

Atropine Inhibition of Echothiophate Cataractogenesis in Monkeys

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• One cynomolgus and three vervet monkeys were treated topically twice daily in one eye with 63 µg of echothiophate iodide and 750 µg of atropine sulfate. The opposite eyes were treated with echothiophate alone. The eyes treated with echothiophate alone all developed posterior and/or anterior subcapsular cataracts within 26 to 66 days. The echothiophate plus atropine-treated cynomolgus eye developed a cataract of much delayed onset and lesser severity compared to its echothiophate-only-treated fellow. None of the echothiophate plus atropine-treated vervet eyes developed cataracts during the 121 (two eyes) or 231 (one eye) days of treatment. After 121 days of echothiophate plus atropine treatment, two vervet eyes were switched to echothiophate alone. Both eyes developed unequivocal posterior and anterior subcapsular lens opacities, one within 51 and one within 71 days after the switch. We conclude that atropine inhibits echothiophate cataractogenesis in vervet and cynomolgus monkeys.

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Chronic topical application of long-acting cholinesterase inhibitors to human eyes can cause characteristic anterior and posterior subcapsular (ASC, PSC) cataracts.¹⁻⁶ Topical application of echothiophate iodide twice daily for several weeks consistently causes similar ASC and PSC cataracts in vervet and cynomolgus monkeys.^{7,8} We report here the inhibition of echothiophate cataractogenesis in these two monkey species by simultaneous topical administration of atropine.

MATERIALS AND METHODS

Animals

Three adult female vervet monkeys (*Cercopithecus ethiops*) weighing 2.8 to 3.6 kg, and one young adult female cynomolgus

monkey (*Macaca fascicularis*) weighing 2.1 kg, were studied.

Drugs

Echothiophate iodide (Phospholine Iodide) was purchased commercially and diluted to a 0.5% solution with the accompanying diluent. The 0.5% echothiophate iodide solution had pH = 6.39 and osmolality = 435 mOsm. Atropine sulfate, medicinal grade, was added to the echothiophate solution to give an atropine sulfate concentration of 2%, 3%, 4.5%, or 6%. The 0.5% echothiophate iodide plus 6.0% atropine sulfate solution had pH = 6.27 and osmolality = 610 mOsm. Solutions were refrigerated at 4°C and used within two weeks of preparation. (The stability of echothiophate and atropine in the experimental solutions was tested. Batches of the 0.5% echothiophate iodide, the 6% atropine sulfate, and the 0.5% echothiophate iodide plus 6% atropine sulfate solutions, identical to those used in the animal experiments, and a control solution identical to the others with respect to inactive ingredients but devoid of echothiophate iodide and atropine sulfate^a were prepared in 99.6% D₂O (Aldrich, gold label grade). The four solutions were analyzed in a Bruker 270 MHz proton magnetic resonance spectrometer. All the individual proton resonances for echothiophate and atropine were resolved. Spectra taken one hour, 11 days, 21 days, and 28 days after preparation of

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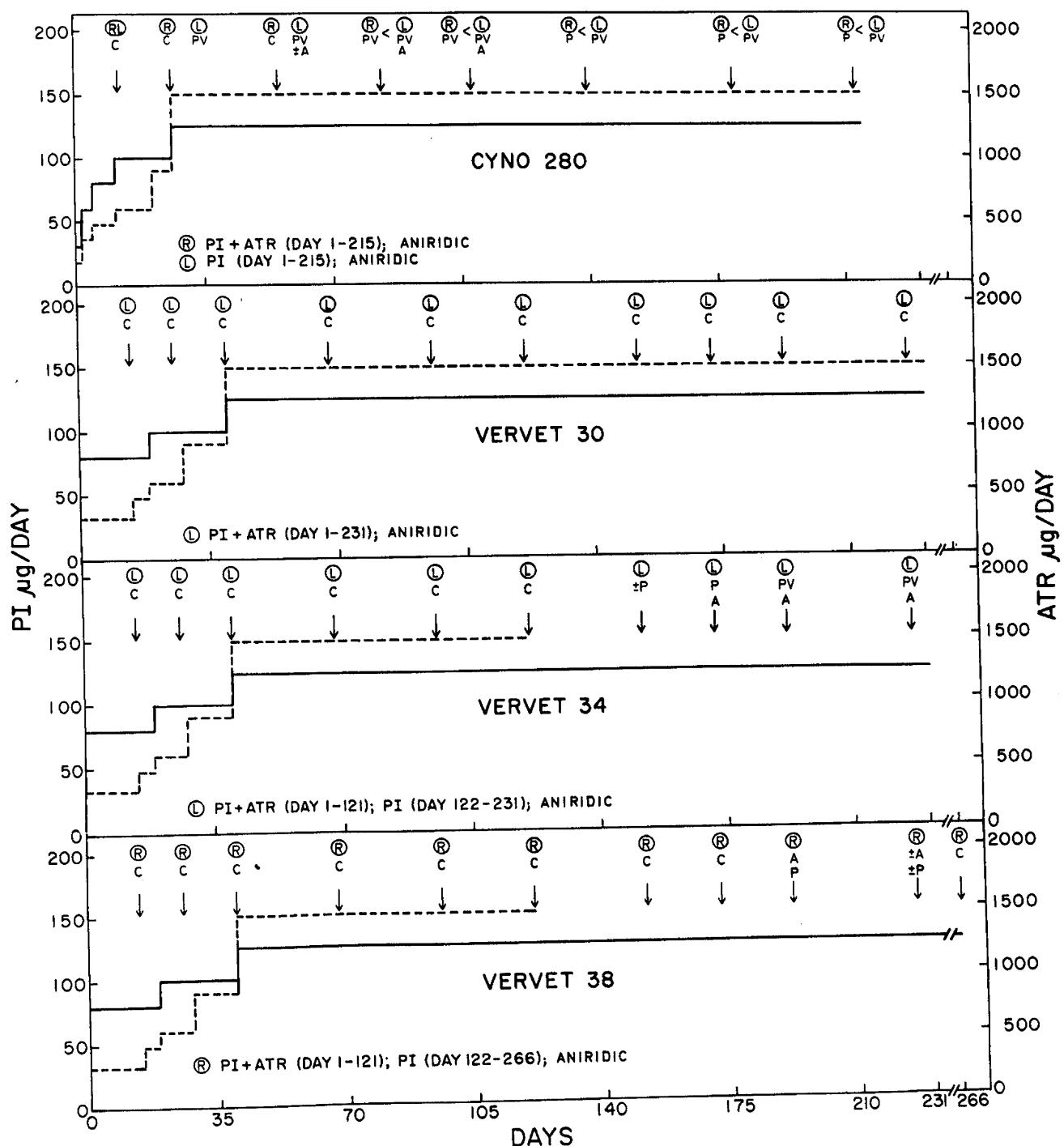


Fig 1.—Treatment protocols and lens findings. Abscissa: days after starting topical treatment. Ordinates: left, echothiophate iodide (PI) dose; right, atropine sulfate (ATR) dose; both drugs in one solution. Solid line = echothiophate iodide dose; broken line = atropine sulfate dose; R = right eye; L = left eye; cynomolgus (CYNO) 280 was treated in both eyes simultaneously; echothiophate was given to both eyes, atropine only to the right eye. The three vervets received echothiophate plus atropine only in the indicated eye. Opposite eyes had previously received echothiophate iodide, 80 µg/day for eight weeks, ending eight months before start of current session, and had all developed cataracts. Arrow indicates slit-lamp examination; P, posterior subcapsular lens changes; A, anterior subcapsular lens changes; V, vacuoles; ±, questionable lens changes; C, clear lens.

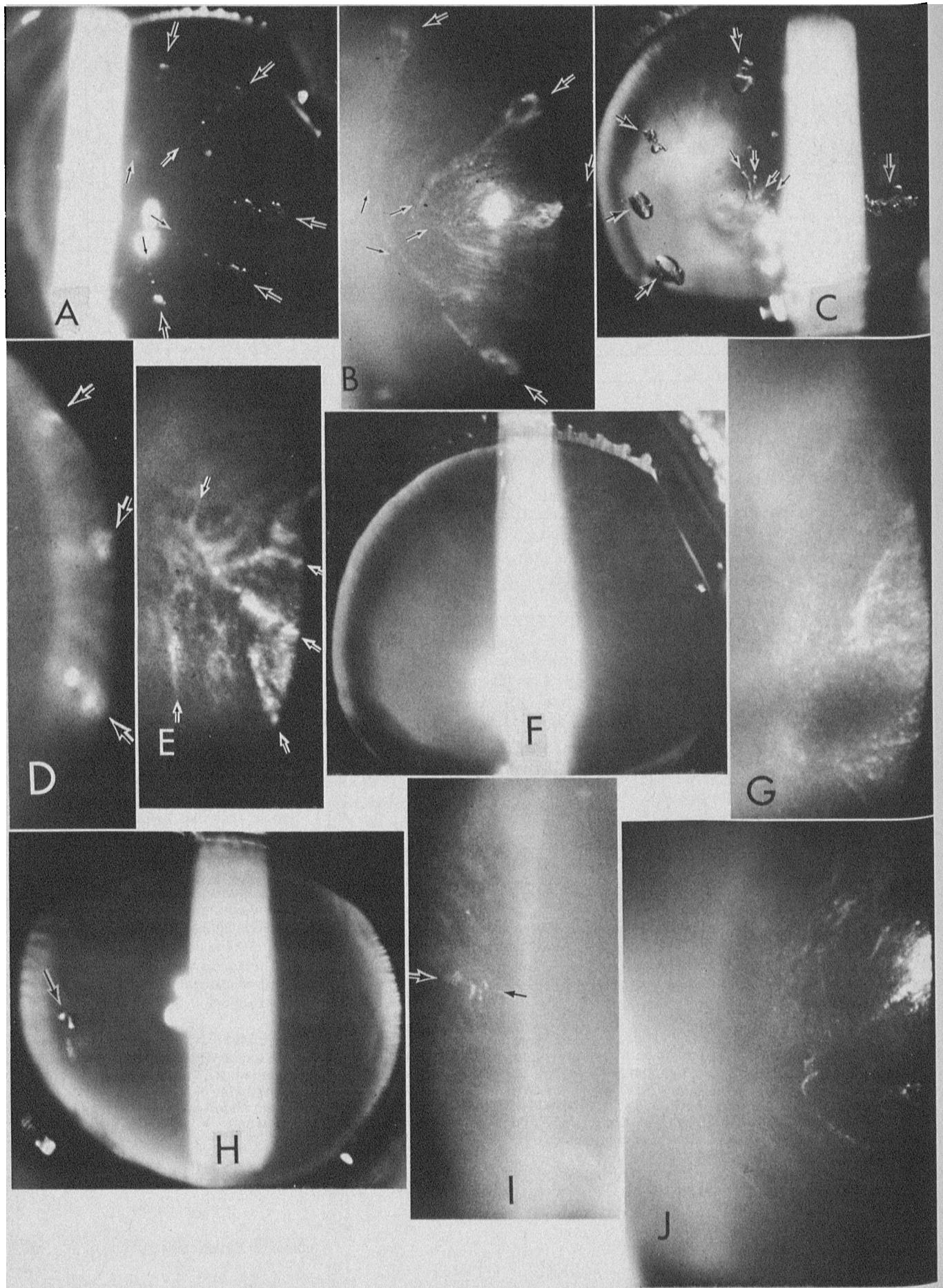


Fig 2.—Slit-lamp photographs of cynomolgus 280. A to G, Left eye (echothiophate); H to J, right eye (echothiophate plus atropine). A and B, OS after 32 days showing midperipheral PSC chains of vacuoles (large arrows) at approximately clock-hourly intervals, continuous with linear opacities (small arrows) converging at posterior pole of lens and delimiting wedges of smaller opacities. C, OS after 58 days, showing midperipheral PSC vacuoles (large arrows) and central PSC vacuoles (small arrows). D and E, OS after 86 days, showing midperipheral PSC chains of vacuoles (D, large arrows) and central PSC opacities, including wedge boundary lines (E, small arrows). F and G, OS after 180 days. F, Midperipheral PSC vacuoles are still present, but have regressed so strikingly that they are no longer visible in retroilluminated lens photograph (compare with A and C). G, Central PSC opacities have also regressed substantially (compare with C and E). H to J, OD after 86 days, showing most pronounced stage of PSC opacities observed during course of echothiophate plus atropine treatment. Opacities were of later onset and much less severity than in opposite echothiophate-treated eye. H and I, Single cluster of midperipheral PSC vacuoles (large arrows; compare with A and C). J, Central PSC opacities (compare with B, C, E, and G).

the solutions showed no evidence for interaction of the drugs nor any evidence of chemical change.) Hexamethonium-bromide, 2% in distilled water, was given intramuscularly.

Total iridectomy¹⁰ was performed bilaterally two months (cynomolgus) or 14 months (verrets) before starting topical treatments.

Refraction was determined periodically with a refractometer (Thorner).¹¹

Slit-lamp examination and photography¹² were performed periodically beginning four to six weeks prior to starting topical treatment.

General anesthesia for topical treatments was intramuscularly administered, methohexitol sodium (Brietal [Britain]; Brevital, comparable US product), 15 mg/kg for the verrets, and intramuscularly administered tiletamine hydrochloride and zolazepam hydrochloride (1:1 mixture by weight) 5 to 10 mg/kg¹³ for the cynomolgus. Refraction was performed under methohexitol alone or under methohexitol followed by intramuscular administration of pentobarbital sodium, 30 to 35 mg/kg.

PROTOCOL AND RESULTS

The three verrets had been previously treated with 40 µg of echothiophate

iodide twice daily in one eye for eight weeks, ending eight months prior to the start of the current session. The opposite eye had been treated simultaneously with an equal volume of identical control solution devoid of echothiophate. The echothiophate-treated eyes had all developed cataracts within 6 to 9.5 weeks after starting treatment while the control lenses had all remained clear.⁷ In the present experiments, the former control eyes were treated with echothiophate plus atropine. Vervet 30 received echothiophate plus atropine for 231 days. Vervets 34 and 38 received echothiophate plus atropine for 121 days, followed by echothiophate alone for the next 110 (vervet 34) or 145 (vervet 38) days. The cynomolgus, which had not been treated previously in either eye, received echothiophate alone in one eye and echothiophate plus atropine in the other for 215 days. Topical echothiophate or echothiophate plus atropine was given twice daily on weekdays (7:15 AM to 7:45 AM; 4:15 PM to 4:45 PM) and once daily on weekends (9 to 9:30 AM).⁸ For the first three months one of the two daily treatments was given under short-acting general anesthesia (see above); thereafter, all treatments were given with the monkeys fully conscious. The initially low echothiophate and atropine doses and the atropine:echothiophate ratio were gradually increased over a few weeks. The plateau doses were 63 µg of echothiophate iodide and 750 µg of atropine sulfate twice daily (echothiophate iodide 0.5%; atropine sulfate 6%). Echothiophate iodide, 63 µg twice daily, had induced cataracts in other cynomolgus within six weeks after starting treatment.⁸

Slit-lamp examination just prior to starting topical treatments revealed clear corneas, anterior chambers, and lenses, except for the PSC and/or ASC cataracts in the previously echothiophate-treated verret eyes.

Figure 1 shows the topical treatment protocols and the lens findings in the four monkeys. All three verret lenses remained clear as long as atropine was given with echothiophate. When atropine was stopped verret 34 demonstrated equivocal PSC lens opacities after 31 days, unequivocal

PSC and ASC opacities after 51 days, and PSC vacuoles after 71 days of echothiophate only treatment. Similarly, after atropine was stopped verret 38 exhibited mild but unequivocal ASC and PSC opacities after 71 days of echothiophate only treatment. The opacities in verret 38 subsequently regressed; they were only questionably present after 107 days and absent after 145 days of echothiophate only treatment.

The cynomolgus demonstrated striking PSC and ASC lens opacities in the echothiophate only treated eye (Fig 2, A to G). The opacities were identical in slit-lamp appearance to those previously described in echothiophate-treated cynomolgus eyes,⁹ and underwent partial regression with time despite continued echothiophate treatment, as has also been described.⁹ In the echothiophate plus atropine-treated eye, lens opacities also developed. However, they were restricted to the PSC region and were of later onset and much less prominence than in the echothiophate only treated eye (Fig 1; Fig 2, H to J). These opacities also regressed partially with continued treatment.

Figure 3 shows refractions performed during the course of the topical treatment to assess the cycloplegic effectiveness of the atropine dose. The nontreated eyes demonstrated reasonably stable baseline refractions.

The initial atropine:echothiophate dose ratios used were sufficient to prevent echothiophate-induced accommodation under deep anesthesia in the echothiophate plus atropine-treated eye of the cynomolgus (note similarity of the refractions in the right eye of cynomolgus 280 on days No. -5 and -1 with those on days No. +12 and +26) but not the verrets (note differences between refractions in the two eyes of verret 30 on days No. +14, +25, +39; verret 34 on day No. +16; verret 38 on day No. +39; compare also refractions on these days with refractions on day No. -1). The highest doses of atropine and echothiophate and the highest atropine sulfate:echothiophate iodide dose ratio (12:1 weight:weight) were started on day No. +40 in the verrets

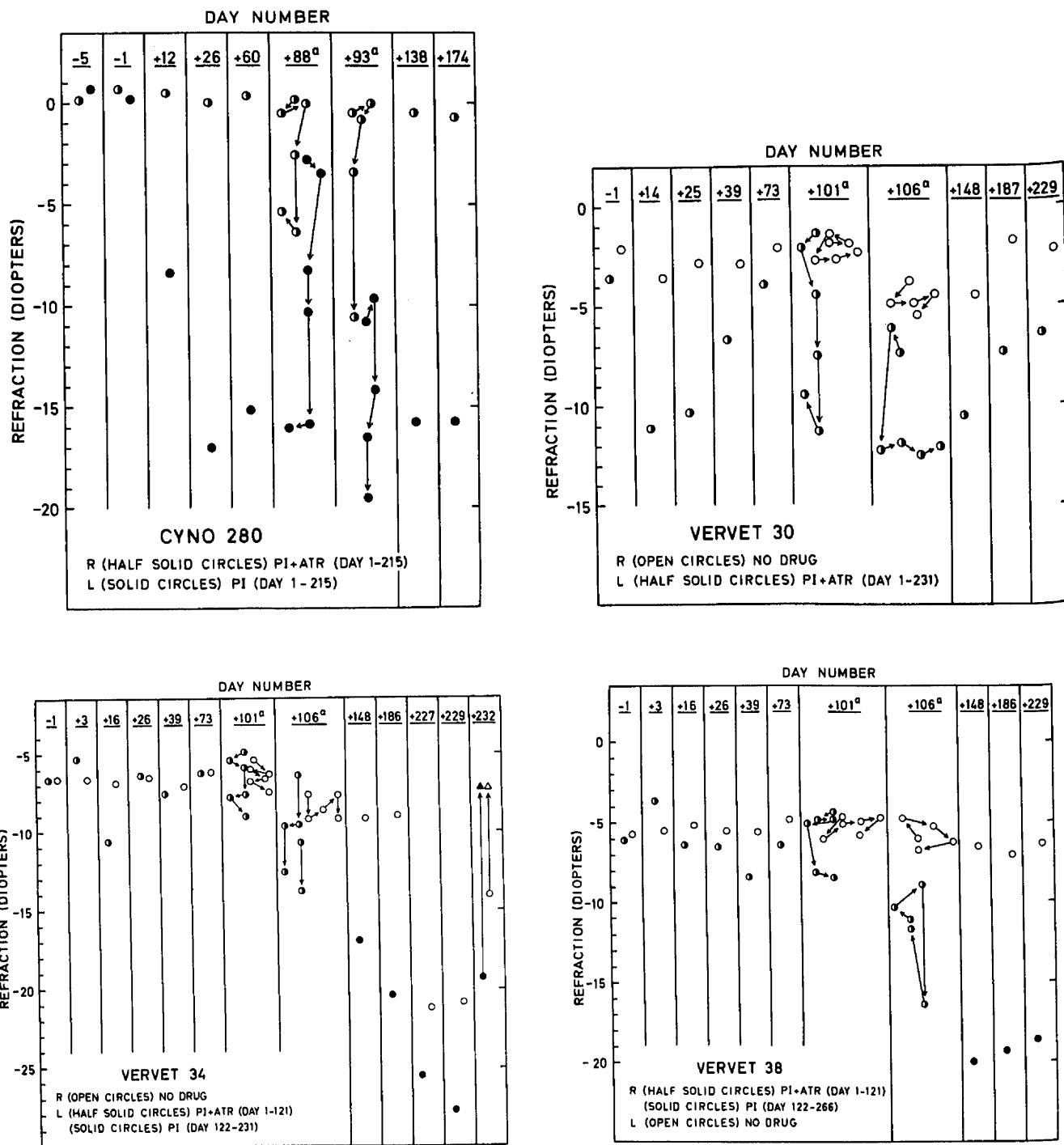


Fig 3.—Refraction experiments. Day No. 0 is day topical treatment was started. Days before topical treatment was started are numbered negatively; days after topical treatment was started are numbered positively. R indicates right eye; L, left eye; PI, echothiophate iodide; ATR, atropine sulfate; a, readings taken 1 to 2.5 hours apart; arrows, sequence of readings. Pentobarbital anesthesia supplemented as necessary (see text). Right eye of vervet 34 accidentally given echothiophate iodide, 10 µg, plus atropine sulfate, 60 µg, on day No. +24, and echothiophate iodide, 63 µg, on day No. +227 prior to refraction. Latter error accounts for high but progressively decreasing myopia on days No. +227, +229, and +232. Note complete reversal of echothiophate-induced myopia in both eyes after ganglionic blockade by intramuscularly administered hexamethonium bromide, 20 mg/kg, on day No. +232 (triangles, refraction after hexamethonium).

and day No. +27 in the cynomolgus. The baseline refractions on days No. +73 and +101 in the vervets and on days No. +60 and +88 in the cynomolgus seemed to indicate that this atropine dose was sufficient to block the cholinergic effect of this echothiophate dose on the accommodative mechanism under deep anesthesia.

The experiments on days No. +101 and +106 in the vervets and on days No. +88 and +93 in the cynomolgus were done to determine the degree of cycloplegia during the entire 9 to 24 hours between successive topical treatments and whether the refraction in the deeply anesthetized animal was likely to be comparable to that in the unanesthetized animal (which might well have very different cholinergic tone). On day No. +101 (or +88), baseline refraction was determined about 90 minutes after the AM topical treatment and five subsequent refractions were determined at intervals of 1 to 2.5 hours, the last refraction being done about 10 hours after the AM topical treatment. On day No. +106 (or +93), the AM topical treatment was omitted, baseline refraction was determined 12 hours after the previous PM topical treatment, and four subsequent refractions were determined at intervals of 1.5 to 2.5 hours, the last refraction being done 20.5 to 21 hours after the previous topical treatment. At the time of each refraction rectal temperature was determined and depth of anesthesia was subjectively evaluated (presence or absence of spontaneous eye or body movements; degree of muscle relaxation; reaction to pinching skin of arm, to being placed in the head-holder, and to digital separation of lids). On both days, the monkey was deeply anesthetized with a single intramuscular dose of pentobarbital sodium (30 to 35 mg/kg) following induction with intramuscularly administered methohexitone sodium (15 mg/kg). No further anesthesia was given until the monkey became so "light" that it exhibited spontaneous movements or resisted the head-holder or digital separation of the lids. Even then, supplemental anesthesia (intramuscularly administered pentobarbital sodium, approximately 10 mg/kg) was with-

held until after the refraction had been performed. Supplemental anesthesia was required once (four experiments) or twice (four experiments) during each of the eight experiments.

In the echothiophate only and echothiophate plus atropine treated eyes, the refractions were less myopic when anesthesia was deep (eye and body movements absent, rectal temperature low) and more myopic when anesthesia was light (eye or body movements present, rectal temperature near normal). These findings pertained to both the 1.5 to 10 hour and 12 to 21 hour posttopical treatment experiments. The nontreated verret eyes showed but little variation in refraction.

The echothiophate only treated eye of the cynomolgus demonstrated near maximum myopia on most occasions after the first few weeks of echothiophate treatment. The previously echothiophate-only-treated eyes of the vervets had maintained near-maximum myopia during the entire course of their previous eight week echothiophate treatment.¹⁰ After the echothiophate plus atropine treated eyes of vervets 34 and 38 were switched to echothiophate alone, their refractions became essentially maximally myopic. In the one animal tested (verret 34), this induced myopia was reversed completely by ganglionic blockade with intramuscularly administered hexamethonium, indicating that the induced myopia was entirely accommodative.

No eye showed aqueous cells or flare, and no animal showed signs of systemic toxicity at any time.

COMMENT

Topical echothiophate iodide, 63 µg twice daily, consistently induces cataracts in cynomolgus⁸ and verret⁹ monkeys. This echothiophate dose or a lower one produced cataracts in the non-atropine-treated eyes of all the monkeys used in the current experiments. With topical atropine sulfate, 750 µg, plus echothiophate iodide, 63 µg, twice daily, cataractogenesis was prevented entirely or was of much delayed onset and diminished severity. Thus, atropine inhibits echothio-

phate cataractogenesis.

Since we had no prior clue as to what atropine dose to try, we arbitrarily decided to use an atropine dose sufficient to completely inhibit echothiophate-induced accommodation in all the animals. The animals had been treated for more than three months before we discovered that the plateau atropine dose we finally selected did not always do this. Under deep barbiturate anesthesia, the echothiophate plus atropine treated eyes exhibited little or no echothiophate-induced myopia at the full atropine dose. However, when barbiturate anesthesia was light, considerable accommodation occurred. We assume that increased cholinergic tone under light as compared to deep barbiturate anesthesia caused release of more acetylcholine which, protected by the echothiophate, accumulated in sufficiently high local concentration to cause substantial accommodation even in the presence of large amounts of the competitive inhibitor atropine. We do not know how often or how much accommodation occurred when the monkeys were not anesthetized. Fortunately, the atropine dose we used, although not sufficient to prevent echothiophate-induced accommodation under all conditions, was large enough to inhibit cataractogenesis. Had the echothiophate plus atropine-treated eyes all developed typical cataracts within the usual time, we would not have known whether atropine was ineffective per se, or whether the atropine dose was merely inadequate.

Our experiments do not define the mechanism of echothiophate cataractogenesis in primates. That atropine is inhibitory at a dose that at least partially prevents echothiophate-induced accommodation may indicate that a cholinergic effect, either mechanical (eg, accommodation itself¹¹) or biochemical (eg, a cholinergically induced, atropine inhibitable change in aqueous humor composition¹²), is involved. However, a direct toxic effect of echothiophate on the lens still cannot be ruled out. Atropine inhibits the incorporation of ³²P from labeled DF ³²P in the nuclear, mitochondrial, microsomal, and superna-

tant fractions of rat liver.¹⁴ If atropine has a similar action in the lens, it might protect the lens against a toxic cataractogenic effect of cholinesterase inhibitors.

The diminution of echothiophate-induced vacuoles and other opacities despite continued echothiophate treatment as seen biomicroscopically does not necessarily mean that the underlying lesion is not progressing. We are seeing early changes, the visi-

bility of which may have little bearing on the longer term prognosis for the health of the lens. Similarly, it is possible that atropine inhibits the optical manifestations of a biochemical lesion it does not prevent.

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Nonproprietary Name and Trademark of Drug

Echothiophate iodide—*Phospholine Iodide*.

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