## CONCLUSION

In summary, it is gratifying to see that the fairly complicated biotransformations of various drugs by different microsomal systems can be well correlated with a few physicochemical constants. The results obtained from the available data suggest that the modification of organic compounds by microsomal enzymes can be understood in terms of their physicochemical properties in a quantitative way. This type of approach in the future may be useful in the understanding of drug metabolism and possibly aid the medicinal chemist in drug design *via* molecular modification to affect biotransformation.

## REFERENCES


(1) B. B. Brodie, J. R. Gillette, and B. N. LaDu, *Ann. Rev. Biochem.*, **27**, 427(1958).

(2) L. E. Gaudette and B. B. Brodie, *Biochem. Pharmacol.*, **2**, 89(1959).

(3) E. J. Lien and C. Hansch, *J. Pharm. Sci.*, **57**, 1027(1968).

(4) C. Hansch, E. J. Lien, and F. Helmer, *Arch. Biochem. Biophys.*, **128**, 139(1968).

(5) Y. C. Martin and C. Hansch, *J. Med. Chem.*, **14**, 777(1971).

(6) C. Hansch, *Drug Metab. Rev.*, **1**, 1(1972).

(7) I. Jannson, S. Orrenius, L. Ernster, and J. B. Schenkman, *Arch. Biochem. Biophys.*, **151**, 391(1972).

(8) D. M. Ziegler, C. H. Mitchell, and D. Jollow, "Microsomes and Drug Oxidation," Academic, New York, N.Y., 1969, pp. 173–188.

(9) C. F. Wilkinson, K. Hetnarski, and T. O. Yellin, *Biochem. Pharmacol.*, **21**, 3187(1972).

(10) C. Hansch and S. M. Anderson, *J. Med. Chem.*, **10**, 745(1967).

(11) W. Levin, E. Sernatinger, M. Jacobson, and R. Kuntzman, *Science*, **176**, 1341(1972).

(12) C. Hansch, *Proc. Int. Congr. Pharmacol.*, **4**, 128(1969).

(13) C. Hansch, in "Drug Design," vol. 1, E. J. Ariens, Ed., Academic, New York, N.Y., 1971, p. 271.

(14) C. Hansch, "Biological Correlations—The Hansch Approach," American Chemical Society, Washington, D.C., 1972, pp. 20–40.

(15) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, **71**, 525(1972).

(16) E. J. Lien and G. L. Tong, *Cancer Chemother. Rep.*, **57**, 251(1973).

(17) E. J. Lien, M. Hussain, and G. L. Tong, *J. Pharm. Sci.*, **59**, 865(1970).

(18) C. Hansch, A. Leo, S. H. Unger, K. H. Kim, D. Nikaitani, and E. J. Lien, *J. Med. Chem.*, **16**, 1207(1973).


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# Effect of Topically Applied Pilocarpine on Tear Film pH


**A. LONGWELL [x], S. BIRSS, N. KELLER, and D. MOORE**



**Abstract** □ Changes in tear film pH were observed during the 1st hr after instillation of pilocarpine in various dosage forms to the rabbit eye. In anesthetized rabbits, with periodic blinking induced electrically, commercial formulations of pilocarpine salts applied as drops or a spray acutely lowered tear film pH by 1.1–1.6 pH units. The pH remained below pretreatment levels for 45–>60 min after instillation. Pilocarpine base, administered continuously at the rates of 20 or 80 μg/hr from ocular therapeutic systems, had little or no effect on tear film pH in this same animal preparation. The reduction in tear film pH produced by pilocarpine eyedrops or spray solution is attributable to the acid pH and buffer capacity of these solutions. Delivery of pilocarpine base without pH change was achieved with ocular therapeutic systems, because the drug (pKa = 7.07) was delivered free, or virtually so, of excipients. These observed differences in tear film pH after application may partially explain the four- to eightfold reduction in total effective pilocarpine dose with ocular therapeutic systems compared to eyedrops or spray, since the cornea is less permeable to ionized than to unionized molecules.



**Keyphrases** □ Pilocarpine—topically applied base and salts, effect on tear film pH, rabbits □ Tear film pH—effect of topically applied pilocarpine, base and salts compared, rabbits □ pH—tear film, effect of topically applied pilocarpine, base and salts compared, rabbits □ Ophthalmic cholinergic agents—pilocarpine base and salts, topically applied, effect on tear film pH, rabbits


The pH of the tear film is of interest to ophthalmic pharmacologists for two reasons. Tear pH is an important factor in the penetration of any topically applied ophthalmic drug that is a weak base, since the cornea is less permeable to ionized than to unionized molecules. In addition, well-buffered acid solutions are known to cause more sensation of stinging or burning to the eye than do weakly buffered acid solutions, because the concentrated acid buffer overwhelms the *in vivo* buffer capacity of tear film.

The major buffering system in extracellular fluids, including tears, is the bicarbonate system. Tear film concentration of bicarbonate is the same as that of plasma. However, the total buffering capacity of tear film is obviously very small, since its volume is only about 7 μl. Thus, the addition of eyedrop solutions such as 1–4% pilocarpine at pH 4–5, in volumes five to 10 times that of the tear film, might be expected to overwhelm, for a time, the buffering capacity of the tears.

Pilocarpine is dispensed as a nitrate or hydrochloride in solutions ranging from 0.5 to 10% (0.025–0.5 *M*); even at the commonly prescribed concentration of 2% (0.1 *M*), a solution of pilocarpine nitrate alone, which has an equilibrium pH of 3.9, is a fairly concentrated buffer. The pH of most pilocarpine ophthalmic solutions, however, is adjusted to 4.5–5.5, but no higher, for reasons of drug stability in solution. The topical application of these low pH solutions of pilocarpine, either in eyedrops or in sprays, should reduce tear film pH for some time after administration.

The purpose of these studies was to compare the magnitude and duration of pH changes in tear film of anesthetized rabbits produced by: (*a*) a spray or various pilocarpine eyedrop solutions; (*b*) solutions of a strong acid (hydrochloric), and (*c*) pilocarpine free base delivered continuously without excipients or exogenous fluid from ocular therapeutic systems at rates that span the range used clinically.

## EXPERIMENTAL

**Animals**—New Zealand White rabbits of either sex, 2.0–3.5 kg, were caged individually and fed and watered *ad libitum*.

**pH Measurements**—A combination electrode pH probe[1] with a sensing bulb diameter of 1.2 mm is easily accommodated in the nasal corner of the lower cul-de-sac. Recordings of pH were made with a digital pH meter[2]. Before and after each experiment, the electrode was calibrated with a pH 7 buffer and checked for linearity with pH 5 and 8 buffers.

Prior to pH measurements in the tear film, rabbits were anesthetized with 0.016 g of thiopental sodium[3]/kg iv. Additional thiopental sodium, injected as needed, maintained anesthesia throughout the experiment as gauged by absence of the blink reflex. After a tracheotomy was performed, the tracheal tube was connected to a respirator with the tidal volume set at less than 20 $cm^3$/stroke and the frequency set at 30–40 strokes/min. The animal's head was immobilized in the upright position, and the pH electrode was set in place. Care was taken to avoid covering the lacrimal punctum with the electrode.

Because lacrimation is at a minimum in the unstimulated anesthetized animal (1), tear production was maintained by electrically induced blinking. A stimulating electrode, inserted through the skin into the palpebral portion of the orbicularis muscle of the upper eyelid, was connected to a stimulator[4]. Every 5 sec, the stimulator induced a blink by applying a pulse of 5–7 v and 20-msec duration. This interval between blinks is considerably shorter than the interval in a conscious rabbit but is characteristic of the blink rate in humans. Tear production in anesthetized rabbits with electrically induced blinking every 5 sec was measured previously (2).

The nasolacrimal duct was cannulated, and fluid collected over 30-min intervals was weighed. The average rate of tear production over 90 min was 0.78 ± 0.18 (*SE*) μl/min. The rate of tear production did not decline during this 90-min period. When pH measurements were made, blinking was stimulated for several minutes before the first pH measurement was taken. Subsequent pH measurements were taken every 5 min. One minute before a pH reading was made, the blink stimulator was turned off, the measurement was made, and blinking was reestablished.

Before any test solution was instilled or any ocular therapeutic system was placed in the eye, baseline pH measurements were taken every 5 min for 20–30 min. When four successive pH readings varied no more than ± 0.05 pH unit, the preparation was considered stable. To date, in 10 out of 82 of these eye preparations, a "constant" level of ±0.05 pH unit was not observed during nine successive 5-min measurements. Such preparations were rejected as "unstable." Once stable tear film pH levels were observed, test solutions or systems were applied; tear film pH was recorded every 5 min for 60 min following application.

**Experimental Design**—Eyes of each experimental animal were treated sequentially, but no rabbit received the same treatment in both eyes. Eight eyes in a control group received no eyedrops or pilocarpine in any form. Two groups of six eyes each received a 50-μl drop of $10^{-2}$ or $10^{-4}$ *M* HCl.

Four groups of six eyes each received solutions of pilocarpine salts and excipients. Four different commercially available pilocarpine solutions were used: Solution A[5], 2% pilocarpine hydrochloride at pH 4.5; Solution B[6], 2% pilocarpine nitrate at pH 4.8; Solution C[7], 2% pilocarpine hydrochloride at pH 5.3, supplied in a spray bottle; and Solution D[8], 2% pilocarpine hydrochloride at pH 5.0. The eyedrops were applied as 50-μl drops, and the spray was applied in a quantity estimated to be about 85 μl. Two groups of six eyes each received ocular therapeutic systems designed to deliver unbuffered pilocarpine base to the tear film at the rate of 20 or 80 μg/hr[9].

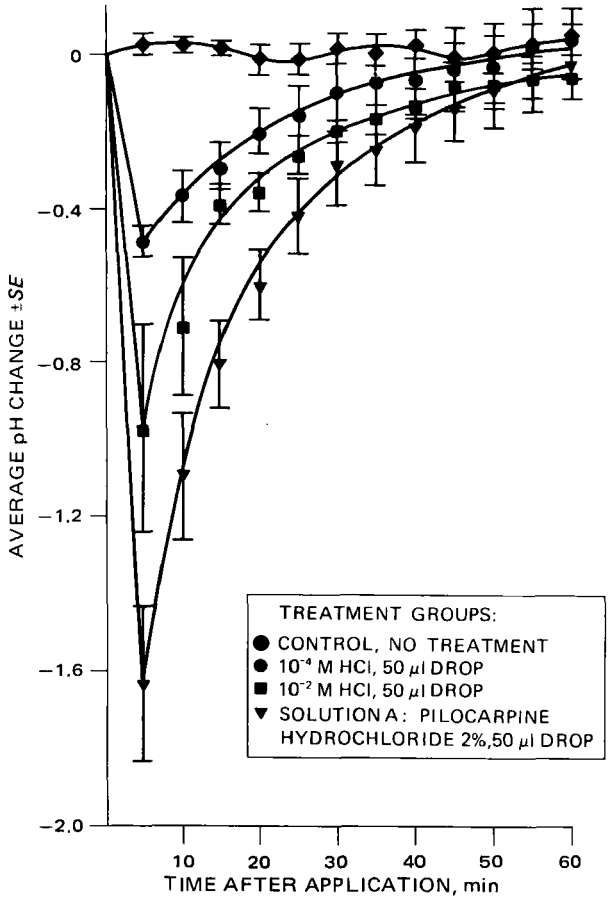


**Figure 1**—*Comparison of effect of instillation of hydrochloric acid on rabbit tear film pH with that of instillation of pilocarpine ophthalmic solution.*

The initial, pretreatment pH values were averaged. For untreated controls, values taken in the first 30 min after insertion of the probe were averaged. Changes from this average baseline pH after each type of treatment, including *no* treatment, were recorded for each rabbit eye. The response to any of the treatment regimens (including no treatment) is expressed as the average, over all animals in the treatment group, of the changes in tear film pH from each animal's baseline period average.

## RESULTS

The undisturbed tear film pH of 56 rabbit eyes measured every 5 min over the 20-min pretreatment period averaged 7.47 ($n$ = 56, $SE$ ± 0.03). This value is very like the average normal human tear film pH of 7.4 (range of 7.3–7.7) (3). The change in pH over a 60-min period in untreated eyes was determined by measuring tear film pH in eight untreated eyes every 5 min for 80 min, averaging the first four values, and comparing the last 12 values with this initial average. The largest range of pH change for individual eyes was +0.18, −0.27 pH unit, observed during the experimental period. Thus, the pH of the normal, undisturbed tear film was quite stable.

The average variation of pH in untreated eyes is shown in Fig. 1. No evidence of drift, either to acid or basic pH, was apparent in tears of untreated eyes.

[1] Microelectrodes, Inc.
[2] Beckman.
[3] Pentothal sodium, Abbott.
[4] Grass S-6.
[5] Isopto Carpine ophthalmic solution, 2%, Alcon Laboratories.
[6] P. V. Carpine Liquifilm ophthalmic solution, 2%, Allergan Pharmaceuticals.
[7] Mistura P, ophthalmic solution sterile, 2%, Lederle Laboratories.
[8] Adsorbocarpine ophthalmic solution USP, 2%, Burton, Parsons and Co.
[9] OCUSERT Pilo-20 ocular therapeutic system, 20 μg/hr, for 1 week, Alza Pharmaceuticals. The 80-μg/hr system is an Investigational New Drug, Alza Research Division of Alza Corp.

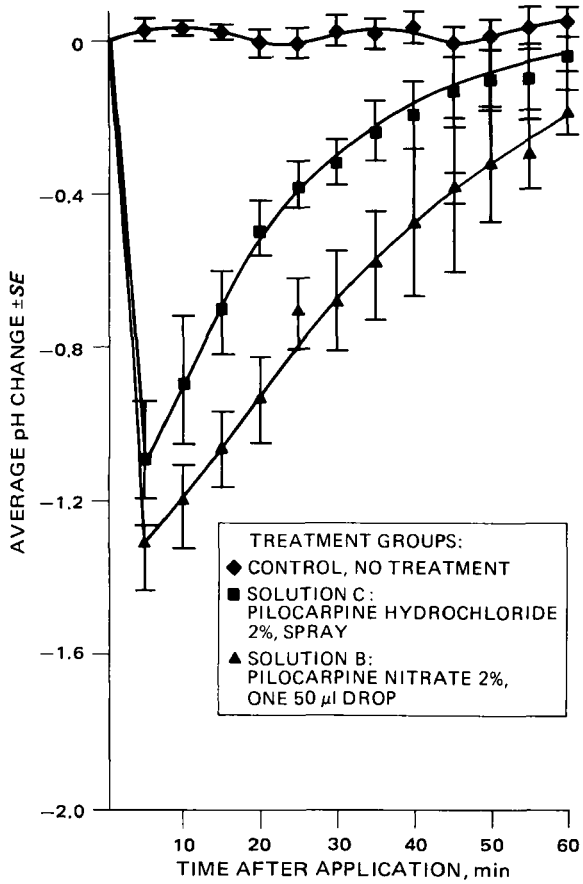


**Figure 2**—*Effect of administration of pilocarpine solutions in drop forms or spray on rabbit tear film pH.*

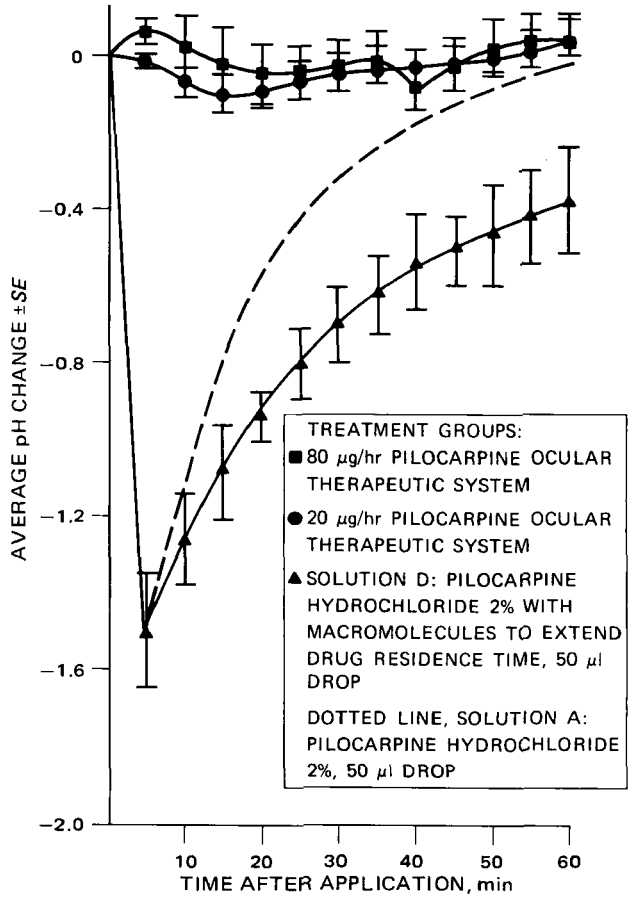


**Figure 3**—*Comparison of effect on rabbit tear film pH of a pilocarpine hydrochloride ophthalmic solution formulated to extend drop residence time with continuous delivery of pilocarpine* via *ocular therapeutic systems. The dashed line indicates the pH change observed in tears when a pilocarpine hydrochloride ophthalmic solution from another manufacturer was applied.*

Figure 1 also compares the perturbations of tear film pH in rabbit eyes produced by a single drop of pilocarpine ophthalmic solution with those produced by the addition of a single drop of either $10^{-2}$ or $10^{-4}$ *M* HCl. The pilocarpine solution produced a change of greater magnitude and longer duration than did the strong acids. For the strong acid, the magnitude and duration of the response were concentration dependent. Within 5 min after addition of a $10^{-4}$ *M* HCl drop, an average decrease of 0.5 pH unit occurred in tear film pH, which returned to the control level at 30 min. Instillation of a drop of $10^{-2}$ *M* HCl solution caused an average pH reduction of 1 pH unit within 5 min; the recovery time was 45 min. The instillation of pilocarpine preparations at pH 4.4–5.3, however, caused a greater and more persistent reduction in tear film pH than did hydrochloric acid alone at pH 4 or even at pH 2 (Figs. 1 and 2).

Following administration of these eyedrop solutions or the spray, the mean maximum pH decrease measured at the 5-min observation time could be correlated with the pH of the bulk solution. Solution C, pH 5.3, caused an average maximum decrease in tear pH of 1.10 pH units; Solution A, pH 4.5, caused a maximum tear pH decrease of 1.63 pH units. Solution B, with an intermediate pH, produced a maximum decrease in tear pH that was intermediate between those produced by Solutions A and C.

In all cases, recovery times were prolonged compared to those observed for the pH 4 hydrochloric acid solution. In contrast to the 30 min required for recovery of tear film pH after $10^{-4}$ *M* HCl, recovery time after a pilocarpine eyedrop ranged from 45 to >60 min. At 60 min after application of Solution B, the tear film pH values were still significantly below pretreatment levels ($p < 0.05$).

Figure 3 compares the effects on tear film pH of two different rates of pilocarpine release, 20 and 80 µg/hr, from ocular therapeutic systems and the effect of Solution D. The latter is a pilocarpine solution made with water-soluble polymers to increase the residence time of the drug in the eye. For comparison, the average curve of Solution A is repeated from Fig. 1. Solution D lowered pH below control levels for more than 60 min ($p < 0.05$), and the average pH change at 60 min was the greatest of all observed changes for that time. In contrast, the fluctuations in pH during 1 hr of continuous delivery of pilocarpine from ocular therapeutic systems were ±0.1 pH unit.

## DISCUSSION

This study compared the magnitude and duration of pH changes in tear film induced by various forms of topical pilocarpine. Solutions were applied at the same volumes or, in the case of the spray, at higher volumes, and under the same conditions as was hydrochloric acid at two different concentrations. These pilocarpine solutions lowered tear pH more than did hydrochloric acid of the same pH. In addition, the duration of the pH change was longer than that produced by a strong acid.

Since this study was carried out in anesthetized animals with artifically stimulated, relatively constant tear flow, some effects of applying drops, sprays, or ocular therapeutic systems to the eye of a conscious animal may be absent. Extensive reflex tearing in response to a burning sensation, common in conscious humans, will probably not be observed, and differences in the rate of tear drainage between conscious and anesthetized animals (1) will affect the absolute rate at which tear film returns to normal pH after instillation of a drop. However, the observed relationships between various forms of ophthalmic medication and acid drops should remain valid under other conditions, *i.e.*, in the conscious animal.

The rate of change of pilocarpine concentration in the tear film is a function of the tear drainage rate. For a given tear production, the concentration of pilocarpine in tear film changes slowly when drainage is slow and vice versa. For drops, when tear film pilocarpine concentration is maximum, presumably at the instant of drop instillation, tear pH is at a minimum, at or below that measured at 5 min after application. Then, concomitant with the subsequent decline in tear pilocarpine concentration, tear pH returns to normal.

Changes in tear film pH alter the rate of ocular penetration of topically applied drugs that are weak acids or bases, because the ratio of their ionized to unionized form is a function of pH and because ionized molecules do not easily penetrate the cornea. Pilocarpine, a case in point, is a weak base, pKa 7.07, but is formulated as a salt in eyedrop preparations. Pilocarpine nitrate at 0.1 *M* in water at 20° has an equilibrium pH of 3.9, but the pH of pilocarpine nitrate ophthalmic formulations is adjusted to 4–5. Altering the pH of tears toward the acid side increases the proportion of protonated pilocarpine to the unionized pilocarpine base in the tears, and the rate of penetration of the drug to the internal eye is expected to decrease.

Anderson and Cowles (4) showed that pilocarpine is a more effective ocular hypotensive agent when administered at pH 6.5 (22% unionized) than at pH 5 (1% unionized). They suggested that this enhanced efficacy at pH 6.5 is a result of increased penetration of pilocarpine due to a greater concentration of the unionized form of the drug. These results suggest that, for a given pilocarpine concentration, more drug will enter the eye if the physiological pH of the tear film can be maintained coincident with drug administration. Unfortunately, the pH of eyedrop solutions must be kept low at present to stabilize the pilocarpine, necessitating larger doses for therapeutic effect than would otherwise be required with more nearly physiological pH.

Fairbairn *et al.* (5) indicated that a more concentrated acid buffer causes a stronger sensation of stinging or burning than does a less concentrated buffer of the same acid pH. Presumably, this sensory effect occurs because the tear film remains at a lower pH for a longer time with the more concentrated buffer. Fairbairn *et al.* (5) recommended minimum buffering of acidic ophthalmic solutions to permit the tear film to return to the physiological pH range rapidly. This conclusion, that the stronger the acid buffer the more subjectively irritating the eyedrop, appears valid for pilocarpine solutions.

Pilocarpine exerts no known topical anesthetic effect, so it should not suppress any stinging sensation caused by a lowering of eye surface pH. The combination of drug and excipients causes at least as great a sensation of burning and stinging as a solution of pharmacologically inactive salt with identical buffer capacity. Whether or not pilocarpine solutions are subjectively more irritating at lower pH values depends not only on the extent and duration of the induced pH change but also on the relative potential for irritation of the protonated *versus* the base form of drug. This question awaits further investigation.

Hydrochloric acid alone in pH 4 solutions produced smaller decreases in tear film pH, and the recovery time to pretreatment tear film pH was shorter by 15 min than with any 2% pilocarpine solution tested. Attempts to prolong residence time of pilocarpine in the eye by adding macromolecules to the formulation (Solution D) appeared to prolong the duration of pH change as well.

The results of this study indicate that reduction of tear film pH by pilocarpine eyedrop formulations is not a simple function of solution pH. On the contrary, both the contact time and the acidic buffer capacity of these solutions contribute to the magnitude and duration of lowered tear film pH after an eyedrop of an ophthalmic pilocarpine formulation is instilled.

The continuous delivery of pilocarpine base in the absence of concomitant delivery of acid buffers favors the prevalence of the neutral base form of pilocarpine. The neutral form of pilocarpine should readily penetrate the lipophilic corneal epithelial barrier, in contrast to the protonated form which predominates at pH < 7. This reasoning may partially explain why continuous delivery of pilocarpine is clinically effective at daily doses of one-fourth to one-eighth (6, 7), and occasionally as little as one-fourteenth (8), those usually administered by eyedrops.

## REFERENCES


(1) S. S. Chrai, T. Patton, A. Mehta, and J. Robinson, *J. Pharm. Sci.*, **62**, 1112(1973).

(2) N. Keller, D. M. Moore, and J. Urquhart, "Pilocarpine Kinetics in Rabbit Eye Tear Film during Continuous Administration," presented at ARVO meeting, Sarasota, Fla., Apr. 28, 1976.

(3) R. A. Moses, "Adler's Physiology of the Eye," 5th ed., Mosby, St. Louis, Mo., 1970, p. 21.

(4) R. A. Anderson and J. B. Cowles, *Br. J. Ophthalmol.*, **52**, 607(1968).

(5) W. Fairbairn, W. Shepherd, and S. Eriksen, in preparation, cited by Allergan Pharmaceuticals, *J. Am. Optom. Assoc.*, **40**, 720(1969).

(6) P. Lee, Y. Shen, and M. Eberle, *Invest. Ophthalmol.*, **14**, 43(1975).

(7) M. S. Armaly and K. R. Rao, in "Symposium on Ocular Therapy," I. H. Leopold, Ed., Mosby, St. Louis, Mo., 1973, pp. 80–94.

(8) K. L. Macoul and D. Pavan-Langston, *Arch. Ophthalmol.*, **93**, 587(1975).


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# Bioavailability Assessment under Quasi- and Nonsteady-State Conditions III: Application


**J. V. BONDI, H. B. HUCKER, K. C. YEH, and K. C. KWAN [x]**



**Abstract** □ The applicability of bioavailability assessment at quasi- and nonsteady state is illustrated with data from a study comparing two formulations of amitriptyline hydrochloride in humans. Relative bioavailability as a function of the observed mean plasma concentrations may be expressed in closed form, provided the affected intervals begin and end in the log–linear region. Alternatively, numerical, graphical, and/or electronic computational techniques may be used to simulate the appropriate $[\overline{C}p^{(i)}]_{sim}$, the proximity of which to $[\overline{C}p^{(i)}]_{obs}$ is a function of relative bioavailability and of $\omega$. If a model can be found to fit the data adequately, it would be sufficient that only one sampled interval end in the log–linear phase.



**Keyphrases** □ Bioavailability—amitriptyline hydrochloride, assessment at quasi- and nonsteady state, equations derived □ Amitriptyline hydrochloride—bioavailability assessment at quasi- and nonsteady state, equations derived □ Antidepressants—amitriptyline hydrochloride, bioavailability assessment at quasi- and nonsteady state, equations derived


Previous reports (1, 2) in this series dealt with the theoretical basis for bioavailability assessment at quasi- and nonsteady state and its versatility in accommodating variations in experimental design. The purpose of this report is to apply the proposed technique to data from a study comparing two different formulations of