Preliminary Pharmacokinetic Model of Pilocarpine Uptake and Distribution in the Eye

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Received April 5, 1977, from the *Department of Chemical and Petroleum Engineering and the ‡Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66045. Accepted for publication August 3, 1977.

Abstract □ A pharmacokinetic model that permits prediction of aqueous humor pilocarpine levels following topical application to rabbit eyes was developed. The model is able to account for changes in both instilled solution volume and drug concentration. The model, although simplified, relies mainly on experimentally verifiable and independently measured parameters. Its utility lies in its ability to account quantitatively for the large drainage loss of instilled drug solutions and its predictive ability regardless of the instilled volume or concentration. The framework established by this model will allow further sophistication as more experimental data become available and should be adaptable to other ophthalmic drugs.

Keyphrases □ Pilocarpine—uptake and distribution in rabbit eye, preliminary pharmacokinetic model □ Pharmacokinetics—preliminary model of uptake and distribution of pilocarpine in rabbit eye □ Models, pharmacokinetic—developed for uptake and distribution of pilocarpine in rabbit eye □ Cholinergics, ophthalmic—pilocarpine, uptake and distribution in rabbit eye, preliminary pharmacokinetic model

The large drainage loss of instilled solutions can account for the relatively poor bioavailability of ophthalmic drugs (1), but other precorneal factors also are important in the disposition of topically applied ophthalmic drugs (2). The cornea, through which ophthalmic drugs must pass to reach the eye interior, is a complex membrane presenting several barriers to drug penetration (3–9). In spite of this knowledge, few systematic pharmacokinetic studies have been reported that permit a degree of predictability of the distribution of ophthalmic drugs under various dosing conditions.

BACKGROUND

Pilocarpine is an important ophthalmic drug and has been studied extensively (2, 9–23). Pilocarpine uptake into the rabbit eye is a complex combination of transport away from the cornea by an accentuated flow mechanism, uptake by various tissues, transport through the cornea, and distribution and elimination from the aqueous humor. Each component may be composed of, and/or supplemented by, other less important but still measurable phenomena such as diffusion away from the cornea and eye by transport through the conjunctiva, drug distribution to other tissues from the aqueous humor itself, and increased tear production and drainage rate of pilocarpine from the eye surface. Finally, protein binding in all compartments may affect free pilocarpine transport.

Data for these phenomena will be developed in the future; however, initially, the overall uptake of pilocarpine in the eye is examined to ascertain the major parameters contributing to the relatively small total uptake of topically applied pilocarpine. For that reason, a mathematical model described here, while simplifying somewhat the actual situation, will be a useful framework for future investigations of pilocarpine distribution, uptake, and elimination from the eye.

Although Makoid (24) recently considered a classical pharmacokinetic approach for pilocarpine distribution in the eye, his model did not consider the effects of instilled drop size on pilocarpine uptake. The uniqueness of the proposed model is that it is able to predict the effects of instilled drop size and concentration such that reasonable *a priori* predictions of aqueous humor pilocarpine levels can be made. It is hoped that this model will provide the framework for a more comprehensive model for pilocarpine as well as other drugs as more experimental data become available.

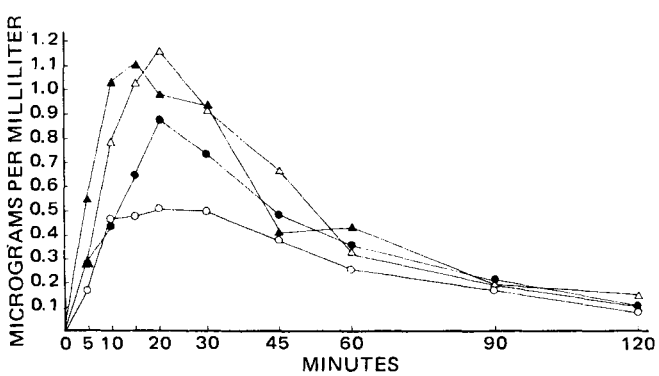


Figure 1—*Aqueous humor concentration–time profiles for pilocarpine nitrate following instillation of 5 (○), 10 (●), 15 (△), and 25 (▲) μl of a 1 × 10^{-2} M solution in rabbits.*

EXPERIMENTAL

Materials—Pilocarpine nitrate was obtained commercially[1] and used as received. Tritiated pilocarpine[2] (specific activity 4.1 Ci/mmole) in ethanol solution was evaporated several times prior to use as previously described (13) to remove any solvent that had become tritiated by exchange.

Male albino rabbits, 60–67 days old, were maintained in standard laboratory animal cages and allowed food and water *ad libitum*.

Computer calculations were performed on a digital computer[3].

Solution Preparation—Pilocarpine nitrate solutions were prepared in pH 6.24 isotonic Sorensen's phosphate buffer and were filtered but not sterile. Procedures for the preparation of tritiated solutions were described previously (13). Solutions were prepared fresh and kept for a maximum of 12 hr.

Aqueous Humor Pilocarpine Concentration–Time Profiles—During the experiments, test animals were kept in restraining boxes in the normal upright position. The head was unencumbered so that all normal eye movements were maintained. Rabbits were unanesthetized in all cases. Accurate volumes of solutions were instilled in rabbit eyes

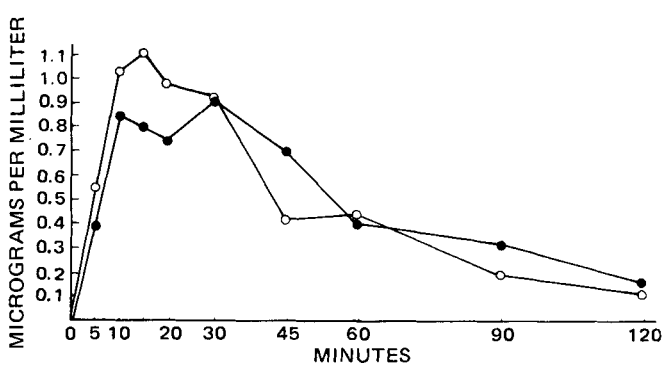


Figure 2—*Aqueous humor concentration–time profiles for pilocarpine nitrate following instillation of 25 μl of a 1 × 10^{-2} M solution (○) and 5 μl of a 1.61 × 10^{-2} M solution (●) in rabbits.*

[1] Sigma Chemical Co., St. Louis, Mo.
[2] New England Nuclear, Boston, Mass.
[3] Honeywell 66/60 at the University of Kansas Computation Center.

Table I—Calculated Areas under Aqueous Humor Concentration–Time Profiles for 25 μl of 1×10^{-2} *M* and for 5 μl of 1.61×10^{-2} *M* Pilocarpine Nitrate in 60-Day-Old Rabbits

Volume Instilled, ml	Concentration Instilled, *M*	Amount of Drug Instilled, μg	*AUC*, μg min/ml
0.025	1.00×10^{-2}	67.82	60.9
0.005	1.61×10^{-2}	21.87	67.2

using a microliter syringe[4]. During instillation, the lower eyelid was pulled slightly away from the globe but was returned to its normal position immediately after instillation.

At various times postinstillation (5, 10, 15, 20, 30, 45, 60, 90, and 120 min), rabbits were sacrificed with an overdose of pentobarbital sodium injected into a marginal ear vein. Eyes were immediately rinsed with distilled water and blotted with tissue, and aqueous humor was aspirated from the anterior chamber. At least 100 μl of aqueous humor was removed in each case. It is important that the elapsed time from animal sacrifice to removal of the aqueous humor sample be as short as possible. In these studies, the time period needed to sacrifice the animal and obtain aqueous humor from both eyes was less than 1 min.

Aqueous humor, 100 μl, was transferred[5] to a scintillation vial[6] containing 5 μl of prerefrigerated liquid scintillation cocktail[7]. After storage in the dark at room temperature for at least 24 hr, samples were counted[8]. Counts were then converted to micrograms of pilocarpine per milliliter of aqueous humor using suitable standard and blank corrections.

Aqueous humor concentration profiles for 5-, 10-, 15-, and 25-μl instilled volumes of 1×10^{-2} *M* pilocarpine were reported previously (25). Since these profiles were used to check the validity of the described pharmacokinetic model, they are reproduced in Fig. 1[9].

From the previous study (25), it was concluded that a considerable decrease in instilled volume coupled with a slight increase in instilled concentration should result in the same amount of drug reaching the eye interior as if much larger volumes were administered. By using a simple calculation, it was shown (25) that a 5-μl dose of 1.61×10^{-2} *M* pilocarpine nitrate should result in the same area under the curve as a 25-μl dose of 1.00×10^{-2} *M* pilocarpine nitrate. This experiment was performed, and the profiles are illustrated in Fig. 2. By using the trapezoidal rule with extrapolation to infinity, the areas under these two profiles were calculated (Table I).

MODEL

Development—The eye is viewed here as consisting of two major compartments. The first compartment represents the precorneal area. Its initial volume consists of the normal resident tear volume plus the volume of the instilled drop. There is a constant production and outflow

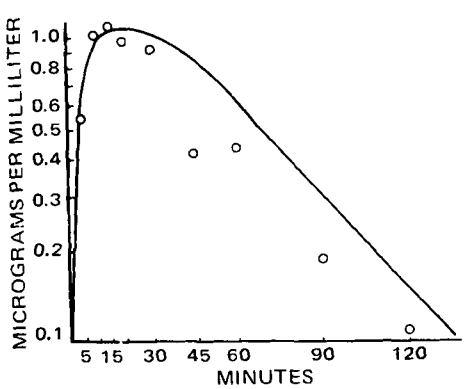


Figure 3—*Comparison of model-predicted (—) and experimental (○) aqueous humor concentrations following instillation of 25 μl of 1×10^{-2} M pilocarpine in rabbits.*

[4] Hamilton Co., Reno, Nev.
[5] Biopette, Schwarz/Mann, Orangeburg, N.Y.
[6] Mini vials, ICN, Isotope and Nuclear Division, Cleveland, Ohio.
[7] Aquasol, New England Nuclear, Boston, Mass.
[8] Beckman LS-150 liquid scintillation counter, Beckman Instruments, Fullerton, Calif.
[9] For clarity, standard error bars have been omitted. These values are reported in Ref. 25. Each point represents the mean of at least eight determinations.

Table II—Parameters Important to the Pharmacokinetic Model

Parameter	Value	Reference
V_0	7.5 μl	1
V_{AH}	311 μl	26
Q_T	0.66 μl/min	1
K	$(0.25 + 0.0113\ V_D)\ \text{min}^{-1}$	1
A	2 cm^2	26
L	0.035 cm	26
K_D	3.675×10^{-4} μl/cm min	
K_{el}	7.5 μl/min	

of tears from this compartment, and the drainage of instilled fluid is a function of drop size (1).

The second compartment represents the aqueous humor, which received pilocarpine by diffusion through the cornea. Various elimination mechanisms from the aqueous humor are lumped together into a single first-order elimination term. Although more mechanistic studies are in progress and some have been reported (9), this preliminary model views the cornea as a single homogeneous structure. In addition, no loss from the precorneal area by conjunctival absorption is included (2). A schematic representation of the model is shown in Scheme I.

The volume of fluid initially present in the precorneal area is a variable that depends on the size of the instilled pilocarpine drop. A flow balance (Eq. 1) is made where it is assumed that the densities of the tear fluid and drug solutions are those of water. It is also assumed that the amount of pilocarpine diffusing through the cornea does not significantly affect the tear fluid volume:

$$\begin{matrix}\text{rate of} \\ \text{change} \\ \text{in fluid} \\ \text{volume}\end{matrix} = \begin{matrix}\text{rate of} \\ \text{flow in}\end{matrix} - \begin{matrix}\text{rate of} \\ \text{flow out}\end{matrix} \qquad \text{(Eq. 1)}$$

Equation 2 shows the representation of the flow balance for pilocarpine in tear fluid:

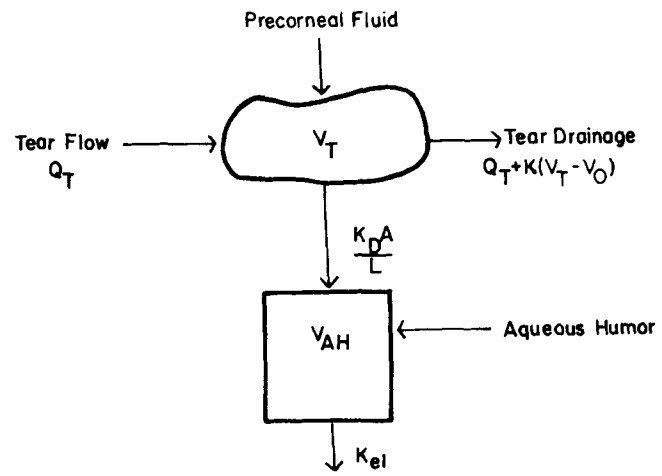
$$\frac{dV_T}{dt} = Q_T - [Q_T + K(V_T - V_0)] \qquad \text{(Eq. 2)}$$

at $t = 0$ and $V_T = V_D + V_0$ and where V_T is the total volume in the precorneal area at any given time, T; Q_T is the normal tear production rate; K is a proportionality constant that is a function of the instilled drop size, V_D(1); and V_0 is the normal resident tear volume. The expression assumes that the tear production rate is not significantly altered by the instilled fluid. The drainage out of the eye is basically that which would normally occur plus a term that is proportional to the actual volume present less the amount normally there.

This differential equation can be solved directly and analytically, noting that at the instillation time the total drop volume is equal to the volume of tears normally there plus the instilled drop size. Solving the expression yields Eq. 3, which represents the total volume of the eye fluid as a function of time:

$$V_T = V_D e^{-Kt} + V_0 \qquad \text{(Eq. 3)}$$

This expression is identical to that derived previously (1).



Scheme I

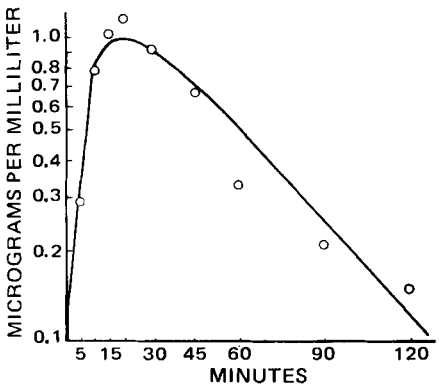


Figure 4—*Comparison of model-predicted (—) and experimental (O) aqueous humor concentrations following instillation of 15 μl of 1 × 10^{-2} M pilocarpine in rabbits.*

A mass balance is now written for pilocarpine in the tear fluid:

$$\begin{array}{l}\text{rate of}\\ \text{change in}\\ \text{mass in}\\ \text{tear fluid}\end{array} = \begin{array}{l}\text{rate of}\\ \text{transfer in}\\ \text{by flow}\end{array} - \begin{array}{l}\text{rate of}\\ \text{loss by}\\ \text{drainage}\end{array} - \begin{array}{l}\text{rate of}\\ \text{loss by}\\ \text{diffusion into}\\ \text{aqueous humor}\end{array} \quad \text{(Eq. 4)}$$

or, mathematically:

$$\frac{dV_TC_T}{dt} = 0 - [Q_T + K(V_T - V_0)]C_T - \frac{K_DA}{L}(C_T - C_{AH}) \quad \text{(Eq. 5)}$$

where C_T is the concentration in the tear fluid, K_D is the specific permeability rate, A is the cornea area, L is the cornea thickness, and C_{AH} is the concentration in the aqueous humor.

By taking the indicated derivative and substituting the value of V_T found into this expression, a simplified relationship results:

$$\frac{dC_T}{dt} = \frac{-Q_TC_t - \frac{K_DA}{L}(C_T - C_{AH})}{V_De^{-Kt} + V_0} \quad \text{(Eq. 6)}$$

This expression assumes that there is no appreciable loss into the conjunctiva and that the diffusion into the aqueous humor is linear. Finally, a similar mass balance is written for the aqueous humor compartment:

$$V_{AH}\frac{dC_{AH}}{dt} = \frac{K_DA}{L}(C_T - C_{AH}) - K_{el}C_{AH} \quad \text{(Eq. 7)}$$

where V_{AH} is the aqueous humor volume and K_{el} is the lumped first-order clearance parameter from the aqueous humor. Equations 6 and 7 can be solved numerically relatively easily, despite their involved nature, by the use of a digital computer.

To be able to evaluate the results of the model, certain parameters must be estimated. Table II lists the various parameters and the sources of these data. The instilled drop size of the pilocarpine solution is a variable quantity. Finally, the initial pilocarpine concentration is given by:

$$C_0 = \frac{V_DC_D}{V_D + V_0} \quad \text{(Eq. 8)}$$

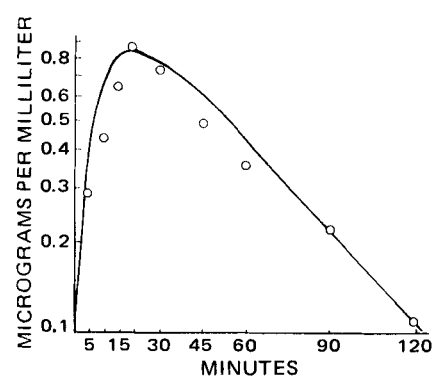


Figure 5—*Comparison of model-predicted (—) and experimental (O) aqueous humor concentrations following instillation of 10 μl of 1 × 10^{-2} M pilocarpine in rabbits.*

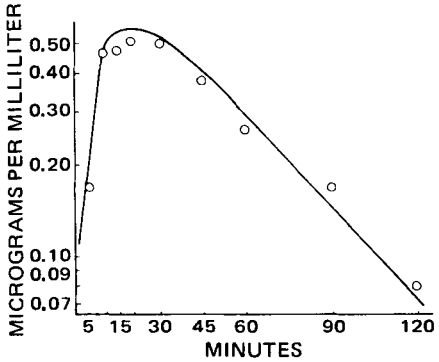


Figure 6—*Comparison of model-predicted (—) and experimental (O) aqueous humor concentrations following instillation of 5 μl of 1 × 10^{-2} M pilocarpine in rabbits.*

where C_D is the pilocarpine concentration in the instilled fluid. Thus, all parameters are measured, except the quantities K_D and K_{el}, which are estimated by applying the model to the data from an experiment where 15 μl of 1.00 × 10^{-2} M pilocarpine solution is instilled and then monitoring the aqueous humor concentration as a function of time. Thus, these two parameters are measured by experiment and evaluated in terms of the model. All of the other parameters are estimated from independent experiments.

Applications—Figures 3–6 show the results of the model applied to four different drop sizes of 1 × 10^{-2} M pilocarpine instilled into rabbit eyes. The model demonstrates that the aqueous humor pilocarpine concentration can be predicted reasonably well (r = 0.82 for the 15-μl instilled volume) over a range of instilled volumes. This result indicates that the drainage mechanism, which this model accounts for, is very important in explaining ultimate aqueous humor drug concentrations. The results are relatively poor at times greater than 1 hr, especially for a 25-μl drop, because of the lack of inclusion of a surrounding tissue of distribution (*i.e.*, the lens) in the model. This factor will be included as data become available. The present results certainly indicate the importance of precorneal considerations. The model is, however, deficient in its treatment of drug transport in the eye interior. Nevertheless, the results are good in terms of peak height and general trends for all drop sizes compared to the 15-μl base experiment, although the fit is not excellent in a least-squares sense.

In addition, as speculated previously (25), the model indicates that considerable reduction in drop size coupled with slight increases in drug concentration can permit dosage reduction without sacrifice of drug concentration in the eye interior. This situation is demonstrated in Fig. 7, which shows the results of an experiment where 25 μl of 1 × 10^{-2} M pilocarpine was instilled compared to an experiment where 5 μl of 1.61 × 10^{-2} M pilocarpine was instilled. The instilled volume was reduced by a factor of five and the overall dose of pilocarpine was reduced by about 70% without appreciable sacrifice of drug concentration in the aqueous humor. Figure 7 also shows that the model predicts these data reasonably well.

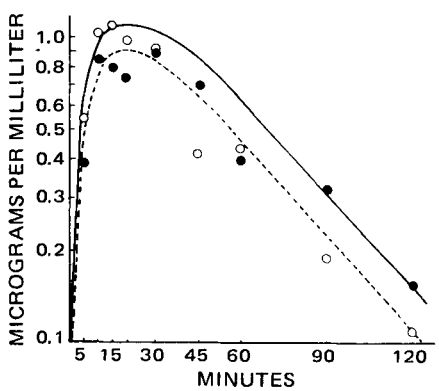


Figure 7—*Aqueous humor concentration–time profiles following instillation of 25 μl of 1 × 10^{-2} M pilocarpine (O, experimental data; and —, model prediction) and 5 μl of 1.61 × 10^{-2} M pilocarpine (●, experimental data; and - - -, model prediction).*

DISCUSSION

The data in Fig. 1 show that, for a constant instilled concentration, aqueous humor levels decrease as the instilled volume is decreased. However, aqueous humor drug levels do not decrease in proportion to dose size. For example, decreasing the instilled volume from 25 to 5 μl, a fivefold dosage reduction, results in only about a twofold reduction in the area under the curve of aqueous humor concentration *versus* time. Previous attempts at quantitation of pilocarpine uptake and distribution could not have predicted such an occurrence since available data on tear and instilled solution drainage were not quantitatively considered (1). The model developed here takes this factor into account and is, therefore, able to make reasonable predictions even when instilled volume and/or concentration are altered.

As has been mentioned, the data from Fig. 1 imply that a considerable decrease in instilled volume coupled with a slight increase in instilled concentration should result in the same amount of drug reaching the eye interior as if much larger volumes were administered. The data of Fig. 2 confirm this result, and Fig. 7 shows that the model also predicts it. It is confirmed that the model is sensitive to changes in both instilled volume and/or concentration. Such predictive capability has not previously been available.

Although the mathematics presented here are a simplification of the actual situation, the framework is now available that will allow refinement and addition of more sophisticated experimental data as they become available. Specifically, conjunctival absorption, protein binding, tissue distribution, and more mechanistic data on corneal absorption are needed. As such information becomes available, the treatment presented here can be refined to quantitate it. Furthermore, once all relevant parameters have been identified, the model should lend itself to quantitation of other ophthalmic drugs in addition to pilocarpine.

REFERENCES

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

(2) T. F. Patton and J. R. Robinson, *ibid.*, **65**, 1295 (1976).

(3) H. Benson, *Arch. Ophthalmol.*, **92**, 313 (1974).

(4) C. Dohlman, *Invest. Ophthalmol.*, **10**, 383 (1971).

(5) K. C. Swan and N. G. White, *Am. J. Ophthalmol.*, **25**, 1043 (1942).

(6) A. Kupferman, M. V. Pratt, K. SucKewer, and H. M. Leibowitz, *Arch. Ophthalmol.*, **91**, 373 (1974).

(7) A. Tonjum, *Acta Ophthalmol.*, **52**, 560 (1974).

(8) T. Iwata, M. Uyama, and K. Ohkawa, *Jpn. J. Ophthalmol.*, **19**, 139 (1975).

(9) J. Sieg and J. R. Robinson, *J. Pharm. Sci.*, **65**, 1816 (1976).

(10) D. H. Abramson, J. Coleman, M. Forbes, and L. A. Franzen, *Arch. Ophthalmol.*, **87**, 615 (1972).

(11) D. H. Abramson, S. Chang, and D. J. Coleman, *ibid.*, **94**, 914 (1976).

(12) P. C. Barsam, *Am. J. Ophthalmol.*, **73**, 742 (1972).

(13) S. S. Chrai and J. R. Robinson, *ibid.*, **77**, 735 (1974).

(14) P. P. Ellis, *Surv. Ophthalmol.*, **16**, 165 (1971).

(15) T. S. Friedman and T. F. Patton, *J. Pharm. Sci.*, **65**, 1095 (1976).

(16) K. Green and S. J. Downs, *Arch. Ophthalmol.*, **93**, 1165 (1975).

(17) L. S. Harris, T. W. Mittag, and M. A. Galin, *ibid.*, **86**, 1 (1971).

(18) D. L. Krohn and J. M. Breitfeller, *Invest. Ophthalmol.*, **13**, 312 (1974).

(19) R. Lazare and M. Horlington, *Exp. Eye Res.*, **21**, 281 (1975).

(20) F. J. Macri and J. J. Cevario, *Invest. Ophthalmol.*, **13**, 617 (1974).

(21) D. A. Newsome and R. Stern, *Am. J. Ophthalmol.*, **77**, 918 (1974).

(22) M. C. VanHoose and F. E. Leaders, *Invest. Ophthalmol.*, **13**, 377 (1974).

(23) T. F. Patton, Ph.D. thesis, University of Wisconsin, Madison, Wis., 1975.

(24) M. C. Makoid, Ph.D. thesis, University of Wisconsin, Madison, Wis., 1976.

(25) T. F. Patton, *J. Pharm. Sci.*, **66**, 1058 (1977).

(26) "The Rabbit in Eye Research," J. H. Prince, Ed., Charles C Thomas, Springfield, Ill., 1964.

ACKNOWLEDGMENTS

Supported by grants from the University of Kansas General Research Fund and the Children's Eye Care Foundation (CECF-RF-1976–77) and by University of Kansas Biomedical Sciences Support Grant RR-07037.

The authors are grateful to Mr. Ted S. Friedman for technical assistance.

Kinetics of Drug Transport and Concurrent Metabolism in Human Cell Cultures I: Theory

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Received October 27, 1976, from the *College of Pharmacy, University of Michigan, Ann Arbor, MI 48104.* Accepted for publication April 28, 1977.

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Abstract □ A method for evaluating the passive permeability and single-step metabolism of drugs in suspension cultures of mammalian cells was formulated assuming linear kinetics. It was assumed that the metabolizing enzymes are driven by endogenous substrates present in steady-state quantities. The presence of the drug in radiolabeled tracer quantities was assumed to cause only a small perturbation from the endogenous steady-state operating point. The time course solutions for the drug and its metabolite are given in terms of macroscopic constants, and their physical interpretations are given in terms of metabolic and transport parameters.

Keyphrases □ Drug transport kinetics—evaluated in suspension cultures of mammalian cells, equations derived □ Metabolism, single step—evaluated for drugs in suspension cultures of mammalian cells, equations derived □ Kinetics—drug transport and concurrent metabolism in suspension cultures of mammalian cells, equations derived

The study of drug kinetics at the cellular level represents a microcosm of the traditional field of pharmacokinetics. Such a study, which includes cellular absorption, elimination, and metabolism *in vitro*, might be regarded as academic and possibly not as relevant as clinical studies *in vivo*. However, the ultimate resolution of the cancer problem and certain persistent viral infections may depend on knowledge of chemotherapy and kinetics at the cellular level. If the tumor problem is epigenetic and potentially reversible, as some investigators believe (1), then the ab-