complexes were 0.22 and 0.16 M^{-1} for caffeine–*p*-cresol and theophylline–*p*-cresol, respectively, at 308 nm (1). On the other hand, the molar absorptivities of these complexes at the intermolecular hydrogen-bonded OH stretching vibration band, 3370 cm^{-1}, were $\sim 3 \times 10^3$ and $\sim 4.3 \times 10^3$ for caffeine–*p*-cresol and theophylline–*p*-cresol, respectively.

The present work supported the theory that hydrogen bonding is a type of charge transfer phenomenon (8–10).

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Pilocarpine Ocular Distribution Volume

Keyphrases □ Pilocarpine—ocular volume of distribution □ Volumes of distribution—ocular distribution of pilocarpine □ Ophthalmic drugs—pilocarpine, ocular volume of distribution

To the Editor:

It is well established that drugs introduced into the body by various routes distribute to and equilibrate with the blood as well as numerous body tissues. Thus, the apparent volume of distribution has become a useful parameter in explaining the drug concentrations achieved in the blood after dosing and for determining the extent of absorption of various agents administered in different formulations or dosage forms.

Similarly, apparent distribution volumes also are useful in describing the ocular disposition characteristics of drugs administered topically to the eye. To make meaningful statements regarding the bioavailability of an ophthalmic preparation, it is necessary to know the apparent volume of distribution for that drug in the eye.

As with volumes of distribution obtained for drugs administered by other routes, various factors can affect apparent volumes of distribution of ocular drugs. These factors include protein binding, tissue distribution, pigmentation of the eye, and, of course, any pharmacological action the drug itself might exert. Therefore, the following discussion is limited to pharmacokinetic data obtained in albino rabbits of approximately the same weight.

Conrad and Robinson (1) developed a method of quantitating apparent ocular drug distribution volumes in experimental animals by injecting drug directly into the anterior chamber and monitoring the decline in the aqueous humor concentration as a function of time. The data from the study were used subsequently[1] (2, 3) to quantitate and describe the disposition of drugs in the eye.

Conrad and Robinson (1) determined the apparent volumes of distribution and elimination rate constants for both a therapeutically active substance (pilocarpine) and a nondistributing species (inulin). From the relationship:

$$Cl = V_d K \qquad \text{(Eq. 1)}$$

and the values obtained for inulin, a clearance of 4.59 μl/min from the aqueous humor was obtained. Since inulin neither binds nor distributes into the tissues and exits the anterior chamber solely *via* aqueous humor drainage, this value should represent normal aqueous humor turnover. The calculated value is consistent with values reported by other investigators (4, 5) for aqueous humor turnover in rabbits. Similarly, unless an agent is known to decrease aqueous humor turnover, the value of 4.59 μl/min also should represent the slowest possible clearance of any compound from the aqueous humor.

Following intracameral injection, the aqueous humor concentration of pilocarpine declined monoexponentially with an apparent first-order elimination rate constant of 0.059 min^{-1}. Based on the reported volume of distribution of 575 μl, a clearance value of 33.9 μl/min was obtained for pilocarpine. Since pilocarpine is known to facilitate aqueous humor outflow, it is not surprising that the elimination rate constant for pilocarpine and, in turn, the clearance were greater than those observed for inulin.

In a recent study by Makoid and Robinson (6), the decline of pilocarpine concentration in the aqueous humor following topical dosing was multiexponential when followed for 12 hr. Conrad and Robinson (1) only followed the aqueous humor concentration of pilocarpine for 1 hr after direct intracameral injection. The effect that this apparent discrepancy might have in determining the ocular volume of distribution for pilocarpine led us to examine further the decline in the aqueous humor concentration following direct injection into the aqueous humor.

For this study, the experimental procedure of Conrad and Robinson was repeated with pilocarpine of a higher specific activity (19.7 mCi/mg). In the original study, Conrad and Robinson (1) measured aqueous humor levels of pilocarpine at 5, 10, 15, 20, 30, and 60 min after injection, but they stated that the accuracy of their 60-min time point was in doubt. In our study, the 30-min point was excluded; however, a 25-min point was added, and sampling was extended to include 45-, 60-, 90-, and 120-min points.

Figure 1 shows the results of this study when 10 μl of 1×10^{-4} *M* pilocarpine was injected intracamerally. The

[1] M. C. Makoid and J. Cobby, presented at the APhA Academy of Pharmaceutical Sciences, Anaheim meeting, April 1979.

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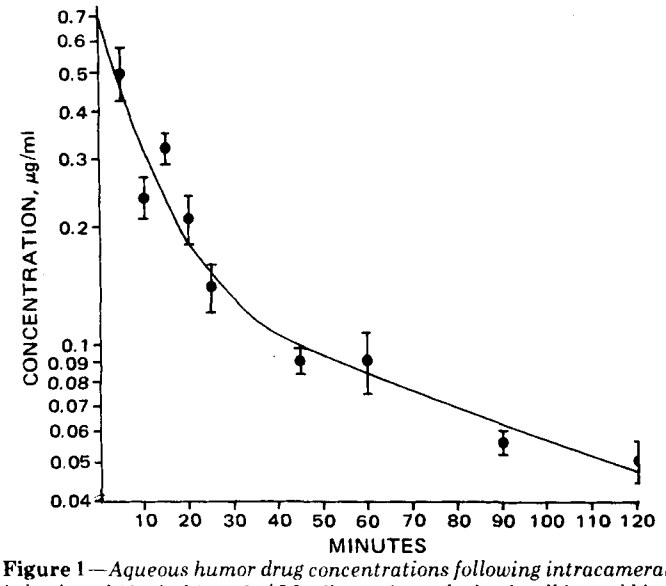

Figure 1—*Aqueous humor drug concentrations following intracameral injection of 10 μl of 1 × 10^{-4} M pilocarpine solution in albino rabbits. Points represent the mean of at least seven determinations. Bars represent the standard error of the mean. The solid line is the nonlinear regression line of the data fitted to a biexponential equation using the NONLIN program.*

absolute pilocarpine levels obtained are in reasonable agreement with those of Conrad and Robinson for the first 25 min. However, when later time points are considered, the curve clearly is not monoexponential. When the data reported here for the first 25 min are fitted to a monoexponential equation, an apparent volume of distribution and an elimination rate similar to those of Conrad and Robinson are obtained. However, due to the curvature noted at later time points, the short sampling period reported by Conrad and Robinson evidently resulted in an underestimation of the apparent ocular distribution volume for pilocarpine and represents a nonequilibrium apparent volume of distribution. This interpretation was mentioned by Conrad and Robinson but was rejected by reference to unpublished data.

The concept of a changing volume of distribution with time is, of course, not new and is expected with tissues that equilibrate slowly (7). Preliminary studies in this laboratory indicate that the partitioning of pilocarpine between aqueous humor and some ocular tissues is, in fact, a slow process. In addition, due to the effect of pilocarpine on aqueous humor turnover, it is to be expected that the slope of the pilocarpine decline from aqueous humor following intracameral injection would not remain constant over time. Ongoing experiments in this laboratory indicate that if pilocarpine levels are followed for times longer than those shown in Fig. 1, the slope becomes even more shallow. Therefore, a combination of factors, slow equilibration along with pilocarpine's pharmacological effect, makes it difficult to assign precise values to the ocular volume of distribution or, in turn, to the clearance of pilocarpine.

From a practical standpoint, apparent volumes of distribution obtained in short-term studies such as those of Conrad and Robinson may suffice for some drugs in describing aqueous humor drug levels in single-dose situations since most of the drug is eliminated quite rapidly. However, such studies usually will not suffice to predict aqueous humor drug levels over a long time or in steady-state situations. Therefore, pharmacokinetic data obtained during ocular multiple dosing or steady-state conditions may require a somewhat different interpretation than those obtained during short duration, single-dose studies.

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Applications of TLC and Bioautography to Detect Contaminated Antibiotic Residues: Tetracycline Identification Scheme

Keyphrases □ Cephalosporins—TLC–bioautographic analysis as contaminants in various tetracyclines □ Penicillin—TLC–bioautographic analysis as contaminant in erythromycin □ Tetracyclines, various—TLC–bioautographic analysis of four cephalosporins as contaminants □ Erythromycin—TLC–bioautographic analysis of penicillin and ampicillin as contaminants

To the Editor:

A method to detect penicillin and ampicillin as contaminants in various tetracyclines and penicillamine was reported previously (1). The method utilizes TLC followed by bioautography and has been applied to other contamination situations and antibiotic standard problems. The cephalosporins are related closely to penicillin in chemical structure, antimicrobial activity, and allergenicity. In addition to the occurrence of allergenic cross-reactivity with penicillin, several cases of apparent primary allergy to cephalosporins in patients not known to be hypersensitive have been reported (2). Minor modification of the assay permitted the separation of four cephalosporin contaminants from seven tetracyclines: demeclocycline, methacycline, minocycline, chlortetracycline, oxytetracycline, doxycycline, and tetracycline.

The original method was followed with several excep-

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