Elastic Fibers in the Rat Exorbital Lacrimal Gland Duct System

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In the rat, each paired subcutaneous exorbital lacrimal gland overlies the retromandibular area. The fibrous cord containing its ducts passes over the masticatory muscles to the temporal canthus. Thus, the lacrimal system must accommodate both jaw and lid movements. To see whether elastic fibers exist to modulate alterations in tension caused by such movements, light and electron micrographs were made of its duct system. The innermost elastic fibers are connected by elaunin fibrils and oxytalan microfibrils to the lamina densa, particularly near the hemidesmosomes. The innermost elastic fibers appear to be longitudinal and about 0.2 µm from the lamina densa. Circumferential fibers exist about 0.8–3.2 µm from that structure. More peripheral fibers of both orientations also exist. Light microscopy of the extraglandular duct demonstrated circumferential fibers near the basement membrane and longitudinal and angular elastic fibers amid the collagenous layers. Some of the longitudinal fibers assume a loose cross-weave. Intraglandularly, as duct size diminishes elastic fibers progressively decrease in number and size until the smallest ducts have none. Thus, an elastic gradient exists. It is believed that recoil of the angular elastic fibers aids distension of the large and medium ducts when secretion is great and that recoil of the circumferential ones permits those duct diameters to diminish when secretion does. The longitudinal elastic fibers would allow all but the smallest ducts to recoil from the stretching of much of the exorbital lacrimal duct system accompanying blinking and other facial movements. Invest Ophthalmol Vis Sci 30:2002–2011, 1989

Rat exorbital lacrimal glands are paired, shield-shaped, subcutaneous structures overlying the front of the parotid gland, the retromandibular area and the rear portion of the body of the mandible. Venable and Griffin1 noted that they are oriented perpendicularly to the zygomatic arch, resting on the superficial muscles of mastication. They lie slightly below and just in front of each ear.2 Because these serous, tubulo-acinar glands3 are the largest components of the rodent lacrimal system, presumably they are the major contributors to the secretions moistening the surface of the ipsilateral eye. Each has a gross structure appearing to be its duct that extends between the upper part of the organ near its anterior border and the conjunctiva near the temporal canthus.

Because of the slight displacement of the exorbital lacrimal gland as the mandible moves and the need of its duct to alter its length with eyelid movements, that is, stretching as the lids blink and recoiling as they open, a search for elastic fibers in the duct and the organ itself that might modulate dimensional changes was performed. Their presence was anticipated because in the embryo the lacrimal gland develops as an evagination of the conjunctiva.4 The latter is known to contain elastic tissue,5,6 so it should not be surprising if the epithelial outpouching that becomes the duct and eventually the gland carries with it some of the collagen and elastic fibers of the connective tissue surrounding the bud. This presumably explains not only the present observations but those made by Sundwall in 19167 that elastic fibers exist in the lacrimal gland of the ox. In 1897, Fumagalli8 described an intricate network of what he termed elastic fibers throughout the human lacrimal gland. Although the internal elastic membrane of arteries was stained, his illustrations and description indicate that he often deemed reticulin fibers to be elastic fibers in addition to those meriting such classification.

Materials and Methods

One or both exorbital lacrimal glands were removed from six adult rats of both sexes following anesthesia with intraperitoneally administered sodium pentobarbital. For light microscopic studies,9 eight glands, often with a portion of their gross duct, were excised from five animals and fixed in pho-
phate-buffered neutral formalin, dehydrated and embedded in paraffin. Sectioning was done at 5 μm followed by hydration and staining with a Weigert's resorcinal-fuchsin stain or a modified Tanzer's stain of 0.4% orcein in acidified ethanol with Van Gieson counterstaining.

For electron microscopy, part of the gross extraglandular duct of one adult rat of each sex was fixed for 1 hr in 5% glutaraldehyde and 4% paraformaldehyde in 0.2 M phosphate buffer, pH 7.4. The tissue was postfixed for one hour in 1% osmium tetroxide in 0.2 M s-collidine buffer at pH 7.4, a modified Karnovsky procedure. En bloc staining with 2% aqueous uranyl acetate was followed by dehydration. Transversely and longitudinally oriented pieces were then embedded in epoxy resin. Survey sections (1 μm) were stained with 0.1% toluidine blue in 2% sodium borate. Selected areas were sectioned at 0.08 μm, placed on copper grids and exposed to 2% uranyl acetate in 25% ethanol for 15 min followed by 0.04 M lead citrate for one to 2 min. The thin sections were examined using a JEOL transmission electron microscope (Tokyo, Japan).

Light microscopic measurements of dimensions and distances were made using an ocular reticle that had been calibrated by an objective micrometer.

These studies accord with the ARVO Resolution on the Use of Animals in Research.

Results

Light Microscopy

Histological examination of what is grossly a single cord appearing to be the duct of the rat exorbital lacrimal gland indicates that at least part of the structure contains two ducts of dissimilar size and shape (Fig. 1a).

Most elastin lies near the duct epithelium. Immediately subepithelially, a layer of delicate circumferential elastic fibers about 0.2–0.5 μm in diameter is seen (Fig. 1b). Many larger, longitudinal elastic fibers, up to 1.2 μm in diameter, exist more peripherally in a number of layers (Figs. 1b, 2a). Some angular elastic fibers that extend diagonally or somewhat perpendicularly are more peripheral (Fig. 2b). They are about 0.4–0.8 μm in diameter. Because they do not encompass the duct and are often not truly perpendicular to the basement membrane, they have not been termed radial fibers.

There is also a loose weave of delicate elastic fibers, about 0.2–0.4 μm in diameter, that is occasionally seen close to the base of the epithelium (Fig. 2c). These connect with the surrounding thicker and more numerous longitudinal elastic fibers (Fig. 2c, d). Ducts in which a loose weaveshere is evident may be more relaxed than those that do not display that architecture. Relaxation is indicated by unusually great undulation of collagen fibers (Fig. 2c). Whether this diminished tension existed in situ or developed subsequently is not known.

Within the gland, between the largest ducts whose lumens have a maximum dimension of about 45–75 μm and the smallest ducts, the elastic fibers progressively diminish in number and often in thickness. The main duct and its largest branches have a relatively few, delicate circumferential fibers, often about 0.3–0.4 μm in diameter, usually appearing in a single
layer immediately beneath the basement membrane underlying the pseudostratified columnar epithelium (Fig. 3a). The surrounding longitudinal elastic fibers are thicker, about 0.6-0.8 μm, and more numerous. They are usually arranged in two to four layers among thick longitudinal collagen fibers in an 11-22 μm zone (Fig. 3b).

The medium ducts, whose lumens are about 20-40 μm in diameter, not only have circumferential and longitudinal elastic fibers but may have some angular collagen and small numbers of fine angular as well as cross-woven elastic fibers (Fig. 4a, b). Typically, circumferential elastic fibers are seen near the basement membrane. Longitudinal elastic fibers about 0.4-0.9 μm thick are present in moderate numbers. They are usually in one layer relatively near the basement membrane but additional layers exist in the surrounding collagen (Fig. 4a). Some close to the epithelium assume a loose weave (Fig. 4b) resembling that in the extraglandular portion of the duct (Fig. 2c). That weave disappears around small ducts. Likewise, no angular or circumferential elastic fibers and no or very few longitudinal ones are seen around the small ducts, whether interlobular (Fig. 5a) or intralobular (Fig. 5b). No elastic fibers are seen in the smallest ducts.

In general, elastic fibers appear thin, rather straight and unbranched. Longitudinal elastic fibers are the
most abundant and most constant. They, and those in the cross-weave, are best seen when the ducts are sectioned longitudinally. However, circumferential and angular fibers are most evident in transected ducts. By light microscopy, circumferential elastic fibers and those in the cross-weave are the finest ones.

The lacrimal duct system's elastic component is not nearly as extensive or as highly organized as in arterioles where an internal elastic membrane exists. Its elastic tissue is more like that in another ramifying low pressure system, the venous system, but the fibers are not as close as in venules.

The fibers that stain with elastic stains in the absence of prior oxidation, as at present, are either elastic fibers or delicate elaunin fibrils. By contrast, the even finer oxytalan microfibrils are said to lack elas-

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**Fig. 3.** The largest intraglandular ducts. (a) The first portion of the longitudinally sectioned duct bends on entry. Several delicate vertical (arrowhead) and transected (arrows) circumferential elastic fibers lie close to the epithelial cells. The short vertical fibers are believed to be circumferential elastic fibers in the frontal plane of the figure. Longitudinal elastic fibers are more prominent, particularly at the bend of the duct. Angular collagenous fibers exist at the lower left and above the duct. Clumps of desquamated epithelial cells are in the lumen. Orcein. Bar = 10 μm. (b) Longitudinal section of large duct. Longitudinal elastic fibers are present particularly above the duct. A few vertical circumferential elastic fibers (arrowheads) are seen as well as a few transected ones (arrows). On the right, a suggestion of an elastic weave exists through which the epithelium is seen. Weigert's stain. Bar = 10 μm.

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**Fig. 4.** Medium intraglandular ducts. (a) is stained by Weigert’s and (b) is stained by orcein. (a) Longitudinal section with predominantly longitudinal elastic fibers. Vertical circumferential elastic fibers (arrow) are seen at about five micron intervals. The longitudinal collagen fibers undulate more above than below the duct. A small area of elastic fiber cross-weave is exposed (arrowhead). Bar = 10 μm. (b) Longitudinally sectioned duct with small numbers of similarly oriented elastic fibers (arrow) and a sparse cross-weave of elastic fibers (arrowheads). Angular collagenous and a few angular elastic fibers (*) are perpendicular to the duct wall. Several acinar cells are at the lower left. Bar = 10 μm.
Fig. 5. Small intraglandular ducts stained with orcein. (a) Relatively few longitudinal elastic fibers (arrows) lie near the epithelium of the longitudinally sectioned interlobular duct. The transected metarteriole above the right end of the duct and the venule at its upper left have circumferential elastic fibers (arrowheads). Bar = 10 μm. (b) A longitudinally sectioned intralobular duct has some adjacent longitudinal elastic fibers (arrows). Bar = 10 μm.

tin because they are not visualized by these routine procedures for staining elastic tissue but are seen by electron microscopy.

Electron Microscopy

Electron micrographs of the extraglandular portion of the exorbital lacrimal duct system demonstrate elastic fibers whose cores are surrounded by dark microfibrils as well as having some internal ones (Fig. 6a). The innermost elastic fibers are about 0.2 μm from the lamina densa. They are longitudinally oriented, have gray cores, and are about 0.25–0.3 μm thick (Fig. 6a, b). Examination of other electron micrographs reveals the typical range of diameters to be 0.18–0.64 μm.

More peripherally, about 0.8–3.2 μm from the lamina densa, are circumferential elastic fibers about 0.3–1.1 μm diameter (Fig. 6b). Beyond them are longitudinal and often other circumferential elastic fibers. In the central region of the extraglandular duct, the fibers are oriented longitudinally in several layers about 1.0–2.6 μm apart (Fig. 7). In contrast to the innermost fine elastic layer, the peripheral ones are not joined to the basal lamina and have pale, amorphous cores (Fig. 6a, b). The distances of all layers from the basal lamina vary as the latter undulates. Thus, the closest elastic fibers may be between 0.05 and 0.4 μm from the basal lamina (Figs. 8a, b, 9a). Some elastic fibers in adjacent layers are connected by fibrils (Fig. 8a).

Connections between the innermost elastic fibers and the basal lamina occur particularly where the undulating lamina densa is very close to the elastic fibers. The connection is by two types of fine filaments. Nearest the elastic fibers are the elaunin fibrils, about 0.015–0.03 μm in diameter. These have very tiny, gray elastin cores surrounded by microfibrils (Figs. 8b, 9a). In turn, they ramify as black, oxytalan microfibrils, about 0.008–0.010 μm in diameter, appearing to have no cores except at very high magnification (Fig. 9b, c), where the microfibrils are seen to be tubular with a lumen diameter of about 0.004–0.006 μm. The zone they occupy between the elaunin and the lamina densa is about 0.10–0.20 μm wide.

Other filaments, about 0.005–0.009 μm in diameter, cross the lamina rara interna from the lamina densa to the basal surface of the epithelial cells (Figs. 8b, 9a). These delicate structures are particularly concentrated opposite the hemidesmosomes on the basal surface of those cells. The widths of the lamina densa and of the lamina rara change reciprocally. That of the lamina densa is about 0.03 μm in the interval between hemidesmosomes but increases to about 0.05 μm opposite a hemidesmosome. By contrast, the lamina rara is about 0.07 μm wide and a gray color between hemidesmosomes but narrows to 0.04 μm and is much darker opposite one (Fig. 9a).

That the peripheral fibrils of an elastic fiber are tubular is evident in high magnification views of such fibers near epithelial cells (Fig. 9b). The fibrils have a diameter of 0.010–0.020 μm. The lumen diameter is about 0.004–0.008 μm. The interfibril distance is approximately the same as the fibril diameter (Fig. 9c).

Discussion

In the exorbital lacrimal gland duct system, the grouping of the elastic fibers into several layers with differing orientations resembles the arrangement in blood vessels. However, in the latter many of the numerous fibers are organized into elastic mem-
Fig. 6. Electron micrographs of the longitudinally sectioned extraglandular portion of a lacrimal duct in the vicinity of its basal lamina. (a) A longitudinal elastic fiber with many fibrils and with a grayish core (e) is connected to the overlying lamina densa (*) by more delicate fibrils and microfibrils. Peripheral to this fiber is a group of three transected circumferential elastic fibers. These are larger and have pale elastin cores (E). The fibrils of the elastic fibers have diameters of about 0.05-0.10 μm and many exhibit a 0.013 μm interval beading (arrowhead). Similar prominences are between the numerous transected collagen fibrils as well as between and around the transected elastic fibers. A few lie within their cores. Bar = 0.13 μm. (b) A lower magnification of the preceding. Alternating longitudinal (arrows) and groups of circumferential (c) elastic fibers exist at various distances from the basal lamina of the epithelial cell, a portion of whose nucleus is seen at the upper left. A fibroblast process is at the lower right. Bar = 0.74 μm.

branes. These do not exist in the lacrimal duct system.

The architecture of this system’s elastic components, together with that of the collagen fibers in whose midst they exist, arouses interest as to the possible functions of the variously oriented fibers. That these differences may have physiological importance is believed for three reasons:

1. Angular and circumferential elastic fibers presumably aid changes in duct diameter. The angular elastic fibers would be tensed not only normally but particularly when the ducts toward which they are
oriented narrow. Their subsequent recoil would facilitate and maintain distension of the large and medium ducts when lacrimal secretion is increased, permitting them to serve as reservoirs. Concomitantly, the circumferential elastic fibers would be tensed as they stretch to accommodate the larger lumen size. The ensuing recoil of these circumferential elastic fibers, accompanied by tensing of the angular fibers, would aid the return of those ducts to their resting diameters when secretion diminishes. It is believed that the correspondingly oriented collagen fibers would function similarly to those elastic fibers. For example, as a duct constricts both types of angular fibers would elongate and become more taut and both types of circumferential fibers would shorten and become more lax. Had they not done so, the lengths of all fibers would have been disproportionate to the smaller lumen, if such change in lumen size could even have occurred independent of fiber alterations.

(2) The longitudinal fibers would primarily provide resiliency to the duct system. This would be due not only to the longitudinal elastic fibers\(^\text{15}\) but to the

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**Fig. 7.** Electron micrograph of a longitudinally sectioned extraglandular lacrimal duct at low magnification showing some very fine longitudinal elastic fibers next to the lamina densa along the upper border of the figure. Layers of larger longitudinal elastic fibers lie in undulating, predominantly longitudinal, collagen within 12 \(\mu\)m of the epithelial cell. The two narrow fibers marked (*) are probably one longitudinal fiber whose central portion is out of the plane of the section. Dark, organelle-containing fibroblast processes contact the two large elastic fibers toward the epithelium as well as the very long one farthest from it. Bar = 1.43 \(\mu\)m.

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**Fig. 8.** Electron micrographs of the extraglandular portion of a lacrimal duct at high magnification. (a) Transverse section showing a band of fibrils connecting a small elastic fiber with a more peripheral, larger one that is in contact with several fibroblast processes. The former is connected by microfibrils (arrowhead) to the basal lamina near a hemidesmosome (arrow). Another transected longitudinal collagen fiber is at the lower right. Both fibers have gray cores with faint outlines of tubular fibrils. Bar = 0.25 \(\mu\)m. (b) Longitudinal section showing part of one elastic fiber with a pale gray elastin core (e) surrounded by fibrils which extend between the adjacent white collagen fibers. Some of the dark fibrils are tubular with a gray amorphous core (arrowheads). These are elaunin fibrils. From those near the lamina densa (arrow) tinier oxytalan microfibrils extend to that structure. Other microfibrils are seen traversing the lamina rara to complete the linkage between the innermost elastic fibers and the duct cell. Bar = 0.20 \(\mu\)m.

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longitudinal collagen fibers whose normal laxity\textsuperscript{16} would permit them to extend as the duct stretches during blinking. This would occur because the temporal canthus near which the duct attaches moves forward then when the lids close in blinking and moves backward when they open. As the lids open, the longitudinal elastic and corresponding collagenous fibers, both of which had been stretched by the preceding lid closure, would shorten. The straight elastic fibers would rapidly recoil and the accompanying collagen fibers would regain their usual degree of undulation. As a result, both the extraglandular and the larger intraglandular portions of the duct system would shorten. Length changes would also be expected when other tensile forces, such as those engendered by vigorous scratching of the facial skin or by mandibular movements, act on these superficial lacrimal structures which rest on the muscles of mastication.

(3) As the extraglandular duct is stretched, the long elastic fibers that form a loose weave near the base of its epithelial cells and those of its larger intraglandular branches would become more longitudinal, thereby enhancing support of the epithelium, reinforcing the duct wall and presumably constricting the lumen somewhat as the weave tightens. This would contrast with the looser cross-weaving of elastic fibers noted in relaxed tissues.\textsuperscript{17}

That the longitudinal elastic fibers have immature morphology compared to the circumferential ones is suggested by the smaller and darker cores of the former (Figs. 6a, b, 8a, 9b, c). Such moderately electron-dense areas are said to correspond to young, uncrosslinked elastin.\textsuperscript{18} Although some lighter core areas are present within the longitudinal fibers (Figs. 8b, 9a), even they are not pale.

A gradient of elastic fibers exists within the duct system of the rat exorbital lacrimal gland. Their number and size tend to be maximal in the extraglandular portion of the duct and progressively diminish within the organ. Additionally, the number of layers of elastic tissue decreases. These reductions are accompanied by the elimination of fibers of a particular orientation from various parts of the system, leaving those that presumably would be physiologically more necessary. Because the longitudinal ones are the most numerous and because they comprise the last remaining elastic fibers in the smaller ducts, the ability of these particular fibers to shorten the duct system may be the chief function of its elastic components.

The biophysical property of connective tissue termed compliance ("stretchability"),\textsuperscript{19} which both collagen and the elastic tissue in the gland aid, would be markedly diminished about the most distant radicles of the duct system (the smallest ducts and acini) because of their sparse collagen and essentially absent elastin. Thus, the transmission of forces to those areas would be minimal, sheltering them from such variations in their physical environment. This would contrast greatly with the larger structures of the rat lacrimal duct system in which both types of fibers are abundant.

The dimensional changes in which the elastic fibers participate must in some fashion be transmitted to the duct epithelium and to the neighboring collagen. These will be considered separately. Where elastic tissue is subjacent to the duct epithelium a physical connection exists by means of elaunin fibrils and oxytalan microfibrils. The latter are connected to the basal plasma membrane, particularly at hemidesmosomes\textsuperscript{20} where the cytoplasmic tonofilaments also insert.\textsuperscript{21} This microstructural framework may provide the mechanism by which the elastic fibers' tension and movements could affect the basement membrane and its adjacent duct epithelium, including the width of its lateral intercellular folds.\textsuperscript{22} All would be expected to vary as duct length and diameter change. Thus, this mechanism might affect the movement of water and of ions such as potassium and chloride\textsuperscript{23} to influence the composition of the lacrimal secretion and permit both the ductal epithelium and the underlying connective tissue to move harmoniously in response to displacing or distending forces.

The connection of the elastic fibers with collagen may be by their most peripheral microfibrils penetrating the matrix between adjacent collagen fibrils (Figs. 6a, 8b). Even collagen fibrils appearing to be at some distance from an elastic fiber in the plane of section are bounded by similar-appearing structures (Figs. 6a, 8b). Haust\textsuperscript{24} stated that microfibrils form a continuum common to both collagen and elastic tissue. He likewise noted that the microfibrils have a periodicity of 0.004–0.014 μm. At present, a periodicity of about 0.013 μm has been found (Fig. 6a). This linkage between the elastic and collagen fibers may be the mechanism by which forces exerted on one type of fiber can be transmitted to the other so that coordinate movements occur.

In man, the conjunctiva from which the lacrimal organ arises\textsuperscript{25} contains elastic tissue\textsuperscript{27} that presumably affects conjunctival extensibility.\textsuperscript{6} Likewise, the presence of such tissue in the lacrimal duct system may affect its response to forces. A similar relationship of elastic fibers to the basement membrane beneath epithelium, as has presently been shown, also exists in the skin.

Cotta-Pereira et al\textsuperscript{28} noted the presence of elaunin fibrils superficial to the dermal elastic fibers of human skin. The former ramify as oxytalan microfibrils that anchor the basal lamina and are, as is
Fig. 9. Electron micrographs of the extraglandular portion of a lacrimal duct at very high magnification. (a) Longitudinal section showing part of one longitudinal elastic fiber with a pale gray elastin core (E) surrounded by fibrils. In some of the latter a tiny, gray core (arrowheads) is evident. These are elaunin fibrils. From them, oxytalan microfibrils (*) extend to the lamina densa, particularly opposite hemidesmosomes (arrows). Traversing the lamina rara (**) are many microfilaments, especially at the hemidesmosomes, to complete the linkage between the innermost elastic fibers and the duct cells. A larger elastic fiber (F) and three fibroblast processes occupy the right border of the figure. Bar = 0.094 μm. (b) Cross-section of two adjacent longitudinal elastic fibers showing the tubularity of the peripheral fibrils and how they encompass two intervening transected collagen fibers (asterisks). Both oxytalan (arrowhead) and elaunin (arrow) are tubular. Bar = 0.08 μm. (c) Longitudinal section showing part of one longitudinal elastic fiber whose associated longitudinal fibrils are tubular. Its elastin core is barely evident left of center. The fibrils extending toward the black hemidesmosomes (H) are not clearly seen. Many of the inner collagen fibrils are circumferential and have been transected. Bar = 0.064 μm.

elaunin, fibrotubular (Fig. 9b). Thus, the most immature fibers are near the epidermis and the most mature ones are deeper in the dermis. The arrangement of lacrimal duct epithelium and its underlying elastic fibers is comparable except for the latter being far fewer than in skin. Other similarities are recognizable; for example, the positioning of the superficial elastic fibers close to the basement membrane has been said to aid the attachment of the epidermis to the corium29 and the basement membrane itself has been cited as offering "elastic support" to its attached cells.30 The presently observed microstructural linkage appears to be the mechanism by which such anchorage and support would also exist in the lacrimal system and suggests that this mechanism may be a widespread one for uniting epithelia and their underlying elastic tissue.

Key words: basement membrane, elastic fibers, elaunin, exorbital lacrimal gland, oxytalan

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