FINAL REPORT

The effects of anticholinesterases and atropine derivatives on visual function in human subjects.

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This project has been concerned with the action of anticholinesterases on vision and the degree of antagonism by atropine sulphate. The main test of visual function was the measurement of contrast sensitivity for the detection of a sine wave grating pattern of specified spatial frequency. The following experiments were undertaken on volunteer subjects with their informed consent.

(1) A quantitative study of the effects of 2mm and 3mm artifical pupils compared with the dilated pupil and of external positive lenses of 1, 2 and 4 dioptres was undertaken in homatropinized and natural eyes. Changes in pupil diameter were without effect on contrast sensitivity for the range 0.5-38 c/deg with the exception of a significant reduction caused by the 2mm pupil at 0.5 and 1 c/deg. Defocus had a relatively small effect on contrast sensitivity at 0.5-3 c/deg, causing a 19% reduction per dioptre in the homatropinized eye. At 3-38 c/deg, a stepwise parallel reduction in which higher and lower spatial frequencies were reduced proportionately by 51% per dioptre in the homatropinized eye was recorded.

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19. ABSTRACT continued

Hence defocus is a far more serious consideration than a change in pupil diameter.
(2) The effects on vision of ingestion of the anticholinesterase pyridostigmine bromide (60mg), assessed from pharmacokinetic data to provide at least 20% inhibition of blood cholinesterase over the experimental period of 1½-4½hr, was compared with 60mg lactose on a double blind crossover basis. Contrast sensitivity to stationary oscilloscope-generated gratings of 3-40c/deg showed a small but significant increase of 7% which was consistent with a small reduction in pupil diameter, surmized to cause a small improvement in optical quality. This reduction in pupil diameter was, however, overshadowed by a larger though still non-significant reduction on the second visit to the laboratory compared with the first. Contrast sensitivities to laser interference fringes observed in the Maxwellian view, by which the effects of the optical media are essentially bypassed and thus provide an entirely neural assessment, were unchanged after pyridostigmine. It is concluded that pyridostigmine may be giv as a prophylactic to anticipated exposure to organophosphorus anticholinesterase without a deleterious effect on stationary visual function.
(3) Instillation of the anticholinesterase physostigmine sulphate (0.25% solution) into the e caused a sustained reduction in pupil diameter and a transient increase in accommodation which had a peak at 30 min and had subsided by 90 min. A wide range from nil response to +9 dioptres occurred between subjects and comparisons between 2 families of 3 siblings suggested the possibl involvement of a genetic disposition. Contrast sensitivity to stationary grating patterns of 3-30c/deg and to moving patterns of 0.5-3c/deg was transiently reduced with a time course simil to that of the increase in accommodation. The peak reduction in contrast sensitivity was correlated with the peak amplitude of accommodation, except at 0.5c/deg where contrast sensitivity was essentially unchanged. As well as its action by defocus of the retinal image, physostigmine also reduced contrast sensitivity by a direct action on the central nervous syste as shown by a reduction in contrast sensitivity to laser interference fringes observed in the Maxwellian view, and by reduction of critical fusion frequency.
(4) A single intramuscular injection of 2mg atropine sulphate produced the well known effects of increased heart rate, dryness of the mouth, increased pupil diameter and reduced accommodati range. Visual acuity, stereoacuity, red-green colour balance and reaction time to a visual stimulus were unaffected by atropine, while extra-ocular muscle balance (horizontal heterophori and cyclophoria) underwent a transient change. There was no significant change in contrast sensitivity measurements to stationary sinusoidal grating patterns of spatial frequencies 1-30 c/deg; however contrast sensitivity to moving grating patterns of spatial frequencies 1-5 c/deg showed a sustained reduction which was still present at 6hr post-injection. It is concluded that atropine adversely affects movement detection but not stationary visual function.
(5) An intramuscular injection of 2mg atropine sulphate was given at either 8 min or 120 min prior to instillation of 0.25% physostigmine sulphate eyedrops. In this way, the maximum accommodative change and the concomitant reduction in contrast sensitivity caused by physostigmine coincided with, respectively, the peak plasma atropine concentration or the fully developed mydriasis and reduction of near point accommodation caused by atropine. Atropine at both times did not affect the miosis, the reduction in near point, the increase in accommodatio or the reduction in contrast sensitivity caused by physostigmine. Contrast sensitivity to a phase-reversed grating pattern was actually diminished by atropine, though this was not statistically significant. By contrast, 2% homatropine hydrobromide eyedrops did effectively antagonize physostigmine's actions. This indicates that the rate of delivery of atropine from t intramuscular injection was insufficient to compete against the ocular effects of physostigmine

In conclusion, if visual function were the sole consideration, the standard intramuscular injection of 2mg atropine sulphate as treatment against anticholinesterase poisoning is ineffective. It has a deleterious effect on movement perception at low spatial frequencies if injected prematurely and is ineffective in antagonizing the deleterious effects of an anticholinesterase applied topically to the eye. This is due to delivery of insufficient atropine to counter the anticholinesterase in ocular tissues. In this case, atropine may actually worsen the deleterious effect on movement perception.

SUMMARY

This project has been concerned with the action of anticholinesterases on vision and the degree of antagonism by atropine sulphate. The main test of visual function was the measurement of contrast sensitivity for the detection of a sine wave grating pattern of specified spatial frequency. The following experiments were undertaken on volunteer subjects, with their informed consent.

(1) A quantitative study of the effects of 2mm and 3mm artificial pupils compared with the dilated pupil and of external positive lenses of 1, 2 and 4 dioptres was undertaken in homatropinized and natural eyes. Changes in pupil diameter were without effect on contrast sensitivity for the range 0.5-38 c/deg with the exception of a significant reduction caused by the the 2mm pupil at 0.5 and 1c/deg. Defocus had a relatively small effect on contrast sensitivity at 0.5-3 c/deg, causing a 19% reduction per dioptre in the homatropinized eye. At 3-38 c/deg, a stepwise parallel reduction in which higher and lower spatial frequencies were reduced proportionately by 51% per dioptre in the homatropinized eye was recorded. Hence defocus is a far more serious consideration than a change in pupil diameter.

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quality. This reduction in pupil diameter was, however, overshadowed by a larger though still non-significant reduction on the second visit to the laboratory compared with the first. Contrast sensitivities to laser interference fringes observed in the Maxwellian view, by which the effects of the optical media are essentially bypassed and thus provide an entirely neural assessment, were unchanged after pyridostigmine. It is concluded that pyridostigmine may be given as a prophylactic to anticipated exposure to organophosphorus anticholinesterase without a deleterious effect on stationary visual function.

(3) Instillation of the anticholinesterase physostigmine sulphate (0.25% solution) into the eye caused a sustained reduction in pupil diameter and a transient increase in accommodation which had a peak at 30 min and had subsided by 90 min. A wide range from nil response to +9 dioptres occurred between subjects and comparisons between 2 families of 3 siblings suggested the possible involvement of a genetic disposition. Contrast sensitivity to stationary grating patterns of 3-30 c/deg and to moving patterns of 0.5-3 c/deg was transiently reduced with a time course similar to that of the increase in accommodation. The peak reduction in contrast sensitivity was correlated with the peak amplitude of accommodation, except at 0.5 c/deg where contrast sensitivity was essentially unchanged. As well as its action by defocus of the retinal image, physostigmine also reduced contrast sensitivity by a direct action on the central nervous system as shown by a reduction in contrast sensitivity to laser interference fringes observed in the Maxwellian view, and by reduction of critical fusion frequency.

(4) A single intramuscular injection of 2mg atropine sulphate produced the well known effects of increased heart rate, dryness of the mouth, increased pupil diameter and reduced accommodative range. Visual acuity, stereoacuity, red-green colour balance and reaction time to a visual stimulus were unaffected by atropine, while extra-ocular muscle balance (horizontal heterophoria and cyclophoria) underwent a transient change. There was no significant change in contrast sensitivity measurements to stationary sinusoidal grating patterns of spatial frequencies 1-30 c/deg; however contrast sensitivity to moving grating patterns of spatial frequencies 1-5 c/deg showed a sustained reduction which was still present at 6hr post-injection. It is concluded that atropine adversely affects movement detection but not stationary visual function.
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INTRODUCTION

The aim of the present study has been to determine the effects of anticholinesterases on vision and assess the protective value of an intramuscular injection of 2mg atropine sulphate, which is the standard treatment against anticholinesterase poisoning (Anon, 1972). Anticholinesterases have their action by inhibition of the enzyme acetylcholinesterase which terminates the action of the neurotransmitter acetylcholine, thus potentiating its action. Physostigmine (eserine) was the first anticholinesterase to be discovered and its actions in causing constriction of the pupil and a "loss of vision", which may be attributed to spasm of accommodation, were described by Thomas Fraser of Edinburgh University in 1863 (Fraser, 1863). Such anticholinesterases are termed to be of the reversible type since their actions are relatively transient due to regeneration of the tissue cholinesterase. A much more potent variety consists of the irreversible organophosphorus anticholinesterases which, in addition to their great potency, form a stable phosphonated complex with cholinesterase, resulting in effects of very long duration. The compounds which now have the code letters GA (Tabun), GB (Sarin), GD (Soman) and VX were synthesized in secrete by the German War Department during the Second World War with a view to their employment as poisonous gases (Anon, 1972). Alkylated fluoridated organophosphorus anticholinesterases, of which DFP (diisopropylfluorophosphate) is the most notable, were also synthesized during the Second World War in the UK (Kilby and Kilby, 1947) and have later proved to be useful commercially as insecticides.

The systemic effects of organophosphorus anticholinesterases have been reported in detail (eg. Sidell, 1974) but those on vision are less

well understood. A pin-point pupil of as small as 1mm and blurring of vision are generally described (Anon, 1972; Grob, 1956; 1963; Grob and Harvey, 1958; Cullumbine, 1963; Smith, Stavinoha and Ryan, 1968; Wood, 1950). By contrast, the data for reversible anticholinesterases are more detailed. Douglas Argyll Robertson, a colleague of Thomas Fraser, described that pupil diameter was constricted by 75% after 12 hr and the amplitude of accommodation was +5D after 30 min after instillation of a physostigmine extract to the eye (Argyll Robertson, 1863). Rengstorff (1970) has produced data for the time-course of miosis and increase in accommodation and described that application of 2 drops had a greater effect than 1 drop of proprietary 0.5% physostigmine salicylate.

The consequences of the actions of anticholinesterases on the eye and visual system may be subdivided as follows:

Pupil diameter

Pupil diameter has marked effects on image quality as shown by psychophysical studies with the Landolt C (Jenkins, 1963), the Snellen test (Tucker and Charman, 1975) and sinusoidal grating patterns (Arnulf and Dupuy, 1960; Campbell and Green, 1965; van Meeteren, 1974; Charman, 1979; and Bour, 1980). Objective measurements have also been made of the line-spread function by scanning photo-electrically the faintly reflected fundal image of a line of light, from which the modulation transfer characteristics of the optical media at different spatial frequencies can be calculated (Westheimer and Campbell, 1962; Campbell and Gubisch, 1966; Charman and Jennings, 1976). With monochromatic light, in a perfectly diffraction-limited system, image contrast declines approximately linearly with spatial frequency until the cut-off frequency determined by a/λ (a is pupil diameter and λ is wavelength) is

reached (Westheimer, 1964). However, for the human eye, additional marked attenuation of contrast occurs, particularly in the range of intermediate spatial frequencies, due to the effects of geometrical aberrations. The aberrations have been assessed for the human eye by van Meeteren (1974) who concluded that, in white light, chromatic differences of focus were most important. Others have, however, rated the effects of spherical aberrations as being of equal importance to chromatic aberrations (Gubisch, 1967). More recently, a new perspective on *monochromatic* aberrations was presented by Howland and Howland (1976; 1977) who derived the magnitudes of the different aberrations from drawings made by their subjects of a grid pattern viewed through 2 crossed cylindrical lenses. This approach was continued by Walsh and Charman (1985) who photographed the retinal image and derived phase as well as amplitude relationships. The important finding was that two sets of aberrations, hitherto unmeasured and tacitly assumed to be of negligible importance viz. coma and cylindrical aberrations (van Meeteren, 1974), were shown to predominate at all diameters of pupil.

The aberration effects are reduced as pupil diameter decreases until an approximately diffraction-limited system is attained at pupil diameters of 2.0-3.0 mm (Arnulf and Dupuy, 1960; Bour, 1980; Campbell and Green, 1965; Campbell and Gubisch, 1966; van Meeteren, 1974; and Howland and Howland, 1977). Further reductions in pupil diameter to below the optimal value lead to increased effects of diffraction from around the edge of the pupil. Comparison between theoretical predictions of the in-focus retinal image quality based on the effects of both aberrations and diffraction and actual measured data for different pupil diameters revealed close agreement (van Meeteren, 1974).

Defocus

The theory of the effects of defocus is relatively more complex compared with that for different pupil diameters. Defocus leads to an accelerated fall in image contrast with increasing spatial frequency as illustrated in Fig 1 which is taken from Hopkins (1955). Above 0.25D (n=2 in Fig 1), the transfer function of a diffraction-limited system is predicted to cross the abscissa to give contrast reversal, with the reversal occurring at lower spatial frequencies for greater defocus.

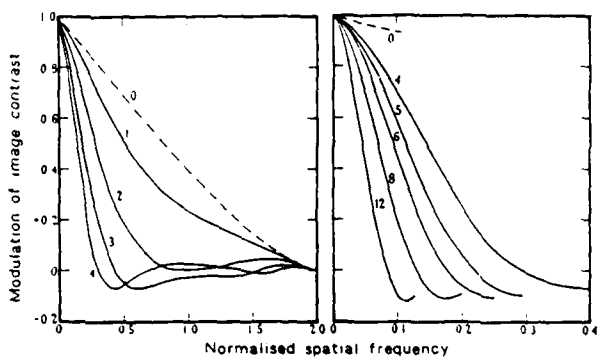

Fig. 1 Computations by Hopkins (1955) of the effects of defocus on the modulation transfer function for a diffraction-limited optical system. Spatial frequency is normalized so that the value of 2.0 corresponds to the cut-off value for the system (this cut-off is given by $(1.746 \times 10^4 a)\lambda^{-1}$ c deg^{-1} for the eye, where a is the *pupil* diameter in mm and λ is the wavelength in nm). The figures on the curves give the defocus in terms of the parameter n, where the corresponding wavefront aberration is $w_{18} = (n/\pi)\lambda$. A value of $n = 1$ corresponds to a *defocus* of approximately 0.125 D when the eye has a pupil diameter of 3.3 mm and the wavelength is 555 nm. Note the expanded abscissa on the right hand graph. Reproduced with permission from Hopkins (1955).

The agreement between theoretical predictions and measurements for a simple glass lens is very close (Hopkins, 1962), though for measurements of contrast sensitivity and optical quality of the human eye, however, the correspondence is not so close. Contrast reversal *per se* is not a cause for concern in that contrast is detected by the visual system irrespective of phase, but minima of zero contrast have not been

reported and image contrast generally is higher than predicted (Campbell and Green, 1965: Charman, 1979). On the other hand, the measurement of line spread functions do tend to show close agreement with predicted results (Charman and Jennings, 1976).

The deleterious effects of defocus are generally described as being more severe for higher spatial frequencies with an absence of effect at very low spatial frequencies (Campbell and Green, 1965; and Regan, Silver and Murray, 1977). Qualifications must be added due to the fact that Campbell and Green's observations were made at a small (2mm) pupil diameter and Regan et al's results were part of a larger study into multiple sclerosis and involved only a limited range of spatial frequencies with one level of defocus in the natural eye. Contrast sensitivities at low spatial frequencies are, however, attenuated with a larger pupil diameter (Green and Campbell, 1965), the use of which also gives rise to a further complication when spherical aberration is present. The refractions required to bring different spatial frequencies into focus differ eg. focus of high spatial frequencies may require an *additional +0.8D compared with low spatial frequencies* (Green and Campbell, 1965). The visual system is also able to tolerate a degree of defocus blur. Hopkins (1962) has calculated that, for a diffraction-limited system, image contrast deteriorated rapidly when the Rayleigh criterion of $\lambda/4$ (approximately 0.03D) was exceeded while psychophysical measurements of tolerance of defocus blur indicate a larger value of 0.20-0.25D (Whiteside, 1957; Campbell and Westheimer, 1958).

Central actions

Anticholinesterases also have definite actions on the central nervous system, even when applied as eyedrops, as shown by Alpern and Jampel (1959) who demonstrated that physostigmine eyedrops caused a significant reduction in critical fusion frequency which was independent of defocus and was not reproduced by instillation of pilocarpine. Visual thresholds in the central visual field are elevated by both physostigmine eyedrops and Sarin vapour (Gazzard and Price Thomas, 1975). Sarin, given as vapour but not as eyedrops, elevated dark adaptation thresholds, especially that of rods, which was a long lasting effect (Rubin and Goldberg, 1957; Rubin, Krop and Goldberg, 1957). The central action was furthermore confirmed by the finding that Sarin's action was antagonized by atropine sulphate which crosses the blood-brain barrier but not by atropine methyl nitrate which does not (Rubin and Goldberg, 1958). In all studies, the effects of anticholinesterases were independent of the miosis which they caused. The possibility also exists, in theory at least, that the central actions of physostigmine may also be contributed to by its non-neural actions in reducing intraocular pressure resulting from constriction of the pupil or by causing a generalized hyperaemia (Leopold, 1961).

Atropine

The usefulness of atropine sulphate in anatagonizing the systemic actions of anticholinesterases was recognized by Thomas Fraser (1870), while the antagonism of physostigmine extract given as eyedrops by atropine was described by Argyll Robertson (1863). (Fraser's work, though published later, actually predated that of his colleague Argyll Robertson to whom he provided the extract, called "Eseria").

Atropine is now well known for its anatagonism of the neurotransmitter acetylcholine at muscarinic receptors which occur throughout the body. Its effects are widespread, causing increased heart rate, depressed motility of the gastro-intestinal tract, inhibition of micturition, reduced secretions of the salivary glands, gastro-intestinal tract, bronchioles and sweat glands, dilation of the pupils by blocking of the circular smooth muscle of the iris and paralysis of accommodation by blocking of the ciliary muscles (Martindale, 1982; Headley, 1982). In sulphate form, atropine also crosses the blood-brain barrier to cause giddiness and ataxia.

Atropine is used as a premedication in surgery to obviate vagal inhibition of the heart and to inhibit salivary, gastro-intestinal and pulmonary secretions. It is also employed to counter poisoning by organophosphorus anticholinesterases. As part of their standard equipment, UK servicemen carry 3 self-inject syringes each containing 2mg atropine sulphate in 1ml saline, this being the standard first aid measure against cholinesterase poisoning (Miles, 1955; Anon, 1972). Pilots involved in crop spraying with organophosphorus insecticides a e also reported to administer atropine to counter contamination by the insecticides (Dille and Smith, 1964). There arises the question of what would be the effects of premature injection of atropine. Much of the early work concentrated on the effects on physical performance with less attention paid to visual function. Moylan-Jones (1969) showed that an injection of 6 mg atropine reduced soldiers' work rate assessed by the volume of earth excavated whereas shooting ability was unimpaired. However, due to the increasing importance of rapid detection of objects on radar displays, alignment of sophisticated weaponry and the

considerable demands involved in flying aircraft at high speed and low altitude, even the smallest decrement in visual function may be of significance. Previous visual assessments have been based on the Snellen test but, as Ginsburg, Evans, Sekuler and Harp (1982) have shown, this is not as accurate a predictor of visual performance as the more recently introduced test of measuring contrast sensitivity to sinusoidal grating patterns.

Following an intramuscular injection of 1 mg atropine sulphate, the plasma concentration rises to a peak after 30 min and then declines gradually with a measurable amount still remaining at 4hr (Berghem, Bergman, Schildt and Sorbo, 1980). Similar results were reported for a 2mg intramuscular injection which was monitored over 60min (Kalser and McLain, 1970). The results of this study indicated a close correlation between plasma atropine concentration and heart rate, though Mirakhur (1978) and Baker, Adams, Jampolsky, Brown, Haegerstrom-Portnoy and Jones (1983) reported that the peak effect occurred at 1hr Ocular effects are much longer lasting: dilation of the pupil and impairment of accommodation persisted beyond 4 hr (Herxheimer, 1958; Rozsival and Cigánek, 1978; Mirakhur, 1978; Baker et al, 1983), thus making close tasks requiring accommodation very difficult (Moylan-Jones, 1969; Rozsival and Cigánek, 1978). The effects on distance vision seem rather slight: no change in Snellen acuity was noted (Cullumbine, McKee and Creasey, 1955; Miles, 1955; Rozsival and Cigánek, 1978), contrast sensitivity over 1-20 c/deg was unaffected except at 5 and 20c/deg when it was significantly reduced at 4 hr post-injection (Baker et al, 1983).

Contrast sensitivity to moving grating patterns has, however, not been tested. Stereoacuity was unaffected although the test did not measure below 10 sec arc compared with expected normal values of *circa* 2 sec arc (Anderson and Weymouth, 1923). Visual reaction times were reported to be impaired in some cases but not in others (Miles, 1955; Holland, Kemp and Wetherell, 1978).

Rational of present study

(1) Artificial pupils and defocusing lenses

A study of the effects of artificial pupils and external defocusing lenses was undertaken prior to examination of the action of anticholinesterases. Of particular interest to us was to make measurements which could be used practically to assess the effects of defocus and pupil diameter under natural circumstances. Several reasons exist why this cannot be done from existing data. One factor is the relatively small number of subjects studied in any depth - 2 by Campbell and Green (1965) and one by Charman (1979). Howland and Howland (1977) who studied a total of 55 eyes reported a 4 fold range of variability in contrast modulation at 20 c/deg, thus emphasizing the need for a relatively large sample. We have, therefore, carried out our measurements on a total of 12 subjects in order to go some way to meeting this point. Previous studies also used a cycloplegic drug to remove the fluctuations in accommodation (Campbell, Robson and Westheimer, 1959) and pupil diameter in order that these might be controlled precisely experimentally. This may of its own account adversely affect contrast sensitivity measurements and should the cycloplegic drug diffuse into the retina, neuronal activity may also be affected. Therefore, we took the precaution of repeating our experiments

in a number of subjects without homatropine to check against such possible effects. In our experiments, pupil diameter was studied in the *absence* of compensation for changes in retinal illumination, as usually practised, to reproduce what occurs in natural viewing conditions. Likewise, defocus was studied at small pupil diameters to reflect the well known link between accommodation and pupil diameter. Our method of assessment was to measure contrast sensitivity to sinusoidally modulated grating patterns generated on a cathode ray tube (CRT), since this provides a more complete description of visual function. This study has been published in full as Kay and Morrison (1987a).

(2) Ingestion of pyridostigmine

The effects of systemic anticholinesterase on vision was studied by determining the effects of ingestion of 60mg pyridostigmine bromide. Pyridostigmine is a synthetic, quaternary inhibitor of the enzyme cholinesterase which terminates the action of the neurotransmitter acetylcholine. It binds at the esteratic and anionic sites of the cholinesterase molecule. The bond which is formed between the esteratic site and the carbamyl group of pyridostigmine is hydrolysed relatively rapidy thus resulting in a relatively short lasting inhibition of the cholinesterase molecule (Bowman and Rand, 1980). By contrast, the organophosphorus anticholinesterases form a very stable phosphonated link with the esteratic site resulting in a very long lasting inhibition of cholinesterase. The consequence of substantial cholinesterase inhibition is inevitably death due to respiratory or cardiac failure (Grob, 1956; 1963; Grob and Harvey, 1953). Protection against organophosphorus anticholinesterase poisoning is, however, afforded by prior treatment with a carbamate anticholinesterase in a dose which has

no significant actions *per se*, which was first described for physostigmine given prior to injection of an otherwise lethal dose of DFP, in the cat (Koster, 1946). In this respect, pyridostigmine is one of the most effective treatments against poisoning by Soman in guinea pigs (Gordon, Leadbeater and Maidment, 1978) even though it is reported not to penetrate into the brain following an intramuscular injection, in the rat (Birtley, Roberts and Thomas, 1966). It is proposed that the carbamate anticholinesterase occupies a proportion of cholinesterase active sites thus preventing binding by the organophosphorus anticholinesterase which is, in time, excreted from the body. Dissociation of the carbamate-cholinesterase complex then releases free cholinesterase (Koelle, 1946) for which only a relatively small proportion of the total cholinesterase seems to be required for normal function. This action of the carbamate may be sufficient to preserve life. There is a considerable species difference: for instance, pyridostigmine has practically no protective effect in rats (Gordon et al, 1978), though a protective action of pyridostigmine against Soman has been demonstrated in primates (Dirnhuber and Green, 1978; Dirnhuber, French, Green Leadbeater and Stratton, 1979).

Our concern was to test whether ingestion of 60mg pyridostigmine, the maximum dose tolerated by the gastro-intestinal tract (Dr R.I. Gleadle, personal communication), has a significant effect on vision as assessed by measurement of contrast sensitivity to a sinuoidal grating pattern of specified spatial frequencies. Hitherto, the available data appear to be those of Borland, Brennan, Nicholson and Smith (1985).

For repeated ingestion of 30 mg every 8hr for 3 days by 4 subjects during which they were assessed by tests of psychomotor function. They mentioned without elaboration that there was no effect on contrast sensitivity.

The time course of our experiments was determined by the time-course of blood cholinesterase inhibition following pyridostigmine absorption, which was made available by Dr. R.I. Gleadle for ingestion of 30mg with a single determination for ingestion of 60mg (Fig 2). We have displaced the graph for 30 mg to the 60mg point. The period of interest was when blood cholinesterase is inhibited by 20%, when the protective effect of prior pyridostigmine administration is optimal (R.I. Gleadle, personal communication). This condition is fulfilled for 30 mg from 1 to 5hr after ingestion. There a considerable range in the safety ratio of the carbamate, so the percentage inhibition of cholinesterase need not be precise. The duration of action for human subjects is, in fact, similar to the duration of the protective effect of an injection of pyridostigmine against Soman in guinea pigs (Gordon et al, 1978). Visual function was assessed by two methods. First, measurements of contrast sensitivity to grating patterns generated by conventional cathode ray tube (CRT) in which the display is focused by the eye and thus subject to optical aberrations were made. Second, measurements were obtained in response to laser interference fringes observed in the Maxwellian view in which the target is focused in approximately the plane of the pupil by a microscope objective (the Maxwellian lens) (Morrison and McGrath, 1985). The virtue of this display is that, since it is not refracted by the eye and is thus independent of optical aberrations, it provides an assessment of neural function.

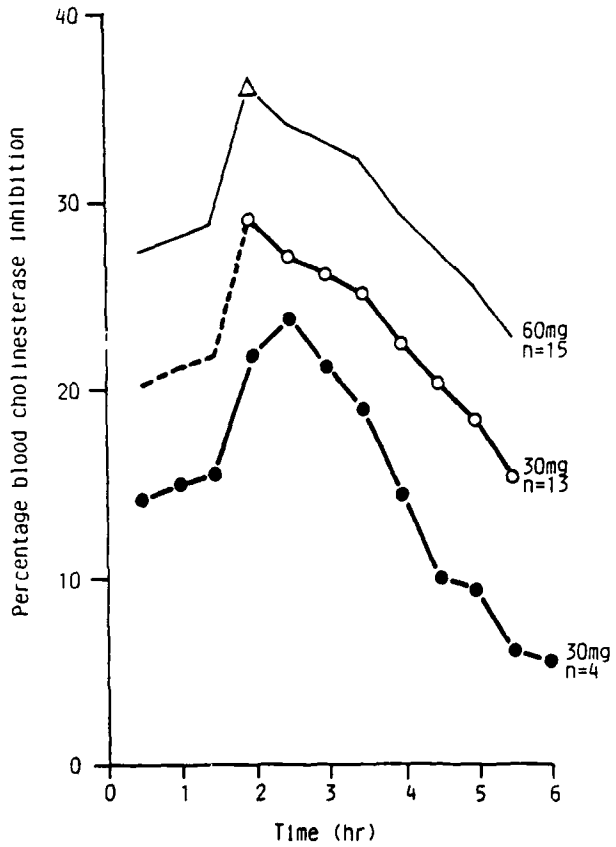

Fig.2 Time course of inhibition of blood cholinesterase after ingestion of pyridostigmine. Lower graph shows complete data for 4 subjects after ingestion of 30mg. Middle graph is also after ingestion of 30mg but for 13 subjects from 2hr onwards. Broken line is transposed part of n=4 curve. Upper graph shows single determination for 15 subjects at 2hr after ingestion of 60mg (△). Graph from n=13 data has been transposed through this point (from CDE. Porton Down, unpublished data, by permission)

(3) Physostigmine eyedrops

The effects of the reversible anticholinesterase, physostigmine sulphate, given as eyedrops was tested since it is known to evoke a marked reduction in pupil diameter and an increase in accommodation (Rengstorff, 1970). Instillation of eyedrops is, however, complex (Shell, 1982). The normal tear volume is some 7μL compared with a typical drop volume of 50-70 .. Provided blinking is prevented, the cul de sac is capable of holding some 30μL. Initially, some 80% of the instilled fluid may be drained rapidly through the naso-lacrimal canal, whence it enters the bloodstream. The rate of drainage is actually greater for greater instilled volumes, leading in turn to the possibility of appreciable systemic absorption of the drug. This may provide another route, in addition to transcorneal absorption, by which physostigmine eyedrops have their central actions.

We have determined the effects of physostigmine eyedrops on visual function by measurement of pupil diameter, accommodation for distance, near point accommodation and contrast sensitivity to a range of spatial frequencies of stationary and moving grating patterns to which the human visual system is sensitive (Kulikowski and Tolhurst, 1973). The central actions of physostigmine were assessed by measurement of contrast sensitivity to laser interference fringes observed in the Maxwellian view (Campbell and Green, 1965) and of critical fusion frequency. A preliminary account of part of this work has been presented previously (Kay and Morrison, 1987b).

(4) Atropine injection

We have undertaken a more comprehensive study of the effects of atropine on visual function, which encompasses the measurement of contrast sensitivity over an extended range of spatial frequencies for stationary sinusoidal gratings patterns up to 30 c/deg and for moving grating patterns up to 5 c/deg which is the optimal range for movement detection (Kulikowski and Tolhurst, 1973; Tolhurst, 1973). Stereoacuity, red-green colour balance, reaction time and choice reaction time to a visual stimulus and extra-ocular muscle balance have also been measured. This work has been published as Kay and Morrison (1987c).

(5) Physostigmine eyedrops and atropine injection

Next, we used physostigmine eyedrops against which the effectiveness of intramuscular injection of 2mg atropine sulphate was tested. First, atropine sulphate was injected some 8 min prior to physostigmine eyedrops, in order that the peak plasma atropine concentration shown to occur at 30 min post-injection (Berghem et al, 1980) coincided with the peak action of physostigmine on contrast sensitivity and defocus (Kay and Morrison, 1987b). Second, atropine sulphate was injected some 120 min prior to physostigmine to allow its ocular actions in dilating the pupil and reducing near point accommmodation to develop fully (Kay and Morrison, 1987c). Third, physostigmine was tested against prior instillation of homatropine hydrobromide eyedrops, this being preferred to atropine which has a much more persistent action. A communication of part of this work has been made previously (Kay and Morrison, 1987b).

METHODS

Common measurements

First, each subject underwent the Snellen test and if additional lenses were required for optimal acuity which was 6/5 or 5/4 depending on whether the viewing distance was 6 or 5 m, respectively, these were worn throughout the experiments. In each trial, pupil diameter was recorded by photography at regular intervals. The pupil diameter in the horizontal plane was taken as the mean of two exposures. Near point was taken as the mean of 3 readings measured with the RAF rule. Measurements prior to physostigmine instillation were made with a 2mm artificial pupil to take account of the anticipated miosis. The amplitude of accommodation for distance was measured as the strength of spherical negative lens required to bring the bottom line of the Snellen chart into focus.

Contrast sensitivity to the oscilloscope display

This was the mean of 5 readings obtained with the ascending method by which the subject increased the contrast of the grating display from a uniform field until the grating pattern was just visible. This gives results very similar to those obtained by the more time consuming method of determining contrast sensitivity for 50% detection of the grating pattern (Morrison and Reilly, 1986). The grating display was generated on a Tektronix 606B monitor of screen luminance 10.2 cd/m^2 and peak wavelength 520nm. The time base was provided from the time base output of a Tektronix 5103 oscilloscope running at 0.5msec/div. The grating display was generated by modulation of a raster, formed by a 770kHz triangular wave fed into the Y amplifier of the monitor, by a sine wave fed into the Z amplifier. The 5103 oscilloscope was also used to monitor

the frequency and amplitude of this sine wave. The contrast of the grating pattern was determined from the calibration graph of contrast against the peak to peak amplitude of the Z modulation voltage, obtained by the psychophysical method of Campbell and Green (1965). The subject controlled the amplitude of this sine wave by means of a control unit incorporating a ten turn potentiometer. The experimenter was able to control independently the sensitivity of this unit, thus precluding any tendency of the subject to arrive at contrast threshold by counting the number of turns of the potentiometer. Spatial frequencies 8-30 c/deg and visual acuity were measured at 2.86m where the CRT screen subtended 2 deg arc, while spatial frequencies 1-8 c/deg were viewed at 1.43m where the CRT screen subtended 4 deg arc. This was done in order to maintain the number of cycles below the limit at which the performance of the CRT declined and above the number at which contrast sensitivity was determined by the number of grating cycles rather than by their spatial frequency (Hoekstra, van der Groot, van der Brink and Bilsen, 1974). The combination of measurements from near and far viewing distances into a common contrast sensitivity function had previously been established by Campbell and Robson (1968). Contrast thresholds were also measured to "moving" sinusoidal grating patterns of spatial frequencies 1-5 c/deg. These were generated by phase reversal at 5.5Hz of a grating pattern giving rise to an apparent movement of the pattern (Kulikowski, 1971), known as the phi-phenomenon, which is an effective stimulus for movement detection (Kulikowski and Tolhurst, 1973; Tolhurst, 1973).

In experiments (1) and (2), an older set of apparatus was used, consisting of a Telequipment DM53S oscilloscope with P31 phosphor (peak emission 520nm) and screen luminance 1.4cd/m^2 was employed. For this,

the far viewing distance was 3m, when the screen subtended 1.5 deg, and the near viewing distance was 1m with a screen subtense of 4.5 deg.

Assessment of neural function

Measurements were made of contrast sensitivity to laser interference fringes observed in the Maxwellian view, thus bypassing the effects of the optical media. The field diameter was 7 deg and intensity 3 log units above photopic threshold, which was comfortable for long-term viewing. The apparatus which is described fully elsewhere (Morrison and McGrath, 1985) consisted of a Mach-Zehnder interferometer in which two collimated beams from a helium-neon laser (wavelength 632nm) were combined to produce interference fringes on the retina when viewed through a X2 Leitz objective. By combining the interference beam with a uniform beam of same intensity but polarized at 90 deg, it was possible to increase the contrast of the interference fringes by rotation of a Glan-Thompson calcite polarizer (extinction better than 10^{-6}) in proportion to $\sin^2\theta$ where θ is the angle of rotation. The intensities of the interference beam and the diffuse beam were made equal by adjustment while monitoring with a purpose-made photodiode-amplifier. Spatial frequency which is proportional to the pathlength difference between the 2 laser beams was varied by translation of one of the front silvered mirrors of the interferometer as described in Morrison and McGrath (1985). The spatial frequency of the display was measured precisely by counting the number of cycles with a X100 phase contrast objective and was determined by photography to be 0.75 times the number of cycles within the phase ring of the objective.

Specific Protocols

(1) Artificial pupils and defocus

Prior to the study proper, each subject underwent a familiarization run consisting of spatial frequencies 10, 20 and 30 c/deg. Our experience was that once a subject had successfully practised the highest spatial frequency, he/she became attuned to the method and was reasonably consistent in judgements thereafter. Consistency was actually tested in a series of control experiments described later.

Experimental Protocol

The same standard protocol was followed by all subjects. Five measurements of contrast threshold were made at each of 10, 20, 30, 38, 35, 25, 15 and 10 c/deg viewed at 3m and 8, 3, 1, 0.5 and 5 c/deg at 1m. These were collected for the following conditions.

(a) Natural pupil diameter and optimal accommodation.

(b) Viewing with a 3.0mm artificial pupil and optimal accommodation.

(c) 2.0mm artificial pupil and optimal accommodation.

(d) 2.0mm artificial pupil and +2.0D additional defocus.

(e) 3.0mm artificial pupil and +2.0D additional defocus.

(f) 3.0mm artificial pupil and +4.0D additional defocus.

(g) 3.0mm artificial pupil and +1.0D additional defocus.

(h) Repeat measurements at 10, 20 and 30 c/deg with natural pupil and optimal accommodation.

The artificial pupil was worn in a trial frame which also carried the additional lenses when these were required.

Alternatively, one drop of 2% homatropine hydrobromide solution (Evans Medical Ltd) was instilled, which maximally dilated the pupil and paralyzed accommodation after 20-30 min. With the aid of a purpose-made

Snellen chart, the subject was optimally refracted to within 0.12D for viewing at 1m and 3m, in accordance with the comments of Charman (1979) on the importance of accurate refraction. Then experiments (3)-(8) were repeated, except that (8) was with the dilated pupil. Experimental time was some 6hr with an additional 1-1½hr allowed for breaks.

Control Experiments

Since our experiments were of a demanding nature, though no undue discomfort was ever reported during them, and since we could find no reports in the literature of the consistency of contrast sensitivity measurements bar Ginsburg and Cannon (1983)'s study on different methods, we decided to undertake a set of control measurements. Contrast thresholds were measured at 10, 20, 30, 25, 15, 5, 3, 1 and 8 c/deg to the CRT display at 4 one hourly intervals and once more on a second day. These experiments were undertaken by 13 subjects, 4 of whom also took part in the main series of experiment.

(2) Ingestion of pyridostigmine

This experiment was undertaken by 14 male volunteers ages 18-40 yr. Each subject undertook 2 experimental trials at the same time of day to avoid the possible complication of diurnal changes in brain acetylcholine content, as demonstrated for rats (Hanin, Massarelli & Costa, 1970), and at least 1 hr usually 2 hr, after a light meal. No tea, coffee or tobacco was allowed during the experimental period. On each occasion, the subject ingested a capsule containing either 60mg pyridostigmine bromide (Roche) or 60 mg lactose. These were prepared by the Pharmacy, Western Infirmary, Glasgow. The tests were done on a double blind crossover basis in which neither the subject nor the experimenters were aware of the sequence in which the capsules were

taken. The keys were made available only after all 14 pairs of experiments had been completed. Photographs of pupi' diameter were taken every 30 min under dim illumination when the pupil was well dilated, in order that an effect of pyridostigmine would be more readily discerned.

Experimental Protocol

The time-course of the experimental trials was dictated by the absorption kinetics of pyridostigmine shown in Fig 2. The aim was to complete the measurements while blood cholinesterase was inhibited by at least 20%. The sequence of each experimental run was as follows:

0-1hr Absorption of pyridostigmine- no measurments.

1-1½hr Familiarization determinations of contrast sensitivity to CRT and laser interferometer displays at 10, 20 and 30 c/deg. These were not included in the analysis.

1½-2½hr Contrast sensitivity measurements to CRT display at 10, 20, 30, 35, 38, 25, 15 and 8 c/deg at 3m viewing distance and 5, 3, 1, 6 and 8 c/deg at 1m, in that order.

2½-2¾hr Short break.

2¾-4¾hr Contrast sensitivity measurements to laser display at same spatial frequencies as for CRT.

4¾-4¾hr Repeat measurements for CRT display at 10, 20 and 30 c/deg.

(3) Physostigmine eyedrops

The actions of physostigmine eyedrops were recorded in 12 subjects ages 20-28, of whom 7 were male and 5 were female. Each subject received 2 drops of 0.25% physostigmine sulphate (Evans Medical Ltd) on three separate occasions (except for one subject who completed 2 trials only). After receiving the drops, the subject was restrained from blinking for several minutes to ensure adequate absorption.

Two complete control runs were completed prior to administration of physostigmine eyedrops. In order to obtain the time course of the action of physostigmine, measurements were made in short trials lasting *circa* 20 min. Following physostigmine, 3 tests were made at 20 min intervals and were followed by 4 tests at 30 min intervals. In Trial 1, contrast sensitivity was measured for stationary grating patterns of 3, 10, 20 and 30 c/deg, generated by CRT while in trial 2, contrast sensitivity was measured to grating patterns of 0.5, 1, 2 and 3 c/deg, phase-reversed at 5.5Hz.

In trial 3, contrast sensitivity, taken as the mean of 5 readings to stationary grating patterns of spatial frequencies 4, 15 and 25 c/deg, was measured to the laser interference fringes. Critical fusion frequency was also measured as the mean of 3 readings obtained with the Visual Function Tester of Genco and Task (1984), which was on loan from the US Air Force Office of Scientific Research. This consists of a circular field of 5 deg diameter illuminated by a yellow light-emitting diode and viewed through collimating eyepieces. The frequency of modulation of the display was adjusted until fusion was just observed and the frequency noted from a digital display. The control measurements were made with a 2mm artificial pupil for comparison with the post-physostigmine results when a miosis of some 2mm was anticipated. Since the measurements in Trial 3 were more time consuming, they were repeated at 30 min intervals during the 3hr period following instillation of physostigmine eyedrops.

(4) Injection of atropine

Prior to the experiments, each subject received a routine medical examination by a Medical Officer who also gave the injection of 2.0 mg atropine sulphate in 1ml saline (McCarthys Ltd) into the upper arm. He also remained on-call for the duration of the experiment and discharged the subject after the experiment had been completed. Two major hazards were identified with respect to the atropine injection. First, for the period immediately following the injection, the subject was likely to be susceptible to a vaso-vagal attack resulting in fainting. Thus, for the first 15 min following the injection, the subject was instructed to rest until the heart rate which was monitored continually by palpation started to increase. Second, a possible risk existed in those subjects with an abnormality of the ventricular conducting system eg. the accessory bundle of Kent when an increased risk of ventricular fibrillation existed. In cases of doubt, an electrocardiogram was taken. In fact, no adverse effects other than those anticipated were encountered and all subjects were discharged as satisfactory after the experiment, though driving of a motor vehicle was expressly forbidden.

Throughout all the experiments, no tea, coffee, any drink with additives, or tobacco was permitted, though in the longer (8 hr) experiment, some food together with water, milk or fruit juice was permitted. In all, 13 male subjects ages 21-40yr participated in a total of 23 experiments.

Experimental procedures

In the course of the experiments, heart rate was measured by palpation and pupil diameter by photography every 30 min. The other measurements were taken every hour. Near-point was taken as the mean of

four readings recorded with the RAF rule. Extra-ocular muscle balance was determined with the Visual Function Tester. The subject views the target at infinity through collimating eyepieces. In the test for heterophoria, the right eye views a grid calibrated vertically and horizontally in prism dioptres. On pressing the control button, a point of light appears in the left visual field, the coordinates of which on the grid give both horizontal and vertical heterophoria. In the test for cyclophoria, both eyes view a bullseye pattern to ensure correct fusion of the images in horizontal and vertical planes. The left eye also views an arrow and the right eye a circular scale calibrated in degrees. The amount of cyclophoria *ie.* rotation of one eye with respect to the other is given by the reading on the scale. For both tests, the mean of 4 readings was taken.

Visual acuity was measured as the highest spatial frequency of sinusoidal grating pattern which was resolvable. This is related to Snellen acuity since 30 c/deg is equivalent to 6/6 vision, 36 c/deg to 6/5 vision and 42 c/deg as 6/4 vision. The mean of the best 2 readings from 4 trials was taken as the visual acuity. Stereoacuity was measured as the smallest displacement of a vertical needle in front of or behind 2 vertical needles viewed at 2m against a black background, as described by Anderson and Weymouth (1923). Judgements were made by the subject of the position of the central needle for 15 different positions in the range 3.25mm in front and 3.75mm behind in 0.5mm steps, each repeated 3 times. Stereoacuity was calculated as half the distance between the positions in front and behind the lateral needles when 3 out of 3 correct scores were made. Red-green colour discrimination was determined by the Pickford-Nicolson Anomaloscope (Pickford and Lakowski, 1960) by

which the subject was required to adjust the proportion of red and green light on a scale from 0-80 to determine the range within which there was no perceptible difference from a yellow reference field. The mean of 4-6 determinations was made. For normal subjects, the range lies between 33.6 - 41.2 (99% confidence limits).

Reaction times to a visual stimulus were tested with a purpose-made light emitting diode (*led*) display coupled to a digital latency meter accurate to the nearest 0.1msec. Reaction time was measured as the time to respond to illumination of the red *led*. The choice reaction time involved the response to illumination of one of three *leds* each of which had its own response button. In each case, the mean of 10 determinations was taken.

Contrast sensitivity was measured in response to the following:
(a) Stationary patterns of 10, 20, 30, 25, 15, 8, 10, 5, 3, 1 and 8 c/deg in that order. First, this was done as 4 hourly repeats in order to determine the effects of repeat measurements. On a second occasion, at the same time of day, the injection of 2mg atropine was made after one control set of measurements and followed over 3 hourly sets of measurements. These experiments were undertaken by 13 subjects and heart rate, pupil diameter and near point were also measured.
(b) Moving patterns of 1, 2, 3 and 5 c/deg. Two control sets of measurements were taken prior to injection of 2mg atropine, after which 6 sets of determinations were made at hourly intervals. This study which was done by 8 subjects also included the measurement of heart rate, pupil diameter, near point, extra-ocular muscle balance, visual acuity, stereoacuity, red-green balance and reaction times.

(5) Physostigmine eyedrops and injection of atropine

Each subject underwent a cycle of tests of visual function, which lasted 15-20 min, before and after each of the following treatments:

(i) Intramuscular injection of 2 mg atropine sulphate at 8 min prior to 2 drops of 0.25% physostigmine sulphate eyedrops.

The interval of 8 min was the minimum which could reasonably be allowed due to the need for the subject to rest after the injection. In most subjects, a bradycardia arises immediately after the injection and, at this time, the subject is most susceptible to syncope: once the heart rate had began to increase, physostigmine eyedrops were instilled. Prior to atropine and physostigmine, two sets of control measurements were made. Then, following the treatment, 3 sets of measurements were made in the first hour post-physostigmine and were followed by 4 further sets at half hourly intervals.

(ii) Intramuscular injection of 2 mg atropine sulphate at 120 min prior to 2 drops of 0.25% physostigmine sulphate eyedrops.

Two sets of measurements were made prior to and following atropine. After physostigmine, 3 sets of measurements were made in the first hour and were followed by 2 further sets at half hourly intervals.

(iii) Ocular instillation of 3 drops of 2% homatropine hydrobromide at 60 min prior to 2 drops of 0.25% physostigmine sulphate eyedrops.

Following 3 sets of control measurements, 3 drops of homatropine was given, each at 5 min intervals. Then three further sets of measurements were made prior to the instillation of physostigmine eyedrops. After physostigmine, 3 sets of measurements were made in the first hour and were followed by 4 further sets at half hourly intervals.

Each cycle of measurements consisted of the following: pupil diameter by photography, near point accommodation with the RAF rule, amplitude of accommodation as the power of spherical lens required to bring the bottom line of the Snellen chart viewed at 5m into focus, contrast sensitivity for stationary sinusoidal grating patterns of spatial frequencies 10, 20 and 30 c/deg, and contrast sensitivity for a sinusoidal grating pattern of 3 c/deg, phase reversed at 5.5Hz.

Data Analysis

Comparisons between test and control periods for the same set of subjects were made with the paired t-test using the Minitab package (Ryan, Joiner and Ryan, 1985). Best fitting linear regression equations were calculated and comparisons of their slopes and intercepts were made, where appropriate, according to Draper and Smith (1981).

RESULTS

(1) Artificial pupils and external lenses.

Contrast sensitivity was measured after homatropine eyedrops in 12 subjects and in the natural eye in 8 subjects. The former consisted of 10 male and 2 female ages 18-40 yr : no differences were observed between male and female subjects nor was there an age-related trend in the group as a whole.

Contrast sensitivity to CRT display

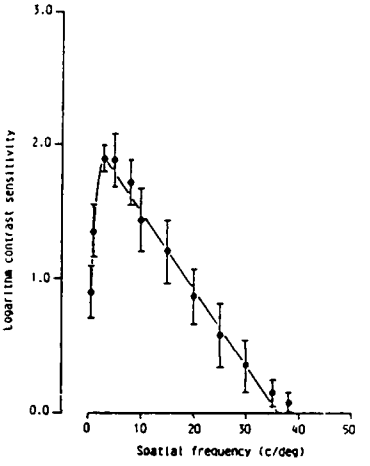

Fig. 3. Contrast sensitivities (on logarithmic scale) to CRT showing the geometric mean ± SD for 12 observers for viewing with natural 6 mm pupil.

The contrast sensitivity function for viewing with the natural pupil of some 6mm diameter consisted of a peak of mean value 81 units (range 62-182) at 3-5c/deg with fall-offs at lower and higher spatial frequencies. The high frequency fall-off was best fitted by logarithmic-linear plot as illustrated in Fig 3, with a cut-off frequency of typically 35-38 c/deg. For each subject the contrast sensitivity

function was of the same form as that in Fig 3, though there was variation in the absolute contrast sensitivity values as shown by the standard deviation bars. For any individual the spread of individual data points was within ±0.1 log units (Morrison and McGrath, 1985).

Homatropine eyedrops

These caused the pupil to dilate from typically 6mm to 8mm under our illumination conditions. The additional refraction required for optimal vision was +0.25D at 3m and +0.75D at 1m *ie.* somewhat less than the expected values of +0.33D and +1.0D, respectively. Comparison of Fig 3 (natural pupil) and Fig 4A (homatropine) indicates that homatropine eyedrops were without effect when the additional lenses were being worn. This is also apparent from the regression equations for 3-38c/deg presented in Table 1: there was no significant difference between the intercepts or between the slopes for the 2 sets of data ($P>0.25$). When paired t-tests were made for the highest spatial frequency detected, no significant difference was observed ($P>0.25$). Paired t-tests before and after homatropine for 0.5, 1.0 and 3 c/deg also failed to reveal a significant difference ($P>0.25$).

Pupil Diameter

In the homatropinized eye, viewing with the 3mm artificial pupil tended to improve contrast sensitivity whilst the 2mm pupil tended to worsen it, compared with the fully dilated pupil (Fig 4A). Over the range 3-38 c/deg, the intercepts and slopes of the regression equations for 3mm and 2mm pupil were, however, not significantly different from those for the dilated pupil ($P>0.25$ in each case). This lack of significance is also apparent from the regression equations listed in Table 1. At 0.5 and 1.0 c/deg, the paired t-test showed that contrast

sensitivities for the 3mm pupil were not significantly different from those for the dilated pupil ($P>0.25$); however, those for the 2mm pupil were significantly reduced ($P<0.01$, in each case).

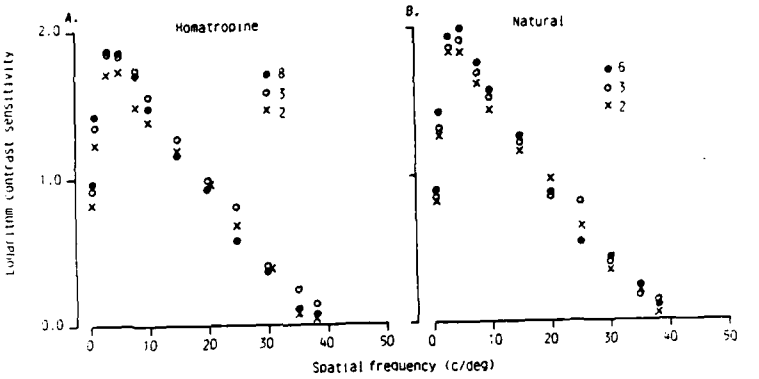

Fig. 4. Effects of pupil diameter on mean logarithmic contrast sensitivities in the homatropinized (A) and natural eye (B). Pupil diameter in mm is indicated.

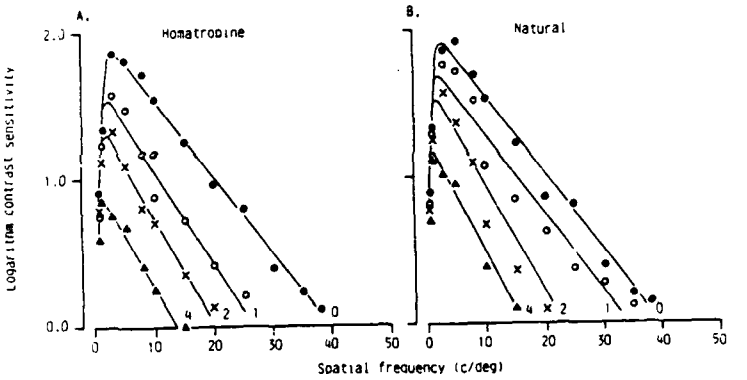

Fig. 5. Effects of positive defocusing lenses on mean logarithmic contrast sensitivities for viewing with the 3 mm artificial pupil in homatropinized (A) and natural (B) eyes. Defocus in dioptres is given alongside the appropriate contrast sensitivity curves.

TABLE 1. Pupil diameter and contrast sensitivity (3-38c/deg).

Condition	Regression	SD of y	r	P
Pre-homatropine	y=2.08-0.056x	0.08	-0.992	<0.001
Homatropine/8mm	y=2.07-0.058x	0.05	-0.997	<0.001
Homatropine/3mm	y=2.06-0.052x	0.06	-0.996	<0.001
Homatropine/2mm	y=1.94-0.052x	0.05	-0.996	<0.001
Natural/6mm	y=2.15-0.057x	0.12	-0.987	<0.001
Natural/3mm	y=2.07-0.053x	0.08	-0.992	<0.001
Natural/2mm	y=2.00-0.052x	0.05	-0.996	<0.001

where y is logarithm contrast sensitivity and x is spatial frequency

TABLE 2. Defocus and contrast sensitivity (3-38c/deg).

Condition	Regression	SD of y	r	P
After homatropine:				
0.0D/3mm	y=2.06-0.052x	0.06	-0.996	<0.001
+1.0D/3mm	y=1.69-0.063x	0.10	-0.980	<0.001
+2.0D/3mm	y=1.44-0.068x	0.07	-0.986	<0.001
+4.0D/3mm	y=0.96-0.068x	0.04	-0.986	<0.01
0.0D/2mm	y=1.94-0.052x	0.05	-0.996	<0.001
+2.0D/2mm	y=1.56-0.076x	0.10	-0.979	<0.001
Natural eye:				
0.0D/3mm	y=2.07-0.053x	0.08	-0.992	<0.001
+1.0D/3mm	y=1.79-0.053x	0.15	-0.968	<0.001
+2.0D/3mm	y=1.64-0.072x	0.19	-0.950	<0.001
+4.0D/3mm	y=1.26-0.077x	0.07	-0.982	<0.001
0.0D/2mm	y=2.00-0.052x	0.05	-0.996	<0.001
+2.0D/2mm	y=1.71-0.072x	0.18	-0.956	<0.001

In the natural eye, much the same results were obtained as those for the homatropinized eye (Fig 4A) viz. the 3mm pupil improved marginally and the 2mm worsened marginally contrast sensitivity compared with the natural 6mm pupil (Fig 4B). The regression equations for 3-38 c/deg had intercepts and slopes which were not significantly different ($P>0.25$) (Table 1). Contrast sensitivity was unaffected at 0.5c/deg with 2mm and 3mm pupils (paired t-test, $P>0.25$) but a significant reduction did occur with 2mm and 3mm pupils at 1c/deg ($P<0.05$). It is, therefore, concluded that changes in pupil diameter without compensatory changes in retinal illumination have no significant effect on contrast sensitivity in both natural and homatropinized eyes.

Defocus

Increasing defocus by positive spherical lenses with the 3mm artificial pupil had an increasingly deleterious effects on contrast sensitivity in homatropinized (Fig 5A) and natural eyes (Fig 5B). In both cases the impression is of a step-wise downward shift of the 3-38 c/deg segment of the contrast sensitivity function with a smaller decrease below 3 c/deg. Essentially similar results were also obtained for the 2mm pupil with optimal focus and with the +2.0D lens. The regression equations for 3-38 c/deg are presented in Table 2. For increasing defocus, the very marked reductions in the intercepts of the regression equations were significantly different when compared with optimal focus in homatropinized and natural eyes ($P<0.001$). The only exception was with +1.0D lens in the natural eye when the apparent reduction in intercept was not significantly different ($P>0.25$).

Comparison of the slopes of the regression lines shows an apparent increase in negative slope with increasing defocus indicating that higher frequencies were more severely affected (Table 2). However, comparison of the regression equation for each defocus with that for optimal focus showed the slopes not to be significantly different ($P>0.25$, in each case). The same absence of a significant difference between slopes was also present in each one of the individual results which are listed in Table 3.

In the homatropinized eye, where defocus was precisely controlled, the subjects fell into groups. Subjects 1 and 2 (the authors) had slopes which were essentially unchanged with defocus. At the opposite end of the spectrum, subjects 10-12 had slopes which increased stepwise as defocus increased in the same way as Campbell and Green (1965)'s results, shown as "C and G" (a value of $m=-0.046$ was also obtained for +0.5D). For subjects 3-8, the trend was for the slope to increase with +1.0D and then to remain constant or decrease with greater defocus. Viewing with the natural eye was not without difficulties since continual shifts of focus were reported, presumably as the accommodation mechanism hunted for optimal focus. In general, the variability of the data was appreciably greater than in the homatropinized eye as shown in Fig 5B and Table 2. Subject 2 again had reasonably constant slopes with increasing defocus. Subjects 3 and 4 showed a monotonic increase while subjects 1, 5, 6, 8 and 10 were characterised by an increase followed by a fall in slope at +4.0D. The data of Regan et al (1977) shown as "R, S and M" indicate an increase in slope for +1.0D. For the data of Campbell and Green, and Regan et al, and in Table 3, none of the regression lines with defocus were significantly different from optimal focus ($P>0.25$).

TABLE 3. CRT contrast sensitivity slopes at different defocuses for viewing with 3mm pupil.

	Homatropine				*Natural*			
	0.0D	**+1.0D**	**+2.0D**	**+4.0D**	**0.0D**	**+1.0D**	**+2.0D**	**+4.0D**
1. CDK	-0.054	-0.058	-0.059	-0.054	-0.063	-0.078	-0.095	-0.054
2. JDM	-0.049	-0.044	-0.042	-0.050	-0.063	-0.060	-0.048	-0.069
3. GW	-0.050	-0.061	-0.058	-0.067	-0.051	-0.052	-0.074	-0.124
4. AR	-0.048	-0.066	-0.058	-0.066	-0.048	-0.048	-0.069	-0.072
5. RO	-0.070	-0.085	-0.090	-0.083	-0.060	-0.075	-0.083	-0.077
6. JMI	-0.050	-0.061	-0.060	-0.059	-0.043	-0.069	-0.088	-0.076
7. HMD	-0.052	-0.073	-0.067	-0.059				
8. RR	-0.053	-0.074	-0.088	-0.080	-0.060	-0.067	-0.093	-0.069
9. DQ	-0.060	-0.043	-0.059	-0.081				
10. SON	-0.045	-0.059	-0.071	-0.085	-0.064	-0.068	-0.082	-0.075
11. LCC	-0.050	-0.058	-0.064	-0.073				
12. VK	-0.047	-0.055	-0.076	-0.093				
C and G	-0.048	-0.056	-0.060					
R, S and M					-0.064	-0.093		

(Units: log units per cycle per degree)

Increased defocus in the homatropinized eye caused a significant reduction in contrast sensitivity at 0.5 and 1.0 c/deg with the 3mm pupil ($P<0.02$) but not with the 2mm pupil ($P>0.1$). In both 2mm and 3mm pupils, contrast sensitivity at 3c/deg was significantly reduced with defocus ($P<0.001$). Slight differences occurred with the natural eye: at 0.5 and 1.0 c/deg, +1.0D defocus did not cause a significant reduction ($P>0.1$) *ie.* similar to 3-38 c/deg. With greater defocus, contrast sensitivity was significantly reduced with the 3mm pupil ($P<0.02$) but not with the 2mm pupil ($P>0.25$).

Control experiments

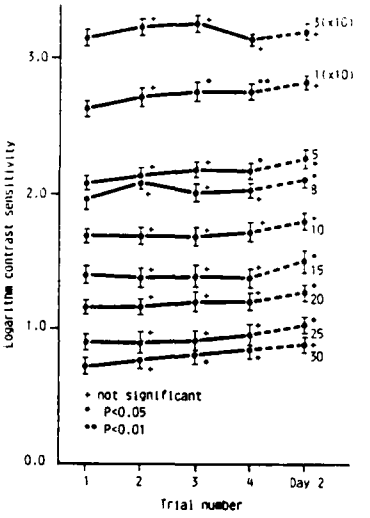

Fig.6 Reproducibility of contrast sensitivity measurements. Mean ± SE for 13 observers at spatial frequencies stated (c deg^{-1}) and four consecutive hourly trials. Significance levels refer to comparisons by paired *t*-test between a given trial and trial 1. Day 2 is compared with trial 4.

The results of systematically measuring CRT contrast sensitivities over 1-30 c/deg at 4 one hourly intervals and on a second day in 13 subjects, 4 of whom had completed the previous experiments, are shown in

Fig 6. The significance levels of paired t-tests between a given trial and the first trial are shown. On day 1, there were no significant changes in contrast sensitivity except for the 3rd and 4th trials at 1,5 and 30 c/deg when a significant improvement occurred. Taking the contrast sensitivity function as a whole from 3-30 c/deg, there were no significant changes for intercept and slope between the first and subsequent trials (P>0.25). Comparisons between the 4th trial on day 1 and the trial on day 2 showed a significant improvement on day 2 at all spatial frequencies except 1 and 30 c/deg (Fig 6). These results were consistent with the reproducibility of measurements at 10, 20 and 30 c/deg at the start and end of the experimental session in the above experiments and also in those reported below (section 2).

(2) Ingestion of pyridostigmine

Contrast sensitivity

Of the 14 subjects to undertake the experiments, one subject experienced such difficulty in making judgements on the first visit, though not on the second, that his results were discarded. For each subject, the form of the contrast sensitivity function for the CRT and laser displays was similar to that of the mean results shown in Fig 7A and B, respectively. The contrast sensitivity function to the CRT display had a peak at 3/5 c/deg with fall-offs at lower and higher spatial frequencies, with a maximum resolution of 35-40 c/deg. The contrast sensitivity function to the laser display was of similar shape but with a peak at 3-10 c/deg and a resolution in excess of 45 c/deg.

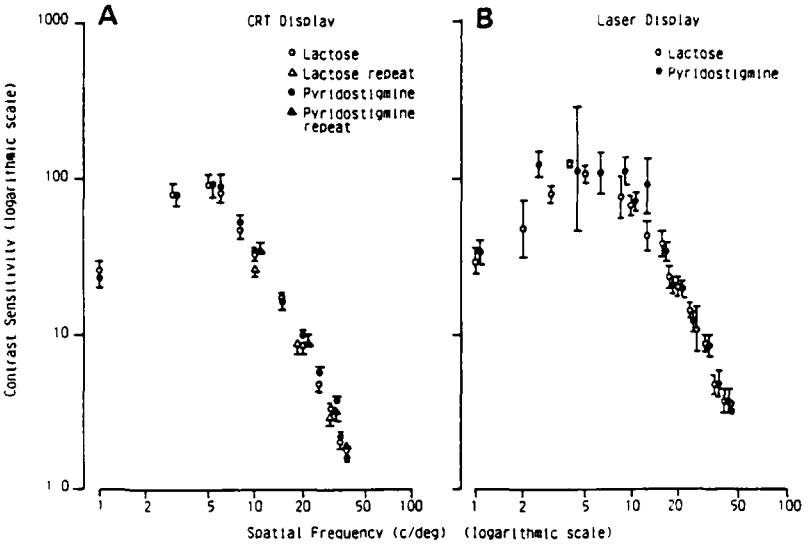

Fig.7 Effects of ingestion of 60mg pyridostigmine on contrast sensitivity to CRT display (A) and laser display (B) compared with ingestion of 60mg lactose. Mean ± S.E. (n=13).

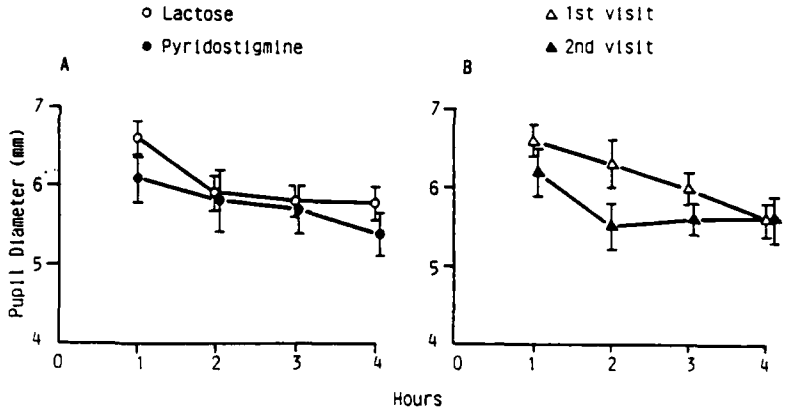

Fig.8 Effects of ingestion of 60mg pyridostigmine on pupil diameter compared with 60mg lactose (A). For comparison, pupil diameters on 1st and 2nd visits are shown (B). Mean ± S.E. (n=10).

Since it is highly improbable that any one spatial frequency would be affected *per se* and not its immediate neighbours, comparisons were made of the high spatial frequency limb at 3 c/deg and above after ingestion of pyridostigmine and lactose. The intercepts and slopes of the regression equations for the CRT and laser displays were not significiantly different after pyridostigmine and lactose in every subject ($P>0.25$). At 1 c/deg and 3c/deg, comparisons, made by eye, since the number of readings (5 in each subject) hardly warranted statistical analysis, also indicated no discernible difference after pyridostigmine and lactose.

For the mean data, the peak contrast sensitivity for the CRT display was 93 units after pyridostigmine and 91 units after lactose. For the laser display, the peak was 126 units in both cases. The high frequency cut-offs for the CRT display were 39.8 c/deg after pyridostigmine and 39.6 c/deg after lactose. For the laser display, the corresponding values were 53.5 c/deg and 53.4 c/deg, respectively. None of these values were significantly different ($P>0.25$). The regression equations over 3-40 c/deg for the CRT display were:

Lactose $y=2.06-0.052x$ SD $y=0.11$ $r=-0.983$ ($P<0.001$)

Pyridostigmine $y=2.11-0.053x$ SD $y=0.09$ $r=-0.988$ ($P<0.001$)

For the laser display, the regression equations were:

Lactose $y=2.19-0.041x$ SD $y=0.08$ $r=-0.988$ ($P<0.001$)

Pyridostigmine $y=2.30-0.043x$ SD $y=0.12$ $r=-0.978$ ($P<0.001$)

where y is logarithm contrast sensitivity and x is spatial frequency

For both CRT and laser displays, the slopes and intercepts for lactose and pyridostigmine equations were not significantly different ($P>0.25$). However, when the data for 3-40 c/deg were compared by paired t-test, a signficant increase of 0.031 log units (7%) after pyridostigmine occurred for the CRT display ($P<0.02$) but not for the laser display ($P>0.25$). At 1 and 3 c/deg, there was no significant change for either display ($P>0.10$). Comparisons between the first and second groups of measurements to the CRT display at 10, 20 and 30 c/deg showed no signficant differences for either pyridostigmine or lactose ($P>0.23$), indicating that there had been no change in criterion during the course of the day.

Pupil diameter

Comparisons were made with the paired t-test for pupil diameters after pyridostigmine and lactose in 10 subjects for whom a satisfactory series of photographs was obtained (Fig 8A).

There is an indication of a reduction after pyridostigmine, though this was not statistically significant ($P>0.25$). However, comparisons of pupil diameter for first and second visits indicated a more marked reduction though, again, this was not significant ($P>0.25$) (Fig 8B).

(3) Physostigmine eyedrops

Eleven subjects completed 3 trials and one person completed 2 trials. (This latter instance was due to severe headaches arising after the second trial, as opposed to the eyeache commonly reported during the experiments). No differences were present between the results of male and female subjects.

Pupil diameter

This constricted from a control diameter of 6mm down to 2mm within 30min (Fig 9A). A slight recovery was apparent by 3 hr though a complete return to normal did not occur until the second day after the experiment. The diameter of the companion eye remained unchanged during the trial, indicating the absence of significant systemic absorption of physostigmine. The mean results reflect very closely those for individual subjects.

Near point accommodation

The control near point accommodation with the 2mm artificial pupil was some +8D and this increased by +4.5D at 30 min after physostigmine and was still elevated by +2D at 180 min after physostigmine (Fig 9B).

Accommodation for distance

A transient increase in the amplitude of accommodation occurred to a mean of +5 to +6D at 30 min which subsequently returned to normal by *circa* 90 min as shown by the mean results for Trials 1-3 (Fig 9C). Considerable variation, however, was present in the individual results as shown in Table 4.

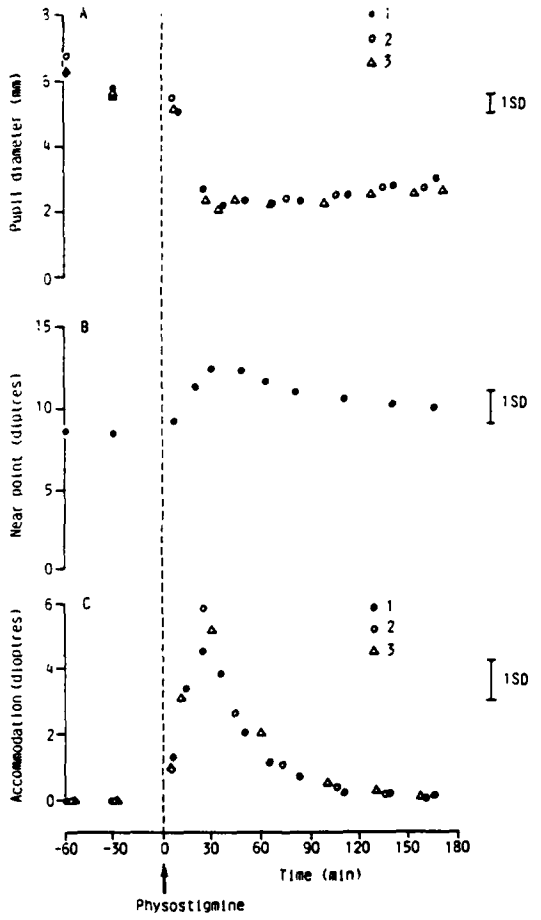

Fig. 9 Action of 0.25% physostigmine sulphate eyedrops on pupil diameter (A), near point accommodation (B) and accommodation for distance (C). Mean data from 12 subjects for 3 trials (A and C) and 1 trial (B) are shown. Mean value of individual standard deviations is shown (SD).

TABLE 4. Effect of physostigmine on accommodation for distance.

Subject	Sex	Age	Peak increase in accommodation (dioptres)		
		(yr)	Trial 1	Trial 2	Trial 3
1. SW	M	28	9.0	10.0	6.0
2. DO	M	20	9.0	8.5	7.5
3. CK	F	24	8.0	9.0	8.0
4. GM	M	24	8.0	8.5	7.5
5. AB	F	25	7.0	8.5	9.0
6. SO	M	23	7.0	1.25	6.0
7. AM	F	20	4.5	4.0	6.0
8. LM	F	22	3.0	3.5	-
9. CH	M	23	1.5	6.0	4.5
10. JG	F	20	0.75	1.5	3.5
11. JR	M	24	0.5	4.0	1.5
12. MF	M	22	0.5	0.75	0.5

The responses may be arranged into three levels viz. 7-9D (subjects 1-6), 1.5-4.5D (subjects 7-9) and 0.5-0.75D (subjects 10-12). Some uneveness in the responses of individual subjects in the 3 trials is present eg. subjects 6 and 9, which may have been due to inadequate instillation of the eyedrops on one of the trials, but on the whole the classification is reasonably reproducible. Those subjects who accommodated appreciably also complained of an ache behind the eye which subsided as the accommodation returned to normal. There was no correlation between the absence of a strong accommodative response and the amount of tear formation or of eye colour. We did, however, investigate the responses of 2 families of 3 siblings (Table 5).

TABLE 5. Comparison between siblings.

	Family 1					Family 2				
		Sex	Age (yr)	Miosis (mm)	Defocus (D)		Sex	Age (yr)	Miosis (mm)	Defocus (D)
1	DO	M	20	1.8	9.0	SR	M	17	1.9	4.5
2	KO	M	21	1.6	6.0	DR	F	17	1.9	4.5
3	SO	M	23	1.9	7.0	JR	M	25	1.8	0.5

The above results indicate a similarity in the amplitude of accommodation within each family viz. family 1 showing strong accommodation and family 2 showing moderate or weak accommodation. By contrast, the miosis caused by physostigmine was similar for the 2 families. This lends some support to the possibility that the response to physostigmine eyedrops may be affected by a genetic trait, though it would be desirable to investigate the matter more extensively.

Contrast sensitivity to grating patterns

In general, contrast sensitivity underwent a transient reduction with the nadir at 30 min and complete recovery by 90 min for both stationary (Fig 10A) and phase-reversed (Fig 10B) grating patterns. The reduction at 0.5 and 1.0 c/deg was noticeably smaller than at the higher spatial frequencies. When expressed in terms of a percentage change, contrast sensitivity at 2, 3, 10, 20 and 30 c/deg showed a fall to *circa* 20% of the pre-physostigmine control value with almost identical time courses in each case. The reduction in contrast sensitivity and its time-course at 3c/deg were the same irrespective of whether the grating pattern was stationary or phase-reversed ($P>0.14$)

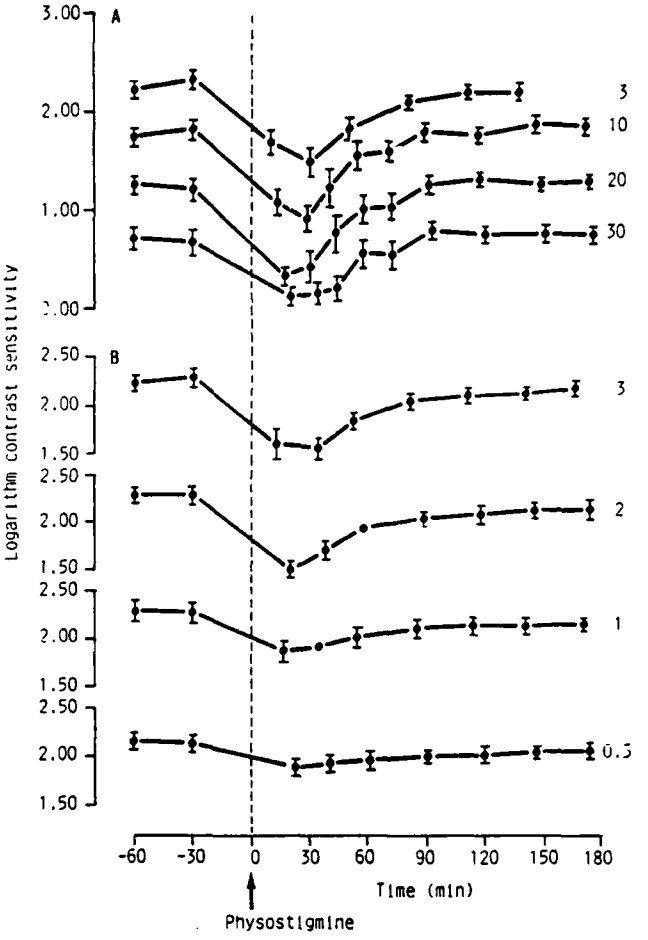

Fig.10 Effects of 0.25% physostigmine sulphate eyedrops on contrast sensitivity to CRT grating patterns of (A) 3, 10, 20 and 30 c/deg (stationary) and (B) 0.5, 1, 2 and 3c/deg (phase-reversed at 5.5Hz). Mean ± S.E. for 12 subjects.

indicating that physostigmine's action was not affected by movement of the target. It was notable that at 0.5, 1, 2 and 3 c/deg, contrast sensitivity returned to only some 80% of the pre-physostigmine value even after 180 min ($P<0.01$) (Fig 10B).

The similarity between the time course of the change in contrast sensitivity and accommodation shown in Figs 10 and 9C, respectively, and the smaller change in contrast sensitivity after physostigmine at 0.5 and 1.0 c/deg suggest that the reduction in contrast sensitivity may have been caused by defocus of the retinal image. Comparison between the peak amplitude of accommodation and the maximal reduction in contrast sensitivity expressed as a percentage of the pre-physostigmine control showed a strong negative correlation at 1, 2, 3 and 10 c/deg ($r=-0.93$, -0.93, -0.87, and -0.74, respectively: $P< 0.006$). At 20 and 30 c/deg, a negative correlation was also apparent, though the large number of points when the display was not visible at maximum contrast precluded statistical analysis. At 0.5 c/deg, no significant relationship existed between accommodation and contrast sensitivity ($r=-0.38$, $P=0.22$)

Assessment of neural function

After physostigmine, contrast sensitivity to the laser interference fringes of 4, 15 and 25 c/deg was transiently reduced with the nadir at 30 min and full recovery by 120 min (Fig 11A). The reduction was most marked at 15 and 25 c/deg where it amounted to 30% and 60% control value, respectively ($P<0.05$). At 4c/deg, a significant reduction only occurred at 15 min post-physostigmine ($P<0.05$) while, at 76 min, a significant increase was actually present ($P=0.05$). Since the miosis persisted beyond the reduction in contrast sensitivity, this may be excluded as the cause. The reduction, however, had a similar time course to the increase in accommodation. Defocus *per se* would not affect the perception of the interference fringes, though the circular aperture containing the interference fringes would, however, be defocused. In order to test this as a possible explanation for the transient reduction

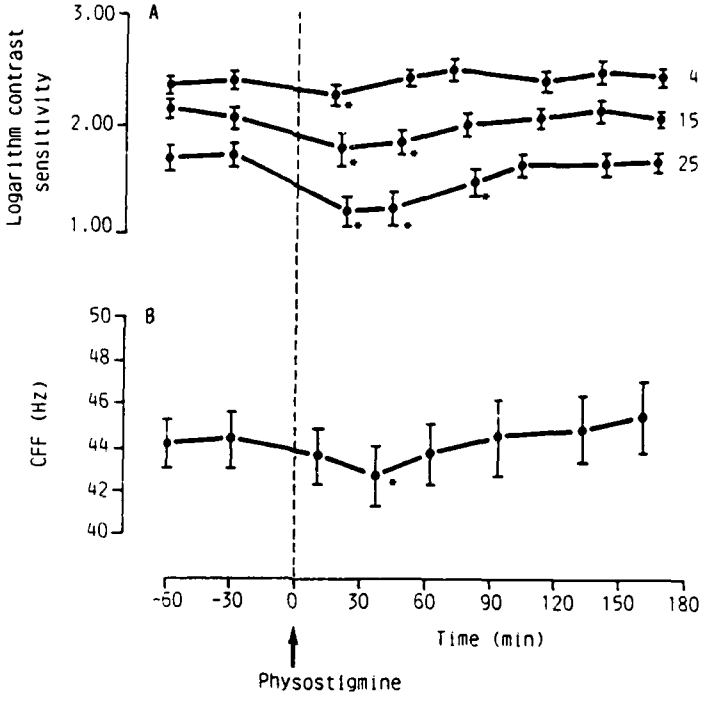

Fig.11 Effects of 0.25% phystostigmine sulphate eyedrops on contrast sensitivity to stationary laser interference fringes of 4, 15, and 25 c/deg (A) and critical fusion frequency (B). Mean ± S.E. for 11 subjects. Asterisk denotes significant reduction from control values ($p<0.05$).

in contrast sensitivity, measurements at 4, 15 and 25 c/deg were made for optimal focus and with +7D defocus. The measurements were very similar ($P>0.20$), thus excluding defocus as a cause of the reduction. Support for a neural action also comes from measurements of critical fusion frequency which were transiently but significantly reduced by some 2Hz at 30 min after physostigmine ($P=0.03$) (Fig 11B).

(4) Intramuscular injection of atropine

The most common effect of the intramuscular injection of 2mg atropine sulphate was dryness of the mouth with difficulty in swallowing, coupled with tiredness and general lassitude. Central effects in the form of dizziness, unsteadiness in walking and difficulty in concentrating were reported in 10 out of the 21 experiments. Difficulty in micturition was reported by 2 subjects. In 3 subjects who underwent both sets of experiments, the discomforture was less on the second occasion.

Heart rate changed in each subject in the manner shown in Fig 12A. Immediately following the injection, a transient bradycardia was recorded which was more pronounced in some subjects than in others. This soon transformed into a pronounced tachycardia consisting of an approximately 50% increase in resting heart rate occurring at 20-40 min post-injection, which was followed by a steady decline back to control values at 4-5hr post-injection. This time course, thus, follows closely the plasma concentration of atropine sulphate (see Introduction).

The ocular effects followed a longer time course. Pupil diameter increased by about 1mm after 2hr post-injection while, after 4hr, the change ceased to be significantly different from control values (Fig 12B). The depth of accommodation, measured as the near point, declined by some 1.5D by 1-2 hr post-injection and was still significantly reduced at 5-6hr post-injection (Fig 12C). These effects may, therefore, be attributed to binding of atropine to ocular smooth muscle.

Atropine had very little effect on visual function as shown in Table 6 which shows, for clarity, the mean value for each task plus the mean of the standard errors to give an indication of spread of data.

Visual acuity remained steady at 42-43 c/deg, stereoacuity was constant at *circa* 3 sec arc, red-green balance had a constant range of *circa* 38.5- 39.5, and reaction time and choice reaction time showed no significant change, though there was a numerical increase of 30-40 msec in the mean choice reaction time which was not statistically significant. Horizontal heterophoria and cyclophoria were, however, significantly changed at 1 and 2hr post-injection, respectively, while vertical heterophoria was unaffected. Seven out of eight subjects became less esophoric after atropine while the remaining subject became more exophoric ie. the common direction of change of alignment was towards exophoria by some 0.8 prism dioptres.

Table 6. Effect of atropine on visual performance.

	C2	*A1*	*A2*	*A3*	*A4*	*A5*	*A6*	*SE*
Visual acuity (c/deg)	43.7	43.9	43.5	42.3	42.7	43.3	43.1	0.8
Stereoacuity (sec arc)	2.8	2.6	2.6	2.4	3.1	2.9	2.2	0.3
Red-green balance	39.0 -39.7	38.9 -39.5	38.7 -39.6	38.7 -39.2	38.9 -39.6	38.7 -39.4	38.6 -39.2	0.3
Reaction time (msec)	452	468	453	473	464	470	453	43
Choice reaction time (msec)	591	623	630	629	635	601	591	39
Cyclophoria (deg arc)	1.94	1.25	1.06*	1.87	1.62	2.06	1.81	0.49
Horizontal phoria (prism dioptres)	3.67	2.83*	3.37	3.62	3.52	3.92	4.06	0.55
Vertical phoria (prism dioptres)	0.27	0.37	0.41	0.50	0.26	0.48	0.30	0.14

C2: second control measurements A1 - 6: hourly periods post-atropine
* value significantly different from C2 by paired t-test ($P<0.05$)

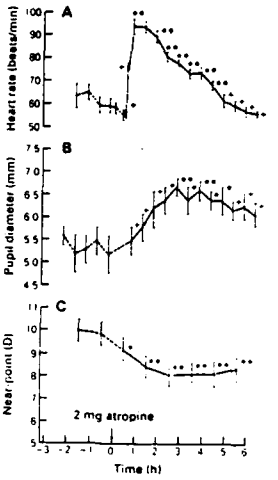

Fig.12 Effects of intramuscular injection of 2 mg atropine on (a) heart rate, (b) pupil diameter and (c) near-point. Each point shows the mean and s.e.m. for 8 subjects. The significance levels indicated are for the paired *t*-test between the test period and the period immediately preceding the injection. **, $P < 0.01$; *, $P < 0.05$; +, n.s.

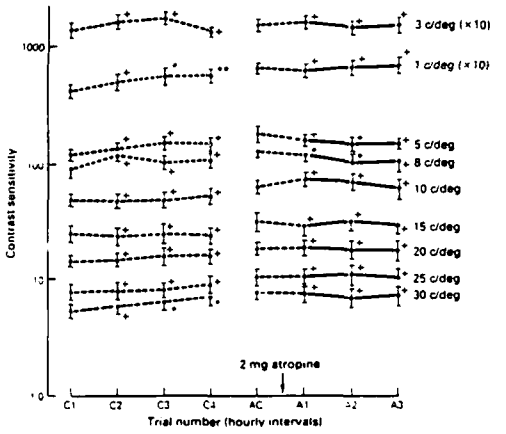

Fig.13 Effects of atropine on contrast sensitivity (logarithmic scale) to sinusoidal grating patterns of the spatial frequencies stated. Left, a series of 4 control measurements (C1–C4) repeated at hourly intervals, each point shows the mean and s.e.m. for 13 subjects. Right, one control measurement (AC) followed by post-atropine measurements (continuous line). Significance levels are for paired *t*-test against the first measurement in series (C1 or AC). **, $P < 0.01$; *, $P < 0.05$; +, n.s.

Contrast sensitivity to stationary patterns

In the control experiments which are the same as those reported separately in Fig 6, contrast sensitivity at 1-30 c/deg showed a gradual improvement over 4 one hourly measurements of the order of some 4-7% per set of measurements but this was never statistically significant ($P>0.05$) (Fig 13, left) except at 5 c/deg for 4hr and 30 c/deg for 3 and 4 hr when a significant increase was recorded. Therefore, the normal expectation in repeated measurements would be a gradual improvement in performance. The effects of atropine, however, were minimal. An equivocal deleterious effect of atropine occurred in 3 out of 13 subjects whilst, in the majority, the same gradual improvement as in the control measurements was observed. For the mean data (Fig 13, right), there was no significant difference between a given post-atropine measurement and the appropriate control ($P>0.05$) except at 1 hr post-injection for 5c/deg and at 1 and 2 hr post-injection for 8 c/deg when a significant reduction occurred ($P<0.05$).

Since it is unlikely that only one spatial frequency would be depressed while the remainder was unaffected, it was thought appropriate to test the contrast sensitivity data collectively by regression analysis. Contrast sensitivity (logarithmic scale) was inversely linearly related to spatial frequency over the range 3-30 c/deg. For the pre-atropine control and 3 post-atropine periods (Fig 13, right), the correlation coefficients were better than 0.985 with significance of $P<0.001$. Comparison between pre and post-atropine regression equations for each subject showed an absence of a significant difference in both intercept and slope of the regression equations ($P<0.001$). These results are consistent with the lack of effect of atropine on visual acuity

which remained at 42-43 c/deg (Table 6), It is, therefore, concluded that atropine had no measurable effect on the perception of stationary grating patterns over the range of spatial vision studied.

Contrast sensitivity to moving patterns

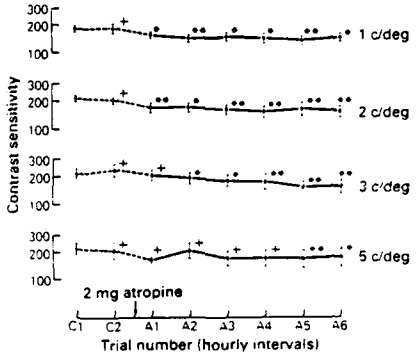

Fig.14 Effects of atropine on contrast sensitivity (logarithmic scale) to sinusoidal grating patterns phase-reversed at 5.5 Hz at the spatial frequencies specified. Each point shows the mean and s.e.m. for 8 subjects. Significance levels are for the paired *t*-test against control period C2. **, $P < 0.01$; *, $P < 0.05$; +, n.s.

Atropine caused a decline in contrast sensitivity at the 4 spatial frequencies studied in 4 out of the 8 subjects tested and at 3 spatial frequencies in 3 subjects. (The eighth subject showed no change). At 1, 2 and 3 c/deg, atropine caused a significant fall in contrast sensitivity of the order of 20-35% at 1-2 hr post-injection and which persisted at 5-6 hr post-injection (Fig 14). At 5 c/deg, a smaller reduction was observed which was significant only at 5 and 6 hr post-injection. Thus, the perception of moving grating patterns was adversely affected by atropine with a time course which was more sustained than that of either heart rate or pupil diameter.

(5) Physostigmine eyedrops and injection of atropine

Initially, 6 subjects who had previously demonstrated a marked increase in accommodation in the 3 trials with physostigmine eyedrops (subjects 1-6 of Table 4) were tested. Then, after one experiment, subject 5 became unavailable and was replaced by a new subject. This person underwent a single control experiment with physostigmine eyedrops alone prior to the atropine experiment. This, thus, necessitated the calculation of two different sets of control data for physostigmine alone for comparison with the results for prior injection of atropine, which was made with the paired t-test.

Atropine at 8 min pre-physostigmine

This had no significant effect on the reduction in pupil diameter which would have been caused by physostigmine alone ($P>0.08$) (Fig 15A), nor on the increase in the near point ($P>0.1$) (Fig 15C) nor on the increase in accommodation for distance ($P>0.1$) (Fig 15E).

The two sets of control contrast sensitivity values for physostigmine-plus-atropine and physostigmine-alone were not identical (Fig 16A and C), probably reflecting slightly different criteria on the two different days, as has been described previously for contrast sensitivity measurements made on different days (Fig 6). Therefore, the results were normalized in both cases against the respective pre-physostigmine control value and compared. In no case did atropine significantly affect the action which physostigmine alone would have had in reducing contrast sensitivity at 10, 20 and 30 c/deg to 20% control value after 30 min, as illustrated by Fig 16A ($P>0.1$). With the 3 c/deg phase-reversed grating pattern, there was actually a tendency for the nadir to be depressed with atropine, from 26% to 13% control, though

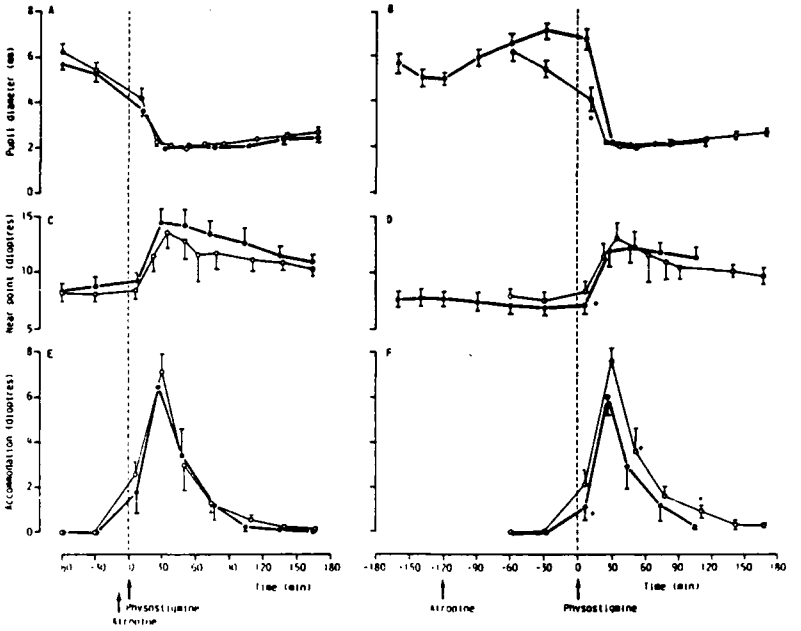

Fig.15 Injection of atropine sulphate (2mg IM) at 8 min (left) and 120 min (right) prior to 0.25% physostigmine sulphate eyedrops (●, ——). Significance of differences from control data for 0.25% physostigmine sulphate eyedrops alone (○, ——) denoted by + (0.1>p.0.05) and * (p<0.05). Mean ± S.E. for 6 subjects.

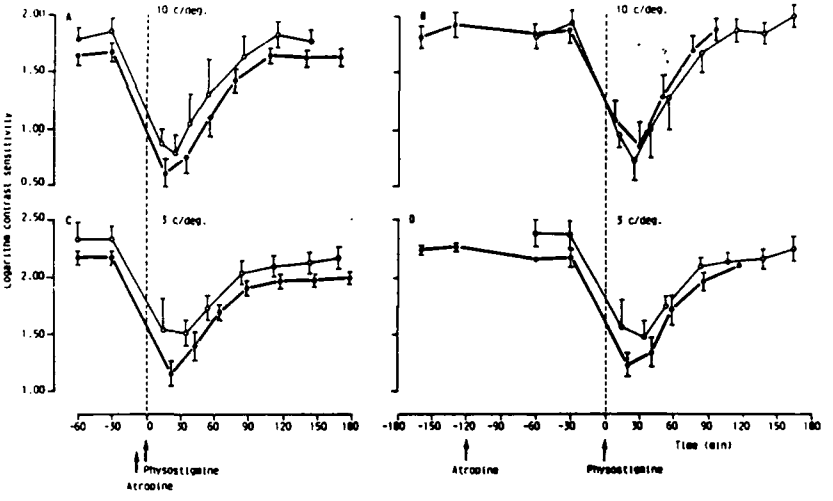

Fig.16 Similar to Fig.15 but showing comparison for 10 c/deg stationary grating pattern (A & B) and 3 c/deg phase reversed grating pattern (C & D). Mean ± S.E. for 6 subjects.

this was not statistically significantly different ($P>0.5$) (Fig 16C). At 90-180 min, contrast sensitivity at 3 c/deg was still significantly reduced at 60% of the control value ($P<0.01$) whereas at 10, 20 and 30 c/deg, it had returned to 100% of the control value ($P>0.17$).

Atropine at 120 min pre-physostigmine

Atropine *per se* caused the following changes: pupil diameter increased from 6 to 7 mm, near point accommodation was marginally reduced by +1D, while accommodation for distance was unaffected (Fig 15B, D and F, respectively). Contrast sensitivity to the 3 c/deg phase-reversed grating pattern was significantly reduced by atropine in both first and second post-atropine trials ($P<0.05$) while, at 30 c/deg, it was unaffected ($P>0.24$). At 10 and 20 c/deg, it was reduced in the first post-atropine period ($P<0.01$) but not in the second period ($P>0.24$) (Fig 16B & D).

Atropine at 120 min generally had no significant effect on the actions of physostigmine. The reduction in pupil diameter to 2mm was unaffected ($P>0.16$) (Fig 15B) as was the increase in near point accommodation ($P>0.35$) (Fig 15D). Accommodation for distance after physostigmine was reduced by atropine by about +1D with marginal significance at 10 min and 45 min ($0.05>P>0.1$), but not at the peak at 30 min ($P>0.1$) (Fig 15F).

The transient reduction in contrast sensitivity at 10, 20 and 30 c/deg after physostigmine, which is illustrated by the results for 10 c/deg (Fig 16B), was not significantly affected by atropine ($P>0.07$). Nor did atropine diminish physostigmine's deleterious effect on the detection of the phase-reversed 3 c/deg grating pattern. In fact, the nadir was reduced from 21% to 11% of the normalized control value,

though this was not significant (P>0.1) (Fig 16D). Contrast sensitivity at 10, 20 and 30 c/deg returned to 100% control after 90 min post-physostigmine (P>0.1); but for 3 c/deg it was still significantly depressed by 40% at 90-180 min (P<0.01) (Fig 16D).

Homatropine pretreatment

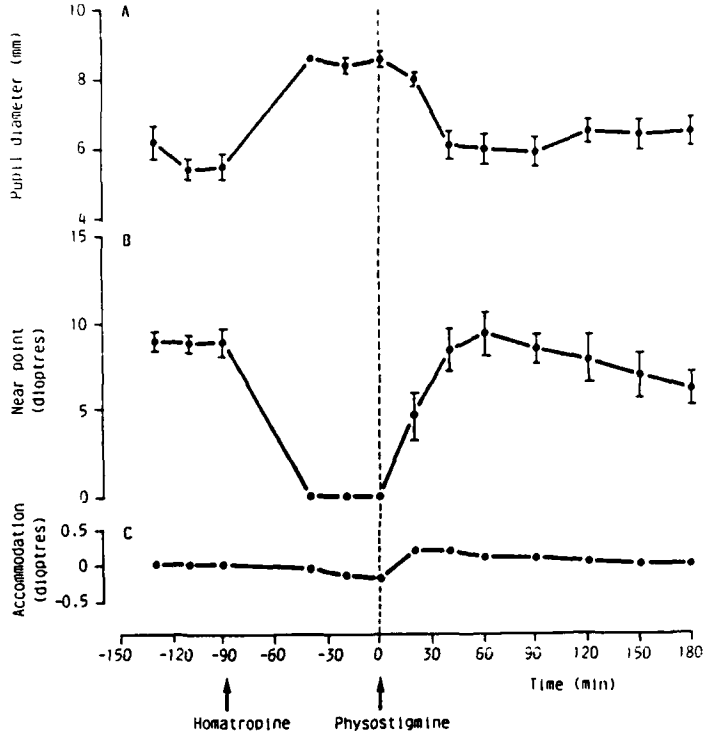

Fig17 Instillation of 2% homatropine hydrobromide eyedrops prior to 0.25% physostigmine sulphate eyedrops and pupil diameter (A), near point accommodation (B) and accommodation for distance (C). Mean ± S.E. for 5 subjects

Homatropine eyedrops applied to 5 subjects caused a dilation of the pupil from 6 mm to 8mm and the abolition of near point accommodation (Fig 17A and B), respectively. The abolition of accommodation for distance necessitated the wearing of a small positive correction by some subjects for optimal distance acuity at 2.86m (Fig 17C). Following physostigmine eyedrops, pupil diameter was reduced to 6 mm which was not significantly different from the control value before homatropine ($P>0.35$). However, at 80 min post-physostigmine, pupil diameter now showed a significant increase over the control value ($P<0.05$). Near point accommodation was increased transiently to *circa* +8D which was not significantly different from normal ($P>0.13$) but, by 180 min post-physostigmine, it had declined significantly ($P=0.04$). Accommodation for distance required a small negative lens at 30 min, indicating that physostigmine had caused a small increase in involuntary accommodation: this had subsided for longer intervals after physostigmine (Fig 17C). These results indicate that physostigmine was still able to exert its actions, though to a much reduced extent.

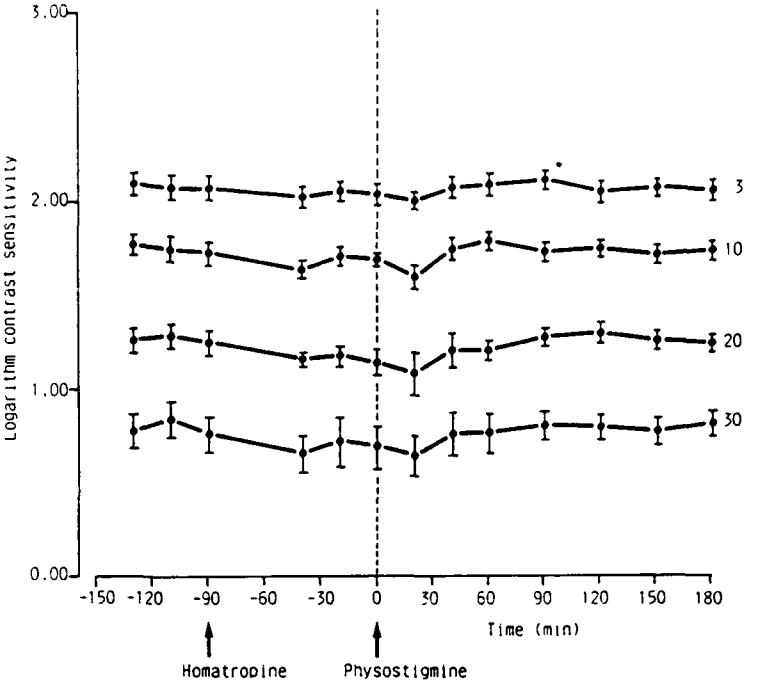

Fig.18 Instillation of 2% homatropine hydrobromide eyedrops prior to 0.25% physostigmine sulphate eyedrops and contrast sensitivity to CRT grating patterns of 3 c/deg (phase-reversed) and 10, 20 and 30 c/deg (stationary). Mean ± S.E.for 5 subjects, with asterisk denoting significant difference from control ($p<0.05$)

Contrast sensitivity to stationary grating patterns of 10, 20 and 30 c/deg and a phase-reversed pattern of 3 c/deg remained effectively unchanged following instillation of homatropine (Fig 18). The marked fall normally seen after physostigmine (Fig 16) was effectively anatagonized by homatropine at all spatial frequencies. Apart from a single value, none of the contrast sensitivities was significantly different from the pre-physostigmine controls ($P>0.12$) (Fig 18).

DISCUSSION

(1) Artificial pupils and external lenses

In the present study, the effects of changes in pupil diameter and defocus on contrast sensitivity have been investigated. The new aspects of this study are that a relatively large sample of subjects was tested and that viewing was done with the natural eye as well as the homatropinized eye. Changes in pupil diameter were made without compensation for changes in retinal illumination, such as occurs in real life, and a relatively wide range of defocuses was studied. By repeating the measurements of contrast sensitivity for the full range of spatial frequencies, reproducibility was found to be good, though would not necessarily have been the case if the measurements had been spread over several days. The interpretation of contrast sensitivities at low spatial frequencies does, however, require some discussion. Spatial frequencies at 8 c/deg and below were tested from the near distance of 1 metre where the display subtended 4.5 deg. It has been demonstrated by McCann, Savoy, Hall and Scarpetti (1974) that the viewing of low spatial frequencies is unaffected by distance and the results from near and far distances may reasonably be combined (Campbell and Robson, 1968). Two other considerations are, however, of importance. First, contrast sensitivity depends upon the number of grating cycles visible as well as spatial frequency. According to Hoekstra et al, 1974), the minimum number of cycles at the screen luminance used in our experiment is 3 cycles. At 1 c/deg when 4 cycles were visible, contrast sensitivity may be expected to be normal, whereas at 0.5 c/deg when only 2 cycles were visible, there would be this additional factor of a reduced number of cycles causing a further reduction in contrast sensitivity. This factor,

however, would be present for all the subjects tested. Second, contrast sensitivity measurements are additionally depressed when the surround is darker than the CRT display, as was the case in our experiments (Estevez and Cavonius, 1976). While this effect would be present for all subjects, it would not be constant throughout our experiments since the effect of defocusing lenses would be to reduce the luminance gradient from CRT to surround. The consequence of this would be to tend to reduce the depressant effect of the defocusing lenses observed in the present study. This factor would, however, be present for all subjects and for viewing an object against a dark background, our results would still be directly relevant.

Pupil Diameter

Changes in pupil diameter were, in general, without significant effect on contrast sensitivity. Given the substantial body of evidence that reduction of the pupil diameter *per se* improves visual resolution (Jenkins, 1963) and optical quality (Arnulf and Dupuy, 1960; Campbell and Green, 1965; Campbell and Gubisch, 1966; Gubisch, 1967; van Meeteren, 1974; Howland and Howland, 1977; Charman, 1979; and Bour, 1980), our result is probably most readily explained by this improvement being annulled by the accompanying reduction in retinal illumination. A similar proposal has been made in respect of the effects of small pupil diameters on visual resolution at low screen inances (Woodhouse, 1975). Our results also confirm those of a previous study in which the wearing of a 3mm pupil was without effect on contrast sensitivity in the natural eye (Morrison and McGrath, 1985). The significant reduction in contrast sensitivity by the 2mm pupil at 0.5 and 1.0 c/deg while the 3mm pupil was without effect is anomalous. If one seeks to explain this on

the basis of reduced retinal illumination, all the evidence indicates that reduced illumination tends to spare the lowest spatial frequencies (Schade, 1956; DePalma and Lowry, 1962; van Meeteren and Vos, 1972). Possibly the increased effects of diffraction at 2mm pupil diameter were sufficient, especially when coupled with reduced retinal illumination, to degrade contrast sensitivity. This is supported by the tendency for contrast sensitivities over 3-38c/deg to be reduced at 2mm compared with 3mm pupil diameter, although the reduction was not statistically significant.

Defocus

This had a profound effect, causing a stepwise reduction in contrast sensitivity with increasing defocus. A number of deviations from predicted theory (Hopkins, 1955) and from the study of Campbell and Green (1965) was, however, apparent. The lowest spatial frequencies were not unaffected though they were reduced less than higher spatial frequencies (Fig 5A and B) while the highest spatial frequencies were not disproportionately affected compared with lower spatial frequencies. The discrepancies with Campbell and Green's work are, however, fairly readily explained. Their study in which low spatial frequencies were not attenuated by defocus was made with a 2mm artificial pupil. With a 7mm pupil diameter low spatial frequencies were markedly reduced (Green and Campbell, 1965) and there is an indication that this was also the case with a 4mm pupil at large defocuses (Charman, 1979). Our results showed a significant reduction with the 3mm but not with the 2mm pupil. The explanation may lie in the depth of focus which would be greater with the 2mm pupil, especially when coupled with the accompanying reduction in retinal illumination which would also increase the depth of focus

(Campbell, 1954), so resulting in a smaller effect of defocus on contrast sensitivity. While a tendency for higher spatial frequencies to have a greater roll-off of contrast sensitivity with defocus was noted in our results, it was never statistically significant on an individual basis or group basis. Campbell and Green had based their conclusion that there was a greater effect on high spatial frequencies from their plot of contrast sensitivity against defocus for a selected range of spatial frequencies. Inspection of this figure (their Fig 6) shows that it depends very much on the precision of the data points for 30 c/deg for large defocus. If, instead the full contrast sensitivity functions for different defocuses (their Fig 7) are analyzed statistically, the regression equations for different defocuses are not significantly different from optimal focus ($P>0.25$, in each case). Further, with 7mm diameter pupil, the downward shift is clearly parallel (Green and Campbell, 1965) as is the case with Charman (1979)'s data over 5-30c/deg for different pupil diameters.

In the homatropinized eye, 3 out of our 12 subjects had an increased roll-off with defocus (though this was not statistically significant), 2 had constant slopes while the majority showed an interesting pattern (Table 3). An increase in slope occurred between optimal focus and +1.0D but, for greater defocus, the slope stayed constant or even decreased. This is contrary to the notion of an increased effect of defocus on higher spatial frequencies which should be a continuous function of defocus. Two explanations for the effect of the +1.0D lens in increasing slope are possible. The simpler is that it is merely the consequence of placing a glass lens before the eye with the result that the additional lens would be without effect on the

slope, which was often the case. While we omitted to exclude this possibility by the use of zero power glass lenses, in another context viz. depth judgements, defocus caused by glass lenses was similar in effect to changes in accommodation caused by a split mirror apparatus by which apparent movement of the target was effected (Morrison and Whiteside, 1984). The second explanation concerns the difference in optimal focus for low and high spatial frequencies, which is particularly apparent at large pupil diameters. At 6-7mm pupil diameter, the difference in focus between high and low spatial frequencies is -0.8D (Green and Campbell, 1965). While this difference disappears at 2mm pupil diameter, presumably due to reduction of geometrical aberrations (Charman and Heron, 1979), some difference may be present with the 3mm pupil diameter used in our study. The entire depth of focus at 3mm pupil diameter and 1mL illumination which is some +0.6D (Campbell, 1958) would be available to offset a defocus error since accommodation is located at the hyperopic point of the depth of focus rather than at the midpoint of the range (Kasai, Kondo, Sekiguchi and Fujii, 1971). Hence a residual difference of some -0.2D may be present at low spatial frequencies which would partially compensate for the external lens at lower spatial frequencies leading to an increase in slope of the contrast sensitivity function. For greater defocus, low and high spatial frequencies would then be defocused to the same degree, resulting in unchanged slopes of the contrast sensitivity function. The conclusion to be drawn is that defocus does not have a greater effect at higher spatial frequencies.

The predicted zero minima which with defocus of +1.0D occur at 6.6 and 10c/deg in the diffraction-limited eye (Smith, 1982) were also not apparent in the present results nor in those of Campbell and Green

(1965). The results of Charman (1979) did show undulations but these were not always coincident with the theoretical minima. Minima are apparent in the results of Bour (1980) though the detection of the grating pattern was done only for 56% and 100% contrast. Nevertheless, given that all the studies employed artificial pupils near to the optimal value for a diffraction-free system, the deviation from theoretical predictions indicates that the optical media of the eye are more complex than a simple diffraction-limited system.

In the present study, similar results were obtained with the natural eye as with the homatropinized eye with certain exceptions. It was notable that, during the experiments, accommodation did not remain constant with the defocusing lens but fluctuated noticeably, presumably as the visual system sought sharp focus. Then another factor adding to the variability is that different individuals respond differently to positive lenses: some relax what accommodation they have, many do not change their level of accommodation at all, while some actually increase their accommodation (Reese and Fry, 1941). Generally, the reduction in contrast sensitivity caused by the +1D lens was not statistically significant and, in 2 out of our 8 subjects who viewed with natural eyes, contrast sensitivities to +1D defocus were indistinguishable from optimal focus, indicating that these belonged to the category of individuals who relaxed their accommodation to the external lens. Hence relaxation of some 0.25D for viewing at 3m and 0.75D at 1m, when added to a depth of focus of 0.6D with the 3mm pupil (Campbell, 1958) and a tolerance to defocus blur of 0.20-0.25D (Whiteside, 1957; Campbell and Westheimer, 1958), would be sufficient to compensate for the external +1D lens. Since the latter 2 factors were also present in the

homatropinized eye, when the +1.0D lens did significantly reduce contrast sensitivity, this highlights the marked effect that even a small degree of defocus might have on the contrast sensitivity function. This is consistent with the prediction of Hopkins (1955) that the optical transfer function will be markedly depressed for defocus of greater than 0.125D (Fig 1).

Predictions of contrast sensitivity

In order that our measurements might be of practical value in predicting contrast sensitivity at our CRT luminance, we derived the best-fitting multiple regression equations in terms of pupil diameter, defocus and spatial frequency. To simplify the analysis, the contrast sensitivity function was separated at the peak of 3c/deg into low and high spatial frequency limbs and analyzed in both natural and homatropinized eyes. The most appropriate equations were:

Homatropine

0.5-3c/deg $y=0.80+ 0.29x_p+ 0.021x_p- 0.09x_D$ $SD_y=0.15$ $r=+0.924$ $(P<0.001)$

3-38c/deg $y=1.99- 0.055x_p+ 0.009x_p- 0.31x_D$ $SD_y=0.11$ $r=-0.981$ $(P<0.001)$

Natural

0.5-3c/deg $y=0.78+ 0.33x_p+ 0.024x_p- 0.05x_D$ $SD_y=0.15$ $r=+0.926$ $(P<0.001)$

3-38c/deg $y=2.05- 0.057x_p+ 0.016x_p- 0.26x_D$ $SD_y=0.15$ $r=-0.972$ $(P<0.001)$

where y is logarithm contrast sensitivity, x_p is spatial frequency (c/deg), x_p is pupil diameter (mm) and x_D is defocus (dioptres).

Further analysis at 0.5-1.0c/deg in the homatropinized eye gave coefficients for pupil diameter and defocus of +0.017 and -0.08 ie. similar to those above, indicating that the inclusion of 3c/deg in the above equations had not biased these terms. In general, pupil diameter had only a very small effect on contrast sensitivity. On the other hand, the effect of defocus was profound, particularly over 3-38c/deg, causing reductions of 51% and 45% per dioptre in homatropinized and natural eyes, respectively. At 0.5-3c/deg, the reductions were smaller viz. 19% and 11% per diopre, respectively. This relative sparing of low spatial frequencies has important consequences. Object detection should be relatively unimpaired since this has been correlated with the peak of the contrast sensitivity function rather than the highest spatial frequency (Ginsburg, 1981; Ginsburg et al, 1982). Stereopsis should also be relatively unimpaired in that fusion of the 2 images can occur with low spatial frequencies even in the presence of conflicting high spatial frequency information (Julesz, 1971). Stereoacuity will, however, be degraded to much greater degree than visual acuity since a full range of spatial frequencies is required for this task (Westheimer and McKee, 1980). Initiation of the accommodative mechanism should also be unaffected but the attainment of sharp focus which depends upon high spatial frequencies (Charman and Heron, 1979) would be impaired. The smaller effect of defocus in the natural eye probably reflects the capacity to relax accommodation in response to defocus.

The deleterious effect of defocus in our study is somewhat smaller than the 65% per dioptre reduction calulated from Campbell and Green's Fig 7 and the 77% per dioptre from Regan at al (1977)'s data. Both sets of investigators used a much higher screen luminance and appear to have used a descending method to determine contrast threshold. In inexperienced subjects this may lead to underestimation of contrast threshold (Ginsburg and Cannon, 1983) and it is noteworthy that Regan et al's subjects had very high values of slopes (Table 4) indicating that, perhaps, their subjects found greater difficulty at higher compared with lower spatial frequencies. In Campbell and Green's case, the data from only 1 subject were presented and thus sampling may have been a factor accounting for this difference.

In simplifying our results, the term for pupil diameter could reasonably be ignored since its effect is so small. Hence, in the mesopic range of illumination, for up to 4 dioptres of defocus, it may be expected that contrast sensitivity will be reduced by half for each dioptre of defocus, for all but the very lowest of spatial frequecies.

(2) Ingestion of pyridostigmine

A small improvement of 7% in CRT contrast sensitivity averaged over 3-40 c/deg was recorded after ingestion of 60 mg pyridostigmine compared with the lactose control. Such a result is a refinement of the result of Borland et al (1985) who intimated that there was no change with repeated doses of 30mg. The increase was not present for contrast sensitivities to laser interference fringes, nor at 1 and 3 c/deg for the CRT display where optical factors have minimal effect. It may, thus, be concluded that the improvement over 3-40 c/deg arose from an improvement in optical quality. The most probable explanation lies in

the possible reduction in pupil diameter caused by pyridostigmine (Fig 8A) since this would result in an improvement in optical quality (Campbell and Green, 1965). An increase in accommodation, as might be conceivable after an anticholinesterase, would only diminish contrast sensitivity (Fig 5). However, the order of change was very small and, in fact, a larger difference in pupil diamter was present between first and second visits (Fig 8B). This may be explained by a greater effect of the sympathetic nervous system on the first visit, perhaps due to the stress of undertaking the measurements for the first time.

There was the problem in designing this study that contrast sensitivity measurements done on different days may be affected by criterion differences (Fig 6). However, to have carried out both pyridostigmine and lactose determinations on the same day would have meant always having the lactose experiment first and the pyridostigmine experiment second to avoid any persisting effects of the latter. It was, therefore, considered preferable to undertake the trial on a double blind basis. A trial with both experiments on the same day would now also be useful. The present study was restricted to the study of stationary visual function. However, we have also demonstrated the deleterious effect of atropine sulphate injected intramuscularly on movement sensitivity at low spatial frequencies (Fig 14). Harding, Kirby and Wiley (1985) reported that intravenous injection of sufficient physostigmine to produce 46% inhibition of blood cholinesterase led to selective depression of the visually evoked response at low spatial frequencies in cat. These data suggest the existence of cholinergic transmission for the preception of low spatial frequencies, upon which pyridostigmine may have an effect in man. A possible derangement of

binocular vision should also be considered since stereoacuity is profoundly affected by a small degree of defocus which did not affect visual resolution (Westheimer and McKee, 1980). Notwithstanding these aspects of vision, which still require to be investigated, the present demonstration of the close correspondence of contrast sensitivities after pyridostigmine or lactose indicates that 60mg pyridostigmine may be used as a prophylactic against poisoning by organophosphorus anticholinesterases.

(3) Physostigmine eyedrops

The rapid onset and sustained time course of miosis caused by instillation of physostigmine eyedrops and the transient increase in accommodation confirm the earlier reports of Fraser (1863), Argyll Robertson (1863) and Rengstorff (1970). The effect of physostigmine in potentiating voluntary accommodative effort, assessed as the near point, was also observed. This is consistent with the enhancement of accommodation accompanying convergence by physostigmine (Fincham, 1955) and the clinical use of anticholinesterases in treating accommodative esotropia (Havener, 1978, p297). While the miosis was pronounced in all subjects after physostigmine, the amplitude of accommodation for distance varied between subjects from practically zero to +10D. A considerable range of up to +7.5D is also apparent from the data of Rengstorff (1970). A range of defocuses was also noted on instillation of the cholinomimetic pilocarpine (Lindstrom, Tredici and Martin, 1968). We have presented some preliminary evidence that the differing responses may be influenced by a genetic trait, though the topic deserves further attention.

Physostigmine also caused a transient reduction in contrast sensitivity to both CRT-generated grating patterns which are focused by the optical media and to laser interference fringes which do not undergo refraction and thus represent a mainly neural assessment. Since these reductions were outlasted by the sustained miosis, this may be excluded as the cause. In the case of the CRT display, defocus of the retinal image resulting from an increase in accommodation was an important factor. This could not, however, be the explanation for the decrease in contrast sensitivity to the laser interference fringes, which may be attributed to a direct neural action of relatively short time-course. In addition, another action of physostigmine was evident for viewing CRT-generated grating patterns of low spatial frequencies when a sustained loss of contrast sensitivity occurred which was not apparent at higher spatial frequencies. This result was reproduced in two further sets of experiments (section 5). The reduction in contrast sensitivity to CRT-generated grating patterns closely followed the time course of the increase in the amplitude of accommodation and a strong negative correlation was present between the contrast sensitivity nadir and maximal accommodative change, except at the lowest spatial frequency. This suggests that defocus of the retinal image, but not the miosis, caused by physostigmine contributed sustantially to the loss of contrast sensitivity. This accords with the results of section (1) which showed that changes in pupil diameter, without compensation for changes in retinal illumination, were without significant effect on contrast sensitivity and that defocus had least effect at very low spatial frequencies. It is, however, not possible to say whether the accommodative changes accounted entirely for the loss of contrast

sensitivity. The presence of an additional, central change is suggested by the results for measurement of critical fusion frequency and contrast sensitivity to laser interference fringes. Critical fusion frequency measured with an approximately constant entrance pupil was significantly reduced, thus confirming the results of Alpern and Jampel (1958) who had additionally shown that critical fusion frequency was not affected by defocus or by instillation of pilocarpine which caused otherwise similar actions to physostigmine. The decrease in laser interference fringe contrast sensitivity was not explicable by either the reduction in pupil diameter which had a more extended time course nor by defocus of the aperture in which the interference fringes were observed. This loss probably arose through trans-corneal absorption of physostigmine rather than systemic absorption since the companion eye's pupil diameter was quite unchanged throughout the experiment.

The site of physostigmine's central action is most probably at the retina which is well endowed with both nicotinic and muscarinic receptors, the latter being more numerous (Hruska, White, Azari and Yamamura, 1978). Higher levels of the visual pathway *per se* appear to be devoid of cholinergic transmission (Hebb and Silver, 1956; Phillis, Tebecis and York, 1967). The identification of the type of cholinoceptor on ganglion cells by iontophoretic studies is somewhat controversial. In cat, the Y ganglion cells which probably subserve detection of moving targets, but not X ganglion cells which subserve spatial resolution, are said to bear excitatory nicotinic receptors (Ikeda and Sheardown, 1982). By contrast, Schmidt, Humphrey and Wässle (1987) have identified excitatory muscarinic receptors on both X and Y cells. Consistent with this is the enhancement of the light-evoked response by physostigmine.

Since, in the present study, physostigmine had two actions, one of short and the other of long duration, both of which were inhibitory, this suggests that the site of action was not at the level of the ganglion cell but at a different population of neurones which had an inhibitory action on ganglion cells. These are probably amacrine cell populations which may be surmized to release either glycine or GABA at the ganglion cells (Ikeda & Sheardown, 1983; Bolz, Frumkes, Voigt and Wässle, 1985; Bolz, Thier, Voigt & Wässle, 1985). Facilitation of inhibitory transmission thus appears to outweigh any potentiation of excitatory transmission at the ganglion cell since contrast sensitivity was reduced by physostigmine.

(4) Intramuscular injection of atropine

In the present study, atropine caused an initial bradycardia followed by a marked tachycardia which had passed by 4-5 hr post-injection. This bradycardia has also been described by Chamberlain, Turner and Sneddon (1967) with 0.6-2.4mg, Kalser and McLain (1970) and Holland, Parkes and White (1975) with 2mg and Mirakhur (1978) with 0.5mg and 1.0mg but not at larger doses. The bradycardia has been attributed to either a stimulatory effect of atropine on vagal centres in the brain or a direct agonist action on the heart before the antagonist action develops (Bowman and Rand, 1980). The latter is consistent with the absence of an effect at higher doses. The increase in pupil diameter persisted up to 4 hr when it appeared to decline to values not significantly different from controls. This agrees with the results of Herxheimer (1958) and Rozsival and Cigánek (1978) who showed a similar decline in pupillary dilation but differs from the results of Mirakhur (1978) where the effect appeared to be still increasing at 6 hr post-

injection. The reduction in the amplitude of accommodation was sustained in the present study, now agreeing with the results of Mirakhur (1978) but differing from those of Herxheimer (1958) and Rozsival and Cigánek (1978) where some recovery from atropine was apparent at 6 hr post-injection. The results of Baker et al (1983) showed sustained changes in pupil diameter and near-point but these were followed only to 4 hr post-injection. It is unlikely that the loss of some 1.5D accommodation which had a similar time course to the pupillary dilation was due to loss of depth of focus since, for the 3mm pupil, this amounts to only 0.5D (Campbell, 1958) and must therefore be attributable to an effect of atropine *per se* on the ciliary body. The effect of atropine on causing a change in horizontal heterophoria and in cyclophoria was a quite short lasting effect which seemed to coincide with the change in heart rate rather than with the changes in pupil diameter and accommodation. This effect may be a non-specific effect on the opening of ion channels at the motor end plate (Wray, 1980) though there is evidence for muscarinic receptors which are blocked by atropine in superior oblique muscle of cat (Sanghvi and Smith, 1969).

In general, the ability to undertake stationary visual tasks was unaffected by atropine. Red-green colour balance remained unchanged which confirms the results of Baker et al (1983). Visual reaction time and choice reaction time were not significantly different, though the latter was extended somewhat. This differs from Miles (1955)'s result that reaction time was increased but choice reaction time was shorter. Baker et al (1983) found no significant difference in a visual search task of stationary objects. Most surprising was the lack of effect of atropine on visual acuity and stereoacuity since these were demanding

tasks requiring considerable concentration. While visual acuity is inversely related to pupil diameter (Westheimer, 1964), the lack of change despite an increased pupil diameter may be attributed to a compensatory increase in retinal illumination (Woodhouse, 1975).

The main new finding from this study is that an intramuscular injection of atropine sulphate adversely affected movement sensitivity at low spatial frequencies without affecting the perception of stationary sinusoidal grating patterns over the range of spatial vision. An adverse effect on movement sensitivity is consistent with the recently reported impaired tracking performance following an injection of 4 mg atropine (Penetar and Beatrice, 1986). The time course of the reduced contrast sensitivity to movement was sustained and did not correspond closely with the pupillary dilation which recovered towards the end of the experimental period. A corollary to our results is that changes in pupil diameter *per se* do not affect contrast sensitivity to stationary grating patterns which confirms the results obtained with homatropine eyedrops (section 1). Therefore, we may conclude that atropine has a selective direct effect on the visual system, causing depression of contrast sensitivity to movement.

Cholinergic inputs are present throughout the visual system but are quite often not involved in the transmission of visual information. The optic nerve is most notable for its absence of choline acetylase (Hebb and Silver, 1956) and neither atropine nor dihydro-β-erythroidine, a nicotinic antagonist, affect reponses of lateral geniculate neurones to stimulation of the optic tract (Phillis et al, 1967), indicating that acetylcholine is not the neurotransmitter released by the axon terminals of retinal ganglion cells. The cholinesterase reaction that is present

in the lateral geniculate body (*ibid*) seems to originate from neurones of the pontine and mesencephalic reticular formation and may be involved in arousal responses (Singer, 1979). The reticular formation also appears to be the source of cholinergic fibres to the visual cortex (*ibid*) which has the lowest concentration of choline acetylase in the entire cerebral cortex (Hebb and Silver, 1956). Iontophoretically-applied acetylcholine mainly enhances the response to visual stimuli without an effect on their specificity (Sillito and Kemp, 1983). Some neurones, particularly in layer 4, were depressed by acetylcholine and their dominance of the visual evoked response would explain its depression at lower spatial frequencies in cat after intravenous administration of a cholinesterase inhibitor (Harding et al, 1983).

In the retina, acetylcholinesterase occurs in the inner plexiform layer as indicated originally by histochemical studies in mouse (Eränkö, Niemi and Merenmies, 1961), being located in a population of amacrine cells with numerous varicosities ending on ganglion cell dendrites of rabbit (Masland, Mills and Hayden, 1984). In cat, cholinergic amacrine cells have been identified in the inner nuclear layer and as displaced cells in the ganglion cell layer (Schmidt et al, 1987). The cholinergic receptors on retinal ganglion cells of chick retina have been reported to be of the nicotinic type (Morgan and Murray, 1982). In cat, Ikeda and Sheardown (1982) described the presence of excitatory nicotinic receptors only on Y cells showing the periphery effect, the X cells being excited selectively by aspartate. Atropine was described as having a non-specific depressant effect when applied in high concentration. By contrast, Schmidt et al (1987) have reported that the resting discharge rate and the light evoked response of both X and Y cells were reduced by

iontophoretic application of hyoscine. The effects of the nicotinic antagonist dihydro-β-erythroidine produced anomalous results; OFF responding cells were inhibited while ON responding cells were *excited*. The balance of evidence therefore suggests that atropine has its action at the retina, probably within the inner plexiform layer. In the previous section, we argued that physostigmine enhanced the efficacy of amacrine cells which were surmized to have cholinergic inputs and released inhibitory transmitters onto the ganglion cell. This effect outweighed any potentiation of the direct excitatory cholinergic inputs onto the ganglion cell. It is at the latter synapse which the action of atropine is surmized to have its dominant effect in selectively depressing movement sensitive ganglion cells. There was, however, no evidence for an inhibitory effect on the spatial resolution function at higher spatial frequencies. While it is unwise to extrapolate without qualification from cat to man, there is an degree of agreement with the results of Ikeda and Sheardown (1982) except that they described the presence of nicotinic rather than muscarinic receptors on Y cells and with Schmidt et al (1987) who described hyoscine sensitive receptors on both X and Y cells. Given that the central actions of hyoscine are generally recognized to be more potent than those of atropine (Herxheimer, 1958; Mirakhur, 1978), it may be that atropine especially in low dosage may discrimate between the ganglion cells underlying the two systems which have also been described in man (Tolhurst, 1973).

It was recommended by Cullumbine et al (1955) that in cases of doubt (concerning cholinesterase poisoning) there should be no hesitation in the administration of 2mg atropine, a view endorsed by Headley (1982). However, in consideration of the adverse effects of atropine on movement

sensitivity, especially when coupled with impaired accommodation, it would seem prudent to exercise the utmost caution in administering any dose of atropine to operators involved in close work entailing the detection of moving objects.

(5) Physostigmine eyedrops and injection of atropine

Atropine sulphate given as a 2mg intramuscular injection was ineffective in antagonizing the effects of physostigmine eyedrops on vision whether given 8 min or 120 min previously. By contrast, prior application of 2% homatropine eyedrops effectively blocked the physostigmine-induced reduction in pupil diameter, the increase in near point accommodation, the increase in accommodation for distance and the accompanying decrease in contrast sensitivity. This indicates that neither the peak concentration of atropine in plasma occurring 30 min after injection nor atropine bound to the ocular tissues which was maximal at 120 min was sufficient to anatagonize the effects of physostigmine. It was noticeable that atropine *per se* reduced contrast sensitivity to the phase-reversed 3c/deg grating pattern while higher spatial frequencies were not consistently affected. Atropine also appears to have augmented slightly the deleterious action of physostigmine on contrast sensitivity to a phase-reversed grating pattern, though the results were not statistically significant. Both results are consistent with the results of the previous section (4) that atropine sulphate (2mg IM) *per se* reduced contrast sensitivity to phase-reversed grating patterns by some 20-30% and with the report of Penetar and Beatrice (1986) that 4 mg atropine sulphate (IM) significantly reduced the ability to detect a moving target.

It is of interest to ascertain the degree to which our results may be extrapolated to organophosphorus anticholinesterases which cause a more marked constriction of the pupil down to 1mm. The change in accommodation, however, remains uncertain. Spasm of accommodation or blurring of vision has frequently been stated to occur (Grob, 1956, 1963; Grob and Harvey, 1958; Cullumbine, 1963; Smith et al, 1968; Wood, 1950); but, of those who have actually measured accommodation, the results are varied. Scholz and Wallen (1946) and Upholt, Quinby, Batchelor and Thompson, (1957) described an appreciable increase in accommodation with DFP and TEPP, respectively, while Kilby and Kilby (1947) reported spasm of accommodation for DFP which actually amounted to only *circa* 2.2D. This may, in fact, be accounted for entirely by the increased depth of focus conferred by a 1mm pupil and the associated reduction in retinal illumination (Campbell, 1958). By contrast, Aldrige, Davson, Dunphy and Uhde (1947) reported unchanged visual acuity with DFP and Rengstorff (1985) reported improved visual acuity after accidental exposure to Sarin of 2 men who were slightly hypermetropic. This improvement was attributed entirely to the increased depth of focus resulting from a miosis of 1mm. So it is by no means clear whether organophosphorus anticholinesterases do effect spasm of accommodation in the way that physostigmine does (Fig 9). If not, then this would be a most remarkable difference. However, alternative explanations exist. Their binding potency may result in considerable binding to the iris without appreciable amounts reaching the ciliary musculature. This would be especially relevant to experimental studies where exposures were to low concentrations of agent. Second, Ruben et al (1957) noted that Sarin applied to the eye directly was remarkably ineffective in elevating the

threshold of dark adaptation which was caused by the vapour. This implies that the cornea may constitute a barrier to absorption of appreciable amounts of Sarin. Hence, those amounts which do penetrate are sufficient to cause miosis but not accommodative spasm. Third, since relatively few subjects have ever been studied, the possible genetic disposition may be of relevance.

Conclusions

In conclusion, when assessed against physostigmine eyedrops, intramuscular injection of atropine is quite ineffective and may actually worsen the perception of a moving target. Hence, if vision were the sole consideration, there would be no advantage in receiving atropine in anticipation of exposure to anticholinesterases.

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