of the cilia seen in our study of the trabecular meshwork have such a configuration. The centriole pair may occur in a cell region marked by definite striations (Fig. 4), but there is no indication that these are associated with the centriole pair. The cell region containing the centriole may be highly filamentous and these filaments may show periodic increased densities, but none of these appear to be true ciliary rootlets. Microtubules are also commonly seen in the cell region containing the centriole pair.

**Discussion.** Although the density distribution of single cilia in the meshwork region could be an artifact of cell distribution, that is not apparent from our data. The mesothelial cells of the uveal and cornescleral meshwork seem to exhibit more types of single cilia and a wider variety of morphologic configuration of these cilia than do other cells described in the literature.

The importance of all the combinations of cilia and centrioles is difficult to approach in a study of pathological material alone. However, it is apparent that the concept of the typical mesothelial primary cilium, one arising only from the centriole pair, does not hold in the case of the trabecular meshwork and into Schlemm’s canal in the cynomolgous monkey (*Macaca irus*), AM. J. Ophthalomol. 73: 760, 1972.


**REFERENCES**


The adrenergic receptors of the intraocular muscles of the human eye. G. W. H. M. VAN ALPHEN.

To the Memory of Dr. Ludwig von Sallmann.

The adrenergic receptors in man were analyzed using isolated sphincter, dilator, and ciliary muscle strips, dissected from eyebank eyes. The dilator is mainly α, the sphincter both α and β, and the ciliary muscle predominantly β adrenergic.

In previous work we analyzed the distribution of the adrenergic receptors of the internal muscles of the eye in three species. The results are shown in Table I.

The receptors in the human eye remain unknown since fresh material is hard to obtain. When it appeared that muscles of cat eyes would still respond after 12 to 24 hours of refrigeration at 4° C, a trial was made with eyebank eyes. It was found that from some eyes muscular responses could be obtained up to four days after death. There were enough eyes that adequately
responded in vitro to cholinergic and adrenergic drugs to map the adrenergic receptors of the iris and ciliary muscles. The fact that 24 hours of refrigeration did not qualitatively alter the responses in the cat suggests similar results with human muscles.

**Materials and methods.** Muscle preparations were obtained from human eyebank eyes which had been enucleated from one to six hours after death. In most of these eyes, the cornea had been removed for corneal transplantation, but the eye was kept refrigerated at 4°C.

Sphincter strips were prepared by excision of one-half of the sphincter region of the iris. A thread was tied to either end. Dilator strips were obtained by two parallel incisions, 3 mm. apart, in a radial direction. One suture was tied at the level of the arterial circle of the iris; another was tied at the iris root. Ciliary muscle preparations were obtained by dissecting the ciliary body from the scleral spur, lens, and choroid. A suture was tied to either end. In the meridional strip one end of the muscle was left adherent to the scleral spur, lens, and choroid. A suture was tied to the choroid at the equator.

Two identical strips were suspended in a 30 ml. bath at a tension of 75 mg. for sphincter and dilator and 250 mg. for ciliary muscle strips. The bath contained oxygenated (95 per cent O₂-5 per cent CO₂) Krebs-Ringer solution maintained at 37°C. Drug effects were measured under isometric conditions by means of Grass force displacement transducers and responses were recorded on a Grass 4 channel recorder.

Acetylcholine (Ach) was used as the hydrochloride; catecholamines (CA): L-epinephrine (E), L-norepinephrine (nE), and L-isoproterenol (Iso) as bitartrate; atropine as sulfate; eserine (Es) as salicylate. Adrenergic blocking agents included dichloroisoproterenol (DCI), propranolol, and phentolamine.

The weight of the salt of the drugs mentioned in the text pertains to the final concentration in the bath (weight per milliliter of bath solution).

**Results. Dilator strips,** when responsive, contracted to epinephrine and norepinephrine (E > nE). They slightly relaxed to 1 μg of isoproterenol but frequently contracted to 10 μg. The contraction to epinephrine and norepinephrine was potentiated by 1 μg DCI and totally blocked by 1 μg phentolamine; in several strips the contractions before phentolamine were even reversed into a slight relaxation. Obviously, the dilator contains predominantly α receptors with some β receptors admixed (see Fig. 1).

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**Table I. Distribution of adrenergic receptors**

<table>
<thead>
<tr>
<th></th>
<th>Dilator</th>
<th>Sphincter</th>
<th>Ciliary muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat</td>
<td>Mainly α</td>
<td>Mainly β</td>
<td>Mainly β</td>
</tr>
<tr>
<td></td>
<td>Some β</td>
<td>Some α</td>
<td>Some α</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Mainly α</td>
<td>Mainly β</td>
<td>Mainly α</td>
</tr>
<tr>
<td></td>
<td>Few β</td>
<td>Few α</td>
<td>Few β</td>
</tr>
<tr>
<td>Monkey</td>
<td>Mainly α</td>
<td>Mainly α</td>
<td>Exclusively β</td>
</tr>
<tr>
<td></td>
<td>Very few β</td>
<td>Perhaps β</td>
<td>No α</td>
</tr>
</tbody>
</table>
Table II. The distribution of the adrenergic receptors in man and other species

<table>
<thead>
<tr>
<th></th>
<th>Dilator</th>
<th>Sphincter</th>
<th>Ciliary muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat</td>
<td>Mainly α, Some β</td>
<td>Mainly β, Some α</td>
<td>Mainly β</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Mainly α, Mainly β, Few α</td>
<td>Mainly α, Few α</td>
<td>Few β</td>
</tr>
<tr>
<td>Monkey</td>
<td>Mainly α, Mainly β, Few α</td>
<td>Exclusively β</td>
<td>No α</td>
</tr>
<tr>
<td>Man</td>
<td>Mainly α, Very few β</td>
<td>α and β in equal amounts</td>
<td>Very few β</td>
</tr>
</tbody>
</table>

**Discussion.** The distribution of the adrenergic receptors of the internal muscles of the human eye closely resembles that of the rhesus monkey. The human dilator, like the monkey dilator, carries predominantly α receptors. Similarly, the ciliary muscle, dissected in either a circular or a longitudinal direction, is exclusively β in the monkey and predominantly β in man. But the human sphincter appears to carry α as well as β receptors in roughly equal numbers; in the monkey the α receptors predominate. The distribution of the adrenergic receptors in man is summarized in Table II; Table I is added to facilitate comparisons.

In the cat and rabbit, the α type predominates in the dilator and the β type in the sphincter. Hence, widening of the pupil by CA’s is probably the result of α activation of the dilator, producing a contraction, and β activation of the sphincter, producing a relaxation. Unfortunately, we cannot presently explain as to why there is no complete synergism between sphincter and dilator in monkey and man.

The ciliary muscle shows a sequence Iso > nE > E which is not a classical β sequence since norepinephrine and epinephrine are interchanged. This same sequence was first found in the cat, then in the monkey, and now in man. Once again such a sequence, although not unusual for a β receptor, is in any event incompatible with α receptors; this is confirmed by β blockade.

A knowledge of adrenergic receptors of the ciliary muscle is of theoretical interest. It may provide clues as to whether or not the muscle participates in facilitation of aqueous outflow or whether it affects accommodation. For example, the observation that in the rabbit aqueous outflow is facilitated by β stimulation cannot be explained by contraction of the ciliary muscle since it carries α receptors. Similarly, since we now know that in man the β receptor predominates, a muscular effect of epinephrine on aqueous outflow becomes unlikely. But it certainly would explain an inhibitory effect of epinephrine on accommodation.

The almost equal contraction of the ciliary muscle of a 2- and an 82-year-old human eye refutes those theories of presbyopia that consider atrophy of the muscle an either essential or contributory factor. On the other hand there is evidence that the ciliary muscle contracts less with advancing age in the living eye. The reason for that I have explained and documented elsewhere: the continuously growing lens, relaxes the muscle and the relaxing muscle appears to contract less to ACh.

There are theories which claim an atrophy of...
disuse in myopes. Again this seems unlikely. The 82-year-old eye did presumably not accommodate for some 20 years; moreover, it happened to be aphakic whereas the fellow eye contained a cataractous lens.

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From the University of Michigan, Ann Arbor, Mich. This work was supported by a grant from the Rackham Foundation. Submitted for publication Dec. 1, 1975. Reprint requests: Dr. G. W. H. M. van Alphen, Department of Ophthalmology, University of Michigan, Ann Arbor, Mich. 48109.

Key words: receptors, adrenergic, cholinergic, intraocular muscle, pupil, ciliary muscle, human eye, accommodation.

REFERENCES


pH-dependent temperature sensitivity of rat lens phosphofructokinase. Hong-Ming Cheng and Leo T. Chylack, Jr.

Rat lens phosphofructokinase (PFK) has been found to be cold-labile at acidic pH, even in the presence of sulfate and inorganic phosphate, two known positive effectors. The inactivation appears to be an irreversible process, but can be prevented by including ATP in the incubating media. The enzyme is relatively stable at pH 8.2 incubated at 0 to 4°, 25°, or 37° C. in the absence of the effectors, but is extremely thermolabile if the pH is lowered to 7.30 or lower. The thermolability is counteracted by many effectors, among them sulfate and ATP are the most effective. The physiologic significance of PFK instability and effector protection in the lens are discussed.

Rat lens phosphofructokinase (PFK) exhibits a number of pH-dependent properties, e.g., inhibitory effect of ATP and interconversion of two PFK forms (I and II). PFK-II is the dominant and stable form at alkaline pH. At neutral or acidic pH, PFK-I dominates and becomes more sensitive to ATP inhibition; yet, at the same time, ATP seems to protect PFK from cold inactivation.

pH-Dependent cold lability has been observed in PFK purified from rabbit muscle and from chicken liver; although the effect of ATP on either enzyme was essentially that of catalysis of enzyme molecular dissociation or re-association.

Studies on the effects of ATP and some effectors on cold lability of lens PFK are instrumental in evaluating the molecular aspects of PFK regulation. In addition, the thermolability of this enzyme is also explored and its relevance to lens physiology discussed.

Materials and methods. Albino rats (100 to 150 grams), purchased from Charles River Breeding Laboratories, Wilmington, Mass., were decapitated, and from the enucleated globes, the lenses were removed and homogenized in 0.05 M Tris-Cl buffer (pH 8.2) containing 0.05 M Na2SO4 and 0.005 M EDTA (Buffer-I) at 0 to 4° C, or in 0.05 M phosphate buffer (pH 6.2) also containing Na2SO4 and EDTA (Buffer-II) at room temperature (25° C.). The homogenates were centrifuged at 96,000 x g for one hour in a Beckman L2-65B ultracentrifuge with rotor No. SW50.1. PFK assays were conducted according to the method described previously.

PFK assays were conducted according to the method described previously.

Results. Stability of PFK. If PFK was prepared in Buffer-II without sulfate and the 96,000 x g supernatant was passed through a Bio-Gel column (usually a Bio-Gel A-1.5 m column, 1.5 by 27.5 cm.) equilibrated with the same buffer at 6° C,