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AGE-RELATED DIFFERENCES IN OPHTHALMIC DRUG DISPOSITION I. EFFECT OF SIZE ON THE INTRAOCULAR TISSUE DISTRIBUTION OF PILOCARPINE IN ALBINO RABBITS

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ABSTRACT

It has previously been documented that substantially different aqueous humour drug levels are observed in rabbits of different ages when the same dose of pilocarpine is instilled into the eye. Also, it has been shown that the aqueous humour volume ratio for rabbits of different ages can be used to predict aqueous humour levels of pilocarpine attained after topical dosing. In the present study, concentrations of pilocarpine in the cornea, aqueous humour, iris-ciliary body, lens, and vitreous humour were determined in both 20-day old and 60-day old rabbits following the topical administration of identical doses of drug. For tissues other than the aqueous humour and iris-ciliary body, consideration of only tissue size differences between rabbits of different ages will not suffice to explain the observed differences in pilocarpine concentration. Any attempt to develop rational age-related dosage modifications for ophthalmic drugs must include a consideration of functional and developmental differences as well as size effects.

KEY WORDS Pilocarpine Ocular tissue concentrations Age differences

INTRODUCTION

It is well known that many structural and functional changes occur within the eye from birth to adulthood. Nonetheless, the effect such changes have on drug disposition in the eye is not well understood. Preliminary investigations in this laboratory[1] have shown that when the same dose of pilocarpine is instilled into

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the eyes of rabbits of different ages, significantly higher levels of drug are attained in the aqueous humour of younger animals. Results of a subsequent study[2] indicated that it was possible to predict, reasonably well, aqueous humour levels of pilocarpine attained following the topical dosing of drug solutions to the eyes of rabbits of different ages, based solely on the aqueous humour volume ratio. That is, it appeared that differences in aqueous humour drug levels, observed between rabbits of different ages, could be attributed simply to size differences in the aqueous humour or anterior chamber volume between rabbits of different ages. The purpose of the present study was to further investigate age-related differences in the ocular disposition of pilocarpine in rabbits of different ages by considering drug uptake and distribution in ocular tissues surrounding the anterior chamber.

The use of the anterior chamber as a sampling compartment has provided a considerable amount of information relative to the corneal penetrability of compounds applied topically to the eye. The area under the aqueous humour concentration *vs* time profile (AUC) has often been used to evaluate the effectiveness of an ophthalmic drug delivery system. Generally, it has been assumed that drug levels achieved in the aqueous humour are reflective of levels of drug attained in the surrounding ocular tissues such as the iris, ciliary body, and lens. For pilocarpine, however, the results of some studies indicate that drug levels achieved in the tissues surrounding the anterior chamber are markedly different from, and often do not parallel, aqueous humour drug levels.[3-5] Since drug receptors are often located in these tissues, it is particularly important to understand the distribution and movement of drugs in these areas.

The studies described below involved the use of two age categories of rabbits. Young rabbits were between 17 and 23 days of age (20-day old rabbits) and older rabbits were between 56 and 65 days of age (60-day old rabbits).

The globe of the rabbit's eye varies considerably with the age of the animal and in the adult rabbit it is relatively large and rather prominent as compared to that of man.[6] At birth, the rabbit's globe is approximately 6 mm in diameter and grows quite rapidly, attaining about $\frac{2}{3}$ of its adult size by the 20th postnatal day.[6] Growth, thereafter, continues at a somewhat slower rate. By the 60th postnatal day, the globe attains about 90 per cent of its adult size which is generally reached between the 14th and 20th week.[6]

In humans, the globe is about 10 mm in diameter at birth[7] and the eye is proportionately larger than in rabbits, being about $\frac{2}{3}$ of its adult size. The rate of eye growth after birth, however, is somewhat slower than that observed in rabbits. In humans, the globe does not attain 90 per cent of its adult size until about 3 years of age.[8]

In this laboratory,[2] based on ocular developmental considerations, 20-day old rabbits have been selected to serve as an animal model for humans at birth. Similarly, 60-day old rabbits have been chosen to represent a 3-year old child, based on the extent of human eye growth by this age.

EXPERIMENTAL

Materials

Pilocarpine nitrate was obtained commercially* and used as received. Radiolabelled ^{3}H(G)-pilocarpine† (specific activity 10·0 Ci mmol) was received in ethanol and evaporated several times prior to use to remove any solvent that had become tritiated by exchange.[9]

Male, New Zealand white rabbits‡ were maintained in standard laboratory cages and prior to experimentation were allowed food and water *ad libitum*.

Solution preparation

Pilocarpine nitrate solutions were prepared in pH 7·38 Sorensen's phosphate buffer.[10] Procedures for the preparation of tritiated solutions were described previously.[9] All solutions were prepared on the day of use and any unused portion was discarded.

RADIOCHEMICAL PURITY AND STABILITY OF THE TRITIUM LABEL

The radiochemical purity of the labelled pilocarpine was reported by the manufacturer to be greater than 97 per cent. This was verified by thin layer chromatography (TLC), spotting 3 μl of ^{3}H-pilocarpine on silica gel G plates§ and developing the plates in 1 : 1, methanol : chloroform.[11] After development, the plates were radiochromatographically scanned‖ and the resulting chromatograms were integrated.¶

The lability of the tritium label to solvent exchange in phosphate buffer (pH 7·38) was investigated at room temperature (RT) and at 33°. This was done by preparing a labelled drug solution which was divided into 500 μl aliquots immediately after preparation. From one of the aliquots, four 50 μl samples were taken and each sample was transferred to a polyethylene mini-vial containing 5 ml of pre-refrigerated liquid scintillation fluid**. These 4 samples were considered to be taken at time zero. The remaining aliquots were either incubated in a water bath at 33° or, for the RT study, kept at ambient temperature.

At various times over a 10 h period (approximately 1, 2, 5, 7, and 9 h) one of the aliquots was evaporated to dryness under a stream of nitrogen and was then reconstituted with 500 μl of 'cold' pilocarpine nitrate. The sample was vortexed and 4 samples were taken as described above. The procedure was repeated with the remaining aliquots at different times over a 10 h period. To minimize

* Sigma Chemical Co., St. Louis, MO.
† New England Nuclear, Boston, MA.
‡ Small Stock Industries, Pea Ridge, AR.
§ Uniplate®, Analtech, Newark, DE.
‖ Packard Model 7230, Downer's Grove, IL. ¶ Packard Model 7240, Downer's Grove, IL.
** Aquasol®, New England Nuclear, Boston, MA.

photoluminescence, samples were stored in the dark for 24 h prior to counting in a liquid scintillation spectrometer*.

For each temperature, the 4 determinations at each time were averaged and counts per minute (cpm) were regressed on time (based on the time of reconstitution). For the RT study the regression coefficient was 107 cpm h^{-1} and the regression coefficient for the study conducted at 33° was −440 cpm h^{-1}. Neither regression coefficient was statistically significantly different from zero at the 95 per cent confidence level.[12] Therefore, it was concluded that there was no appreciable exchange of tritium between pilocarpine and the solvent during the time course of the experiments.

PILOCARPINE CONCENTRATION VERSUS TIME PROFILES

During the experiments, test animals were kept in restraining boxes in the normal upright position. The head of the rabbit was unencumbered so that all normal eye movements were maintained. Drug solutions were administered topically using a microlitre syringe†. The drop was placed at the top edge of the cornea, allowing it to flow downward over the eye and collect in the lower cul-de-sac. Since the typical response of the rabbit is to blink following the instillation of such solutions, no attempt was made to mechanically mix the fluid in the precorneal area after dosing.

In both age categories of rabbits, 25 μl of $1{\cdot}00 \times 10^{-2}$ mol l^{-1}, labelled pilocarpine nitrate were administered. Both eyes of the rabbit were used and the test solution was administered randomly to either the left or right eye first. Generally, the two eyes of an individual animal were used for different time points. At various times post-instillation (5, 10, 15, 20, 30, 45, 60, 90, and 120 min), animals were sacrificed with an overdose of pentobarbital sodium‡ and the aqueous humour, cornea, iris-ciliary body, lens, and vitreous humour were sampled as described below.

The 60-day old rabbits were sacrificed approximately 2 min before the indicated time point by rapid injection of the drug into a marginal ear vein. For 20-day old rabbits, the drug was administered by intraperitoneal injection approximately 4 min before the time point. Immediately after death of the rabbit, the corneas were rinsed and blotted and the aqueous humour was obtained by making a single puncture at the limbus using a 27 G × $\frac{1}{2}''$ needle attached to a 1 ml tuberculin syringe. The tip of the needle was placed in the centre of the anterior chamber and the aqueous humour was aspirated by gently pulling back on the plunger. The eye was then proptosed from the socket by pushing the eyelids downward around the globe. A pair of curved Crille forceps were clamped behind the globe to keep it protruded during the dissection.

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* Model LS-7000, Beckman Instruments, Irvine, CA.
† Hamilton Co., Reno, NV.
‡ Nembutal® sodium, Abbott Laboratories, North Chicago, IL.

A razor blade was used to make a small incision at the edge of the cornea just inside the limbus. One tip of a pair of small dissecting scissors was inserted into the opening and the entire cornea was separated from the sclera by cutting around its circumference. During this procedure, the lens generally extruded and was lifted out of the eye with a pair of forceps. After removal of the cornea, the iris was grasped with a pair of forceps. By gently pulling upward, the iris, ciliary body and the entire vitreous humour could be removed together. While still grasping the iris, a second pair of forceps was used to gently pull the vitreous humour free from the iris-ciliary body. It was then transferred directly to a tared, glass liquid scintillation vial. The iris-ciliary body, cornea, and lens were briefly rinsed and blotted before transferring to similar vials. Since, in the rabbit, the ciliary processes have a base which extends into the iris,[13] a complete separation of the iris and ciliary body is not possible. Therefore, in these studies, no attempt was made to separate these tissues*.

The entire dissection procedure took approximately one minute for each eye and was done as rapidly as possible to minimize any redistribution of drug which might occur over time. Dissection of the first eye was done during the minute prior to the reported sampling time, whereas, the second eye was dissected in the minute following the time point.

Aqueous humour samples were transferred from the collecting syringes to an aluminium planchett and duplicate 50 μl aliquots (60-day old rabbits) or 20 μl aliquots (20-day old rabbits) were transferred to mini-vials containing 5 ml of pre-refrigerated liquid scintillation fluid†. Prior to counting, the samples were stored in the dark for 24 h. The counts for the two samples were averaged and, after correcting for background radiation, cpm were converted to concentration using standard drug solutions.

For the tissue samples, the scintillation vials were re-weighted and the wet tissue weights determined by difference. To the iris and cornea samples, 500 μl of tissue solubilizer‡ were added which was the minimum amount of digestant required to completely cover the tissue in the bottom of the vials. To the vitreous humour and lens samples, 1 ml of tissue solubilizer was added. All of the samples were then tightly sealed with polyethylene lined screw-caps and incubated in a water bath at 55° until digestion was complete. The samples were allowed to cool to room temperature after which 10 ml of liquid scintillation fluid§ were added and the samples were vortexed for approximately 10 s. Before counting, the samples were stored in the dark for a minimum of 48 h which was the time required for subsidence of photo- and chemiluminescence.

Spiked tissues standards were used to convert the counts observed for a particular tissue sample to the amount of pilocarpine present. For each set of

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* In the following discussion any reference to observations made for the iris, implies that the tissue was comprised of both the iris and ciliary body.

† Aquasol®, New England Nuclear, Boston, MA.; ‡ Protosol®, New England Nuclear, Boston, MA.; § Econofluor®, New England Nuclear, Boston, MA.

experiments, individual standards were prepared for each type of tissue. Prior investigations indicated that small weight variations in the tissues did not affect the counting efficiency for a particular type of tissue. Tissue concentrations were then determined based on the wet weights of the individual tissues.

Average wet weights of ocular tissues

The average wet tissue weights of the cornea, iris, lens, and vitreous humour were determined by taking ocular tissue samples from animals of both ages immediately after sacrifice. The tissue samples were carefully dissected from the eye, taking care to obtain the total tissue mass of each particular type. The tissues were blotted and transferred to tared glass vials. The vials were re-weighed and the wet tissue weight was determined by difference.

Table 1. Concentration of pilocarpine in the cornea following the topical instillation of 25 μl of $1{\cdot}00 \times 10^{-2}$ mol l^{-1} pilocarpine nitrate, pH 7·2

Time (min)	μg of pilocarpine per g of cornea*		p†
	20-day	60-day	
5	25·3 (2·5)‡ [10]§	14·5 (1·64) [10]	<0·005
10	35·4 (4·1) [9]	9·24 (0·93) [11]	<0·001
15	26·3 (5·2) [9]	9·57 (1·07) [11]	<0·005
20	21·4 (3·7) [9]	7·00 (1·05) [13]	<0·001
30	16·7 (4·2) [9]	5·63 (0·90) [15]	<0·005
45	5·22 (0·90) [10]	2·52 (0·18) [10]	<0·01
60	4·41 (0·50) [9]	2·00 (0·20) [14]	<0·001
90	1·41 (0·13) [9]	1·17 (0·13) [9]	n.s.
120	0·90 (0·05) [8]	0·81 (0·13) [12]	n.s.

* Concentrations are based upon pilocarpine alkaloid.
† Probability of age-related differences in concentration based on a two-tailed weighted t-test ($\alpha = 0{\cdot}05$).
‡ Numbers in parentheses refer to the standard error of the mean.
§ Numbers in brackets refer to the number of determinations at that time point.
n.s.—Not significant.

RESULTS

The concentration *vs* time profiles for pilocarpine in ocular tissues and fluids observed following the topical instillation of 25 μl of $1{\cdot}00 \times 10^{-2}$ mol l^{-1} pilocarpine nitrate in 20- and 60-day old rabbits are listed in Tables 1–5. Generally, in both ages of rabbits, the highest concentration of drug was observed in the cornea, followed by the aqueous humour, iris, lens, and vitreous humour. Between the two ages of rabbits, at all time points considered, the concentrations of drug observed in each of the individual tissues were higher in the 20-day old rabbits than the corresponding concentrations in 60-day old rabbits.

The overall concentration-time profiles for each individual ocular tissue were analysed for the significance of age-related differences in concentration by

Table 2. Concentration of pilocarpine in the aqueous humour following the topical instillation of 25 μl of $1{\cdot}00 \times 10^{-2}$ mol l^{-1} pilocarpine nitrate, pH 7·2

Time (min)	μg of pilocarpine per ml of aqueous humour*		p†
	20-day	60-day	
5	2·23 (0·25)‡ [10]§	0·84 (0·07) [9]	<0·001
10	4·31 (0·39) [8]	1·78 (0·23) [11]	<0·001
15	5·57 (0·84) [12]	2·02 (0·22) [8]	<0·005
20	5·70 (0·61) [9]	1·78 (0·17) [12]	<0·001
30	4·44 (0·75) [10]	1·97 (0·32) [13]	<0·005
45	2·37 (0·38) [10]	0·95 (0·05) [5]	<0·025
60	2·01 (0·22) [8]	0·60 (0·01) [5]	<0·001
90	0·66 (0·06) [7]	0·56 (0·05) [10]	n.s.
120	0·30 (0·02) [8]	0·25 (0·02) [7]	n.s.

* Concentrations are based upon pilocarpine alkaloid.
† Probability of age-related differences in concentration based on a two-tailed weighted t-test ($\alpha = 0{\cdot}05$).
‡ Numbers in parentheses refer to the standard error of the mean.
§ Numbers in brackets refer to the number of determinations at that time point.
n.s.—Not significant.

Friedman's method for randomized blocks.[12] In the analyses, the mean drug concentration at each time point was used in a two-tailed test ($\alpha = 0{\cdot}05$). For each of the individual tissues and fluids, the overall difference in the concentration profiles between the two age groups was significant ($p < 0{\cdot}01$). Next, a series of t-tests were made and used to determine at which individual time points the age-related differences in concentration were significantly different. The probabilities associated with these differences are given in the last column of Tables 1–5.

Average wet weights of ocular tissues

The average wet weights of ocular tissues from 20-day old and 60-day old rabbits are listed in Table 6. As would be expected, the tissues obtained from 60-day old rabbits were substantially heavier than those sampled from the eyes of

Table 3. Concentration of pilocarpine in the iris-ciliary body following the topical instillation of 25 μl of $1{\cdot}00 \times 10^{-2}$ mol l^{-1} pilocarpine nitrate, pH 7·2

Time (min)	μg of pilocarpine per g of iris-ciliary body*		p†
	20-day	60-day	
5	1·44 (0·21)‡ [8]§	0·36 (0·03) [10]	<0·001
10	3·16 (0·26) [10]	0·62 (0·07) [12]	<0·001
15	1·93 (0·21) [9]	0·80 (0·09) [11]	<0·001
20	1·85 (0·19) [9]	0·60 (0·08) [12]	<0·001
30	1·74 (0·19) [7]	0·72 (0·11) [15]	<0·001
45	0·81 (0·10) [9]	0·41 (0·02) [10]	<0·001
60	0·89 (0·06) [8]	0·36 (0·03) [14]	<0·001
90	0·45 (0·03) [8]	0·23 (0·02) [9]	<0·001
120	0·47 (0·05) [8]	0·29 (0·04) [12]	<0·02

* Concentrations are based upon pilocarpine alkaloid.
† Probability of age-related differences in concentration based on a two-tailed weighted t-test ($\alpha = 0{\cdot}05$).
‡ Numbers in parentheses refer to the standard error of the mean.
§ Numbers in brackets refer to the number of determinations at that time point.

20-day old rabbits. The average wet tissue weight ratio (60 : 20) for the cornea was 2·00; the iris, 2·57; the lens, 1·88; and the vitreous humour, 2·48. The volume of the aqueous humour can be approximated (assuming the globe is spherical) to be 127 μl and 311 μl in 20-day old and 60-day old rabbits, respectively.[2] The ratio for the aqueous humour (60 : 20) is, therefore, about 2·45. Although there are both tissue and animal to animal variations, generally, ocular tissues of 60-day old rabbits are about 2–3 times larger than those of 20-day old rabbits.

DISCUSSION

Although a number of factors may contribute to age-related differences in the ocular disposition of pilocarpine, one factor which is immediately apparent is size differences between the various ocular structures among animals of different

Table 4. Concentration of pilocarpine in the lens following the topical instillation of 25 μl of $1{\cdot}00 \times 10^{-2}$ mol l^{-1} pilocarpine nitrate, pH 7·2

Time (min)	μg of pilocarpine per g of lens*		p†
	20-day	60-day	
5	0·22 (0·04)‡ [17]§	0·063 (0·004) [7]	<0·005
10	0·25 (0·04) [7]	0·061 (0·005) [7]	<0·001
15	0·26 (0·03) [8]	0·058 (0·004) [7]	<0·001
20	0·37 (0·03) [6]	0·072 (0·008) [7]	<0·001
30	0·24 (0·05) [7]	0·074 (0·005) [7]	<0·005
45	0·21 (0·02) [7]	0·080 (0·008) [6]	<0·001
60	0·39 (0·05) [7]	0·097 (0·008) [7]	<0·001
90	0·24 (0·04) [6]	0·069 (0·008) [7]	<0·001
120	0·20 (0·03) [7]	0·11 (0·013) [7]	<0·02

* Concentrations are based upon pilocarpine alkaloid.
† Probability of age-related differences in concentration based on a two-tailed weighted t-test ($\alpha = 0{\cdot}05$).
‡ Numbers in parentheses refer to the standard error of the mean.
§ Numbers in brackets refer to the number of determinations at that time point.

Table 5. Concentration of pilocarpine in the vitreous humour following the topical instillation of 25 μl of $1{\cdot}00 \times 10^{-2}$ mol l^{-1} pilocarpine nitrate, pH 7·2

Time (min)	μg of pilocarpine per g of vitreous humour*		p†
	20-day	60-day	
5	0·071 (0·012)‡ [7]§	0·019 (0·002) [7]	<0·005
10	0·073 (0·013) [7]	0·022 (0·002) [6]	<0·005
15	0·10 (0·02) [8]	0·018 (0·001) [6]	<0·01
20	0·11 (0·02) [7]	0·024 (0·002) [7]	<0·005
30	0·12 (0·02) [5]	0·020 (0·002) [6]	<0·001
45	0·060 (0·008) [7]	0·019 (0·002) [7]	<0·001
60	0·089 (0·010) [7]	0·021 (0·003) [7]	<0·001
90	0·096 (0·032) [7]	0·019 (0·003) [6]	<0·05
120	0·046 (0·005) [7]	0·015 (0·001) [7]	<0·001

* Concentrations are based upon pilocarpine alkaloid.
† Probability of age-related differences in concentration based on a two-tailed weighted t-test ($\alpha = 0{\cdot}05$).
‡ Numbers in parentheses refer to the standard error of the mean.
§ Numbers in brackets refer to the number of determinations at that time point.

Table 6. Average ocular tissue weights of rabbits

Tissue	Wet weight	Range
60-day old*		
Cornea	46·11 mg (0·86)†	44·15–48·36 mg
Iris-ciliary body	41·00 mg (0·69)	39·82–42·94 mg
Lens	234·77 mg (1·80)	230·11–238·98 mg
Vitreous humour	1122·39 mg (9·61)	1094·36–1138·11 mg
20-day old‡		
Cornea	23·10 mg (0·76)	19·89–26·70 mg
Iris-ciliary body	15·96 mg (1·13)	11·93–22·23 mg
Lens	124·92 mg (2·53)	116·02–139·09 mg
Vitreous humour	452·15 mg (40·2)	276·10–652·86 mg

* Average is based on a total of four determinations for all tissues.
† Numbers in parentheses refer to the standard error of the mean.
‡ Average is based on a total of nine determinations for all tissues.

ages. Obviously, if one places a given amount of drug in two different volumes, all other factors being equal, one would expect to obtain a higher concentration of drug in the smaller volume. Similarly, if the same amount of drug is administered to animals of different size, it would be expected, all other factors being equal, that a higher concentration of drug would be observed in the smaller animals.

In order to determine whether the differences in ocular tissue concentrations of pilocarpine observed between 20-day old and 60-day old rabbits following identical dosing could be attributed to size alone, the concentration *vs* time profiles were converted to amount *vs* time profiles. This was accomplished by multiplying the mean tissue concentration at each time point (see Tables 1–5) by the average wet weight of the ocular tissues listed in Table 6 for each age of rabbits. Aqueous humour concentrations were converted to the total amount of drug present in this fluid based on the values for aqueous humour volume given above.

Since the values for drug concentration at a particular time and ocular tissue weights are both mean values, the conversion of drug concentration to drug amount generates a series of values for which the variance cannot be precisely determined. Therefore, it is not possible to quantitatively assess the contribution of size alone to the total age-related differences in ocular drug disposition. The precision of the calculated amounts of drug observed in the tissues as a function of time can be estimated, however, if it is assumed that there is no variation in tissue weight. In general, this assumption appears to be reasonable since the coefficients of variation for the tissue weights are small compared to those for concentration. Based on this assumption, a 95 per cent confidence interval can be determined for the amount of drug present in each of the tissues, at each time point, for both ages of rabbits.

For a particular tissue and time point, if the 95 per cent confidence interval for the amount of drug present in each age group does not overlap, it can be reasonably concluded that the amount of drug present in that tissue, at that time point, was different in the two ages of rabbits. On the other hand, if any portion of the two 95 per cent confidence intervals did overlap, it could be concluded that there was no significant difference in the amount of drug present in the two ages of rabbits, for that tissue, at that time point.

Since the method described above can only provide an estimate of the 95 per cent confidence interval for drug amount (due to the fact that no variation in tissue weight was assumed), it would not be practical to further assign a probability to a difference in drug amount detected by this treatment. It can be used, however, to determine, in a semi-quantitative manner, whether the amount *vs* time profiles for drug in the various ocular tissues are different between the two ages of rabbits. The important thing to keep in mind is that if size were the only factor contributing to the age-related differences in drug concentration, within experimental error, the amount *vs* time profiles for both ages of rabbits would be superimposable.

The amount *vs* time profiles for pilocarpine in the corneas of 20-day old and

60-day old rabbits are shown in Figure 1.* Although the amount of drug in the cornea was slightly higher in the older rabbits at the first sampling time, the difference was not significant. Shortly thereafter, a greater amount of drug was observed in the corneas of the 20-day old rabbits. Although this trend continued until 60 min post-instillation, the difference in drug amount was significant only at the 10 min point. From about 45 min onwards, the amount of drug present in the corneas of the two ages of rabbits were approximately equal and declined roughly in parallel. The 95 per cent confidence intervals at the 90 and 120 min time points did not overlap, however, with the amount of drug present being greater in 60-day old rabbits than in 20-day old rabbits.

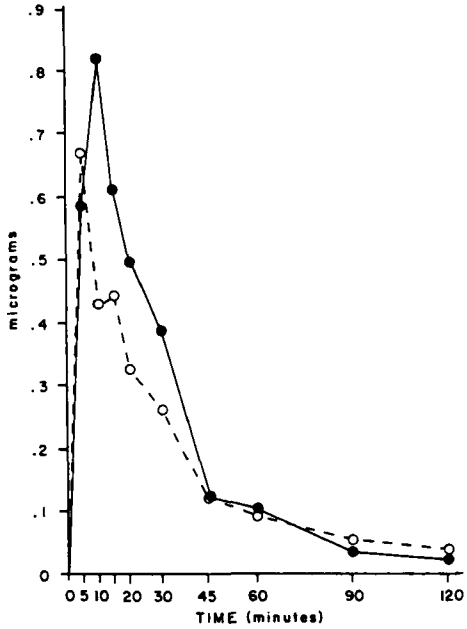

Figure 1. Cornea amount *vs* time profile following the topical instillation of 25 μl of 1×10^{-2} mol l^{-1} pilocarpine nitrate, pH 7·2. Closed circles represent 20-day old rabbits; open circles represent 60-day old rabbits

As can be seen in Figure 2, the total amount of drug in the aqueous humour was nearly identical in the two ages of rabbits at the two earliest sampling times. Thereafter, the total amount of drug in the aqueous humour of 20-day old animals rose slightly above that of the 60-day old animals. In both ages of

* The 95 per cent confidence intervals for the data have been omitted for clarity of presentation. They can, however, be readily determined using the data given in Tables 1 and 6. This is similarly true for the other ocular tissues.

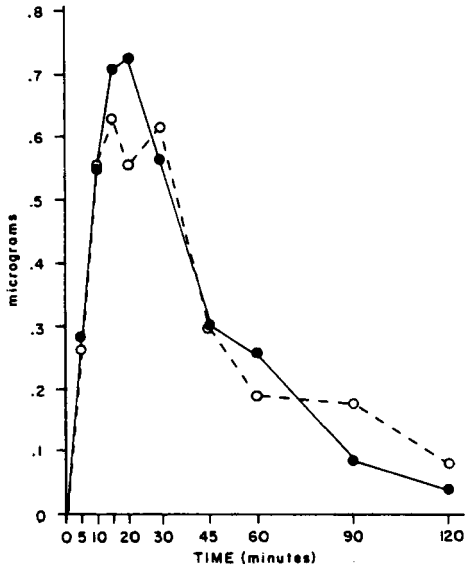

Figure 2. Aqueous humour amount *vs* time profile following the topical instillation of 25 µl of 1×10^{-2} mol l^{-1} pilocarpine nitrate, pH 7·2. Closed circles represent 20-day old rabbits; open circles represent 60-day old rabbits

rabbits, the amount of drug present in the aqueous humour declined very rapidly between 30 and 45 min. Thereafter, the rate of decline slowed somewhat. Differences in drug levels between the two ages of rabbits were significant only at the 90 and 120 min time points, with the amount of drug present in the aqueous

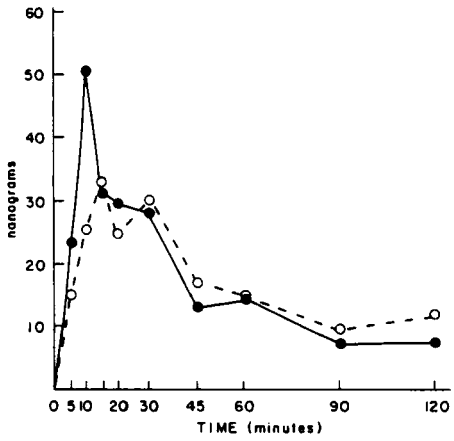

Figure 3. Iris-ciliary body amount *vs* time profile following the topical instillation of 25 µl of 1×10^{-2} mol l^{-1} pilocarpine nitrate, pH 7·2. Closed circles represent 20-day old rabbits; open circles represent 60-day old rabbits

humour of 60-day old rabbits being greater than that present in 20-day old rabbits.

As can be noted from Figure 3, the amount of drug in the iris was substantially greater in 20-day old rabbits than in 60-day old rabbits at very early time points. The amounts became quite similar in the two ages of rabbits between 15 and 20 min post-instillation, and thereafter declined approximately in parallel. The difference in drug levels between the two age groups was significant only at the 10 min time point, with the amount of drug present in the irises of 20-day old rabbits being greater than the amount present in 60-day old rabbits.

If one considers the amount *vs* time profiles for the lenses shown in Figure 4, it is apprarent that drug levels in this tissue were considerably higher in the 20-day old animals, relative to those 60-day old, than would be expected on the basis of size alone. The difference in drug levels was significant at 5 of the 9 time points.

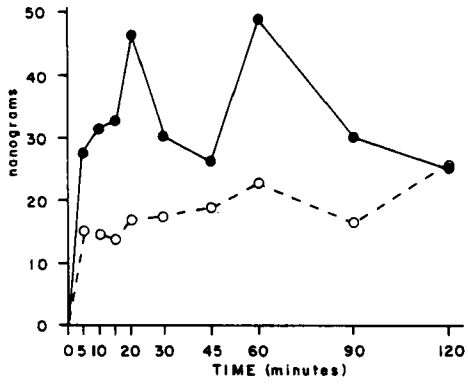

Figure 4. Lens amount *vs* time profile following the topical instillation of 25 μl of 1×10^{-2} mol l^{-1} pilocarpine nitrate, pH 7·2. Closed circles represent 20-day old rabbits; open circles represent 60-day old rabbits

The amount of drug present in the lenses of 20-day old rabbits was greater than the amount present in lenses of 60-day old rabbits at 10, 15, 20, 45, and 60 min post-instillation. As can be seen in Figure 4, the amount *vs* time profile for the lenses of 20-day old rabbits was considerably more erratic than the tissues previously considered.

Similarly, the amount *vs* time profile for pilocarpine in the vitreous humour of 20-day old rabbits also seemed to fluctuate considerably. The difference in amount of drug in the vitreous humour between the two ages of rabbits was significant, however, at only the 15 and 30 min time points. At these times, the amount of drug was greater in the vitreous humour obtained from 20-day old rabbits than that sampled from 60-day old rabbits.

Within an age group, the amount of drug present in each tissue at a particular time point can be summed for all the ocular tissues to obtain the apparent, total

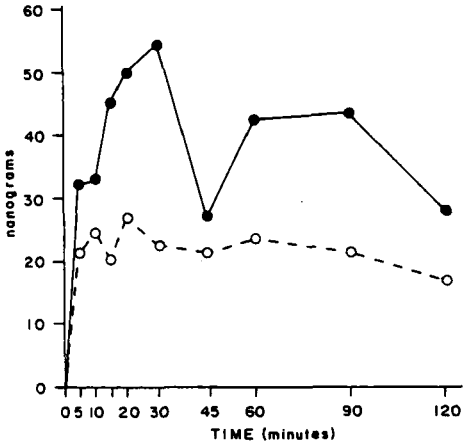

Figure 5. Vitreous humour amount *vs* time profiles following the topical instillation of 25 μl of 1×10^{-2} mol l^{-1} pilocarpine nitrate, pH 7·2. Closed circles represent 20-day old rabbits; open circles represent 60-day old rabbits

amount of drug present in the eye as a function of time. This amount does not, in fact, represent the actual toal amount of drug in the eye since, it is based only on those tissues sampled. It can, however, be considered as an estimate of the total amount of absorbed drug present in the eye at various times post-instillation. The comparative profiles for total amount of drug in the eye as a function of age are shown in Figure 6. From this illustration it can be seen that, overall, it appears

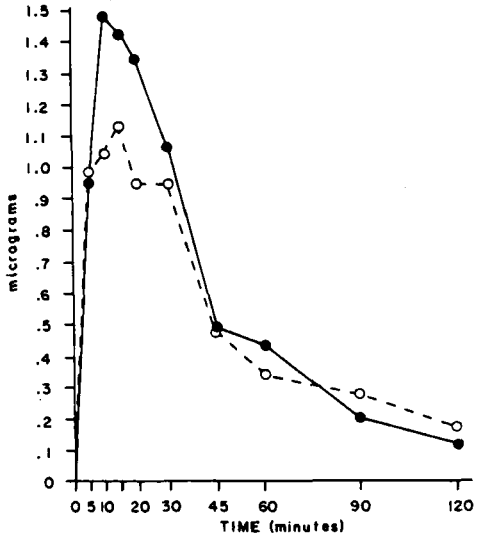

Figure 6. Total amount *vs* time profile in the eye following the topical instillation of 25 μl of 1×10^{-2} mol l^{-1} pilocarpine nitrate, pH 7·2. Closed circles represent 20-day old rabbits; open circles represent 60-day old rabbits

that the total amount of absorbed drug in the eye is somewhat greater in 20-day old rabbits than those 60-days of age.

Based on the mean concentration and mean amount of pilocarpine at each time point, the AUC's were calculated for the individual ocular tissues in each age group of rabbits. The areas ($0 \rightarrow 120$ min) obtained using trapezoidal integration are listed in Table 7. These results indicate that, on the average, the

Table 7. Area* under the concentration *vs* time curves and area under the amount *vs* time curves ($0 \rightarrow 120$ min) following the topical instillation of 25 μl of $1{\cdot}00 \times 10^{-2}$ mol l^{-1} pilocarpine nitrate, pH 7·2, to rabbits of different ages

	Concentration†			Amount‡		
	20-day	60-day	Ratio (20/60)	20-day	60-day	Ratio (20/60)
Cornea	1040	419	2·48	24·0	19·3	1·24
Aqueous humour§	264	109	2·42	33·5	34·0	0·99
Iris-ciliary body	121	48	2·52	1·9	2·0	0·95
Lens	31	9·5	3·26	3·9	2·2	1·77
Vitreous humour	10	2·3	4·35	4·5	2·6	1·73

* Area was calculated by trapezoidal integration.
† Units are μg min gm^{-1}.
‡ Units are μg min.
§ The density of aqueous humour was assumed to be 1 gm ml^{-1}.

concentration of drug observed in the 20-day old rabbits was about 3 times as great as that observed in 60-day old rabbits. When AUC's were calculated for the amount *vs* time profiles, however, the difference in area was substantially reduced for all of the tissues considered. From the last column of Table 7 it can be seen that the AUC's for the amount *vs* time profiles were much more similar between the two ages of rabbits than those determined from the concentration *vs* time profiles. The 20/60-day AUC ratio was near unity for the aqueous humour and iris-ciliary body. The observation for the aqueous humour is in agreement with earlier findings[2] which suggested that differences in aqueous humour levels of pilocarpine observed in rabbits of different ages could be attributed to aqueous humour volume differences.[2] In the cornea, the 20-day AUC was about 25 per cent larger than that calculated for the 60-day old rabbits. The largest relative differences in the AUC's based on the amount *vs* time profiles determined for the two ages of rabbits were seen in the lens and vitreous humour.

Age-related differences in ocular drug distribution can also be examined by considering what fraction of the toal amount of absorbed drug is present in the individual ocular tissues at any given time. These fractions can readily be calculated based on the total amount of drug in the eye, present in each of the ocular tissues sampled. Such considerations are briefly described below.

At early times, as would be expected, the fraction of total drug which is present in the cornea is quite large, accounting for about 60–70 per cent of the absorbed

drug. This fraction declines rather rapidly though as drug passes through this tissue into the aqueous humour. From about 45 min onward, the fraction of total drug present in the cornea remained relatively constant at about 25 per cent. Throughout the duration of these experiments, the fraction of total drug present in the cornea for each age of rabbits was approximately the same.

If one considers the aqueous humour, it can be determined that as the corneal fraction of total drug declines, a corresponding increase in the total fraction of drug in the aqueous humour occurs. For most time points, the fraction of total drug present in the aqueous humour was slightly greater in the 60-day old rabbits than those 20-days of age. Also, in neither age of rabbits did the fraction of total drug present in the aqueous humour level off as was observed in the cornea. In additional, it can be determined that, although the aqueous humour fractions do appear to decline with time, as late as 2 h post-instillation, drug present in the aqueous humour still accounts for over 40 per cent of the total amount of drug present in the eye.

On the other hand, the amount of drug present in the iris accounts for only a very small percentage (generally, $<0{\cdot}04$ per cent) of the total amount of pilocarpine in the eye, regardless of the time point considered. The fraction of total drug present in the iris was quite similar between the two ages of rabbits at all times considered. Unlike either the cornea or the aqueous humour, the fraction of total drug present in the iris showed almost a continual rise, indicating that, during the time course of these studies, pilocarpine accumulates in this tissue.

The same trend of increasing fraction with time was observed in the lens. However, the rise was much steeper than that observed for the iris and at late times, over 10 per cent of the total amount of drug in the eye could be attributed to drug present in the lens. At all times, the fraction of the total amount of drug in the eye present in the lens was higher in the 20-day old rabbits than in the 60-day old rabbits.

A similar trend was observed in the vitreous humour. At early times, fractional differences in the amount of drug present in the vitreous humour were quite small between ages. At early time points, drug present in this fluid also contributed little to the total amount of drug present in the eye. In 60-day old rabbits, a gradual increase in the fraction of total drug present in the vitreous was observed over the 2 h duration of these studies. The rise in vitreous humour drug fraction was more marked in 20-day old rabbits than those 60-days of age and at later time points appeared to make a rather substantial contribution to the total amount of drug present in the eye ($\cong$8–20 per cent).

If the movement of drug within the eye occurs solely via first-order processes, at some time post-instillation, a pseudo-steady state should be attained within the eye. In this post-distributive phase, the fraction of drug present in any compartment should approach a constant value.[14] As is evidenced by considering the fraction of drug present in each of the tissues as a function of time, within the 2 h duration of these studies, pilocarpine does not attain a pseudo-

distribution equilibrium. This is probably due to its slow equilibration with some ocular tissues.[15]

SUMMARY AND CONCLUSIONS

Overall, it appears that size differences in ocular tissues between 20-day old and 60-day old rabbits can account for some of the differences in the ocular tissue concentrations of pilocarpine. A consideration of size differences alone, however, will not suffice to explain all of the observed age-related differences in pilocarpine concentrations in ocular tissues following identical topical dosing of pilocarpine to 20-day old and 60-day old rabbits.

Comparison of the AUC's for the apparent total amount of pilocarpine in the eye *vs* time (see Figure 6) seems to suggest that in 20-day old rabbits a greater fraction of the applied dose is absorbed as compared to 60-day old rabbits. The observed difference in AUC may, however, be due, at least in part, to differences in parameters affecting disposition of drug within the eye. The action of pilocarpine on the regulation of intraocular pressure is often defined in terms of its ability to increase outflow facility and cause vascular dilation.[16] It might be, that differences in receptor sensitivity, as a function of age, could influence the disposition of pilocarpine within the eye.

If differences in absorption, *per se*, are responsible for the differences in the amount of pilocarpine in the eye, as a function of age, this difference could, itself, be due to one or more factors. Of a direct nature, such an observation might be due to differences in corneal permeability. The difference, however, might also be indirectly caused, by differences in pre-corneal fluid dynamics. It might be possible that the eyes of younger rabbits cannot adapt as readily to the instillation of a foreign fluid volume.[17] There may also be differences as a function of age in the resident pre-corneal fluid volume or the rate of normal tear production and turnover. Such possibilities have been suggested by observed differences in the pre-corneal concentration *vs* time profiles observed in 20-day old and 60-day old rabbits following identical dosing of pilocarpine nitrate.[18]

Within the eye, additional factors that might contribute to age-related differences in drug disposition are also operative. These include possible differences in aqueous humour dynamics, drug-protein interaction in ocular fluids and tissues and drug metabolism. Some of these factors have been investigated[18] and other studies are currently in progress. Results of these studies will be reported in subsequent communications.

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