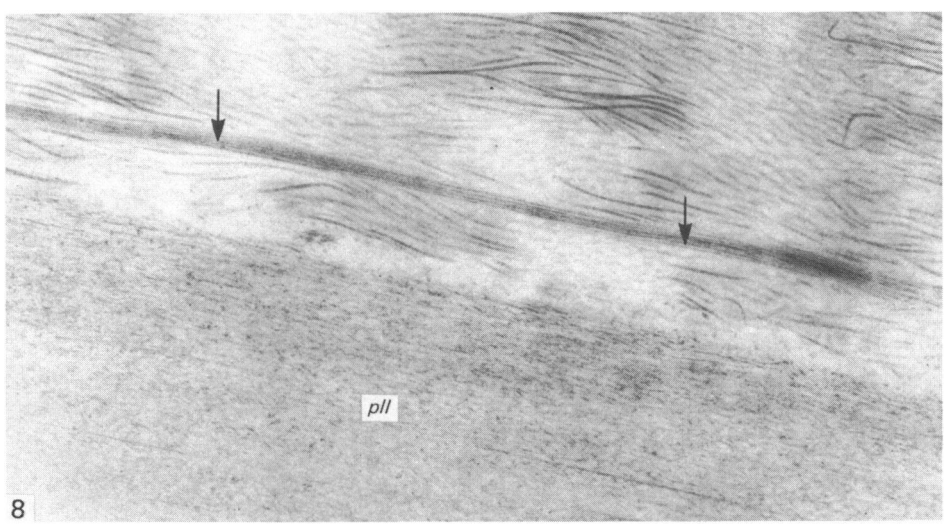


Fig. 7. Electron micrograph of oxytalan fibres cut in transverse section (arrows) lying in close proximity to a keratocyte. ×28200.

Fig. 8. Electron micrograph of an oxytalan fibre (arrows) in longitudinal section lying parallel to the posterior limiting lamina (*pll*). ×24300.

limbal epithelium contained fibres. This zone represented the fusion of the peripheral subepithelial spur with the subconjunctival connective tissue. In maturity, it contained appreciable amounts of elaunin and, in its deepest parts, occasional very thin elastic fibres. The fibre maturation which took place at this site appeared to have been parallel with that in the subconjunctival connective tissue immediately outside the limbus, and all elements of the elastic fibre series, including a number of thin elastic fibres, were present in appreciable numbers.

With the exception of the junctional fibre zone, where fibres tended to run parallel to the posterior limiting lamina, elastic and related fibres were randomly orientated in the limbus of all animals studied.

Sections stained with haematoxylin and eosin, Mowry alcian blue and Gomori trichrome showed no other connective tissue fibre types which might have been confused with oxytalan.

Electron microscopy

Cornea

Electron microscopy generally confirmed the identity of oxytalan fibres seen by light microscopy. Their distribution and orientation within the corneal substantia propria was similarly confirmed. However, owing to the greater magnification, it was possible to appreciate that about a quarter of the fibres lay between the collagen lamellae (Fig. 7). The remaining three quarters were randomly orientated within the lamellae themselves. Oxytalan fibres were usually round in cross section but were of variable diameter, depending on the age of the animal and the area in which the fibre was situated. Longitudinally cut fibres showed little variation in diameter along their length (Fig. 8). Each oxytalan fibre was seen to be made up of bundles of fibrils. The fibrils, which were 10–14 nm in diameter, appeared tubular in cross section and exhibited poorly defined cross striations with an irregular periodicity of between 2 and 4 fibril diameters. The ultrastructural appearance and size of individual fibrils was the same after both methods of fixation (2·3 % glutaraldehyde and half strength Karnovsky). These observations conformed to previously published data on the ultrastructure of oxytalan fibres (Carmichael & Fullmer, 1966; Cotta-Pereira *et al.* 1976; Sheetz *et al.* 1973).

A concentration of long well developed fibrils running parallel to the posterior limiting lamina was seen in the junctional fibre zone (Fig. 8). The lamina itself, however, appeared to contain no oxytalan fibres despite evidence to the contrary from light microscopy. This was the case even in the younger animals studied.

Limbus

In the limbus and immediately adjoining tissues oxytalan fibres were more irregular in both transverse (Fig. 9) and longitudinal section (Fig. 12) when compared

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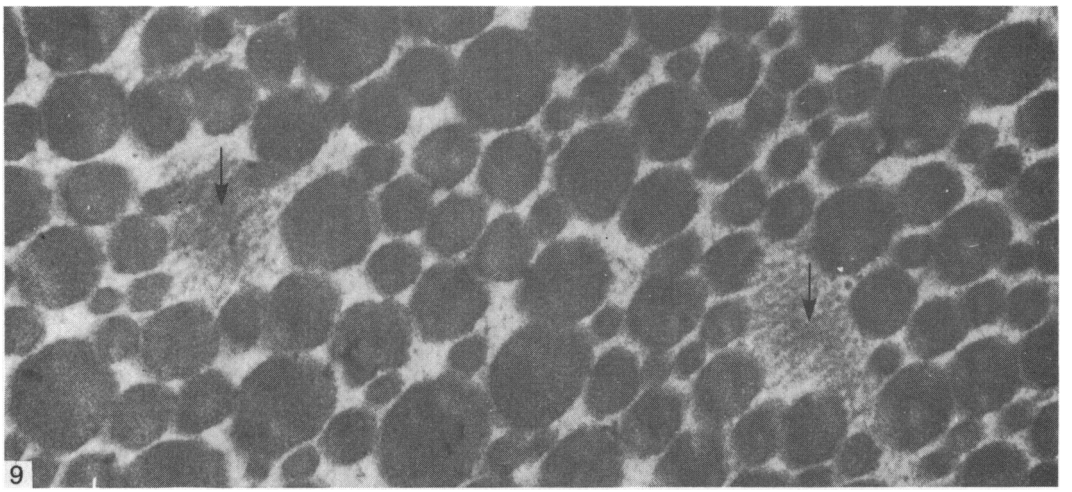
Figs. 9–11. Electron micrographs of elastic fibre types cut in transverse section from the limbal tissue of an adult cat.

Fig. 9. Oxytalan fibres (arrows) comprising microfibrils 11·5–14 nm in diameter. × 71 000.

Fig. 10. Elaunin fibre (arrow) comprising amorphous elastin and 12·5–15 nm diameter microfibrils. × 71 000.

Fig. 11. Elastic fibre (vertical arrow) comprising amorphous elastin and a collar of 12·5–14 nm diameter microfibrils. An oxytalan fibre which has been distorted by an adjacent collagen fibre is also present (horizontal arrow). × 71 000.



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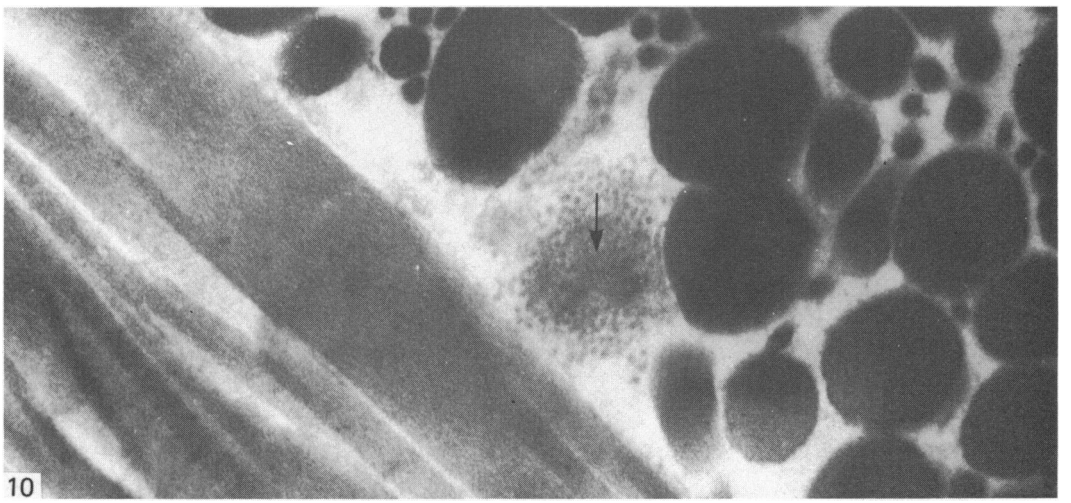

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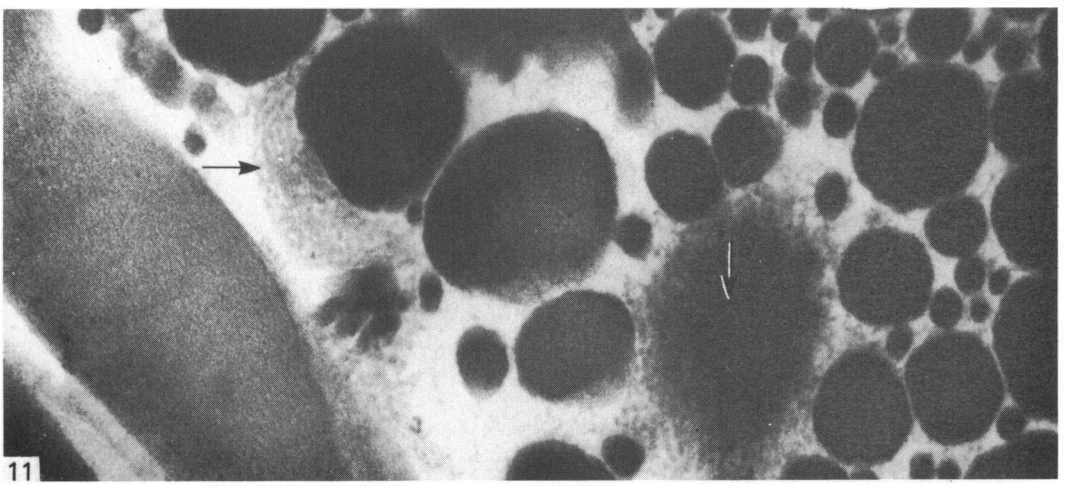

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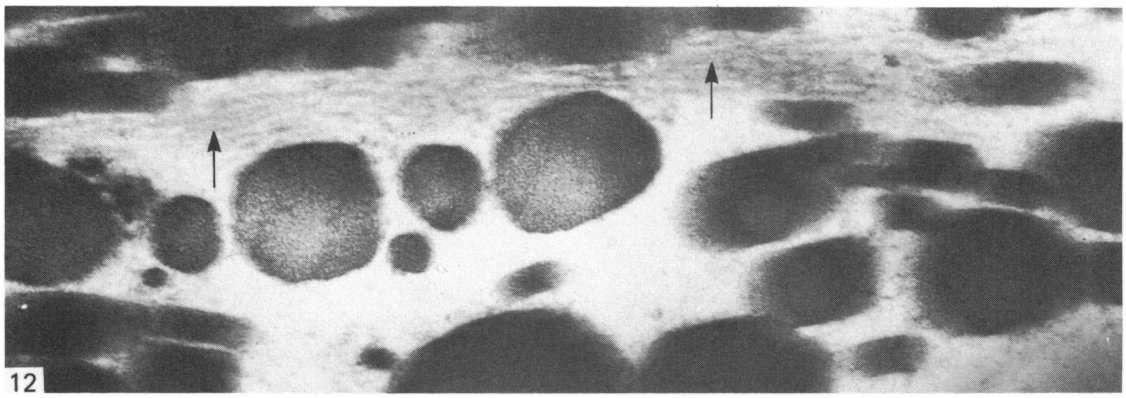

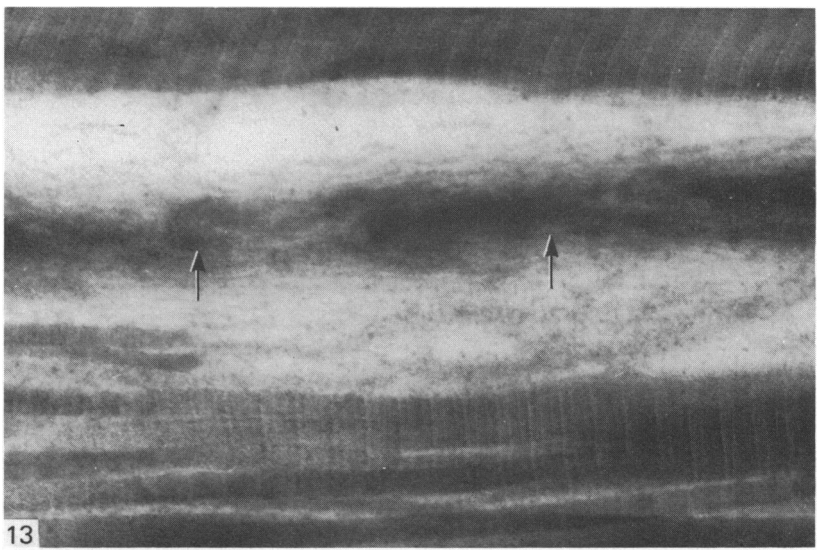

Figs. 12–13. Electron micrographs of elastic fibre types cut in longitudinal section in the limbal tissues of an adult cat.

Fig. 12. Oxytalan fibre (arrows). × 71000.

Fig. 13. Elastic fibre (arrows). × 71000.

to those in the cornea. These fibres often appeared to be distorted by adjacent collagen fibres (Fig. 11) and were generally of greater diameter than those seen in the cornea. Where elements containing more mature elastin were seen in the region of the limbus, these fibres took on a more regular appearance (Figs. 10, 11, 13) and were usually round in cross section. Limbal oxytalan and elaunin fibres (other than in the subconjunctival tissue) ran within, and parallel to, bundles of scleral collagen. In comparison only about a quarter of the fibres in the corneal substantia propria bore any specific relationship to the collagen lamellae.

DISCUSSION

A comprehensive investigation of the presence and distribution of corneal oxytalan fibres in a broad spectrum of mammalian species is lacking. This is the first time such fibres have been demonstrated in the cornea of a non-human species. Alexander &

Garner (1983) have described the presence and distribution of oxytalan fibres in the human cornea and from these observations it is possible to make comparisons between the two species. Significant differences between the feline material examined here and the previously described material from the normal human cornea are apparent and are summarised below.

Corneal fibres of the elastic series

(*A*) *Oxytalan*

Increase in fibre thickness from outer to inner cornea. This occurs in both species.

General fibre thickness. Human material contains thicker fibres when compared to the cat cornea at a similar stage of development.

Fibre orientation. In man, fibres mostly run parallel to and between the lamellae of the substantia propria. In the cat, however, about three quarters of the fibres within the substantia propria, other than in the junctional fibre zone, show a random distribution and orientation. The remainder lie between the collagen lamellae.

Junctional fibre zone. This is present in both cats and humans but appears to be most pronounced in the newborn kitten.

Peripheral subepithelial spur. In feline material, this spur is most pronounced in the juvenile animal but appears to be absent in man.

Fibres within the posterior limiting lamina. Histochemical techniques show the presence of oxytalan fibres in the posterior limiting lamina of both species. Electron microscopical study of this area in cats, however, has failed so far to demonstrate fibres within the lamina.

Age changes. No elastic fibre types are present in the mature human cornea. In the adult cat, however, oxytalan fibres persist in the peripheral cornea, particularly in the junctional fibre zone and the peripheral subepithelial spur.

(*B*) *Mixed elastic fibre types*

Cornea. In the mature cat some elaunin fibres, together with oxytalan, are present in the peripheral subepithelial spur.

Limbus. In the adult cat, all three types of elastic fibre are present at the limbus. The elaunin and elastic fibres present appear to mature from oxytalan, which is seen predominantly in the newborn animal. In man, however, oxytalan fibres present at this site are confined to younger subjects and largely disappear in adults.

Bulbar subconjunctival connective tissue immediately peripheral to the limbus. Material from newborn kittens contains large numbers of oxytalan fibres in this area. However, in the younger group of human subjects which was studied, fibres are not seen. Both adult man and the mature cat appear to have all three types of elastic fibre within the subconjunctival connective tissue.

Figure 14 summarises the relative size and distribution of oxytalan fibres in the cornea and limbus of the cat at birth and maturity.

In this study, techniques similar to those used previously on human material (Alexander & Garner, 1983) have demonstrated that the cat, like man, has oxytalan fibres within the posterior limiting lamina. Using the electron microscope, however, it is not possible to identify them definitively. The discrepancy may have been due to masking of fibres by the homogeneity of glycoproteins within the lamina. Jakus (1956) showed by extraction and digestion techniques that the posterior limiting lamina in a large number of species has regular structural components within its

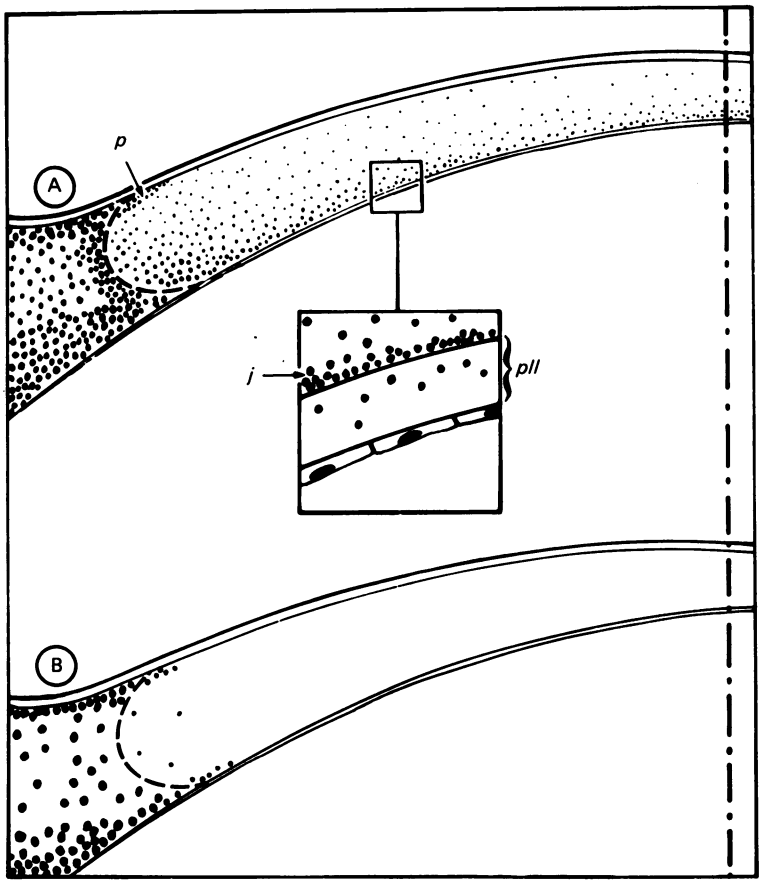

Fig. 14 (A–B). Diagram of the relative distribution and size of oxytalan fibres in sections from (A) newborn kitten and (B) adult cat. *p*, peripheral subepithelial spur; *j*, junctional fibre zone; *pll*, posterior limiting lamina. The number of dots represents the relative numbers of oxytalan fibres. The size of the dots represents the relative thickness of the oxytalan fibres. The broken line represents the axis of symmetry. A magnified inset shows a part of the inner substantia propria, posterior limiting lamina and posterior epithelial monolayer.

substance which are not normally visible in the electron microscope using conventional processing and staining techniques. Oxytalan fibres might well be similarly masked. Heathcote, Eyre & Gross (1982) have been able to identify desmosine and isodesmosine in bovine posterior limiting lamina. These residues were concentrated in a fraction which did not resemble elastin, in which they are normally found. This anomaly may indicate the presence of oxytalan since the microfibrillar component of oxytalan is also a constituent of elastic fibres.

Using electron microscopy, it has been possible to demonstrate that a small but significant proportion of oxytalan fibres, within the corneal substantia propria of the cat, lies between the collagen lamellae. In the present study, however, light microscopy techniques have failed to demonstrate such a specific fibre orientation. The discrepancy is likely to be due to the greater section thickness and limited resolution using conventional wax sections for histology.

Which corneal cell type is primarily responsible for oxytalan fibre synthesis is unclear. Elastic and related fibres are found in a wide variety of locations within

animal tissues and thus a number of different cell types may be capable of synthesising the fibrillar component of the elastic fibre series.

Alexander & Garner (1983) found oxytalan in a semi-continuous layer at the level of the anterior epithelial basal lamina in two corneae from elderly human subjects. No such material has been found in the present study of the cat but the observation in man would suggest that the anterior epithelial basal cells are, in certain circumstances, capable of synthesising oxytalan.

Since fibres are present throughout the thickness of the corneal substantia propria until late adolescence in the cat, the keratocytes may also be capable of oxytalan synthesis. Evidence to support this postulate comes from studies of pathological material in man (Garner & Alexander, 1981).

The presence of oxytalan fibres within and adjacent to the posterior limiting lamina of the newborn and juvenile cat suggests that the posterior epithelium may also contribute to corneal oxytalan synthesis. Pathological evidence to support this argument is seen in cases of Fuchs' endothelial dystrophy in man (Alexander *et al.* 1981). In such cases, a fibrous layer containing oxytalan develops beneath the posterior epithelial monolayer which apparently synthesises it. The origin of fibres within the junctional fibre zone is uncertain. In the newborn animal, these fibres lie closer to the keratocytes. However, in embryonic life when the posterior limiting lamina is as yet unformed, the posterior epithelial cells would lie immediately adjacent to the junctional fibre zone. It is possible, therefore, that these cells may contribute to oxytalan fibre formation during the period before recognisable elements of the lamina are formed. Once the synthesis of the amorphous material which will constitute the adult posterior limiting lamina is initiated, the junctional fibre zone would naturally be displaced away from these cells while still remaining adjacent to, or forming part of, the substantia propria. As a result, the zone would come to lie at the junction of the posterior limiting lamina with the substantia propria. Examination of feline material *in utero* is awaited to clarify this point.

In previously studied pathological cases of human cornea, elastic and elaunin fibre formation occurred only in stromal scar tissue following trauma to the cornea. Of the principal cell types present within the cornea, only keratocytes would therefore seem capable of producing elastin. Studies of pathological conditions in the feline cornea will be necessary to determine whether such cellular synthetic capabilities are similar in human and feline keratocytes.

The specific function, if there is one, of oxytalan fibres in the cornea of the cat remains unclear. Alexander & Garner (1977) and Garner & Alexander (1981) suggest that oxytalan is synthesised in response to stresses altered as a result of pathological states of the cornea. The presence of these fibres in the newborn and juvenile cornea, and their subsequent regression in the adult animal, would tend to support their argument. Considerable ocular growth takes place in the first year of life in the cat and, as a result, changes in corneal curvature (Freeman, 1980) must inevitably lead to altered tissue stress patterns. These altered stresses may well dictate the presence of elastic elements during the neonatal and adolescent period.

SUMMARY

The presence of elastic and related fibres in the corneal and limbal tissues of the domestic cat is described. Various ages of animal from birth to maturity were studied by light and electron microscopy. Oxytalan fibres were present throughout the

new-born kitten cornea. They were seen in greatest concentration both at the junction of the posterior limiting lamina with the inner substantia propria and beneath the peripheral anterior epithelium. With increasing maturity, oxytalan fibres disappeared from all but the most peripheral parts of the cornea where some of the, by now, thicker oxytalan fibres had matured into elaunin. All ages of animals studied showed an abrupt increase in fibre thickness and numbers at the corneal limbus. Oxytalan fibres predominated in the newborn kitten limbus but material from adult animals showed an increased proportion of elaunin fibres. Occasional elastic fibres were also seen in the more superficial region of the adult limbus. An overall decrease in elastic fibre types with increasing maturity was most obvious in the middle limbal layers.

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