Serum enzyme, and more specifically to answer the clinical question of the pilocarpine-hydrolyzing enzyme in human and rabbit sera and to demonstrate the presence of a pilocarpine-hydrolyzing enzyme in the vitreous body of the cat, Acta Physiol. Scand. 84: 261, 1972.


**Pilocarpine hydrolysis: clinical significance. Philip P. Ellis and Katherine Littlejohn.**

Prior investigations in this laboratory have demonstrated the presence of a pilocarpine-hydrolyzing enzyme in human and rabbit sera and ocular tissues. The enzyme was also demonstrated in secondary aqueous humor of humans and rabbits at lower levels than in the respective sera; more was demonstrated in primary aqueous humor. The following studies were undertaken to investigate the clinical significance of the serum enzyme, and more specifically to answer these questions: (1) Does pilocarpine administration alter serum enzyme levels? (2) Does the serum level of the pilocarpine-hydrolyzing enzyme influence the success of pilocarpine therapy in open-angle glaucoma, i.e., are patients with high-enzyme levels less susceptible to pilocarpine control? (3) Is it possible to inhibit the pilocarpine-hydrolyzing enzyme and thereby increase the hypotensive action of pilocarpine. The laboratory methods used in these studies have been previously reported and only deviations from those methods will be noted here. In the animal experiments, New Zealand albino rabbits weighing between 2.7 and 4.0 kilograms were used.

To determine the effect of chronic topical pilocarpine therapy on serum enzyme levels, 2 percent pilocarpine in 0.5 percent hydroxypropyl methylcellulose (Isopto Carpine) was applied to the eyes of six animals. Application regimen consisted of two drops in each eye, three times a day, five days a week for fourteen weeks. Hydroxypropyl methylcellulose, 0.5 percent (Isopto Tears), was applied for the same period to four control animals. Weekly blood samples were drawn by cardiac puncture and serum was analyzed for three weeks prior to the beginning of drug administration to establish baseline serum-enzyme levels. An additional seven blood samples were drawn at intervals of one to two weeks from each animal during the fourteen week period of drug administration.

Serum analysis varied slightly from the previously described procedure. Pilocarpine (2 × 10^{-8} M) was mixed with 0.1 ml. of rabbit serum, incubated 10 minutes, and residual pilocarpine determined. Serum from control animals hydrolyzed 215.78 ± 30.22 (S.D.) μg of pilocarpine per minute per milliliter of serum prior to treatment and 237.83 ± 26.99 μg per minute per milliliter at the end of fourteen weeks of treatment. Serum from treated animals hydrolyzed 218.35 ± 19.36 μg and 233.63 ± 10.66 μg of pilocarpine per minute per milliliter of serum, respectively. The differences are not significant (p > 0.05) and indicate that topical pilocarpine administration had no effect on the serum enzyme levels during the treatment period.

To determine the effect of acute pilocarpine administration, nine rabbits were injected with large doses of pilocarpine. Through trials it was determined that the animals did not survive if more than one-eighth of the lethal intravenous dose in rabbits (21.87 mg. per kilogram per day) was administered subcutaneously for three consecutive days. The daily dose was given in two injections at seven-hour intervals. This regimen produced increased salivation and diarrhea in all animals; death occurred in five animals before blood samples could be drawn on the fourth day. In the four surviving animals 0.2 ml. of serum hydrolyzed 215.29 ± 35.13 μg of pilocarpine per minute per milliliter of serum prior to treatment and 237.83 ± 26.99 μg per minute per milliliter at the end of fourteen weeks of treatment. Serum from treated animals hydrolyzed 218.35 ± 19.36 μg and 233.63 ± 10.66 μg of pilocarpine per minute per milliliter of serum, respectively. The differences are not significant (p > 0.05) and indicate that topical pilocarpine administration had no effect on the serum enzyme levels during the treatment period.

Reports 931
Fig. 1. Mean per cent inhibition of the human serum pilocarpine-hydrolyzing enzyme by inhibitors [I]: △ EMB; ● penicillamine; and ○ EDTA.

Table I. Milligrams of pilocarpine hydrolyzed per 0.8 ml. of human serum

<table>
<thead>
<tr>
<th>Patient source</th>
<th>Control group</th>
<th>Glaucoma group</th>
<th>No pilocarpine therapy</th>
<th>EMB therapy</th>
<th>No EMB therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>4.990 ± 0.597</td>
<td>4.411 ± 0.651</td>
<td>4.776 ± 0.774</td>
<td>4.264 ± 1.103</td>
<td>4.475 ± 0.883</td>
</tr>
<tr>
<td>Pilocarpine therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No pilocarpine therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuberculosis group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EMB therapy</td>
<td>4.264 ± 1.103</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No EMB therapy</td>
<td>4.475 ± 0.883</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are the arithmetic mean ± the standard deviation. Number of samples given in parentheses.

minute per milliliter of serum prior to treatment and 224.33 ± 22.33 µg per minute per milliliter after three days of acute pilocarpine application. This difference is not significant (p > 0.05).

To determine the relationship between glaucoma therapy and pilocarpine-hydrolyzing enzyme levels, a comparison was made of serum samples from nonglaucomatous individuals, from patients with open-angle glaucoma treated with pilocarpine, and from glaucoma patients treated with drugs other than pilocarpine. Although no correlation was found between age and enzyme activity in a large group of normal samples studied earlier, an attempt was made to keep age groups similar in this study. Control samples were obtained from 32 individuals, ages 48 to 76 years, with no history of cardiac disease, cancer, diabetes, or glaucoma. Experimental samples were obtained from patients with open-angle glaucoma. Fifteen of these patients, ages 54 to 84 years, were receiving only pilocarpine therapy; 26 patients, ages 32 to 86 years, were receiving antiglaucoma medications other than pilocarpine. The serum-enzyme activity of each group is shown in Table I. There was no significant difference between glaucoma and control groups or between pilocarpine-treated and nonpilocarpine-treated patients (p > 0.05). This suggests that neither pilocarpine nor other glaucoma medications in the dosages used clinically affect serum levels of the pilocarpine-hydrolyzing enzyme.

In the group of glaucoma patients receiving pilocarpine, there was no significant difference in the serum enzyme levels of patients with controlled disease and those uncontrolled at the time of sampling. Control is defined as intraocular pressure (IOP) of 22 mm. Hg or less, no progressive cupping of the optic disc, nor progressive field loss.

Work by Schonberg and Ellis demonstrated an in vitro inhibition of the pilocarpine-hydrolyzing enzyme in rabbit serum by certain chelating agents. This presented the possibility that a similar inhibitory response in human serum might also increase the ocular hypotensive action of pilocarpine. One of the chelating agents used by Schonberg and Ellis, ethambutol hydrochloride (EMB), is widely used in the treatment of tuberculosis, offering a population in which to study the effect of the usual clinical dose (25 mg. per kilogram of body weight) on the serum enzyme levels and ocular hypotensive action of pilocarpine.

Seventeen tuberculosis patients, ages 19 to 78 years, receiving EMB comprised the experimental group. The control group, ages 23 to 72 years, consisted of seven tuberculosis patients receiving drugs other than EMB and nine healthy adults. Intraocular pressures (IOP) were measured with the Goldmann Applanation tonometer.
line pressures were determined in both eyes, a single dose of 4 per cent pilocarpine (Pilocel) was instilled into the right eye of each patient. Repeat IOP measurements were made at half-hour intervals for two hours and then hourly for two more hours. The decrease in pressure in the treated eye reached maximum levels in both groups between one and two hours, with pressure differences between the treated and untreated eye reaching significant levels at 30 minutes in the control group (p < 0.005) and 60 minutes in the EMB group (p < 0.01). Analysis of the percentage of decline in IOP revealed the two groups differed significantly only at the 30 minute reading. No attempt was made to determine a difference in the duration of the pilocarpine effect between the EMB and control groups. Blood samples were drawn prior to baseline pressure determinations. Serum analysis showed no difference in in vitro enzymatic hydrolysis of pilocarpine between the two groups (Table I).

Since this study indicates that EMB at the usual clinical dose is not effective as an inhibitor of the pilocarpine-hydrolyzing enzyme, further analysis was carried out to determine the approximate serum level at which this inhibitory effect would be observed. In addition to EMB, penicillamine, EDTA, and kanamycin were also studied. Each inhibitor was evaluated on three individual and one pooled serum samples. The basic method of serum analysis was followed except that inhibitors were allowed to react with the serum for 30 minutes prior to the addition of pilocarpine. Total incubation time was 7 hours.

The inhibitor concentration at which 50 per cent inhibition occurred was determined from a plot of mean per cent inhibition of the enzyme against log molar concentration of the inhibitor. The concentrations of the inhibitors producing 50 per cent enzyme inhibition per 0.8 ml. of serum were: EMB, 3.07 x 10^-2 M.; penicillamine, 2.26 x 10^-3 M.; and EDTA, 2.11 x 10^-3 M. (Fig. 1). Normal serum level of EMB obtained two to four hours following ingestion of the usual clinical dose of 25 mg. per kilogram is 5 µg per milliliter (1.8 x 10^-6 M.).

The results of these present studies indicate that the pilocarpine-hydrolyzing enzyme in human serum is probably not clinically significant. There appears to be no relationship between control of clinical glaucoma with pilocarpine therapy and serum levels of the pilocarpine-hydrolyzing enzyme. In rabbits, serum enzyme levels are not altered by chronic or acute pilocarpine application, nor do pilocarpine or other glucosoma medications appear to have any effect on serum enzyme levels in patients with open-angle glaucoma. In the doses employed clinically inhibitors of the pilocarpine-hydrolyzing enzyme of serum do not increase the hypotensive action of pilocarpine.

Isopto Carpine and Isopto Tears were provided by Alcon Laboratories, Fort Worth, Texas. Pilocel was provided by Professional Pharmacal Company, San Antonio, Texas. Ethambutol hydrochloride was provided by Lederle Laboratories, American Cyanamid Company, Pearl River, N. Y.

From the Division of Ophthalmology, University of Colorado Medical Center, 4200 E. 9 Ave., Denver, Colo. 80220. This study was supported in part by an unrestricted grant from Research to Prevent Blindness, Inc. Manuscript submitted for publication July 5, 1973; manuscript accepted for publication Sept. 12, 1973.

REFERENCES

A technique for testing visual function in the presence of opacities.

C. R. CAVONIUS* AND R. HILZ

If two small images of a coherent light source are focused in the pupil of the eye, they produce an interference pattern on the retina that is sinusoidally modulated and thus appears to the subject as a grating, with a spatial frequency that depends on the distance between the images.1 Because the optics of the eye are not used to form the interference pattern, this technique has been advocated as a means for estimating retinal function in patients with whom conventional methods for measuring acuity cannot be used because of opacities in the ocular media or severe optical aberrations.2-4 This technique may be of use as an aid in deciding whether retinal function is normal enough to justify surgery and whether (in Goldmann's words)5 not only the doctor, but also the patient, will benefit from the operation.