

# Experimental Miotic Cataract

## I. Effects of Miotics on Lens Structure, Cation Content, and Hydration

*Joseph Michon, Jr., MD, and Jin H. Kinoshita, PhD, Boston*

Clinical surveys suggest that miotics produce lens opacities. Since such studies examine the diseased eyes of an older age group, interpretation is often difficult. The current study investigates the direct effect of miotics on the isolated rabbit lens in tissue culture. Both demecarium bromide (Humorsol) and echothiophate iodide (Phospholine Iodide) bring about subcapsular vacuoles, though their topographical distribution is different in the case of each drug. Both miotics alter cation and water balance in the lens, causing an increased sodium, decreased potassium, and gain in lens water. The rapidity of onset and the severity of these lesions exhibit a dose dependency. Although a cholinesterase is present at the lens surface, inhibition of the enzyme appears to have no direct role in the pathogenesis of the drug-induced cataract. The meaning of these studies in regard to the clinical situation is uncertain.

IN RECENT years miotics, especially of the anticholinesterase type, have been implicated in the production of lens opacities. In 1956, Muller and associates<sup>1</sup> reported that five patients receiving diethyl p-nitrophenyl phosphate (Mintacol), pilocarpine hydrochloride, and other agents developed cataracts in an extraordinarily rapid fashion. In 1960, Harrison<sup>2</sup> described the case of a young child who developed anterior subcapsular opacities while receiving isoflurophate (Floropryl) for accommodative esotropia. In 1965, Axelsson and Holmberg,<sup>3</sup>

surveyed 181 eyes having open-angle glaucoma and treated from the outset with either echothiophate iodide (Phospholine Iodide) or pilocarpine. They reported a five times greater incidence of significant lens opacities in the echothiophate treated population as compared to the pilocarpine treated group. Recent patient surveys by de Roeth,<sup>4,5</sup> Shaffer and Hetherington,<sup>6</sup> and Tarkkane and Karjalainen<sup>7</sup> have suggested that isoflurophate, echothiophate iodide, and demecarium bromide (Humorsol) are cataractogenic.

However, the presence of glaucoma and the ongoing aging process in these eyes, the difficulty of securing proper controls, and the hazards of retrospective analysis all serve to cloud the issue, and the relationship of miotics to cataractogenesis remains far from settled.

Experimental work has been scant. Diamant<sup>8</sup> has produced anterior lenticular changes in guinea pigs with the intracarotid injection of near lethal doses of anticholinesterase agents. Muller and associates have reported a decreased oxygen consumption in lenses incubated with  $10^{-6}$ M diethyl p-nitrophenyl phosphate or  $10^{-3}$ M pilocarpine, but could not furnish an adequate explanation for their observation. Harris and associates<sup>9</sup> have demonstrated abnormal cation balance in rabbit lenses incubated with  $5 \times 10^{-5}$ M physostigmine.

The present study investigates the direct effect of miotics on the rabbit lens in tissue culture.

### Methods

Rabbits weighing approximately 0.75 kg are killed; their globes excised; and their lenses removed via a posterior approach. The lenses are transferred to a Merriam-Kinsey culture tube

---

Submitted for publication Aug 11, 1967.

From the Howe Laboratory, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston. Dr. Michon is a research fellow in Ophthalmology, Harvard Medical School, Boston.

Read before the meeting of the Association for Research in Ophthalmology, Chicago, Oct 14, 1966.

Reprint requests to the Howe Laboratory, Massachusetts Eye and Ear Infirmary, 243 Charles St, Boston 02114 (Dr. Michon).

and maintained in 10-ml of tissue culture medium (TC 199) 199-bicarbonate medium according to procedures previously described.<sup>10-13</sup> In all studies, the lens of one eye serves as the control tissue while the lens from the contralateral eye of the same rabbit serves as the experimental tissue, being cultured in the presence of the appropriate drug.

In morphologic studies lenses are observed with the naked eye while in the culture tubes; then removed and examined under a dissecting microscope at  $\times 15$  and  $\times 40$  magnification by several observers in a double blind fashion. The degree of lenticular clarity is graded from (4+) clear to (0) cloudy. For histologic sections the lens is fixed in 70% alcohol for two to seven days; embedded in paraffin; sectioned at  $8\mu$  to  $12\mu$ ; and stained with hematoxylin and eosin.

For determination of cation and water content, lenses are blotted on filter paper; weighed; homogenized in 3 ml of 10% trichloroacetic acid; and known aliquots are analyzed for sodium and potassium content by flame photometry.<sup>11</sup> Water content of control lenses is calculated as 67% of the wet weight of the lens.<sup>13</sup> To arrive at the water content of the experimental tissue, the control value is adjusted by the gain in weight of the drug-treated lens.

Assay of lens cholinesterase activity is done according to the method of Ellman et al.<sup>14</sup> Here the rate of hydrolysis of acetylthiocholine by the enzyme is determined by reacting the product, thiocholine, with 5-5' bisdithionitrobenzoate to form a yellow dye, 5-thio-nitrobenzoic acid. This dye absorbs at  $412\mu$  in a spectrophotometer. The extent of cholinesterase inhibition by demecarium bromide is determined by comparing the enzyme activity of drug treated lenses with activity of controls. Calf lenses are used in these studies in order to obtain more accurate quantitation of lens cholinesterase activity than is possible with rabbit lenses.

## Results

**Morphologic Alterations.**—In the presence of demecarium bromide, alterations in the structure of the rabbit lens occur at concentrations through  $5 \times 10^{-5}M$ . The first lesion visible to the naked eye is a haze which covers the entire anterior surface of the lens and is strictly limited to this surface, not extending beyond the equator. With the dissecting microscope numerous, small subcapsular vacuoles are seen at the anterior lens, while the posterior lens is entirely clear. This lesions appears in 16 of 16

lenses treated with varying concentrations of demecarium bromide,  $5 \times 10^{-4}M$  through  $5 \times 10^{-5}M$ . The 16 paired control lenses remain completely clear. In histologic sections the first detectable lesion consists in swollen and disordered epithelial cells (Fig 1). Subsequently the enlarged epithelial cells detach from their normal position next to the capsule; eosinophilic material interposes between the structured cortical fibers and the surface capsule; and rounded bodies suggestive of vacuoles are interspersed at the anterior surface of the lens (Fig 2-4). Despite this extreme damage at the anterior level of the lens, the remainder of the lens including the bow region (Fig 5), and the posterior surface (Fig 6) appear normal. While several of the histologic controls contain artifacts inherent in lenticular preparations, ie, shattered nucleus and curled capsule, none evidence alterations in any way similar to those described for the demecarium bromide treated lenses.

Echothiophate iodide effects an opacity at concentrations through  $10^{-3}M$ . The lesion here is quite different from that of the demecarium bromide treated lens. In all 16 of the echothiophate treated lenses, the first change visible to the naked eye is a hazy ring at the lens equator. Subsequently this ring increases in breadth to extend over the entire



Fig 1.—Anterior rabbit lens, two hours of incubation with demecarium bromide,  $5 \times 10^{-4}M$ . Epithelial cells are swollen and disordered, while cortex and capsule retain normal structure (hematoxylin and eosin,  $\times 480$ ).

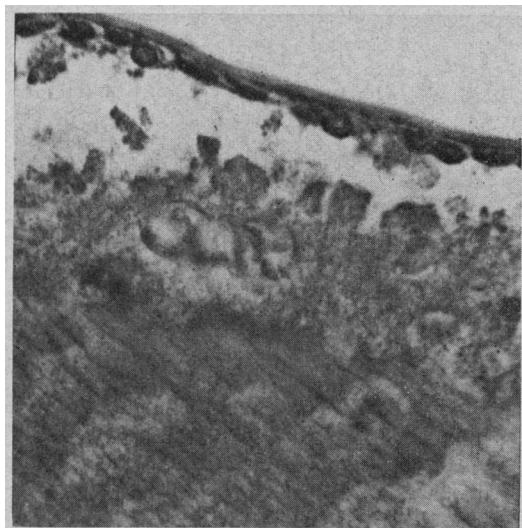


Fig 2.—Anterior rabbit lens, five hours of incubation with demecarium bromide,  $5 \times 10^{-4}$  M. Round vacuole-like bodies appear; swollen epithelial cells detach from the capsule; and eosinophilic material interposes between surface capsule and cortical fibers (hematoxylin and eosin,  $\times 256$ ).



Fig 3.—Anterior rabbit lens, five hours of incubation with demecarium bromide,  $5 \times 10^{-4}$  M. High power view of round, vacuole-like bodies which occur beneath the anterior capsule (hematoxylin and eosin,  $\times 480$ ).

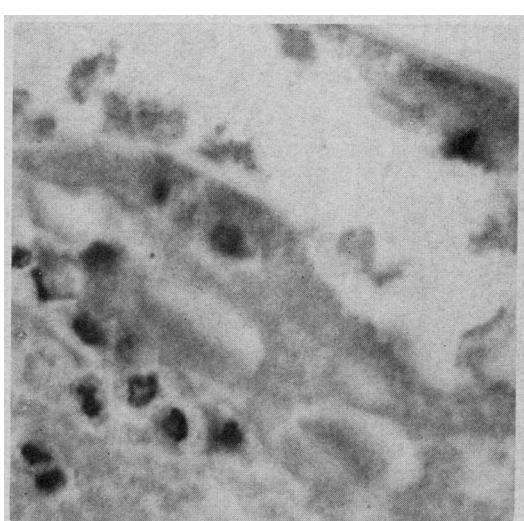


Fig 4.—Anterior rabbit lens, five hours of incubation with demecarium bromide,  $5 \times 10^{-4}$  M. High power view of epithelial cell nuclei which are detached from their normal position next to the capsule. (hematoxylin and eosin,  $\times 480$ ).

posterior surface of the lens. With the dissecting microscope, numerous minute subcapsular vacuoles are visible at the posterior part of the lens while the anterior level of the lens remains entirely normal. No such lesion could be detected in any of the 16 paired controls. In histologic section numerous vacuoles are present at the equatorial region (Fig 7 and 8). Subsequently these extend across the posterior lens (Fig 9) while sparing the anterior lens (Fig 10). The controls show no such change.

In the presence of carbachol the lens remains normal on inspection, examination with the dissecting microscope, and in histologic section even when the lens is incubated for 48 hours at drug concentrations of  $5 \times 10^{-3}$  M.

No alterations can be detected with the naked eye or dissecting microscope at concentrations of  $5 \times 10^{-3}$  M pilocarpine hydrochloride during 48 hours of incubation. However, histologic sections suggest alterations in the epithelial cell layer.

**Alterations in Cation and Water Balance.**—In other tissues the acetylcholine-cholinesterase system and related pharmacologic compounds appear involved in the modification of membrane permeability and the consequent alteration of cation partitioning. For

this reason the effect of miotics on the sodium, potassium, and water balance of the rabbit lens is investigated.

Demecarium bromide at high concentrations,  $10^{-3}$  M for example, rapidly effects a large gain in lens water, an increase in lens sodium, and a decrease in lens potassium (Table 1). A definite effect occurs at the lower concentration  $5 \times 10^{-5}$  M; and a sug-

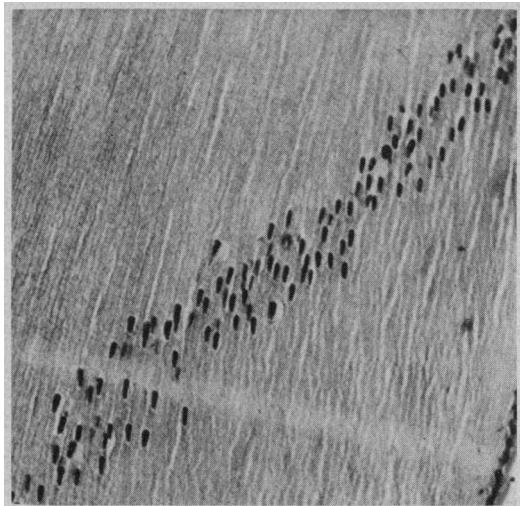


Fig 5.—Equatorial region of rabbit lens, five hours of incubation with demecarium bromide,  $5 \times 10^{-5}$  M. Equatorial region remains normal (hematoxylin and eosin,  $\times 128$ ).

gestion of water gain is present even at  $10^{-5}$  M demecarium bromide.

Similar changes in cation content and hydration occur in lenses incubated for 48 hours in the presence of echothiophate iodide (Table 2). However, here the minimal

Fig 7.—Rabbit lens equator, 18 hours of incubation with echothiophate iodide,  $5 \times 10^{-5}$  M. Numerous vacuoles are present (hematoxylin and eosin,  $\times 128$ ).

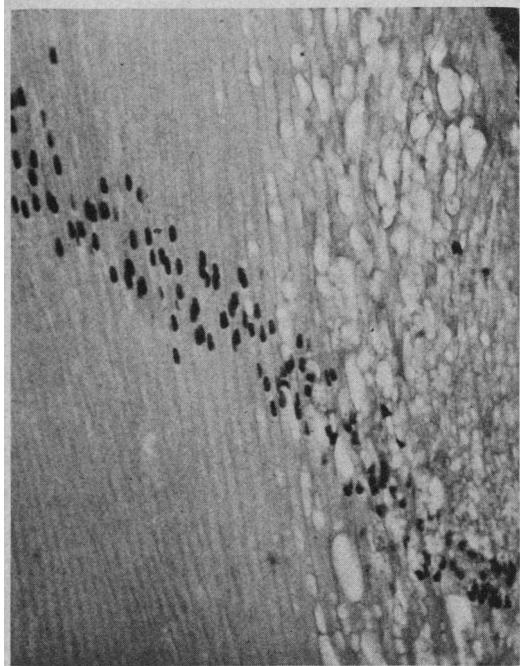


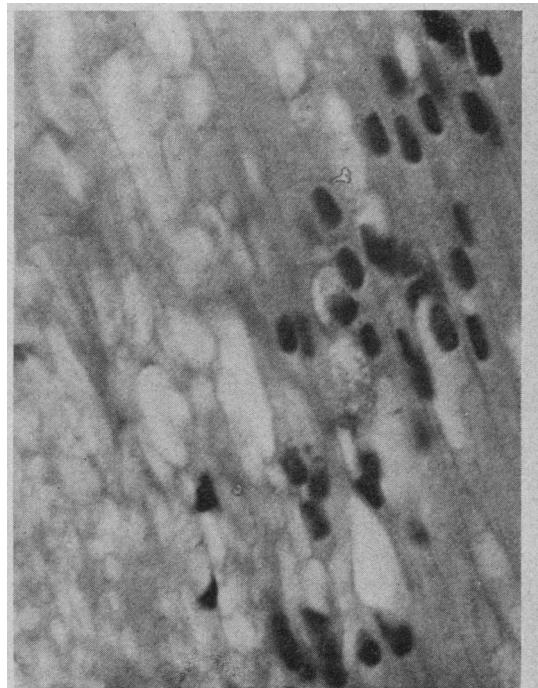
Fig 6.—Posterior rabbit lens, five hours of incubation with demecarium bromide,  $5 \times 10^{-4}$  M. Posterior lens remains normal (hematoxylin and eosin,  $\times 128$ ).

effective dose approximates  $10^{-3}$  M, which is considerably higher than the minimal effective dose of demecarium bromide.

Carbachol is without effect on lens water, sodium, and potassium even at a concentration of  $5 \times 10^{-3}$  M after 48 hours of culture (Table 2).

Finally, pilocarpine produces a slight in-

Fig 8.—Rabbit lens equator, 18 hours of incubation with echothiophate iodide,  $5 \times 10^{-3}$  M. High power view of equatorial vacuoles (hematoxylin and eosin,  $\times 480$ ).



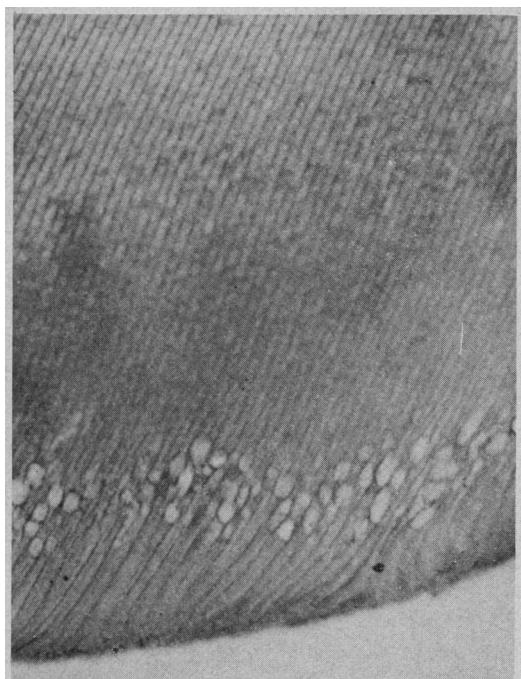


Fig 9.—Posterior rabbit lens, 18 hours of incubation with echothiophate iodide,  $5 \times 10^{-3}$ M. Vacuoles eventually extend across the posterior lens (hematoxylin and eosin,  $\times 256$ ).

crease in lens water at a concentration of  $5 \times 10^{-3}$ M after 48 hours of incubation (Table 2).

**Dose Relationship.**—Both the alterations in structure and the alterations in cation and water content brought about by these agents exhibit a dose-dependency. With the high concentration of  $10^{-3}$ M demecarium bromide an anterior haze appears as early as two hours; with  $5 \times 10^{-4}$ M the clarity

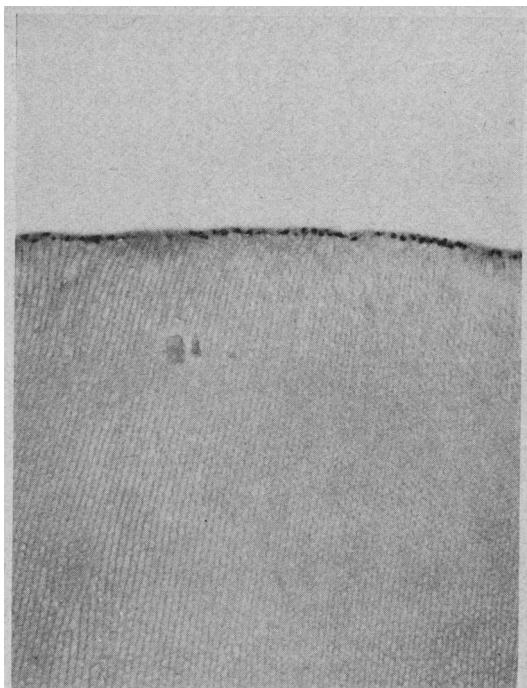


Fig 10.—Anterior rabbit lens, 18 hours of incubation with echothiophate iodide,  $5 \times 10^{-3}$ M. Anterior surface appears normal (hematoxylin and eosin,  $\times 128$ ).

changes at six hours; with  $10^{-4}$ M, 40 hours; and with  $5 \times 10^{-5}$ M, 45 hours (Table 3). In the case of echothiophate iodide, a concentration of  $5 \times 10^{-3}$  alters clarity within 18 hours; whereas at  $10^{-3}$ M the lens does not cloud until 30 hours of exposure (Table 3).

Similarly the severity of lens swelling is dependent upon the concentration of demecarium bromide or echothiophate iodide present; higher concentrations bring about greater water gain in the case of either agent (Table 4).

**Role of Lens Cholinesterase.**—Earlier studies have demonstrated that a cholinesterase occurs in the calf lens.<sup>15</sup> This is a true cholinesterase similar to that found in the erythrocyte and neural tissue, and is closely bound to the lens capsule. A cholinesterase occurs also in the rabbit, swine, and human lens.<sup>5,16</sup>

The next study examines the role of this lens cholinester-

Table 1.—Changes in Cation Levels and Hydration in the Rabbit Lens Exposed to Demecarium Bromide\*

Demecarium (M)	Hours of Incubation	Sodium (mEq/kgHOH)	Potassium (mEq/kgHOH)	Water (mg)
$1 \times 10^{-3}$	24	...	...	+ 50 ± 4
$5 \times 10^{-4}$	24	+ 54 ± 16	+ 51 ± 14	+ 27 ± 7
$1 \times 10^{-4}$	48	+ 5.8 ± 1.1	+ 11 ± 3	+ 4.3 ± 0.5
$5 \times 10^{-5}$	48	...	...	+ 7.5 ± 1.3
$1 \times 10^{-5}$	48	0	0	+ 3.1 ± 0.8
$1 \times 10^{-6}$	48	0	0	0
Average values of control lenses		13.2	136	117

\*Values given represent the mean differences observed between the drug-treated lenses and paired controls,  $\pm$  standard error. The values are based on experiments involving three to nine pairs of lenses. As a point of reference, average values for the incubated controls are listed at bottom.

Table 2.—Changes in Cation Levels and Hydration in the Rabbit Lens Exposed to Miotics\*

Drug	Concentration (M)	Hours of Incubation	Sodium (mEq/kgHOH)	Potassium (mEq/kgHOH)	Water (mg)
Echothiophate	5 x 10 <sup>-3</sup>	24	+7.7 ± 0.7	-20 ± 4	+11 ± 0.7
Echothiophate	1 x 10 <sup>-3</sup>	48	+1.9 ± 0.2	-13 ± 4	+5.8 ± 1.2
Echothiophate	5 x 10 <sup>-4</sup>	48	0	0	0
Carbamylcholine chloride	5 x 10 <sup>-3</sup>	48	0	0	0
Pilocarpine hydrochloride	5 x 10 <sup>-3</sup>	48	0 *	0	+2.2 ± 0.1
Average values of control lenses			13.2	136	117

\*Values given represent the mean differences observed between the drug-treated lenses and paired controls ± standard error. The values are based on experiments involving four to eight pairs of lenses. As a point of reference, average values for incubated controls are listed at bottom.

terase in the alteration of normal lens function brought about by cholinesterase inhibitors. Calf lenses are incubated in control and drug containing media for 24 hours. Optical status is then graded from cloudy (0) to clear (4+) in a double blind manner. Simultaneous studies determine the amount of cholinesterase inhibition brought about by demecarium bromide.

As seen in Table 5, drug levels necessary for production of opacity, 10<sup>-3</sup>M, are at least 1000-fold higher than levels effecting total inhibition of the lens cholinesterase, 10<sup>-6</sup>M. In other words, even though at 10<sup>-4</sup>M through 10<sup>-6</sup>M demecarium bromide the enzyme is completely inhibited, the lens remains clear. Thus no direct relationship appears to exist between inhibition of cholinesterase and alterations of lens clarity.

Similar studies exposing calf lenses to 10<sup>-3</sup>M echothiophate iodide for 24 hours fail to produce an opacity although cholinesterase activity is completely abolished.

### Comment

This study demonstrates that demecarium bromide, echothiophate iodide, and possibly pilocarpine are able to alter the normal structure, cation balance, and hydration of the rabbit lens in tissue culture. In vitro investigations by others have shown that physostigmine likewise upsets cation balance of the rabbit lens,<sup>9</sup> and that the miotics diethyl *p*-nitrophenyl phosphate and pilocarpine decrease oxygen consumption in the human, dog, and swine lens.<sup>1</sup> In vivo studies have demonstrated that cholinesterase inhibitors produce opacities in the guinea pig lens,<sup>8</sup> though results here are ambiguous inasmuch as the state of lid closure is not recorded.<sup>17</sup>

Vulnerability to an adverse influence of miotics appears a common property of mammalian lenses.

Both demecarium bromide and echothiophate iodide cause subcapsular vacuoles in the rabbit lens. Their topographical distribution, however, differs with each drug: demecarium bromide bringing about vacuoles at the anterior level and echothiophate iodide bringing about vacuoles at the equatorial and posterior location. This indicates that the demecarium bromide cataract and echothiophate iodide cataract are distinct entities, and suggests that the term anticho-

Table 3.—Dose Dependency of Cataract Onset\*

Demecarium		Echothiophate	
Molar Concentration	Onset of Opacity	Molar Concentration	Onset of Opacity
1 x 10 <sup>-3</sup>	2-3 hr	5 x 10 <sup>-3</sup>	18
5 x 10 <sup>-4</sup>	6-12 hr	2.5 x 10 <sup>-3</sup>	24
1 x 10 <sup>-4</sup>	40-45 hr	1 x 10 <sup>-3</sup>	30
5 x 10 <sup>-5</sup>	45-48 hr	5 x 10 <sup>-4</sup>	Clear thru 48 hr
1 x 10 <sup>-6</sup>	Clear thru 48 hr	...	...

\*Drug is added at "0" hour. Each notation summarizes observations on four or more lenses. All of the paired control lenses remained clear for 48 hours.

Table 4.—Dose Dependency of Lens Swelling\*

Demecarium		Echothiophate	
Molar Concentration	% H <sub>2</sub> O Gain	Molar Concentration	% H <sub>2</sub> O Gain
1 x 10 <sup>-3</sup>	41.8 ± 3.6	5 x 10 <sup>-3</sup>	8.9 ± 0.5
5 x 10 <sup>-4</sup>	20.5 ± 5.5	1 x 10 <sup>-3</sup>	4.5 ± 0.9
5 x 10 <sup>-5</sup>	6.1 ± 1.1	5 x 10 <sup>-4</sup>	0 ± 0.5
1 x 10 <sup>-6</sup>	2.9 ± 0.8	...	...
1 x 10 <sup>-6</sup>	0 ± 0.5	...	...

\*Each value represents mean increase in water content of drug-exposed lenses ± standard error. The values are based on experiments involving three to nine pairs of lenses. Incubation for 48 hours.

**Table 5.—Effect of Demecarium Bromide on Calf Lens Clarity and Cholinesterase**

Molar Concentration of Demecarium	Clarity at 24 hr	Cholinesterase Inhibition After 1 hr (%)
$1 \times 10^{-3}$ (12)	0+	100
$1 \times 10^{-4}$ (12)	+++	100
$1 \times 10^{-5}$ (6)	+++	100
$1 \times 10^{-6}$ (6)	+++	100
Controls (36)	+++	0

\*0 denotes cloudy lens; +++ denotes clear lens; and number of lenses studied is in parentheses.

linesterase cataract is misleading inasmuch as it implies completely similar mechanisms of cataractogenesis for each drug.

Worthy of comment is a discrepancy between the posterior location of vacuoles in the experimental echothiophate iodide cataract and their anterior location said to occur clinically. The likely explanation is found upon examining the distribution of drug in the two settings. In tissue culture, equal concentrations of drug bathe the anterior and posterior lens; in the clinical setting, however, topical administration would be expected to deliver the highest level of echothiophate iodide selectively at the anterior surface.

Under normal circumstances, the lens maintains an internal environment of low sodium, high potassium, and restricted hydration. In the face of both demecarium bromide and echothiophate iodide the normal partitioning of cations in the rabbit lens changes: sodium concentration increases and potassium concentration decreases. Importantly, a gain in lens water accompanies the shift of cations and serves as an apt explanation for the vacuoles observed with the dissecting microscope and in histologic section. Such events are in no way unique to the lens. For under the stress of similar pharmacologic chemicals, cation distribution also changes in the erythrocytes of the dog, cat, and rabbit<sup>18</sup> and in neural tissue of the chicken.<sup>19</sup>

The drug-induced lesions exhibit a dose dependency. In all cases of the experimental cataract, for example, the higher concentration of demecarium bromide or echothiophate iodide occasions a more rapid alteration of lens clarity. Similarly the degree of lens swelling increases with higher levels of drug or more prolonged exposure. A clinical counterpart appears to exist in Axelsson and

Holmberg's observation that use of 0.25% echothiophate is associated with lens vacuoles more frequently than use of the 0.06% solution of the drug. In this clinical study, however, if the higher dose was occasioned by a more severe glaucoma, then proper interpretation of the data becomes difficult.

Evidence is presented that the lenticular effects of demecarium bromide and echothiophate iodide are explained on some basis other than inhibition of cholinesterase. For there is a marked dissociation between drug levels necessary for alteration of lens clarity and drug levels effecting total inhibition of the enzyme. Additional data support this view. First, the difference in the topographical distribution of vacuoles brought about by demecarium bromide and echothiophate iodide argues against a mechanism of cataractogenesis in common, such as the inhibition of cholinesterase. Second, the lens changes observed by Diamant<sup>8</sup> following the intracarotid injection of long acting cholinesterase inhibitors were of one day's duration, and would not coincide temporally, therefore, with the more extended period of enzyme inhibition. Third, attempts to link enzyme inhibition with the changes in sodium and potassium distribution found in drug-treated erythrocytes have been unsuccessful.<sup>20</sup> Finally, lens cholinesterase is closely bound to the capsule,<sup>15</sup> and this structure can be completely removed by collagenase digestion without affecting the mechanisms responsible for maintaining normal sodium, potassium, and water balance.<sup>21</sup> It must be noted that while these observations discount any direct role for cholinesterase inhibition in cataractogenesis, they do not exclude the possibility that enzyme inhibition in the intact animal may have an indirect influence on the lens.

The meaning of these studies in regard to the clinical situation is uncertain. The concentrations of echothiophate iodide and demecarium bromide required to alter the normal state of the isolated rabbit lens are considerable,  $10^{-3}M$  and  $10^{-5}M$  respectively; and it would appear unlikely that such levels could be attained for any length of time in the aqueous of the posterior chamber. The data might suggest, then, that these agents do no harm in the patient situation. It must be noted, however, that a similar

line of reasoning would deny the existence of steroid cataracts in the human, in light of the massive doses of prednisolone and desoxycorticosterone needed to alter normal function of the rabbit lens in tissue culture.<sup>21</sup> Furthermore, in tissue culture as the time period of drug exposure is prolonged, progressively lower doses of drug prove toxic to the lens. Such data indicate the difficulty of arbitrarily labeling any drug level as harmless when long term exposure is anticipated.

All in all, only a well-controlled, prospective, clinical investigation can yield the final answers. If such a study does indicate that miotics are able to induce cataracts in humans, then the dissociation found between cholinesterase inhibition and cataract production may have practical significance. For it may be possible to regulate dosage such

that the drugs will effectively inhibit cholinesterase and thereby lower intraocular pressure but will not harm the lens.

This work was supported by Public Health Service grants 2-101 NB 05142, NB 06090, and S-K3-17032; and by US Atomic Energy Commission contract AT (30-1) 1368.

Technical assistance was provided by Bill H. C. Tung, Lorenzo O. Merola, Robert C. Kasabian, and Irod Lindsay.

Demecarium bromide, echothiopate iodide, and carbachol were supplied by Merck, Sharp, and Dohme; Ayerst Laboratories; and Alcon Laboratories respectively.

### Generic and Trade Names of Drugs

Pilocarpine hydrochloride—*Pilocar, Pilocel, Pilovisc, Palocarp*.

Isofluorophate—*Floropryl*.

Echothiopate iodide—*Phospholine Iodide*.

Demecarium bromide—*Humorsol*.

### References

- Muller, H.K., et al: Der Einfluss von Pilocarpin und Mintacol auf den Stoffwechsel der Linse, *Ber Deutsch Ophth Ges* **60**:115-120, 1956.
- Harrison, R.: Bilateral Lens Opacities Associated With Use of Diisopropyl Fluorophosphate Eye Drops, *Amer J Ophthal* **50**:153-154 (July) 1960.
- Axelsson, U., and Holmberg, A.: The Frequency of Cataract After Miotic Therapy, *Acta Ophthalm* **44**:421-429, 1966.
- De Roeth, A., Jr.: Lenticular Opacities in Glaucoma Patients Receiving Echothiopate Iodide Therapy, *JAMA* **195**:664-666 (Feb 21) 1966.
- De Roeth, A., Jr.: Lens Opacities in Glaucoma Patients on Phospholine Iodide Therapy, *Amer J Ophthal* **62**:619-628 (Oct) 1966.
- Shaffer, R.N., and Hetherington, J., Jr.: Anticholinesterase Drugs and Cataracts, *Amer J Ophthal* **62**:613-618 (Oct) 1966.
- Tarkkanen, A., and Karjalainen, K.: Cataract Formation During Miotic Treatment for Open-Angle Glaucoma, *Acta Ophthal* **44**:932-939, 1966.
- Diamant, H.: Cataract Due to Cholinesterase Inhibitors in the Guinea-Pig, *Acta Ophthal* **32**:357-361, 1954.
- Harris, J.E.; Gruber, L.; and Hoskinson, G.: The Effect of Methylene Blue and Certain Other Dyes on Cation Transport and Hydration of the Rabbit Lens, *Amer J Ophthal* **47**:387-395 (Jan, pt 2) 1959.
- Kinoshita, J.H., and Wachtl, C.: A Study of the  $C^{14}$ -Glucose Metabolism of the Rabbit Lens, *J Biol Chem* **233**:5-7, 1958.
- Merola, L.O.; Kern, H.L.; and Kinoshita, J.H.: The Effect of Calcium on the Cations of Calf Lens, *Arch Ophthal* **63**:830-835 (May) 1960.
- Kinoshita, J.H.; Kern, H.L.; and Merola, L.O.: Factors Affecting the Cation Transport of Calf Lens, *Biochim Biophys Acta* **47**:458-466, 1961.
- Kinoshita, J.H.; Merola, L.O.; and Hyman, S.: Osmotic Effects on the Amino Acid Concentrating Mechanism in the Rabbit Lens, *J Biol Chem* **240**:310-315, 1965.
- Ellman, G., et al: A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity, *Biochem Pharmacol* **7**:88-95, 1961.
- Michon, J., and Kinoshita, J.H.: Cholinesterase in the Lens, *Arch Ophthal*, (to be published).
- Glick, D.; Lewin, A.; and Antopol, W.: Occurrence of Cholinesterase in the Swine, *Proc Soc Exp Biol Med* **40**:28-32 (Jan) 1939.
- Fraunfelder, F.T., and Burns, R.P.: Effect of Lid Closure in Drug-Induced Experimental Cataracts, *Arch Ophthal* **76**:599-601 (Oct) 1966.
- Holland, W.C., and Grieg, M.E.: Studies on the Permeability of Erythrocytes: III. The Effect of Physostigmine and Acetylcholine on the Permeability of Dog, Cat, and Rabbit Erythrocytes to Sodium and Potassium, *Amer J Physiol* **162**:610-615 (Sept) 1950.
- Strickland, K.P., and Thompson, R.H.S.: On the Mechanism of Potassium Loss From Brain Slices Induced by Cholinesterase Inhibitors, *Biochem J* **60**:468-475, 1955.
- Taylor, I.M.; Weller, J.M.; and Hastings, A.B.: Effect of Cholinesterase and Choline Acetylase Inhibitors on the Potassium Concentration Gradient and Potassium Exchange of Human Erythrocytes, *Amer J Physiol* **168**:658-665, 1952.
- Becker, B., and Cotlier, E.: The Efflux of  $^{82}\text{Rubidium}$  From the Rabbit Lens, *Invest Ophthal* **4**:117-121 (Feb) 1965.