obscure because the endothelium looked normal when biomicroscopically observed. In the W72 group, 21 out of 25 patients achieved 3 days continuous wear (chi-square: \( p < 0.001 \)). The difference between the two groups is even greater when taking into account the reasons which led to premature removal of the W72. One lens was removed from a white eye after 2 days because the patient had to go on an unforeseen trip. In a second case, the margin of the W72 was broken, and the lens was removed because of slight local irritation, the cornea having healed completely without edema or intolerance. A third W72 had to be removed because a traumatic defect near the limbus showed signs of increasing infiltration. After a review of the records it was found that a slight infiltration had existed before soft lens fitting, and thus this patient should not have entered the study. The fourth patient who had his W72 lens removed before the third day demanded this because of pain without observable intolerance.

Although epithelial edema was most marked in the W38 group, it was not totally absent in the W72 group (data not shown). A statistical evaluation of corneal thickness measurements is given in Table I. Fig. 1 shows the variance of corneal thickness measurements immediately after lens removal in both groups.

All complications were effectively treated. Most of the swollen cornea had returned to normal within 1 day following removal of the lens. No permanent injury resulted from soft lens therapy.

Conclusions

1. The highly hydrophilic W72 proved to be superior to the less hydrated W38 in this random, controlled investigation of a clinical model for therapeutic soft lenses. The W72 was clearly better tolerated by the patients. Also corneal swelling after continuous wear was statistically less with the W72 than with the W38.

2. Every known therapeutic soft lens exhibits some barrier function on corneal metabolism, which may or may not be overcome by corneal adaptation. This also holds true, but to a significantly lesser degree, for the W72 in our test system. Tolerance toward the lenses often improved at the end of the wearing time when the corneal defects were healed and metabolism approximately normal again (data not shown).

3. For a rapid clinical investigation of contact lens materials, we suggest the application of a clinical model which allows a sufficient number of comparable patients to be tested under controlled conditions. In our system the investigation of patients with superficial traumatic defects discriminated sharply between the two differently hydrated soft lenses and confirmed the superiority of the W72 lens. We believe that further use of the W72 lens is justified for therapeutic purposes in preference to the W38 lens. Therefore the W72 lens may serve as a reference lens in further comparative studies of the kind reported here.

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REFERENCES


Extraocular muscle fibers: ultrastructural identification of iontophoretically labeled fibers contracting in response to succinylcholine. PAUL BACH-Y-RITA, GUNNAR LENNERSTRAND, JORGE ALVARADO, KRISTIN NICHOLS, AND GREG MCHOLM.

Cat extraocular muscle fibers (from the superior rectus or inferior oblique) were penetrated in vivo with Procion red–filled glass microelectrodes. When stable penetrations were obtained, succinylcholine (Sch), 8 to 20 mg, was injected into the femoral vein. In some fibers, a depolarization-repolarization response was obtained with the same time course (2 min.) as the total muscle contraction. The depolarizing fibers were labeled iontophoretically.

The ultrastructural characteristics of five depolarizing fibers and three control (nondepolarizing) fibers were then studied. The fibers that did not depolarize to Sch had the characteristics of singly innervated cells, whereas those sensitive to Sch had morphological characteristics of multi-innervated fibers.

Extraocular muscles (EOM's) contract when exposed to neuromuscular depolarizing drugs such
as choline, nicotine, decamethonium, and succinyl-cho- 
line (Sch) (see ref. 7), whereas other skele- 
tal muscles and the retractor bulb (an EOM present in some species) become flaccidly para-
lyzed when exposed to Sch. Extraocular muscles 
also differ from most other skeletal muscles and 
the retractor bulb in having a population of 
muscle-fibers with multiple motor nerve endings 
(ref. 2; cf. ref. 3 and 9). Bach-y-Rita, 3 following 
Kuffler and Vaughn-Williams, 8 and Eakin and 
Katz, 7 has suggested that the contraction observed 
in EOM’s results from a summation of the local 
depolarizing effects of Sch on the numerous 
postjunctional membrane areas of these fibers. 
Muscle fibers responding to Sch with membrane 
depolarization sufficiently widespread to be re-
corded by microelectrodes penetrating them ran-
domly were identified in the experiments described 
below. Fibers with no Sch-induced depolariza-
tion in the vicinity of the electrode tip were also 
present. The time course of membrane depolariza-
tion in the former was coincident with that of 
ipsilateral muscle contraction. Depolarizing and 
nondepolarizing fibers were labeled iontophoretic-
ally and examined by electron microscopy to 
determine their morphological and innervational 
characteristics.

In seven cats anesthetized with pentobarbital, 
the superior rectus or inferior oblique muscle 
was separated from the globe but kept attached 
to the orbit, with its blood supply intact. The 
muscle was supported in a stable, vibration-free 
state by a plastic holder placed within the orbit, 
and continuously bathed with 37° mineral oil. 
The ipsilateral medial or lateral rectus muscle 
was also separated from the globe and attached 
to a strain gauge. Recording and labeling tech-
niques have been described elsewhere. 4-10

When a stable intracellular penetration was 
obtained, Sch (8 to 20 μg/kg. body weight) 
was injected into the femoral vein. (Larger doses 
caused so much muscle contraction that electrodes 
were invariably dislodged from the cells. With 
better, smaller doses, intracellular recording was stable, 
but no tension could be recorded.) The time 
required for the depolarization induced by Sch 
and the subsequent repolarization was approxi-
mately 2 minutes. This process was repeated two 
to three times before a cell was labeled. In each 
experiment, at least 50 fibers were penetrated 
and held sufficiently long for preliminary physio-
logical characterization. Since contraction of the 
muscle as a whole often dislodged the electrode 
from the individual fiber under study or damaged 
it to the extent that its resting membrane po-
tential became unstable, only a very small pro-
portion of the cells impaled by the electrodes 
were carried through the entire test. Labeling 
of two or three cells with reproducible ideal 
responses terminated an experiment. In all, 11 
labeled fibers gave positive responses to Sch, 
with depolarization time courses coincident with 
contraction. Four fibers which did not respond 
to Sch were labeled as controls.
Fig. 2. Electron micrographs of three labeled cells (×8,000). Each cell has been identified in cross section (a) and longitudinal section (b). 1a and 1b, Type 5 multi-innervated cell from the orbital region (diameter 11 μ; Z-line 1,000 Å). 2a and 2b, Type 4 multi-innervated cell from the global region (diameter 18 μ; Z-line 1,000 Å). 3a and 3b. Global singly innervated cell (diameter 26 μ; Z-line 600 Å).
Fig. 1 illustrates examples of the responses to the administration of Sch observed reproducibly in fibers selected for morphological study. The fiber shown in A became depolarized following the administration of Sch, and its depolarization and repolarization coincided with the rise and fall in tension recorded in the ipsilateral lateral rectus. The cell in B maintained a stable resting potential and did not respond to Sch in the vicinity of the electrode tip. The membrane potential values shown are typical for uninjured small fibers in cat EOM's. In this study, the range of membrane potentials for Sch-positive fibers was 25 to 62 mv, and for control fibers, 32 to 70 mv.

To prepare labeled fibers for electron microscopy, the entire muscle was fixed in situ by dripping 3 percent paraformaldehyde buffered to pH 7.4 with sodium cacodylate for 30 minutes and then removed from the orbit and placed in the same fixative for an additional 2 hours. Labeled fibers were isolated from the rest of the muscle by teasing with fine needles under a dissecting microscope. Each labeled cell, together with five to 10 adjoining fibers, was processed for electron microscopy by conventional techniques as previously described. Initially, a cholinesterase stain was used in an effort to visualize endplates on labeled cells directly and specifically. However, ultrastructural damage to cells dissected completely free from adjoining fibers was frequently encountered, and the tendency of cells to break at the label discouraged us from subjecting our small yield of cells with unequivocal physiological data to this procedure. Labeled fibers were identified by light microscopy in cross sections at the site of the Procion red injection. Passage of dye iontophoretically resulted in local morphological alterations. The cell membrane was often broken, and intracellular proteins were coagulated by current for 200 to 400 μm on either side of the microelectrode entry site. Once an area free of current damage was reached, thin sections were made for electron microscopy. After examination of cross sections, the same cells were sectioned longitudinally.

Eight of the 15 labeled fibers were suitable for morphological study. Dye spots in the remaining fibers could not be visualized, or the cells sustained severe morphological damage during dissection. Of these eight, five responded to Sch with depolarization and were identified as multi-innervated. Two of these were from the muscle's orbital layer, and three were from its global region. The three fibers which did not respond to the application of Sch were identified as singly innervated cells of the global region. The classification of each labeled cell as singly innervated or multi-innervated was based on its ultrastructural characteristics (Fig. 2). Previous studies in the cat have shown that two of the five cell types present in EOM's are multi-innervated, and physiological evidence for multi-innervation has been obtained. Multi-innervated cells are small (10 to 20 μm in diameter) and have sarcomeres characterized by the thickest Z-line (1,000 to 1,200 Å). The myofibrils are separated by a poor to moderately developed sarcoplasmic reticulum and transverse tubular system. The mitochondrial content of the multi-innervated cells most common in the global region (type 4) is rather scanty, but those found most frequently in the orbital layer (type 5) have many long mitochondria extending the length of two and often three sarcomeres. Singly innervated cells are somewhat larger (20 to 30 μm) and often display a well-developed sarcoplasmic reticulum and transverse tubular system. In addition, these cells have thin Z-lines (400 to 600 Å) and discrete, thin myofibrils.

The fibers that did not respond to Sch in the vicinity of the electrode tip had the attributes of singly innervated cells, whereas those sensitive to Sch had the morphological characteristics of multi-innervated cells. Two of the Sch-positive fibers from the global region were typical examples of the multi-innervated cell found there most frequently—type 4. One of the Sch-positive fibers from the orbital region was identified as type 4 also. The remaining orbital Sch-positive fibers resembled the other depolarizing fibers in size and Z-line thickness but were distinct in having long and numerous mitochondria. They were categorized as type 5.

The singly innervated fibers identified in these experiments did not depolarize, and the retractor bulbi and other singly innervated muscles do not contract when exposed to Sch. The idea that Sch-induced contraction of EOM's results from the combined effects of the depolarized endplate regions of multi-innervated fibers receives support, in that the absence of Sch depolarization and contraction in singly innervated cells indicates that these responses are widespread only in the multi-innervated cells. In singly innervated cells the probability of placing an electrode tip close enough to the junctional membrane to record Sch depolarization is very low, and only a very small proportion of the contractile apparatus is in this membrane's proximity. The distribution of endplates along the length of multi-innervated cells provides a substantially larger electrode "target" of depolarizing membranes and presents this depolarization to contractile elements throughout the fibers.

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REFERENCES

Hydraulic flow conductivity of the vitreous gel. IRVING FATT.

The hydraulic flow conductivity of rabbit and bovine vitreous gel has been measured by a method that gives better results than the one previously used. The vitreous gel has a hydraulic flow conductivity that would be expected for a highly hydrated connective tissue. The "pore" size (the spaces available for water flow) are estimated to be about 4,000 A in diameter.

The physical properties of the vitreous gel have been only rarely measured although these properties are needed for a complete description of nutrient transport in the eye. Fatt and Hedbys calculated the vitreous humor outflow via the sclera by assuming that the vitreous gel offered little or no resistance to flow when compared to the sclera. An estimate of hydraulic flow conductivity of the vitreous gel would aid in evaluating the validity of their assumption.

Fatt has used an assumed hydraulic flow conductivity of the vitreous gel to calculate the relative roles of diffusion and hydraulic flow in transporting dissolved species within the vitreous chamber of the eye. A measured value of hydraulic conductivity would put this calculation on a firmer base.

Finally, hydraulic flow conductivity of the vitreous gel could be used to estimate the maximum size of particles or macromolecules that can move through this gel.

Brubaker and Riley measured the rate at which water was expressed from a cylindrical mass of rabbit vitreous gel placed on a cellulose acetate membrane and compressed by a thin rubber membrane stretched over the top. They found a flow rate per unit pressure of 0.072 µl min.⁻¹ (mm. Hg)⁻¹ when vitreous gel was on the membrane, whereas for water alone on the membrane this value was 0.084 µl min.⁻¹ (mm. Hg)⁻¹. The dimensions of the water- or gel-filled cell were height, 0.65 cm., and area, 2.87 cm.² (R. F. Brubaker, personal communication). From these data it is possible to calculate the hydraulic flow conductivity of the gel. Hydraulic conductivity is defined as the permeability to liquid flow, k, divided by the viscosity, ν, of the liquid to give the term k/ν. The hydraulic conductivity is then the proportionality term that relates flow rate to area and pressure drop per unit thickness of a membrane (see equation 2).

Application of Darcy's law to the system of vitreous gel and cellulose acetate membrane is straightforward and leads to the following equation:

\[ \frac{k}{\nu} = \frac{\Delta P_m}{L} \left( \frac{\Delta V_m}{\Delta P_m} \right) \]

where \( \Delta P_m \) and \( \Delta P \) are pressure drops across membrane and vitreous gel, respectively, at any given flow rate and \( L \) is the height of the vitreous gel cylinder. The term \( (k/\nu)_m \) is calculated from the water data by means of the following equation:

\[ (k/\nu)_m = \frac{q_v}{A \Delta P_m} \]

where \( A \) is the membrane area (same as the vitreous gel area) and \( q_v/\Delta P_m \) is the water flow