Atropine Reduces Experimental Myopia and Eye Enlargement Via a Nonaccommodative Mechanism

Neville A. McBrien, Hadi O. Moghaddam, and Anne P. Reeder

Purpose. To determine whether the muscarinic antagonist atropine effectively reduces or prevents experimentally induced myopia via a nonaccommodative mechanism.

Methods. Chicks were monocularly deprived (MD) of pattern vision by placement of a translucent occluder over the left eye. In two of the three MD groups, chicks received a series of intravitreal injections of atropine (n = 8) or saline vehicle (n = 8) with MD. Control groups (n = 8) of chicks were employed to assess the effects of MD, intravitreal injections, and drug effects.

Results. In sham-injected or saline-injected MD chicks, 8 days of MD produced —18.5 D and —20.9 D of experimental myopia, respectively. In atropine-injected MD chicks, 8 days of MD produced only —2.8 D of experimental myopia. This significant reduction in experimentally induced myopia in atropine-injected MD chicks was associated with a marked reduction in the relative axial elongation of the deprived eye (0.21 mm) when compared to saline-injected or sham-injected MD chicks (1.04 mm and 1.00 mm). This reduction in axial length in atropine-injected MD chicks was predominantly the result of a reduction in vitreous chamber elongation, although a reduction in anterior segment depth also was observed. Mean equatorial diameter was significantly reduced in atropine-injected MD chicks compared to saline-injected and sham-injected MD chicks, although to a lesser extent. Control experiments demonstrated that intravitreally injected atropine did not reduce carbachol-induced accommodation or light-induced pupil constriction in the skeletal intraocular muscles of the chick eye.

Conclusions. These findings demonstrate that chronic administration of the muscarinic antagonist atropine prevents experimentally induced myopia in chick via a nonaccommodative mechanism. Invest Ophthalmol Vis Sci. 1993;34:205-215.

Deprivation of pattern vision in human infants (e.g., ptosis, hemangioma of the lid) has been found to cause an axial elongation of the deprived eye and high degrees of myopia.1-4 It also has been demonstrated in several animal species that monocular deprivation (MD) of pattern vision in neonates produces an axial elongation of the eye, resulting in high degrees of myopia.5-7 These results have been found to occur in chicks,5-7 tree shrews,8,9 cats,10 gray squirrels,11 marmosets,12 and monkeys.13,14

One mechanism that has been proposed as a causative factor in myopia development in humans and in animal models is that of accommodation.15-18 Support for this suggestion comes from results of studies that have employed cycloplegic agents such as atropine to retard the development of myopia. In adolescent humans, daily administration of 1% atropine was found to prevent the progression of juvenile myopia in the treated eye.19,20 Although less conclusive results also have been observed, especially with weaker cycloplegics.21 In animal models, atropine administration also
has been found to prevent or reduce the development of experimentally induced myopia. Young\textsuperscript{22,26} found that monkeys (Macaca nemestrina) restricted to a near viewing environment reliably developed myopia that could be halted if animals were treated with topical 1% atropine eye drops three times a day.

Raviola and Wiesel\textsuperscript{18} found that chronic atropine administration to lid-sutured stump tail Macaque monkey eyes prevented the development of experimental myopia, although they also found that atropine did not prevent myopia in a different species of monkey (rhesus). In lid-sutured tree shrews, chronic administration of atropine was found to prevent the development of axial myopia,\textsuperscript{17} and although a recent study\textsuperscript{23} found topical atropine did not block myopia in tree shrews, results in our laboratory using intravitreally injected atropine in tree shrew did block myopia (McBrien and Reeder, unpublished observations), suggesting a dose-dependent effect. In all the studies with positive findings, it was concluded that atropine effectively prevented or retarded the progression of the myopia because of its cycloplegic effect on smooth ciliary muscle, which blocked the accommodative function of the eye. This has led to the development of feedback models of refractive development that incorporate a pivotal role for accommodation.\textsuperscript{15,17}

In recent years, several investigations have further elucidated the possible underlying mechanisms responsible for altering coordinated ocular growth in the absence of form vision. Studies have shown that blocking communication between the eye and higher centers—by optic nerve section in chicks\textsuperscript{34,35} and monkeys\textsuperscript{18} or blockade of retinal ganglion cell action potentials in tree shrews\textsuperscript{26} and chicks\textsuperscript{27}—does not prevent the development of experimentally induced myopia. Other studies have shown that deprivation of only part of the visual field results in local areas of elongation and myopia in the eye.\textsuperscript{28,29} It also has been demonstrated that experimental myopia can be induced in a species that does not possess a functional accommodative system\textsuperscript{11} or where accommodation has been blocked by bilateral lesioning of the Edinger-Westphal nucleus.\textsuperscript{30} Recently, it has been shown in chick that experimentally induced myopia is associated with increased synthesis of scleral protein,\textsuperscript{31,32} DNA,\textsuperscript{31,32} and proteoglycans\textsuperscript{32,33} and with increases in total scleral collagen content.\textsuperscript{32} This indicates that active growth of the sclera, as opposed to passive stretching, is taking place. The above findings, which point toward local ocular control of postnatal eye growth, question the role of accommodation as a major causative factor of experimental myopia and suggest that the mechanism by which atropine prevents or reduces myopia may be nonaccommodative.

In primate and mammalian models of experimental myopia, atropine invariably blocks the accommodative function of the eye, because of its effect at muscarinic receptors in the ciliary muscle.\textsuperscript{34} As a result, in these species it has been difficult to address whether atropine has its effect on myopia development via a nonaccommodative route. However, because the intraretinal muscles of the chick eye are striated and contain predominantly nicotinic receptors,\textsuperscript{35,36} atropine (a muscarinic receptor antagonist) should not produce cycloplegia or pupil dilation. The present study sought to determine whether atropine effectively reduces or prevents experimentally induced myopia via a nonaccommodative mechanism in chick.

## MATERIALS and METHODS

### Animals

One-day-old hatched chicks (Rhode Island cross) were delivered and kept on a 12 hr light/12 hr dark cycle in a temperature-controlled environment. Food and water were available ad libitum. Chicks were allocated to one of six experimental groups on the basis of whether they were monocularly occluded (MD) and whether they received intravitreal injections of either atropine or saline. Each of the six groups contained eight chicks. All investigations concerning animals adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

### Treatment Protocols

Initial pilot experiments indicated that topical application of 1% atropine sulfate drops daily produced no significant difference in experimentally induced vitreous chamber elongation and myopia for atropine-treated (n = 6) compared to saline-treated (n = 6) MD chicks. Because of this finding, it was decided to apply atropine via intravitreal injection to enhance the possibility that an effective dose would reach potential target sites. On day 6 after hatching, chicks were given an intravitreal injection of 7 µl of phosphate buffered atropine (calculated concentration at retina, 250 – 300 µmol/l, equivalent to 0.01%) or phosphate buffered saline under halothane (2–3.5%) anesthesia. Intravitreal injections were carried out by surgically widening the palpebral aperture on the temporal side of the left eye, inserting a sterile 30 G needle (connected to a microsyringe via sterile tubing), and injecting the drug or vehicle control into the vitreous. The temporal opening then was closed with a single surgical suture, and gentacin ointment was applied to the injected area to prevent infection. Chicks in the MD groups then had a translucent hemispheric occluder placed over the injected eye. This initial injection procedure was repeated every 48 hr over 8 days, resulting in four intravitreal injections per animal. It was nearly always possible to find the same injection site on subsequent
sessions. The occluders were renewed after every injection for MD animals and were never off for more than 15 min, during which time the animal was anesthetized.

As a control for the effect of intravitreal injection, one group of chicks (sham-injected) underwent halothane anesthesia, opening of the palpebral aperture, and mechanical pressure from, but without insertion of, the needle on the sclera, on exactly the same schedule as MD chicks who had intravitreal injections. Two groups of chicks had intravitreal injections of atropine or saline without MD to control for drug and injection effects, respectively. One group of chicks underwent neither intravitreal injection nor monocular occlusion but were housed in identical conditions and had optical and structural measures taken. Thus, the six groups were sham-injected MD, saline-injected MD, atropine-injected MD, atropine-open, saline-open, and normal-open, with eight chicks in each group.

### In Vivo Optical and Structural Measures

Optical and structural measures were taken 8 days after the first injection. Chicks were anesthetized with ketamine (50 mg/kg) and xylazine (3.5 mg/kg), and body temperature was maintained (at 37°C) via a heating pad. Supplementary doses of anesthetic were given as required. All refractive and structural measures were taken after cycloplegia. Because of the predominantly striated nature of chick intraocular muscles, cycloplegia was achieved using five 25 μl drops of vecuronium bromide (2 mg/ml) spaced at 1 min intervals. The cornea was pre-treated with topical proparacaine HCl (0.5%) to enhance penetration of the drug. Measurements were begun 30 min after the final drop, at which time there was full pupil dilation and no evidence of pupil reactions. Corneal curvature was measured using a modified one-position keratometer fitted with a brighter light source and a +8 diopter lens to extend the scale. Eight readings were taken of both the horizontal and vertical meridians of the cornea for each eye. Readings were converted to corneal radius (mm) using a calibration equation derived by measuring ball bearings of known radii. Refraction was measured to the nearest 0.5 D by streak retinoscopy on the horizontal and vertical meridians at a working distance of 33 cm. Values were converted into measures of ocular refraction at the corneal plane by correcting for the effect of the correcting lens (held 5 mm from the cornea) and the working distance. Horizontal and vertical measures were averaged to obtain the spherical equivalent refraction. No correction was made for the effect of eye size on retinoscopy measures.

In vivo measurements of the axial dimensions of the structural components of the eye, along the optic axis, were obtained by A-scan ultrasonography. A 10 MHz, 6.35 mm diameter ultrasound transducer focused at 22 mm and driven by a Panametrics 5052 pulser/receiver was coupled to a 15 mm perspex stand-off that was perfused continuously with 0.9% saline (flow rate 0.8 ml/min). The stand-off was positioned by hand so the saline column contacted the anesthetized cornea (0.5% proparacaine HCl) without any applanation. Waveform echoes passed from the pulser/receiver into a LeCroy (Geneva, Switzerland) 9400 digital storage oscilloscope (sample rate 100 megasamples/s). To enhance the signal-to-noise ratio, each stored waveform was the average of 20 single incoming waveforms. Six stored waveforms from independent positionings of the transducer were collected for each eye and transferred to an Opus (Reading, UK) PCV (AT) computer for subsequent measurement. Conversion of time to distance employed previously published values for the chick eye.

### In Vitro Measures and Histology

After all the in vivo optical and structural measures were completed, the animal was deeply anesthetized with sodium pentobarbital and the eyes were enucleated. Digital caliper measurements of the medial/lateral and superior/inferior equatorial diameters and the axial length were taken. The eye then was weighed to the nearest 10 μg. To determine whether the drug treatment altered the physical integrity of the retina, eyes from atropine-injected and saline-injected chicks were placed in fixative (phosphate buffered 2.5% glutaraldehyde) for 24 hr and dehydrated via graded alcohols and embedded in epoxy resin for histologic evaluation. Semi-thin sections (2 μm) of the posterior pole were cut and examined at the light microscopy level.

### Pupil and Accommodation Measures

Evidence for the presence of extra-synaptic muscarinic receptors recently was presented for chick iris. Although these receptors are more prominent in the embryo, they also are present after hatching. A similar mixed cholinergic receptor population is suggested to be present in chick ciliary muscle. In light of these findings, it was important to determine whether atropine had any measurable effect on accommodation or pupil responses; thus, two control experiments were performed. Age-matched chicks were given intravitreal injections of atropine (n = 5) or saline (n = 5), as described above. Horizontal and vertical pupil diameters were measured using a small operating microscope with a measuring graticule eyepiece at ×10 magnification. Readings were taken before the intravitreal injection at a luminance of 100 lux and approximately 60 min after the injection. Further post-injection measures were taken after an initial 10 min period of dark adaptation at luminances of 10, 50, 100, 500, 1000, and 2000 lux, in an ascending pattern, with 3 min dark
adaptation between readings at different luminances. The investigator who took the readings was unaware of the experimental treatment (atropine or saline).

To determine the effect of atropine on accommodative amplitude, another two groups of chicks were given intravitreal injections of atropine (n = 5) or saline (n = 5). One hour after intravitreal injection, the chicks were anesthetized with ketamine and xylazine, and baseline measures of the refractive state of injected and contralateral control eyes were taken using streak retinoscopy. A-scan ultrasonography was taken on both eyes of each chick using the procedure described above, except that only three averaged waveforms were recorded for each eye. Carbachol 10% (nicotinic and muscarinic agonist) then was topically applied to the injected eye via corneal iontophoresis. The cornea was pre-treated with topical proxymetacaine HCl (0.5%) to enhance penetration of the drug; then a 2.5% agar button of 5 mm diameter containing carbachol 10% was placed on the center of the cornea. The negative electrode was attached to the skin, and the positive electrode was gently applied to the agar button for 20 sec at a current of 200 μA. Because of the rapid and relatively short-lived response to pharmacologic stimulation of chick-striated ciliary muscle, all measures were recorded within 10 min of topical application of carbachol. The pupil diameter of the carbachol-treated eye was measured over the first 3 min post-application at 30 sec intervals. Five min after the carbachol was applied, the refractive state was measured in the horizontal and vertical meridians with a streak retinoscope. A-scan ultrasonography then was taken on the injected eye as described above, and readings were obtained 9 min (± 1) min after topical carbachol was applied. Once measures were completed on the injected eye, retinoscopy and ultrasound were taken again on the contralateral control eye.

Statistical Analysis

For analysis of differences between groups, parametric statistics were employed (analysis of variance [ANOVA], t-test) because there was no evidence of a skewed distribution.

RESULTS

Refractive differences for all six experimental groups are shown in Figure 1 and Table 1. In sham-injected chicks (n = 8), monocular deprivation of pattern vision for 8 days produced a significant myopia in the deprived eye of -18.5 ± 2.9 D (mean ± SEM) when compared to the contralateral open control eye. In saline-injected MD chicks (n = 8), a relative myopia between deprived and control eyes of -20.9 ± 1.2 D was observed. In atropine-injected MD chicks (n = 8), a relative myopia of -2.8 ± 1.5 D was observed. One-way ANOVA revealed a highly significant difference in experimentally induced myopia between MD groups (F = 24.5, P < 0.001). Duncan’s multiple comparison test revealed significant differences between the atropine-injected MD group and both the saline-injected MD group (P < 0.001) and sham-injected MD group (P < 0.001), whereas there was no significant difference between the sham-injected and saline-injected MD groups. Thus, the muscarinic antagonist atropine, administered as described, almost completely eliminated experimentally induced myopia in treated chicks. Receptive differences between experimental and control eyes in groups that received intravitreal injections of atropine or saline, but not MD, were not significantly different from receptive differences between right and left eyes of normal binocular chicks. Cycloplegic refraction (vercuronium bromide) as measured by streak retinoscopy. Chronic atropine administration almost completely eliminated the experimental myopia produced by monocular deprivation. n = 8 in each group. Error bars = 1 SEM.

FIGURE 1. Differences in ocular refraction between open control eyes and deprived eyes in monocularly deprived chicks and between open control and injected or right and left eyes of binocularly open chicks. Cycloplegic refraction (vercuronium bromide) as measured by streak retinoscopy. Chronic atropine administration almost completely eliminated the experimental myopia produced by monocular deprivation. n = 8 in each group. Error bars = 1 SEM.

The major structural cause of the experimentally induced myopia in sham-injected and saline-injected MD chicks was enlargement of the vitreous chamber, axially and equatorially, as found in previous studies. Vitreous chamber depth (Fig. 2A; Table 1), as measured by A-scan ultrasonography, showed a relative elongation in the deprived eye of sham-injected and saline-injected MD chicks of 0.86 ± 0.10 mm and 0.92 ± 0.06 mm, respectively. In atropine-injected MD chicks, the deprived eye showed a relative vitreous chamber elongation of only 0.38 ± 0.05 mm, compared to the contralateral open control eye. There was a highly significant difference in vitreous chamber elongation between MD groups (ANOVA, F = 16.28, P < 0.001). There was no significant difference in vitreous chamber depth between injected and control eyes for atropine-injected (P = 0.80) or saline-injected
Corneal Equatorial Equatorial Equatorial Atropine Reduces Experimental Myopia

Atropine-injected (+0.13 ± 0.06 mm) and saline-injected (+0.11 ± 0.04 mm) MD chicks developed deeper anterior segment depths (corneal thickness plus anterior chamber depth) in the deprived eye compared to the fellow control eye (Fig. 2B; Table 1), whereas atropine-injected MD chicks developed shallower anterior segment depths in the deprived eye (−0.17 ± 0.05 mm). Comparison of differences for anterior segment depth between deprived and control eyes in MD groups revealed a highly significant difference (F = 11.01, P < 0.001). One-way ANOVA also revealed significant differences in anterior segment depth between eyes in binocularly open groups (F = 4.07, P < 0.05) as a result of atropine-injected open eyes developing shallower anterior segment depths than their fellow control eyes (−0.09 ± 0.02 mm). No significant differences were observed in corneal curvature between experimental and control eyes of MD or binocular animals (F = 2.33, P = 0.06), although the atropine groups were the only groups in which the experimental eye had a flatter corneal curvature. No differences in lens thickness were found between deprived and control eyes of MD chicks or between injected and control eyes for binocularly open chicks for any experimental group (F = 0.56, P = 0.73).

Combining interocular axial dimensions, obtained by A-scan ultrasonography, to give axial length measures revealed marked differences between atropine-injected MD chicks and the other MD groups (Fig. 2C; Table 1). Atropine-injected MD chick eyes developed a relative axial elongation of only 0.21 ± 0.15 mm, compared to their fellow control eye, whereas saline-injected MD chick eyes underwent a relative axial elongation of 1.04 ± 0.06 mm and sham-injected MD chick eyes underwent an axial elongation of 1.0 ± 0.14 mm.

Equatorial dimensions, as measured with digital calipers, also showed differences for atropine-treated MD chicks compared to saline-injected or sham-injected MD chicks. Atropine-injected MD chicks underwent less enlargement of the superior/inferior equatorial dimension (0.22 ± 0.07 mm) than did saline-injected (0.36 ± 0.06 mm) or sham-injected (0.38 ± 0.08 mm) MD chicks, although this enlargement was not statistically significant (F = 1.46, P = 0.25). However, measurement of the medial/lateral equatorial dimension revealed a significant reduction in the

### Table 1. Cycloplegic Ocular Refraction and Axial Dimensions of All Deprived (Dep) and Open Control Eyes of Monocularly Deprived (MD) Chicks and Right and Left Eyes of Binocularly Open Chicks

<table>
<thead>
<tr>
<th></th>
<th>Sham-Injected MD</th>
<th>Saline MD</th>
<th>Atropine MD</th>
<th>Atropine Open</th>
<th>Saline Open</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinoscopy (D)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dep</td>
<td>−15.3 ± 2.6</td>
<td>−17.3 ± 1.2</td>
<td>+0.7 ± 1.5</td>
<td>+4.9 ± 0.5</td>
<td>+3.4 ± 0.5</td>
<td>+2.6 ± 0.3</td>
</tr>
<tr>
<td>Open</td>
<td>+3.2 ± 0.6</td>
<td>+3.6 ± 0.7</td>
<td>+3.5 ± 0.3</td>
<td>+3.0 ± 0.5</td>
<td>+3.0 ± 0.3</td>
<td>+3.0 ± 0.2</td>
</tr>
<tr>
<td>Corneal radius (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dep</td>
<td>3.15 ± 0.04</td>
<td>3.29 ± 0.03</td>
<td>3.29 ± 0.04</td>
<td>3.29 ± 0.02</td>
<td>3.20 ± 0.03</td>
<td>3.21 ± 0.03</td>
</tr>
<tr>
<td>Open</td>
<td>3.18 ± 0.02</td>
<td>3.22 ± 0.03</td>
<td>3.25 ± 0.03</td>
<td>3.17 ± 0.02</td>
<td>3.20 ± 0.03</td>
<td>3.22 ± 0.03</td>
</tr>
<tr>
<td>Corneal thickness (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dep</td>
<td>−0.03 ± 0.04</td>
<td>−0.02 ± 0.01</td>
<td>0.04 ± 0.02</td>
<td>0.06 ± 0.02</td>
<td>0.00 ± 0.02</td>
<td>−0.01 ± 0.01</td>
</tr>
<tr>
<td>Anterior segment (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dep</td>
<td>1.70 ± 0.08</td>
<td>1.65 ± 0.03</td>
<td>1.44 ± 0.05</td>
<td>1.51 ± 0.03</td>
<td>1.48 ± 0.02</td>
<td>1.49 ± 0.02</td>
</tr>
<tr>
<td>Open</td>
<td>1.57 ± 0.04</td>
<td>1.54 ± 0.02</td>
<td>1.61 ± 0.03</td>
<td>1.60 ± 0.03</td>
<td>1.53 ± 0.03</td>
<td>1.51 ± 0.02</td>
</tr>
<tr>
<td>Lens thickness (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dep</td>
<td>2.34 ± 0.08</td>
<td>2.28 ± 0.07</td>
<td>2.27 ± 0.07</td>
<td>2.37 ± 0.07</td>
<td>2.23 ± 0.06</td>
<td>2.21 ± 0.02</td>
</tr>
<tr>
<td>Vitreous chamber (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dep</td>
<td>6.36 ± 0.17</td>
<td>6.35 ± 0.19</td>
<td>6.04 ± 0.19</td>
<td>5.71 ± 0.14</td>
<td>5.31 ± 0.10</td>
<td>5.30 ± 0.04</td>
</tr>
<tr>
<td>Open</td>
<td>5.50 ± 0.18</td>
<td>5.45 ± 0.13</td>
<td>5.66 ± 0.15</td>
<td>5.66 ± 0.15</td>
<td>5.32 ± 0.10</td>
<td>5.29 ± 0.05</td>
</tr>
<tr>
<td>Axial length (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dep</td>
<td>10.40 ± 0.27</td>
<td>10.27 ± 0.24</td>
<td>9.76 ± 0.25</td>
<td>9.60 ± 0.22</td>
<td>9.02 ± 0.16</td>
<td>9.01 ± 0.06</td>
</tr>
<tr>
<td>Open</td>
<td>9.40 ± 0.23</td>
<td>9.23 ± 0.21</td>
<td>9.55 ± 0.23</td>
<td>9.61 ± 0.24</td>
<td>9.06 ± 0.17</td>
<td>9.02 ± 0.07</td>
</tr>
<tr>
<td>Equatorial diameter (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dep</td>
<td>12.56 ± 0.16</td>
<td>12.73 ± 0.17</td>
<td>12.48 ± 0.17</td>
<td>12.30 ± 0.08</td>
<td>12.23 ± 0.13</td>
<td>12.45 ± 0.09</td>
</tr>
<tr>
<td>Open</td>
<td>12.38 ± 0.10</td>
<td>12.03 ± 0.16</td>
<td>12.26 ± 0.13</td>
<td>12.21 ± 0.09</td>
<td>12.26 ± 0.10</td>
<td>12.48 ± 0.09</td>
</tr>
<tr>
<td>(sup/infr; mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dep</td>
<td>0.38 ± 0.08</td>
<td>0.36 ± 0.06</td>
<td>0.22 ± 0.07</td>
<td>0.09 ± 0.05</td>
<td>−0.04 ± 0.08</td>
<td>−0.03 ± 0.03</td>
</tr>
<tr>
<td>Open</td>
<td>0.52 ± 0.09</td>
<td>0.69 ± 0.10</td>
<td>0.29 ± 0.08</td>
<td>0.04 ± 0.05</td>
<td>0.06 ± 0.11</td>
<td>−0.04 ± 0.02</td>
</tr>
<tr>
<td>(Med/Lat; mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dep</td>
<td>0.52 ± 0.09</td>
<td>0.69 ± 0.10</td>
<td>0.29 ± 0.08</td>
<td>0.04 ± 0.05</td>
<td>0.06 ± 0.11</td>
<td>−0.04 ± 0.02</td>
</tr>
<tr>
<td>Open</td>
<td>0.69 ± 0.10</td>
<td>0.82 ± 0.11</td>
<td>0.30 ± 0.08</td>
<td>0.10 ± 0.04</td>
<td>0.14 ± 0.05</td>
<td>−0.07 ± 0.02</td>
</tr>
<tr>
<td>(average; mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dep</td>
<td>0.64 ± 0.01</td>
<td>0.64 ± 0.02</td>
<td>0.64 ± 0.02</td>
<td>0.63 ± 0.01</td>
<td>0.67 ± 0.01</td>
<td>0.67 ± 0.02</td>
</tr>
<tr>
<td>Open</td>
<td>0.64 ± 0.01</td>
<td>0.64 ± 0.02</td>
<td>0.64 ± 0.02</td>
<td>0.63 ± 0.01</td>
<td>0.67 ± 0.01</td>
<td>0.67 ± 0.02</td>
</tr>
</tbody>
</table>

In all cases, the left eye received the intravitreal injection or deprivation. n = 8 in all groups. Figures are means ± SEM.
amount of enlargement in the deprived eye of atropine-injected MD chicks (0.29 ± 0.09 mm) compared to saline-injected (0.69 ± 0.10 mm) or sham-injected (0.52 ± 0.09 mm) MD chicks (F = 4.92, P < 0.02). Averaging the equatorial dimensions (Fig. 2D; Table 1) also resulted in significant differences between atropine MD chicks (0.25 ± 0.06 mm) and saline (0.52 ± 0.07 mm) or sham (0.44 ± 0.08 mm) MD chicks (F = 5.72, P < 0.05). Digital caliper measures of axial length confirmed in vivo ultrasound measures by showing a significant reduction in axial elongation in atropine MD chicks.

Histologic evaluation of the retina (posterior pole) at the light microscope (magnification ×1000) level revealed no apparent toxic effects on the retina of atropine-treated eyes when compared to saline-treated eyes (Fig. 3).

Intravitreally injected atropine did not cause a reduction in the direct pupil response to light (Fig. 4). Pupil area was not significantly different between atropine-injected chicks (n = 5) and saline injected (n = 5) controls for illuminance levels ranging from 10–2000 lux (P > 0.17 at all levels). Corneal iontophoresis of 10% carbachol induced the same degree of accom-
Atropine Reduces Experimental Myopia

**DISCUSSION**

The finding that the muscarinic antagonist atropine almost completely prevents the development of experimentally induced myopia is not a new finding. Previous studies, however, usually concluded that atropine was preventing myopia development by blocking accommodation.\(^{17,18,22}\) However, because no significant differences in carbachol-induced accommodation or pupillary constriction to light were observed between atropine- and saline-injected chicks, the present study demonstrates that chronic atropine administration prevents experimentally induced myopia in chick via a nonaccommodative mechanism.

The reduction in axial myopia was accounted for mainly by a reduction in vitreous chamber elongation in the deprived eye—the major structural alteration in experimentally induced myopia in chick. However, it is
It is interesting that although atropine effectively prevented the excessive enlargement of the eye in MD chicks, it had little effect on ocular growth in binocularly open animals. There was no effect on growth of the vitreous chamber in open animals, although there was a small but significant reduction in anterior segment depth. This finding would suggest that growth of an eye deprived of pattern vision and normal ocular growth may be mediated by different mechanisms that do not depend on the same retinal signals, as previously suggested.44

Atropine has been found to effectively reduce the excessive elongation of the eye and consequent myopia in other animal models of experimental myopia (ie, monkey,18,22 tree shrew17) and has been shown to effectively prevent the progression of human juvenile myopia.19,20 It seems reasonable to assume that the mode of action by which atropine prevents excessive elongation of the eye and myopia is similar in the various species investigated. The present investigation has demonstrated that, contrary to previous hypotheses,17,18,25 the mechanism by which atropine reduces or prevents myopia is nonaccommodative. If accommodation does not play a major role in myopia development, the question arises regarding how atropine effectively prevents the excessive elongation of the eye in myopia. Acetylcholine is a retinal neurotransmitter,45-47 and the retina contains a cholinergic amacrine cell population48-50 that includes muscarinic acetylcholine receptors.51-53 It is possible that atropine acts by which atropine prevents excessive elongation of the eye. Evidence suggests that dopamine may regulate cholinergic neurotransmission in the retina57 and that dopaminergic cells are driven excitatorily by cholinergic synapses in the retina.58 It seems feasible that inhibition of experimentally induced axial elongation of the eye by muscarinic antagonists and dopamine agonists is controlled by similar neuromodulatory mechanisms within the retina.

Particularly pertinent to the present study is a recent report42 that found that the nonspecific muscarinic antagonist atropine blocked deprivation-induced axial elongation and that the M1 selective antagonist pirenzepine had similar effects. In addition, M2 and M3 selective antagonists did not prevent axial elongation. This supports a retinal locus, because M1 muscarinic receptors are located in neural tissue59 as opposed to intraocular muscle, where M3 receptors predominate.60 Information on retinal acetylcholine

interesting that there also was a reduction in anterior segment depth in atropine-injected MD eyes when compared to their contralateral control eye. This reduction, which also contributed to reducing the observed myopia, indicates that atropine effectively prevents or attenuates MD-induced axial enlargement of the anterior and posterior segments of the chick eye.

The finding that ocular enlargement was attenuated in the axial and—albeit to a lesser extent—in the equatorial dimension is contrary to a previous report of pharmacologic control of experimentally induced myopia using atropine.42 Stone et al reported that muscarinic antagonists attenuated ocular enlargement exclusively in the axial dimension, with no effect in the equatorial dimension. This is similar to their earlier findings with dopamine agonists. These apparently conflicting results may be the result of methodologic differences between the two studies. The present study used a Rhode Island cross chick; deprivation was achieved by translucent occluder, lasted for 8 days, and induced 0.89 mm axial elongation and 0.69 mm equatorial elongation in MD vehicle control chicks. In contrast, Stone et al.42 using White Leghorn chicks and 2 wk deprivation via lid-suture, induced only 0.35 mm axial elongation and 0.84 mm equatorial elongation (all measures from digital caliper readings for comparability). In addition to differences in induced ocular enlargement in control MD animals for the two studies, Stone et al.42 used a subconjunctival route for drug delivery that would result in a lower final concentration of atropine at the retina than would result from the intravitreal route used in the present study. It is possible that higher doses of atropine are needed to attenuate experimentally induced equatorial enlargement in chick than are required to attenuate axial enlargement.

**FIGURE 5.** Change in lens thickness and amount of accommodation induced by corneal iontophoresis of 10% carbachol in chick eyes after intravitreal injection of atropine or saline. No significant difference in lens thickness changes ($P = 0.77$) or induced accommodation ($P = 0.70$) were observed between atropine- and saline-injected chick eyes. $N = 5$ in each group. Error bars = 1 SEM.
content in experimental myopia is needed to help substantiate a role for retinal muscarinic receptors in the control of ocular growth in myopia. If substantiated, M1 selective antagonists such as pirenzepine could prove to be a more acceptable treatment modality than atropine for preventing human myopia progression. Pirenzepine—compared to atropine—has only limited effect on the amount and particularly the duration of pupil dilation, with minimal reduction in accommodation amplitude in mammalian intraocular musculature (tree shrew; McBrien NA, Cottriall CL, and Reeder AP, unpublished observations). Furthermore, pirenzepine is a safe and well-tolerated drug that has been used widely to treat peptic ulcer disease in humans.

Interestingly, it recently has been shown that atropine and pirenzepine can inhibit physiologic growth hormone release in normal human subjects. Although this is thought to involve the inhibitory effect of acetylcholine on somatostatin release from the hypothalamus, the precise mechanism by which cholinergic blockade influences growth hormone secretion is unclear. Because recent investigations have demonstrated increased synthesis of scleral tissue in experimentally induced myopia in chick, it is feasible that atropine may work by inhibiting release of some yet unidentified growth factor in eyes deprived of form vision, thus preventing myopia progression. Now that it is known that the effective locus of action of cholinergic antagonists in reducing myopia progression is not the accommodative system, subsequent investigations should help clarify the site and role of cholinergic control of ocular growth.

Key Words
accommodation, atropine, chick, experimental myopia, muscarinic antagonists

Acknowledgments
The authors thank Drs. Margaret Woodhouse, Chris McCormack, and William Hodos for critically reading the manuscript; an anonymous reviewer for providing helpful comments; and Lisa Thomas and Christine Dunn for secretarial assistance.

References


46. Ikeda H, Sheardown MJ. Acetylcholine may be an excitatory transmitter mediating visual excitation of ‘transient’ cells with the periphery effect in the cat retina: Iontophoretic studies in *Ciba Foundation Symposium 155.* Chichester, UK: Wiley; 1984:45–57.


Atropine Reduces Experimental Myopia