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Age-related changes in esterase activity in rabbit eyes

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Summary

Thus far, the influence of age on the bioavailability of topically applied ophthalmic drugs has been studied by considering age-dependent changes in membrane permeability and protein binding. The specific objective of this research was to determine variation of ocular esterase activity with age and pigmentation of the rabbit. This was achieved by monitoring the fluorescence intensity as a function of time upon incubating ocular tissue homogenates of albino and pigmented rabbits of various age groups with α-naphthyl acetate, both in the presence and absence of esterase inhibitors. In general, esterase activity was the highest in the iris-ciliary body followed by the cornea and then the aqueous humor. Of the age groups studied, peak ocular esterase activity was reached at 6 weeks in the albino rabbit, and beyond 6 weeks in the pigmented rabbit. In the 6-week-old group the albino rabbit exceeded the pigmented rabbit in ocular esterase activity, whereas in the 12-week-old group it was the pigmented rabbit that exceeded the albino rabbit in ocular esterase activity. The conclusion was that in establishing a dosage regimen, age-dependent changes in ocular esterase activity must be considered in conjunction with age-dependent changes in the other factors that also help determine the amount of active drug ultimately reaching its receptor.

Introduction

Because of the differences in ocular physiology existing between the infant and the adult, the pharmacokinetics of topically applied ophthalmic drugs in these age groups can conceivably be different. The known physiological differences are the surface area available for drug absorption, the integrity of the structural membranes,

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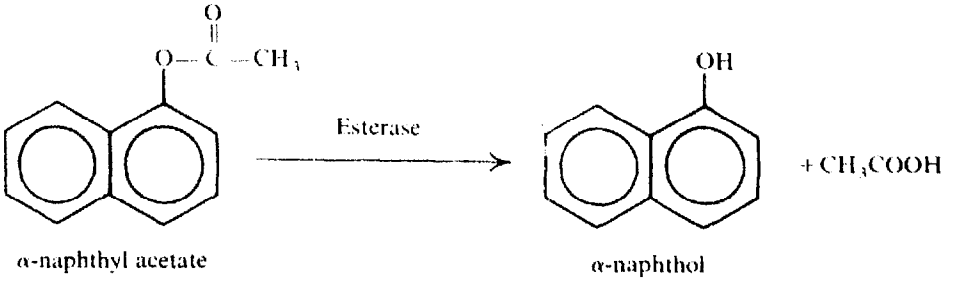
and tear flow rate and aqueous humor volume within which the drugs distribute (Bettman, 1981; Apt and Cullen, 1964; Miller and Patton, 1981). Because ophthalmic drugs can gain entry to the systemic circulation following topical application (Anderson, 1980) and because the ability to do so may vary with age, toxic side-effects have been noted in infants following the administration of adult doses of certain ophthalmic drugs (France and France, 1973). With albino rabbits as a model, Patton (1977) has verified an approximate, theoretical method which can be used to adjust the dose such that the toxic systemic side-effects of ophthalmic drugs are indirectly minimized. However, there are instances whereby infants and young children appear to be non-responsive to doses of certain drugs known to be effective in the adult (Helveston and Ellis, 1980). Possibly this observation can be explained by differences in the extent of protein binding in tears or aqueous humor, tear flow rate, solution drainage, as well as metabolism of the drugs by enzymes in the eye.

Until recently, there have been few reports on drug metabolism in the eye (Shichi and Nebert, 1980; Das and Shichi, 1981). In that metabolism can both elicit and terminate the pharmacological activities of drugs, it constitutes an important aspect of drug action. It follows that if age-related differences in activity and distribution of drug-metabolizing enzymes in the eye exist, age-related differences can be expected to exist in the response to topically applied ophthalmic drugs.

This report concerns the determination of changes in overall esterase activities in the cornea, iris-ciliary body and aqueous humor of 3-, 6- and 12-week-old albino rabbits and 6- and 12-week-old pigmented rabbits. The rationale of studying esterase activities in both types of rabbits was to investigate whether differences in esterase activities seen in the ocular tissues of adult rabbits (unpublished data) are evident as early as 6 weeks of age.

The esterases are thought to metabolize drugs containing ester linkages such as pilocarpine, atropine and dipivalyl epinephrine. In this study, α-naphthyl acetate was chosen as the model substrate for two reasons. First, it has been shown to be a substrate for most esterases (Ecobichon and Kalow, 1962), as well as for peptidases and carbonic anhydrase which also possess esterase activity (unpublished data). Second, at this stage of the study screening for esterase activity rather than identifying specific esterases was of primary concern. An advantage offered by this substrate is the attendant simple methodology. At an excitation wavelength of 317 nm and emission wavelength of 470 nm, it is practically non-fluorescent when



Scheme I. Hydrolysis of α-naphthyl acetate

compared to its hydrolytic product, α-naphthol. Consequently, the progress of esterase-mediated hydrolysis, and therefore esterase activity, can be monitored by noting the increase in fluorescence with time. Scheme I presents the stoichiometry of the reaction.

During preliminary investigation two other substrates—dipivalyl epinephrine and ethyl *p*-aminobenzoate—were found to be unsuitable to screen for esterase activity using fluorescence techniques. Apparently, epinephrine, the hydrolytic product of dipivalyl epinephrine, was subjected to further metabolism by the catecholamine-inactivating enzymes also present in the tissue homogenates (Waltman and Sears, 1964). In the case of ethyl *p*-aminobenzoate, it was subjected to interference by the proteins in the homogenates as both substances absorb and emit at the same wavelengths, 258 nm and 350 nm, respectively.

Materials

Male, albino and pigmented rabbits, aged 3, 6 and 12 weeks, were purchased from ABC Rabbitry (Pomona, CA) [1]. Acetazolamide, α-naphthol, β-naphthylamine, carbonic anhydrase (from rabbit erythrocyte), and porcine liver esterase (Type I) were purchased from Sigma Chemicals (St. Louis, MO); diisopropylfluorophosphate (DFP) from Calbiochem-Behring (San Diego, CA); and α-naphthyl acetate, L-leucine-β-naphthylamide, eserine sulfate, neostigmine bromide, and *p*-chloromercuribenzoate from United States Biochemicals (Cleveland, OH). They were used as received.

Methods

(1) Preparation of substrate solutions

(a) α-Naphthyl acetate

A 2×10^{-3} M solution of α-naphthyl acetate was prepared by first dissolving 0.0372 g of the compound in 10 ml of 95% ethanol followed by dilution to 100 ml with an isotonic Tris buffer at pH 7.4. From this stock solution 1.5×10^{-5} M and 6×10^{-5} M solutions were prepared for the enzymatic hydrolysis studies. These concentrations covered the range of drug concentrations typically encountered in ocular tissues.

The 10 ml of alcohol was found to be necessary to maintain α-naphthyl acetate in solution. While preliminary data indicated that esterase activity was reduced in the presence of ethyl alcohol, it was decided that this did not invalidate determination of esterase activity in the various ocular tissues so long as the relative activity was of interest.

[1] Studies were not conducted with 3-week-old pigmented rabbits because they were not available in the southern California area.

(b) L-Leucyl-β-naphthylamide (LNA) solutions

L-Leucyl-β-naphthylamide, a peptidase substrate, was selected to screen for peptidase activity and to correct the observed esterase activity for the contribution due to peptidases. The peptidases cathepsins have been found in the eye (Hayasaka and Hayasaka, 1978), and shown to possess esterase activity (Krish, 1971).

A 4.67×10^{-4} M solution of L-leucyl-β-naphthylamide was prepared by first dissolving 0.0114 g of the compound in 10 ml of 95% ethanol followed by dilution to 100 ml with a 0.0612 M Tris buffer at pH 8.2. This pH was found to optimize peptidase activity (Wolff and Resnick, 1963).

(2) Fluorescence intensities of α-naphthol, α-naphthyl acetate, β-naphthylamine and L-leucyl-β-naphthylamide

The wavelengths of maximum excitation and emission were determined for each compound using a Cary 219 spectrophotometer and an Aminco-Bowman spectrophotofluorometer, respectively. They were as follows.

Compound	λ_{ex} (nm)	λ_{em} (nm)
α-Naphthol	317	470
β-Naphthylamine	277.5	412

The corresponding esters were found to be non-fluorescent at the wavelengths chosen.

The fluorescence intensity was found to vary linearly with α-naphthol concentration over the 10^{-7}–10^{-5} M concentration range and with β-naphthylamine over the 6×10^{-8}–5×10^{-7} M concentration range. Potential interference of the fluorescence intensity of α-naphthol by α-naphthyl acetate was determined by spiking 1×10^{-6} M solutions of α-naphthyl acetate with α-naphthol whose concentration ranged from 0.1 to 1×10^{-6} M. This procedure was repeated with 1.5×10^{-5} M LNA solution to which was added 1.5×10^{-4} M β-napthylamine. At these concentrations, neither α-naphthyl acetate nor LNA quenched the fluorescence due to α-naphthol and β-naphthylamine, respectively. Thus, the increase in fluorescence intensity in subsequent enzymatic hydrolysis experiments could only be due to the hydrolytic products.

(3) Preparation of ocular tissues for enzymatic hydrolysis studies

Rabbits of various age groups, approximately 10 of each, were kept in restraining boxes in a normal upright posture. They were sacrificed by a rapid injection of a 30% sodium phenobarbital solution into a marginal ear vein. After withdrawing 10–15 ml of blood via intracardiac puncture, the corneal surfaces were rinsed with saline and blotted dry. About 150 μl of aqueous humor were aspirated using a 27-gauge × 0.5 in. needle attached to a 1 ml tuberculin syringe, and the cornea, iris

and ciliary body [2] were removed sequentially using a surgical scalpel.

Immediately following their removal, the tissues were transferred to homogenization vessels and the aqueous humor and blood to culture tubes. All were placed in an ice bath. The tissues were homogenized in about 10 ml of ice-cold isotonic potassium chloride solution intermittently for about 80 s using a motor-driven tissue grinder (Polytron). The homogenate was centrifuged at 755 × g in a refrigerated (4°C) Sorvall centrifuge and the supernate saved for enzymatic hydrolysis studies. The blood was allowed to coagulate, and the fibrin clot and blood cells removed by centrifugation under the same conditions as for ocular tissue homogenates. The protein content of each supernate was then determined using a protein-dye binding assay (Bradford, 1976) with rabbit serum albumin as the standard. Because no attempt was made to free membrane-bound esterases, the esterase activity determined in this study can only be interpreted as that due to soluble esterases. Nevertheless, preliminary studies showed that at least in the case of iris and ciliary body, the extent and rate of α-naphthyl acetate hydrolysis were the same whether the homogenate or supernate was used.

During the aspiration procedure it was possible that the aqueous humor was contaminated with esterases from the cornea and iris-ciliary body, thus providing a false indication of the actual esterase activity present in the aqueous humor. However, the probability of this occurring was low, as evidenced by the response of the esterases in the aqueous humor to various modulators of esterase activity being different from that seen in the cornea and iris-ciliary body (Figs. 4 and 5).

Because of the related concern that some of the esterase activity seen in the iris and ciliary body was due to esterases in the blood efficiently perfusing these tissues, the serum derived from the blood collected earlier from the rabbits was incubated with α-naphthyl acetate as detailed in the next section. The resulting hydrolytic profile, shown in Fig. 1, was different from that for the iris and ciliary body, suggesting that the bulk of esterase activity observed in the iris and ciliary body was due to indigenous esterases, but not to those present in the blood there. Table 1 shows that the contribution of serum esterases to total esterase activity in these tissues increased with age in the albino rabbit while decreasing with age in the pigmented. Moreover, in the age groups studied, the pigmented rabbit was more dependent than the albino rabbit on serum esterases for esterase activity in its iris-ciliary body.

(4) Enzymatic hydrolysis of various substrates in the absence of their inhibitors

Prior to initiating enzymatic hydrolysis studies, the presence of esterases in selected ocular tissues/fluids was ascertained by electrophoresis on 7.5% polyacrylamide gels and staining for esterases (Ecobichon and Kalow, 1962) and peptidases (Felgenhauer and Glenner, 1966).

[2] The iris and ciliary body were removed as one structure and hereafter will be referred to as iris-ciliary body.

TABLE 1

ESTIMATES OF PERCENT OF ESTERASE ACTIVITY IN IRIS-CILIARY BODY DUE TO SERUM ESTERASES [a]

Strain	Age (weeks)	% Due to serum esterases	
		αNA [b] = 1.5×10^{-5} M	αNA = 6×10^{-5} M
Albino	3	5.5	5.5
	6	8.9	9.4
	12	13.6	– [c]
Pigmented	6	43.1	36.0
	12	25.0	18.9

[a] Calculated by dividing the initial rate of hydrolysis of α-naphthyl acetate in serum to that in the iris and ciliary body, both values normalized to protein concentration.

[b] α-Naphthyl acetate.

[c] Not determined.

(a) α-Naphthyl acetate

To 3 ml of 1.5×10^{-5} M or 6×10^{-5} M α-naphthyl acetate solution in a fluorescence cuvette were added 100 μl of an ocular tissue supernate, aqueous humor or serum. After mixing, the fluorescence intensity was monitored at ambient temperature every 25 s for the first 200 s and every 100 s for the next 400 s. This brief time interval corresponds to the duration over which ocular absorption is essentially complete (Sieg and Robinson, 1976). From a calibration plot of fluoresence intensity vs α-naphthol concentration, the initial hydrolysis rate in $M \cdot s^{-1}$ was calculated from the initial slope of a plot of fluorescence intensity vs time followed by normalization to protein concentration. No chemical hydrolysis of α-naphthyl acetate occurred during enzymatic hydrolysis, as evidenced by the lack of increase in fluorescence intensity with time in the control solution, which contained 3 ml of α-naphthyl acetate and 100 μl of 1.17% KCl. Between 3 and 4 determinations were made for each ocular tissue/fluid of a rabbit.

(b) L-Leucyl-β-naphthylamide

One-hundred microliters of an ocular tissue supernate, aqueous humor, or serum, were incubated with 2.6 ml of an activator solution [3] at 45°C for 5 min, conditions reported to optimize peptidase activity (Wolff and Resnick, 1963). After cooling the mixture to room temperature, 300 μl of 95% ethyl alcohol were added. Hydrolysis was initiated by adding 100 μl of a 4.67×10^{-4} M LNA solution to this mixture. The fluorescence intensity was monitored at ambient temperature every 100 s for 600 s. From a plot of fluorescence intensity vs β-naphthylamine concentration, the initial hydrolysis rate in $M \cdot s^{-1}$ was calculated from the initial slope of a plot of

[3] The activator solution was 0.0768 M in Tris, 1.28×10^{-4} M in $CoCl_2 \cdot 6H_2O$, 0.128 M in $MgSO_4 \cdot 7H_2O$ and 2.01×10^{-4} M in $MnCl_2$. It was adjusted to pH 8.2 with 3 N HCl. This solution was reported by Wolff and Resnick (1963) to be optimal for peptidase activity.

fluorescence intensity vs time followed by normalization to protein concentration. No chemical hydrolysis of LNA occurred during enzymatic hydrolysis, as evidenced by the lack of increase in fluorescence intensity with time in the control solution, which contained 3 ml of LNA and 100 μl of 1.17% KCl. Between 3 and 4 determinations were made for each ocular tissue/fluid of a rabbit.

(5) Enzymatic hydrolysis of α-naphthyl acetate in the presence of inhibitors

In order to ascertain that the observed esterase activity was due to esterases, the hydrolysis of α-naphthyl acetate was conducted in the presence of various inhibitors of esterase activity. They were acetazolamide (Diamox), a carbonic anhydrase inhibitor; eserine sulfate (ES), neostigmine bromide (NBr), and diisopropylfluorophosphate (DFP), all cholinesterase inhibitors; and *p*-chloromercuribenzoate (PCMB) and ethylenediamine tetracetic acid (EDTA), modulators of carboxylesterase and arylesterase activity. With the exception of acetazolamide, which was employed at a concentration of 2×10^{-5} M, the concentration of all inhibitor solutions was 1×10^{-3} M. None of these concentrations altered the fluorescence due to α-naphthol.

One-hundred microliters of tissue supernates were incubated with 2.9 ml of an inhibitor solution for 15 min prior to initiation of hydrolysis by adding 100 μl of a 4.5×10^{-5} M α-naphthyl acetate solution. The fluorescence intensity was monitored as a function of time in the same manner as those experiments which did not involve inhibitors. It was shown in a separate experiment that substrate inhibition did not occur at a substrate concentration of 1.5×10^{-5} M, the concentration at which esterase inhibition was studied. This is because the initial hydrolytic rate continued to increase as the concentration was increased to 6×10^{-5} M (Figs. 2 and 3).

Results and Discussion

There have been few studies on ocular esterase activities in newborns, infants or adults, despite the potential usefulness of such information in the prediction of susceptibility of each age group to ester-type compounds such as atropine, pilocarpine, dipivalyl epinephrine, and organophosphate insecticides. The purpose of this study was to investigate variation of esterase activity with age in the albino and pigmented rabbit eye. Fig. 1 shows that when α-naphthyl acetate, the model substrate, was incubated with various ocular tissues/fluids, the fluorescence intensity increased with time, indicating that esterase activity was present. This esterase activity can be derived from true esterases like acetylcholinesterase, peptidases like cathepsin, and carbonic anhydrase, all of which are known or said to be present in the eye (Hayasaka and Hayasaka, 1978; Lönnerholm, 1974; Petersen et al., 1965; Wistrand and Garg, 1979). With L-leucyl-β-naphthylamide as the substrate, peptidase activity could only be detected in the iris-ciliary body, one of the few ocular tissues enriched in the peptidases called cathepsins (Hayasaka and Hayasaka, 1978). Even then, the peptidase activity was a mere 1/400 times the esterase activity

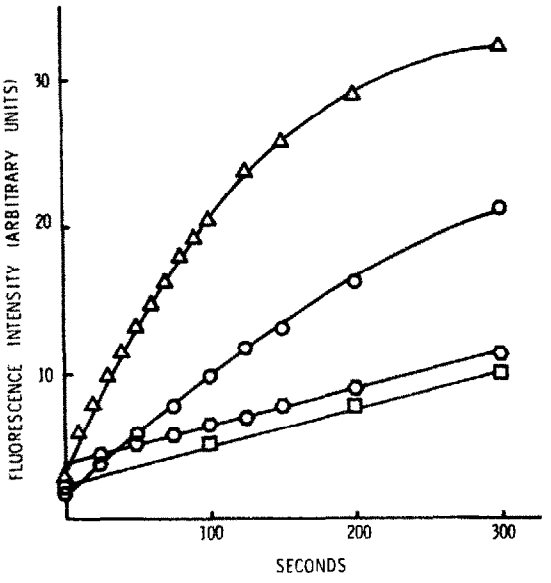


Fig. 1. Hydrolysis of α-naphthyl acetate (1.5×10^{-5} M) in the supernates of the cornea (○), iris-ciliary body (△), aqueous humor (□), and serum (o) of a 3-week-old albino rabbit. The fluorescence intensity was determined in an Aminco-Bowman spectrophotofluorometer with λ_{ex} at 317 nm and λ_{em} at 470 nm, multiplier = 0.1 and sensitivity = 40.

detected by incubating α-naphthyl acetate under the same experimental conditions [4], suggesting that peptidase contributed insignificantly to total esterase activity.

Besides peptidase, carbonic anhydrase also exhibits esterase activity (Pocker and Stone, 1967). When the ocular tissue homogenates were incubated with acetazolamide, a specific carbonic anhydrase inhibitor, reduction in esterase activity was evident in only the cornea and the iris-ciliary body. These are the tissues known to contain carbonic anhydrase (Lönnerholm, 1974; Wistrand and Garg, 1979). In the albino rabbit this reduction was evident in the 3- and 6-week-old groups, while in the pigmented rabbit this was evident in the 12-week-old group only. In preliminary experiments employing the same experimental conditions as outlined in the Methods section, acetazolamide was found to totally inhibit the hydrolysis of α-naphthyl acetate by rabbit erythrocyte carbonic anhydrase while causing no inhibition of hydrolysis of the same ester by porcine liver esterase. On this basis, it was estimated that at most 30% of the observed esterase activity could be ascribed to carbonic anhydrase. Additional, related information is shown in Table 2.

The presence of true esterases in the rabbit eye, possibly acetylcholinesterase ar d pseudocholinesterase (Petersen et al., 1965), was revealed by a reduction in the observed esterase activity in the presence of such cholinesterase inhibitors as eserine sulfate, neostigmine bromide and diisopropylfluorophosphate. Because *p*-chloromercuribenzoate, an agent that acts on free sulfhydryl groups in a non-competitive fashion, altered esterase activity and also because free sulfhydryl groups have been shown to be non-essential for acetylcholinesterase activity, other esterases such as

[4] Data not shown.

TABLE 2

PERCENT OF ESTERASE ACTIVITY DUE TO CARBONIC ANHYDRASE (CA) [a]

Strain	Age (weeks)	% of esterase activity due to CA		
		Cornea	Iris-ciliary body	Aqueous-humor
Albino	3	10	31	12
	6	19	22	– [b]
	12	– [b]	– [b]	– [b]
Pigmented	6	– [b]	– [b]	– [b]
	12	– [b]	24	– [b]

[a] Based on percent of reduction in esterase activity in the presence of acetazolamide.
[b] An increase rather than a reduction in esterase activity in the presence of acetazolamide was seen.

aryl esterases (Augustinsson, 1964) may also be present in the eye.

In the albino and pigmented rabbits studied, the combined effect of these multiple esterases is to generate a rank order such that the esterase activity is the highest in the iris-ciliary body followed by the cornea and then the aqueous humor. This is illustrated in Figs. 2 and 3 for albino and pigmented rabbits, respectively. This rank order of esterase activity, with a few exceptions, also holds in the presence of various modulators of esterase activity. This is shown in Figs. 4 and 5. Such a variation in drug-metabolizing enzyme activity with tissue has recently been reported

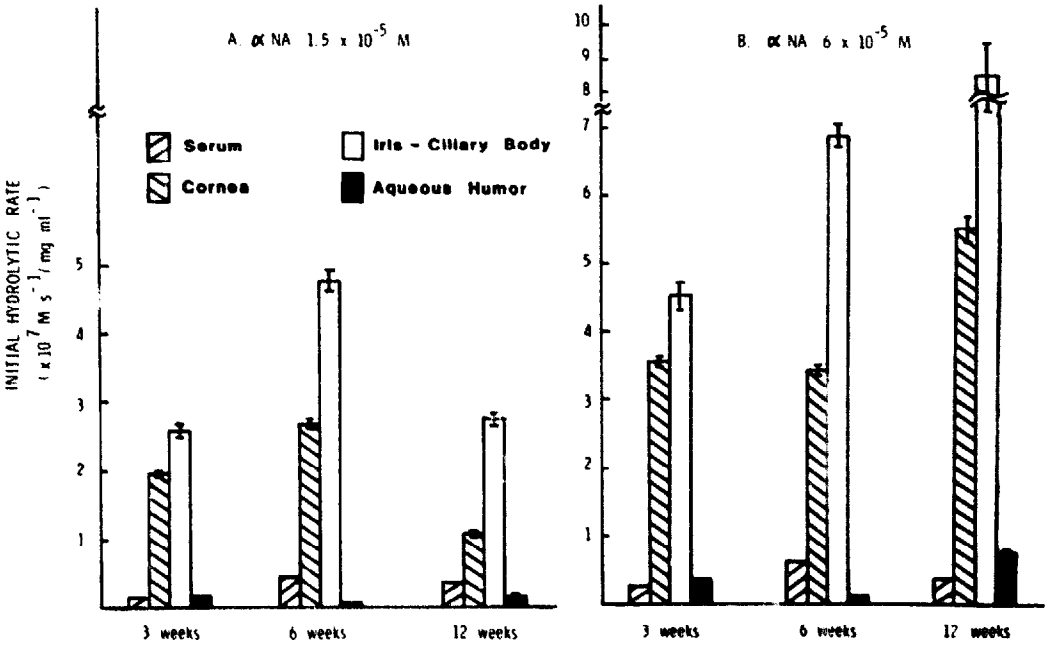


Fig. 2. Esterase activity, expressed as initial hydrolytic rate, in the serum (▨), cornea (▩), iris-ciliary body (□) and aqueous humor (■) of 3-, 6- and 12-week-old albino rabbits. Except for the serum, approximately 20 eyes were used for each tissue or fluid. Error bars represent standard error of the mean. Key: αNA, α-naphthyl acetate.

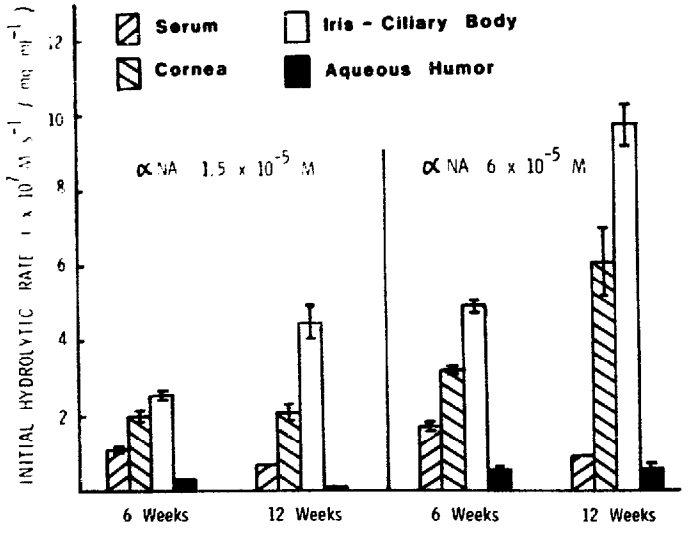


Fig. 3. Esterase activity, expressed as initial hydrolytic rate, in the serum (▨), cornea (▧), iris-ciliary body (□) and aqueous humor (■) of 6- and 12-week-old pigmented rabbits. Except for the serum, approximately 20 eyes were used for each tissue or fluid. Error bars represent standard error of the mean. Key: αNA, α-naphthyl acetate.

for arylhydrocarbon hydroxylase and UDP-glucuronyl transferase activities in the bovine eye, with the activities being the highest in the ciliary body (Das and Shichi, 1981). The significance of these findings is that drugs containing ester linkages would be subjected to esterase-catalyzed hydrolysis in the eye, so that less of them

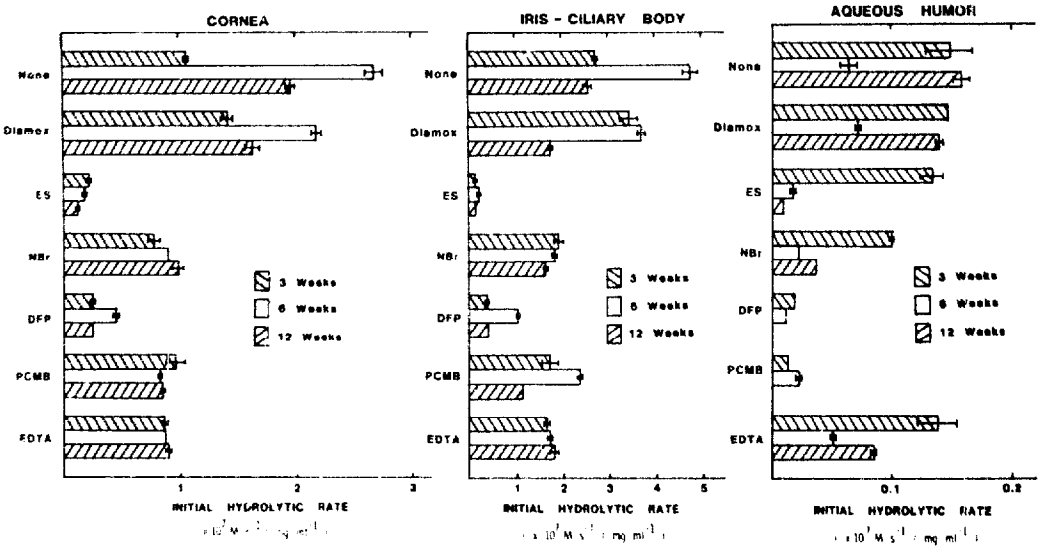


Fig. 4. Esterase activity, expressed as initial hydrolytic rate, in the cornea, iris-ciliary body and aqueous humor of albino rabbits, ages 3, 6 and 12 weeks. Approximately 20 eyes were used for each tissue or fluid. Error bars represent standard error of the mean. Key: Diamox, acetazolamide; ES, eserine sulfate; NBr, neostigmine bromide; DPF, diisopropylfluorophosphate; PCMB, *p*-chloromercuribenzoate; EDTA, ethylenediamine tetracetic acid.

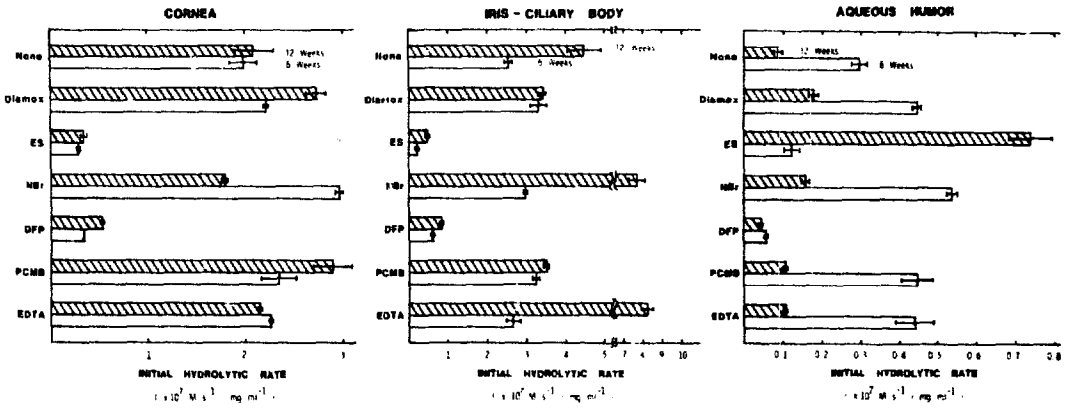


Fig. 5. Esterase activity, expressed as initial hydrolytic rate, in the cornea, iris-ciliary body and aqueous humor of pigmented rabbits, ages 6 and 12 weeks. Approximately 20 eyes were used for each tissue or fluid. Error bars represent standard error of the mean. Key: Diamox, acetazolamide; ES, eserine sulfate; NBr, neostigmine bromide; DPF, diisopropylfluorophosphate; PCMB, *p*-chloromercuribenzoate; EDTA, ethylenediamine tetracetic acid.

would be available for interaction with their target tissues. For the topical route, these drugs would be hydrolyzed while permeating the cornea as well as upon entering the aqueous humor which distributes them to tissues such as the iris and ciliary body. For the systemic route, they would encounter esterases in the iris and ciliary body shortly after their transfer across the blood–aqueous barrier, and would encounter additional esterases in the remaining ocular tissues to which they are distributed. Clearly, the relative activity of esterases in these sites of drug entry—cornea and iris-ciliary body—would be one of the key factors in determining the relative effectiveness of ocular drugs reaching the eye from the respective route of administration. Moreover, if one or more of these factors shows age-dependent changes, this relative effectiveness would show a corresponding age dependency.

On the basis of esterase activity alone and on the assumption that the data obtained in rabbits can be extrapolated to humans, infants would be expected to respond to ophthalamic drugs, those containing ester linkages in particular, differently than adults. The reason is that, at least in the rabbit, there is a definite variation in ocular esterase activity with age. This is shown in Figs. 2 and 3 for the albino and pigmented rabbits, respectively. Broadly speaking, in the albino rabbit, the activity is the highest in the 6-week followed by the 3-week and then the 12-week old group. This finding is in accord with that of Hayasaka and Shiono (1982) on age-dependent variations in the activities of acid phosphatase, β-glucuronidase and arylsulfatase in the rabbit eye. In the pigmented rabbit, however, the esterase activity in the 6-week-old group is either equivalent to or less than that in the 12-week group. This observation implies that ocular esterases develop and age at different rates in albino and pigmented rabbits.

Recently, Lee et al. (1980) reported that pilocarpine, a lactone, was metabolized more extensively and rapidly in the pigmented than in the albino rabbit eye [5],

[5] The rabbits employed by Lee et al. (20) were 14–15 weeks old

suggesting a dependence of metabolic enzyme activity with pigmentation. This hypothesis is supported by the findings of the present study. With α-naphthyl acetate as the substrate, the esterase activity in the cornea and iris-ciliary body of the 12-week-old pigmented rabbit is approximately twice that in the albino rabbit. Quite unexpectedly, this pattern is reversed in the 6-week-old group. Obviously, in order to attain a given pharmacological response, more drug needs to be administered to compensate for the expected reduction in amount of intact drug as a result of metabolism. Additional compensation is required if drug can also be lost to the pigments in the iris-ciliary body. Thus, the 12-week-old pigmented rabbit requires a larger dose than the albino rabbit because of more extensive metabolism and pigment binding. In contrast, the 6-week-old pigmented rabbit may have the same dose requirement as the albino rabbit so long as pigment binding in the pigmented rabbit reduces intact drug to the same extent as the more extensive metabolism in the albino rabbit. This possibility is currently under investigation in our laboratory by administering identical doses of dipivalyl epinephrine to both strains of rabbits. For the time being, on the assumption that the rabbit is an adequate model for humans in terms of ocular esterase activity, brown-eyed teenagers may not necessarily have a larger dose requirement than their blue-eyed counterparts.

While this study demonstrates a dependency of ocular esterase activity on the tissue, age and pigmentation of the rabbit, the basis for these variations has yet to be revealed. For instance, it is not known whether a higher level of esterases or the presence of intrinsically more active esterases is responsible for the high esterase activity in the iris and ciliary body. Likewise, it is not known if the differences in esterase activity existing between the albino and pigmented rabbits arise from different types of esterases, each with its intrinsic activity and unique to either the albino or pigmented rabbits. To determine the basis for these variations, studies are now in progress to fractionate and characterize the esterases with respect to substrate specificity, substrate inhibition, product inhibition, and optimum pH for esterase activity. Results of these studies will be reported in subsequent communications.

In conclusion, age-related changes in ocular esterase activity, as demonstrated in this study, is another factor that must be considered when evaluating age-related changes in ocular drug bioavailability as has been reported for pilocarpine (Miller and Patton, 1981). Other factors influencing ocular drug disposition, which may also show age-related changes, include corneal membrane permeability, tear and aqueous humor dynamics, and drug–protein binding in ocular tissues and fluids. These factors may either offset or reinforce one another in determining the amount of active drug ultimately reaching its receptor.

Acknowledgements

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