the recent report citing WGA-mediated inhibition of epithelial migration during corneal wound healing.8

Corneal pretreatment with WGA produced a 150% increase in the binding of GCL to the cornea. Interestingly, corneal pretreatment with WGA also enhanced the binding of DPCL (34%), which contains no WGA receptors. WGA is a basic protein (isoelectric point at pH 8.7); thus, it is positively charged at physiologic pH. Bound to the corneal surface, WGA may reduce the electrostatic repulsion between the negatively charged DPCL and cell surface, allowing somewhat greater binding. In the absence of WGA there was no difference in corneal binding by DPCL and CCL. Therefore our data suggest that WGA mediates specific binding of CCL to the corneal surface.

At 90 min after drug delivery, liposomes effected a significant enhancement of both corneal uptake and flux of carbachol, despite continuous "tear" flow. No increase was observed at 30 min, during which time unentrapped drug penetrated the cornea. This demonstrates that in the presence of liposomes, unentrapped drug (about 60% for the multilamellar and 90% for the unilamellar preparation) is transported across the cornea at the same rate as the free drug control. We had previously reported that the presence of liposomes did not affect the corneal permeability of free penicillin G, another water-soluble drug.1

The exact mechanism(s) of liposome-mediated enhancement of carbachol flux remains to be determined. However, we propose that the increased transcorneal flux to the aqueous analog seen at 90 min was attributable to: (1) the flux of drug initially liposome-entrapped, and (2) the inhibition of drug reflux due to the continuing presence of drug at the epithelial surface. Despite different levels of drug entrapment, unilamellar and multilamellar liposomes enhanced drug flux to the same extent. This might be explained by the small size of unilamellar liposomes, about 25 nm, allowing closer apposition of liposome to cell membrane, thereby facilitating more efficient drug transfer.

The data reported here suggest that under physiologic conditions, liposomes may be a suitable drug delivery vehicle for enhancement of transcorneal drug flux.

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Key words: liposome, topical drug delivery vehicle, wheat germ agglutinin, rabbit cornea, transcorneal carbachol flux

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Sequential samples of serially reconstructed muscle fibers visualized by electron microscopy, cytoplasmic inclusion bodies were seen in 4.5% of 1187 samples through multiply innervated fibers that vary systematically in diameter along their length; inclusion bodies were also seen in 0.8% of 354 samples through multiply innervated fibers of constant diameter. Cytoplasmic inclusion bodies were not seen in 1738 samples through singly innervated fibers. These data suggest that such inclusion bodies may occur preferentially in multiply innervated fibers. The present findings are not compatible with previous suggestions that such cytoplasmic inclusion bodies may be indicative of a pathologic or aging process. These findings are consistent with previous suggestions that such inclusion bodies are to be considered as normal structures in extraocular muscle. (INVEST OPHTHALMOL. VIS SCI 23:533-538, 1982.)

Cytoplasmic inclusion bodies, similar to those previously described in extraocular muscles (EOM) of humans,1-4 were observed in our study of muscle fiber types in the orbital surface of rabbit extraocular muscle.5-7 This report describes the distribution of such cytoplasmic inclusions among those muscle fiber types.

Methods. This study was based on the serial analysis of individual muscle fibers, as described in detail elsewhere.5-7 In brief, the superior rectus muscle of a prepubertal New Zealand white rabbit was embedded whole in Epon and serially sectioned at about 5 to 10 μm. These thick sections were available both for light microscopy and ultrathin sectioning. Four fascicles located at the middle of the orbital layer width were photographed at 20 to 40 μm intervals, thus permitting 93 contiguous fibers to be followed and reconstructed end-to-end.

These 93 fibers were categorized according to their innervation and diameter: 61 were classified as being singly innervated fibers (SIFs) and 32 were classified as being multiply innervated fibers (MIFs). The MIFs were differentiated according to their diameter characteristics. Ten MIFs were of an essentially constant 10 μm diameter (10 μ MIF). Twenty-two MIFs varied systematically in their diameter, being about 5 μm toward the middle of their length and about 15 μm along their proximal and distal segments (5-15 μ MIF).

The 93 fibers were sequentially examined by electron microscopy (EM) in 68 samples distributed along the extent of the orbital surface layer. The locations of those 68 EM samples are shown in Fig. 1, with reference to the respective extents of the individual fibers, and in Fig. 2, with reference to the pattern of fiber diameter variation. (The 68 sampling locations employed in this study are the same as those shown in a previous article,7 except for five samples moved about 100 μm from previous locations.)

There were 1738 EM samples of the SIFs, 354 EM samples of the 10 μ MIFs, and 1187 EM samples of the 5-15 μ MIFs. Each of these 3279 EM fiber samples was examined for the presence of cytoplasmic inclusion bodies. In the following descriptions, locations along the muscle are referred to in terms of distance in millimeters from the muscle origin (proximal to distal direction) and a given location is noted as MM followed by that number of millimeters (e.g., MM 4.2 indicates a location 4.2 mm from the origin).

Results. Numerous cytoplasmic inclusion bodies were observed. These structures were composed of a homogeneous matrix of flocculent or fine-grained material of low density, studded with granular foci of increased density (Figs. 3 and 4). These cytoplasmic inclusions were not membrane bound, tended to be ovoid in shape, ranged in length from about 0.5 to 2.5 μm, and were characteristically subsarcolemmal. The granular foci of increased density tended to be round, of a poorly demarcated outline, about 450 to 650 Å in diameter, and spaced at about 1000 to 1500 Å. In some of these inclusion bodies, dense particles of about 50 to 250 Å diameter were sporadically dispersed within the flocculent matrix.

A total of 63 such inclusion bodies were observed in 57 of the fiber samples (Fig. 1), six of these samples having two inclusions each. In the 5-15 μ MIFs, these inclusions occurred in 54 of the 1187 samples (4.5%); in the 10 μ MIFs they occurred in three of the 354 samples (0.8%). (Such inclusions were also seen in four other fibers that had been previously identified as "pseudo-orbital" MIFs.)7 Cytoplasmic inclusion bodies were not seen in any of the 1738 samples through the SIFs.

These inclusion bodies did not appear to be evenly distributed along the length of the 5-15 μ MIFs. They were not seen in any of the 391 samples taken within a 5.8 mm region located distally of MM 4.2 and proximally of MM 10.0 (Fig. 1). Proximal of this 5.8 mm region they were seen in 22 of the 273 samples (8.1%), and distal of this 5.8 mm region they were seen in 32 of the 523 samples (6.1%) through the 5-15 μ MIFs (Fig. 1). This 5.8 mm region (MM 4.2 to 10.0) corresponds roughly to that portion of the 5-15 μ MIFs where their fiber diameter tends to be smallest (Fig. 2).

Discussion. In this study cytoplasmic inclusion bodies were observed with moderate frequency in MIFs but were totally absent from the SIFs. In assessing the absence of inclusion bodies from the...
Fig. 1. The 93 horizontal lines represent the reconstructed fibers, grouped according to the three fiber categories within the four fascicles. The 68 vertical lines indicate the sequential EM sampling locations along those fibers. The filled circles indicate those fiber samples within which the cytoplasmic inclusion bodies were observed.

SIFs, it will be noted that although 1000 of the SIF samples occur within the 5.8 mm region (MM 4.2 to 10.0) where such inclusions were not seen in any of the fibers, there are an additional 738 SIF samples (358 proximal and 380 distal of that 5.8 mm region) wherein no inclusions were found.

The apparent preferential localization of cytoplasmic inclusion bodies in MIFs raises the possibility that they may be a reflection of cellular processes that are specific to that fiber type. Previous descriptions of cytoplasmic inclusion bodies in human extraocular muscle did not address the
The question of the fiber type in which such inclusions were found.\(^1\)\(^-\)\(^4\)

The present findings suggest that there is a selective paucity of cytoplasmic inclusions in the region of 5-15 \(\mu\) MIFs where they are of smallest diameter. The region of minimal diameter in 5-15 \(\mu\) MIFs also displays a selective paucity of membrane-glycogen complexes, a quite different variety of cytoplasmic inclusion that has been described in these fibers.\(^7\) (Membrane-glycogen complexes are also absent from SIFs in this muscle layer.\(^7\)) In their region of minimal diameter, these 5-15 \(\mu\) MIFs were found to display an essentially \textit{Fibrillenstruktur} morphology and a relatively higher mitochondrial content, in contrast to their regions of maximal diameter where these fibers were found to display an essentially \textit{Felderstruktur} morphology and a relatively lower mitochondrial content.\(^5\)\(^-\)\(^6\)

The cytoplasmic inclusion bodies observed in this study seem comparable to inclusion bodies described in four previous studies\(^1\)\(^-\)\(^4\) of EOM. Some differences might be noted, however, both among those four descriptions and between some of those descriptions and the present findings. Some authors\(^1\)\(^-\)\(^3\) describe such inclusion bodies as having a background matrix composed of a finely granular particulate material. Others\(^4\) describe this background matrix as being formed of filaments ("fine fibrils"), although the filamentous nature of the matrix is said to show up more clearly in some of these structures than in others.\(^4\) Some authors\(^5\) describe the granular foci of increased density as being formed by clusters of the particular background material. Others\(^4\) maintain that the periodic dense foci represent a convergence of filaments, although this is said to require high magnification to be recognized.\(^4\) In the present study of cytoplasmic inclusion bodies, we did not find convincing evidence that filaments are a significant component of the background matrix or of the periodic dense foci in these structures. However, our data may have been limited in this regard, insofar as we did not employ plate magnifications higher than \(\times 15,000\).

There are also discrepancies with regard to the size of the granular foci of increased density within these cytoplasmic inclusions. One report\(^1\) describes and presents electron micrographs of granular densities with a diameter of 200 to 270 Å. Three reports\(^2\)\(^-\)\(^4\) present electron micrographs of dense granular foci having a diameter of about 500 to 600 Å. In the present study, dense granular foci of 450 to 650 Å diameter were characteristic of the
cytoplasmic inclusion bodies, and dense particles of about 150 to 250 Å were also seen in some of these inclusions.

The cytoplasmic inclusion bodies observed in this study appear to be different from flocculent or fine-grained inclusion bodies that do not display granular foci of density, such as seen in normal

and abnormal EOM and in abnormal skeletal muscle. The presently observed cytoplasmic inclusions also appear to be different from inclusions that are essentially filamentous, such as those seen in normal and abnormal EOM and in abnormal skeletal muscle.

It has been suggested that cytoplasmic inclusion

Fig. 3. Typical subsarcolemmal cytoplasmic inclusion body composed of a fine-grained flocculent matrix studded with granular foci of increased density (heavy arrow). Very few small dense particles (fine arrow) are also seen within the matrix. (x 45,000.)

Fig. 4. This cytoplasmic inclusion body with granular dense foci (heavy arrow) is similar to that seen in Fig. 3, except that numerous small dense particles (fine arrow) are discernible within the background matrix. (x 45,000.)
bodies similar to those seen in this study are indicative of a pathologic state or may be related to the aging process. Others maintain that such inclusions in EOM are normal and unrelated to aging. The present observation of such cytoplasmic inclusions in normal prepubertal rabbit is compatible with the latter opinion.

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Preferential looking acuity obtained with a staircase procedure in pediatric patients.

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Visual acuity of infants and young children with ophthalmologic disorders was assessed by adapting a transformed up-down staircase to preferential looking (PL) procedures. Eighty-five percent of pediatric patients between 11 days and 5 years of age were tested successfully. Acuity of infants and young children with normal eyes obtained by the PL staircase procedure agreed well with acuities obtained previously by the method of constant stimuli. In children with anisometropia, differences in acuity between eyes varied systematically with the amount of anisometropia. Monocular acuities of untreated patients with strabismus did not always agree with fixation preference. In general, test results from pediatric patients with structural ocular abnormalities were consistent with the severity of the disorder. By means of serial measurement of PL acuity, the therapy of patients with amblyopia was monitored. In our patients, anisometropic amblyopia affected grating acuity differently than did strabismic amblyopia, as others have reported in older patients with these conditions. Our results indicate that the PL staircase procedure provides a useful measure of visual acuity in pediatric ocular disorders that can complement the clinical evaluation of infants and young children.

Assessment of visual acuity has been proposed as an aid in the detection and management of ocular disorders in infants. Preferential looking (PL), a technique based on infant looking behavior, has been used to study visual acuity in infants. Several methods of acuity estimation by PL have been proposed but have disadvantages for clinical application. The method of constant stimuli (MCS) is too lengthy for most clinical purposes. The diagnostics stripes procedure, developed for clinical screening, does not yield a direct estimate of acuity and hence is not sensitive enough, at least in young infants, to detect or follow the course of ocular disorders. A recently reported quick method has unusual psychometric properties.