Table IV—Apparent [a] Contribution of Hydroxyl Groups to Net Thermodynamic Parameters for the Interphase Transfer of Tetracyclines in a Two-Phase System at 37°

Functional Group	π [b]	$\Delta F_{G\ \Delta F}$ [c], cal/mole	$\Delta H_{G\ \Delta H}$ [c], cal/mole	$\Delta S_{G\ \Delta S}$ [c], cal/mole degree	$T\Delta S_{GT\ \Delta S}$ [c], cal/mole
C-5 hydroxyl [d]	0.170	−239	1041	4.1	1271
C-6β hydroxyl [e]	−0.789	1121	3025	6.1	1891

[a] Apparent values because the composition of species is not constant for each tetracycline at pH 5.5. [b] The log of substituent constant calculated using Eq. 3. [c] The functional group free energy, enthalpy, entropy, and entropic energy of partitioning calculated using Eq. 4 and the net thermodynamic parameters of Table III; the net value of one derivative is subtracted from a second derivative to obtain the functional group thermodynamic contribution. [d] Obtained by subtracting tetracycline data from oxytetracycline data. [e] Obtained by subtracting doxycycline data from oxytetracycline data.

Substitution of a hydroxyl group at C-5 promotes partitioning by decreasing the apparent free energy of partitioning through an entropy-dominated effect. Shifting the hydroxyl group to C-6β, however, inhibits partitioning due to an enthalpy-dominated gain in the apparent free energy of partitioning. This small variation in position of the hydroxyl group from C-5 to C-6β results in substantial changes in the energy contributions of the hydroxyl group, as estimated by subtracting the C-5 hydroxyl data in Table IV from the C-6β data. For this shift in hydroxyl group, ΔF = 1360 cal/mole, ΔH = 1984 cal/mole, ΔS = 2.0 cal/mole degree, and $T\ \Delta S$ = 620 cal/mole. These observations suggest the fundamental importance of desolvation and resolvation processes in forming the activated complex for interphase transfer. In moving the hydroxyl group from C-5 to C-6β, a shift that probably accentuates the hydrophilic contribution of the hydroxyl group and diminishes the hydrophobic interaction of the tetracycline derivative with 1-octanol molecules in forming the activated complex, a ninefold decrease in the apparent partition coefficient is observed.

As shown in Tables III and IV, the addition of a hydrophilic substituent such as a hydroxyl group to a tetracycline derivative does not necessarily decrease the apparent partition coefficient. When tetracycline is converted to oxytetracycline by adding another hydroxyl group at C-5, the apparent partition coefficient increases because of a negative apparent $\Delta F_{G\ \Delta F}$ contribution. However, in converting doxycycline to oxytetracycline by adding an additional hydroxyl group at C-6β, the apparent partition coefficient decreases because of a positive apparent $\Delta F_{G\ \Delta F}$ contribution. For both examples, an additional hydroxyl group is substituted on the tetracycline derivative, but the effect on the apparent partition coefficient varies due to the fundamental influence on solute–solvate interactions in forming the activated complex for interphase transfer.

The data provide additional support for the mechanisms of solute transfer previously proposed (1, 2). While the simple model system used does not simulate the complexity of biological membranes, the similarity in intermolecular forces governing the transfer of solute across liquid–liquid and liquid–membrane interfaces suggests that studies such as these may furnish insight into *in vitro* and *in vivo* interphase transfer processes.

REFERENCES

(1) G. E. Schumacher and J. B. Nagwekar, *J. Pharm. Sci.*, **63**, 240 (1974).

(2) *Ibid.*, **63**, 245 (1974).

(3) W. C. Barringer, W. Shultz, G. M. Sieger, and R. A. Nash, *Am. J. Pharm.*, **146**, 179 (1974).

(4) L. J. Leeson, J. E. Krueger, and R. Nach, *Tetrahedron Lett.*, **18**, 1155 (1963).

(5) J. L. Colaizzi and P. R. Klink, *J. Pharm. Sci.*, **58**, 1184 (1969).

(6) T. F. Chin and J. L. Lach, *Am. J. Hosp. Pharm.*, **32**, 625 (1975).

(7) H. Terada and T. Inagi, *Chem. Pharm. Bull.*, **23**, 1960 (1975).

(8) E. G. Remmers, G. M. Sieger, and A. P. Doerschuk, *J. Pharm. Sci.*, **52**, 752 (1963).

(9) H. S. Frank and M. W. Evans, *J. Chem. Phys.*, **13**, 507 (1945).

(10) S. A. Howard, M. A. Farvar, A. Suzuki, and W. I. Higuchi, *J. Pharm. Sci.*, **58**, 1325 (1969).

(11) J. T. Davies, *J. Phys. Colloid Chem.*, **54**, 185 (1950).

(12) C. Hansch and A. Steward, *J. Med. Chem.*, **7**, 691 (1964).

(13) G. L. Flynn, *J. Pharm. Sci.*, **60**, 345 (1971).

ACKNOWLEDGMENTS

Abstracted in part from a thesis submitted by D. M. Lei to Wayne State University in partial fulfillment of the Master of Science degree requirements.

Kinetic and Thermodynamic Aspects of *In Vitro* Interphase Transfer of Tetracyclines II: Influence of Divalent Metal Salts

G. E. SCHUMACHER ˣ and EDWARDS E. LINN

Received December 23, 1977, from the *College of Pharmacy and Allied Health Professions, Wayne State University, Detroit, MI 48202.* Accepted for publication April 20, 1978.

Abstract □ With a two-phase *in vitro* model composed of an aqueous pH 5.5 buffer and 1-octanol, the kinetics of the interphase transfer of tetracycline derivatives were examined in the presence and absence of calcium and magnesium salts, and the contribution of some functional group substituents to the "apparent" free energy changes for partitioning of tetracyclines was evaluated. Only small changes were observed in k_f, k_b, K_w^0, and apparent functional group free energy changes, $\Delta F_{G\ \Delta F}$, in the presence of divalent metals as compared to values observed in the absence of these metals. Introduction of the C-6β hydroxyl group on the tetracycline nucleus decreased the apparent K_w^0 because of a positive apparent $\Delta F_{G\ \Delta F}$ contribution, whereas introduction of C-5 hydroxyl, C-6α methyl, or C-7 chloro groups increased the apparent K_w^0 through negative apparent $\Delta F_{G\ \Delta F}$ contributions.

Keyphrases □ Tetracyclines—partitioning in aqueous-octanol system, kinetics and thermodynamics, effect of divalent metal salts □ Partitioning—tetracyclines in aqueous-octanol system, kinetics and thermodynamics, effect of divalent metal salts □ Kinetics—partitioning of tetracyclines in aqueous-octanol system □ Thermodynamics—partitioning of tetracyclines in aqueous-octanol system □ Metal salts, divalent—effect on partitioning of tetracyclines in aqueous-octanol system □ Antibacterials—tetracyclines, partitioning in aqueous-octanol system, kinetics and thermodynamics, effect of divalent metal salts

The absorption of a tetracyclines is depressed in the presence of antacids and dairy products (1). Yet no evidence has been reported that calcium and magnesium ions, commonly present in antacid preparations, are capable of

0022-3549/78/1200-1717$01.00/0

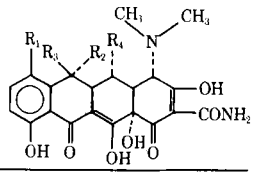


Table I—Structures and Physical Data for the Tetracyclines

Tetracycline	R_1 (C-7)	R_2 (C-6α)	R_3 (C-6β)	R_4 (C-5)	Apparent Dissociation Constants at 25° pK_1	pK_2	pK_3	Apparent Partition Coefficient at 37° and pH 5.5[a]
Tetracycline hydrochloride (8)[b]	H	CH_3	OH	H	3.3	7.7	9.7	0.074
Oxytetracycline hydrochloride (8)	H	CH_3	OH	OH	3.3	7.3	9.1	0.129
Chlortetracycline hydrochloride (8)	Cl	CH_3	OH	H	3.3	7.4	9.3	0.439
Demeclocycline hydrochloride (17)	Cl	H	OH	H	3.3	7.2	9.4	0.317
Doxycycline hyclate (17)	H	CH_3	H	OH	3.4	7.7	9.7	0.947

[a] Expressed in terms of the ratio of solute concentrations in 1-octanol–aqueous pH 5.5 phosphate buffer phases as obtained from interphase transfer experiments. [b] Denotes reference number.

Table II—Kinetic Parameters for the Interphase Transfer of Tetracyclines in the Presence[a] and Absence of Divalent Metal Salts at pH 5.5 and 37°

Tetracycline	k_f, hr^{-1} Absence of Metal	Presence of Calcium	Presence of Magnesium	k_b, hr^{-1} Absence of Metal	Presence of Calcium	Presence of Magnesium
Tetracycline hydrochloride	0.017	0.024	0.020	0.229	0.349	0.312
Oxytetracycline hydrochloride	0.017	0.015	0.014	0.133	0.125	0.130
Chlortetracycline hydrochloride	0.116	0.039	—	0.264	0.113	—
Demeclocycline hydrochloride	0.111	0.079	—	0.350	0.271	—
Doxycycline hyclate	0.288	0.271	0.215	0.304	0.319	0.276

[a] The molar ratio of tetracycline to divalent metal salt was 1:10.

chelating tetracycline derivatives at pH values achieved after administering antacids (2, 3). Therefore, some other mechanism may be responsible for the impaired absorption of tetracyclines (4).

Previously (5–7), a liquid two-phase *in vitro* system to study the kinetic and thermodynamic aspects of the interphase transfer of sulfonamides and tetracyclines was described. The system is applied here to investigate the influence of divalent metal salts on the kinetic and thermodynamic parameters for the partitioning of various tetracycline derivatives.

EXPERIMENTAL

The apparatus, the method of performing the diffusion experiments, and the procedure for determining the partition coefficients were discussed previously (5).

Materials—The following reagents were the highest quality obtainable: tetracycline hydrochloride[1] (98% potency), oxytetracycline hydrochloride[1] (91% potency), doxycycline hyclate[1] (86% potency), chlortetracycline hydrochloride[2] (92% potency), demeclocycline hydrochloride[2] (92% potency), 1-octanol, monobasic and dibasic sodium phosphates, sodium chloride, calcium chloride, magnesium chloride, and hydrochloric acid.

Diffusion Experiments—To study tetracycline diffusion from aqueous to 1-octanol phases, 5.2×10^{-4} *M* solutions[3] of each tetracycline derivative were prepared in aqueous phosphate buffer adjusted to pH 5.5. The same conditions were observed for tetracycline solutions also containing 5.2×10^{-3} *M* calcium chloride or magnesium chloride. All solutions were adjusted to an ionic strength of 0.15.

The studies were performed at 23, 30, and 37° using water baths maintained at ±0.5°. Absorbance was measured with a UV spectrophotometer at 375 nm for chlortetracycline and demeclocycline, 360 nm for tetracycline and oxytetracycline, and 355 nm for doxycycline.

[1] Pfizer and Co., Brooklyn, N.Y.; potency supplied by company.
[2] Lederle Laboratories, Pearl River, N.Y.; potency supplied by company.
[3] All tetracycline derivatives were used as the hydrochloride salts except for doxycycline hyclate, which is the hydrochloride hemiethanolate hemihydrate. Therefore, doxycycline contributes a small quantity of ethyl alcohol ($\sim 10^{-4}$ *M*) to the aqueous buffer phase whereas the other derivatives do not. This variable may have a small influence on the thermodynamic parameters observed for doxycycline.

RESULTS AND DISCUSSION

General Considerations—The structures of the three tetracyclines, their reported macrodissociation constants, and their apparent partition coefficients in pH 5.5 phosphate buffer–1-octanol are summarized in Table I.

The tetracyclines exhibit a complex dissociation scheme of 15 microionization steps resulting from the three acidic moieties present (8, 9). A pH value of 5.5 was selected for the aqueous phase to: (*a*) ensure maximum partitioning of the tetracycline derivatives as observed previously (9, 10) within a range including pH 5.5, (*b*) minimize the epimerization of the various tetracyclines at the C-4 dimethylamino group commonly observed at lower pH values (11), and (*c*) achieve a pH within the range observed after administration of antacid preparations. It is unresolved whether the zwitterionic species (3, 9), the unionized species (10), or both forms of the tetracyclines are capable of partitioning across the interface. Maximum partitioning has been observed in the general pH range of maximum zwitterionic concentration (9).

The concentration of tetracyclines used for the interphase transfer experiments, 5.2×10^{-4} *M*, is within the concentration range achieved in the stomach when the common dose of 250 mg is administered. The concentration of divalent metal salts used, 5.2×10^{-3} *M*, simulates a

Table III—Apparent Partition Coefficients for the Interphase Transfer of Tetracyclines in the Presence[a] and Absence of Divalent Metal Salts at pH 5.5 and 37°

Tetracycline	Apparent K_w^{0}[b] Absence of Metal	Presence of Calcium	Presence of Magnesium
Tetracycline hydrochloride	0.074	0.069	0.064
Oxytetracycline hydrochloride	0.129	0.120	0.108
Chlortetracycline hydrochloride	0.439	0.345	—
Demeclocycline hydrochloride	0.317	0.292	—
Doxycycline hyclate	0.947	0.850	0.779

[a] The molar ratio of tetracycline to divalent metal salt was 1:10. [b] As defined by Eq. 1.

Table IV—Apparent [a] Functional Group Free Energy Contributions for Partitioning of Tetracyclines in the Presence [b] and Absence of Divalent Metal Salts at pH 5.5 and 37°

Functional Group	π [c]			$\Delta F_{G\ \Delta F}$ [d], cal/mole		
	Absence of Metal	Presence of Calcium	Presence of Magnesium	Absence of Metal	Presence of Calcium	Presence of Magnesium
C-6α methyl [e]	0.141	0.074	—	−201	−105	—
C-5 hydroxyl [f]	0.238	0.247	0.223	−338	−350	−315
C-7 chloro [g]	0.773	0.705	—	−1097	−1000	—
C-6β hydroxyl [h]	−0.870	−0.850	−0.862	1233	1250	1222

[a] Apparent values because the composition of species is not constant for each tetracycline at pH 5.5 [b] The molar ratio of tetracycline to divalent metal salt was 1:10. [c] The log of the functional group constant calculated using Eq. 2. [d] The functional group free energy of partitioning calculated using Eq. 2. [e] Obtained by subtracting demeclocycline data from chlortetracycline data. [f] Obtained by subtracting tetracycline data from oxytetracycline data. [g] Obtained by subtracting tetracycline data from chlortetracycline data. [h] Obtained by subtracting doxycycline data from oxytetracycline data.

gastric concentration achieved with the commonly administered dosage of antacids. Under these study conditions, the tetracycline to divalent metal salt molar ratio is 1:10.

The five tetracycline derivatives (Table I) were chosen because they are commonly used in practice; they demonstrate small structural changes, facilitating the study of the contribution of discrete functional groups; and they exhibit greater than a 10-fold range in the apparent partition coefficient.

Kinetic and Partitioning Data—The kinetic scheme for the reversible interphase transfer of the tetracyclines in the two-phase system, as well as the methods for obtaining the kinetic and thermodynamic data, were discussed previously (5).

The kinetic parameters for the interphase transfer of the tetracycline derivatives in the presence and absence of calcium and magnesium salts are summarized in Table II. For all derivatives except tetracycline, the forward rate constants, k_f, for transfer from aqueous to 1-octanol phases were decreased in the presence of the divalent metals. These changes were generally small, although they were statistically significant ($p < 0.05$; t test for independent means, nine determinations for each experiment). The backward rate constants, k_b, for transfer from 1-octanol to aqueous phases were also, again with the exception of tetracycline, subject to small but statistically significant decreases in the presence of the metals. Of course, the specific kinetic parameters (Table II) depend on the cell design and stirring rate used in the interphase transfer experiments. The interphase transfer rates are influenced by the thickness of the aqueous diffusion layer, which, in turn, depends on the agitation rate (12).

The apparent partition coefficients for the interphase transfer of the tetracyclines in the presence and absence of the divalent metal salts at pH 5.5 are summarized in Table III. Changes in the presence of metals generally represented small to negligible decreases; only the variations observed for chlortetracycline and doxycycline were statistically significant ($p < 0.01$). Apparent partition coefficients were calculated using:

$$K_w^0 = \frac{k_f}{k_b} = e^{-\Delta F/RT} \qquad \text{(Eq. 1)}$$

Perhaps the changes observed in the k_f, k_b, and K_w^0 values in the environment of metals result from the presence of the divalent cations at the interface. The cations may alter the structural order of water and octanol molecules in the boundary region, decreasing the escaping tendencies of these molecules into the interface and resulting in changes in desolvation and resolvation of solvent molecules associated with the solute during formation of the activated complexes for interphase transfer (6). In studying the partitioning of phenothiazines across a water–octanol interface, large changes in the apparent partition coefficient were observed when various salts were added to the system (13). These changes were attributed to the influence of the salts on water-structuring effects in hydrophobic interactions and ion-pair formation.

"Apparent" Contribution of Functional Group Substitution to Energy Changes for Partitioning—The method of Hansch (14, 15) was used to estimate the contribution of functional groups to the free energy of partitioning of tetracycline derivatives. With this approach, the contribution of individual substituents is calculated by comparing the partitioning of the "parent" compound to that of a derivative differing by only a single substituent group:

$$\pi = \log (K_w^{0\prime\prime}/K_w^{0\prime}) = \Delta F_{G\ \Delta F}/-2.303RT \qquad \text{(Eq. 2)}$$

where π represents the log of the functional group contribution factor (the log of $K_w^{0\prime\prime}/K_w^{0\prime}$); $K_w^{0\prime\prime}$ and $K_w^{0\prime}$ denote the partition coefficients for the derivative and parent compounds, respectively; and $\Delta F_{G\ \Delta F}$ represents the functional group free energy contribution. When comparing the tetracycline derivatives, only a qualitative interpretation of the data is possible since the slight variation in pK values results in a small change in the ratio of ionized to unionized species at pH 5.5. This change in species composition may in itself alter the apparent partition coefficient and, thus, the free energy data observed. Because of this limitation, the data in Table IV are denoted as "apparent" values.

Introduction of the C-6β hydroxyl group on the tetracycline nucleus decreased the apparent partition coefficient due to a positive apparent $\Delta F_{G\ \Delta F}$ contribution leading to an increase in the free energy of partitioning. Introduction of C-5 hydroxyl, C-6α methyl, or C-7 chloro substituents, however, increased partitioning due to negative apparent $\Delta F_{G\ \Delta F}$ contributions leading to decreases in the free energy of partitioning. Contributions of each of these functional groups were essentially unchanged in the presence and absence of the divalent metal salts.

Tetracycline demonstrated a smaller apparent partition coefficient than its analogs oxytetracycline and chlortetracycline, three derivatives containing the C-6β hydroxyl group, because tetracycline lacks the additional substituent providing a negative apparent $\Delta F_{G\ \Delta F}$ contribution possessed by oxytetracycline and chlortetracycline. Chlortetracycline exhibited a larger apparent partition coefficient than demeclocycline because the addition of a methyl group at C-6α for chlortetracycline promotes partitioning through a negative apparent $\Delta F_{G\ \Delta F}$ contribution. The hydrophobic effect of methyl group substitution was demonstrated previously (5, 15, 16). Converting tetracycline to chlortetracycline by adding a C-7 chloro group resulted in a greater negative apparent $\Delta F_{G\ \Delta F}$ contribution than did the conversion of tetracycline to oxytetracycline by addition of a C-5 hydroxyl group. This result demonstrates the inductive effect of the chloro substituent as well as the more hydrophilic character of the hydroxyl group (16).

A small variation in the placement of the hydroxyl group, shifting from C-6β in tetracycline to C-5 in doxycycline, yielded a large increase in the apparent partition coefficient through a substantial decrease in the apparent $\Delta F_{G\ \Delta F}$. This small change in substituent site demonstrates the fundamental importance of desolvation and resolvation processes in forming the activated complexes for interphase transfer; the potential for hydrophobic interaction between 1-octanol and tetracycline molecules is expected to be greater for deoxycycline than tetracycline because of the larger hydrophobic surface on the tetracycline nucleus when the hydroxyl group is substituted at C-5 rather than C-6β (7).

The relatively small changes in kinetic, thermodynamic, and spectral[4] properties observed at pH 5.5 for the tetracycline derivatives in the presence and absence of divalent metal salts provide indirect evidence *in vitro* that the decreased absorption *in vivo* of some tetracyclines in the presence of magnesium and calcium ions is not the result of chelation between these solutes.

REFERENCES

(1) P. J. Neuvonen, *Drugs*, **11**, 45 (1976).

(2) L. A. Mitscher, A. C. Bonacci, A. K. Hacker, and T. D. Sokoloski, *Antimicrob. Agents Chemother. 1969*, **1970**, 111.

(3) T. F. Chin and J. L. Lach, *Am. J. Hosp. Pharm.*, **32**, 625 (1975).

(4) K. Kakemi, H. Sezaki, H. Ogata, and T. Nadai, *Chem. Pharm. Bull.*, **16**, 2200 (1968).

(5) G. E. Schumacher and J. B. Nagwekar, *J. Pharm. Sci.*, **63**, 240 (1974).

(6) *Ibid.*, **63**, 245 (1974).

[4] No changes in the wavelength of maximum absorbance were observed for any of the tetracycline derivatives over the 350–425-nm range in the presence and absence of calcium and magnesium salts.

(7) G. E. Schumacher and D. M. Lei, *J. Pharm. Sci.*, **67**, 1715 (1978).
(8) L. J. Leeson, J. E. Krueger, and R. Nach, *Tetrahedron Lett.*, **18**, 1115 (1963).
(9) J. L. Colaizzi and P. R. Klink, *J. Pharm. Sci.*, **58**, 1184 (1969).
(10) H. Terada and T. Inagi, *Chem. Pharm. Bull.*, **23**, 1960 (1975).
(11) E. G. Remmers, G. M. Sieger, and A. P. Doerschuk, *J. Pharm. Sci.*, **52**, 752 (1963).
(12) S. A. Howard, M. A. Farvar, A. Suzuki, and W. I. Higuchi, *ibid.*, **58**, 1325 (1969).
(13) K. S. Murthy and G. Zografi, *ibid.*, **59**, 1281 (1970).
(14) C. Hansch and A. Steward, *J. Med. Chem.*, **7**, 691 (1964).
(15) G. L. Flynn, *J. Pharm. Sci.*, **60**, 345 (1971).
(16) J. M. Diamond and E. M. Wright, *Proc. R. Soc. B*, **172**, 273 (1969).
(17) W. Barringer, W. Schultz, G. Sieger, and R. Nash, *Am. J. Pharm.*, **146**, 179 (1974).

ACKNOWLEDGMENTS

Abstracted in part from a thesis submitted by E. E. Linn to Wayne State University in partial fulfillment of the Master of Science degree requirements.

GLC Determination of Nanogram Quantities of a New Analgesic, Nefopam, in Human Plasma

D. SCHUPPAN *, C. S. HANSEN, and R. E. OBER ˣ

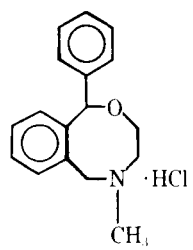
Received June 6, 1977, from the *Drug Metabolism Group, Riker Laboratories, Inc., a Subsidiary of 3M Company, St. Paul, MN 55101.* Accepted for publication April 27, 1978. *Present address: Klinikum der Johann Wolfgang Goethe Universität, 6000 Frankfurt Main 70, West Germany.

Abstract □ A sensitive and specific method was developed for the quantitative GLC determination of plasma nefopam levels. The method includes a multiple-step solvent extraction of the analgesic drug and the internal mass standard, orphenadrine. The accuracy, expressed as the relative error, was −4, 6, 6, and 4% at 20, 40, 70, and 130 ng/ml, respectively. The precision, expressed as relative standard deviation, was 17, 7, 3, and 5% at these same concentrations, respectively. Quantitation of nefopam in human plasma is possible down to 20 ng/ml with a 2-ml plasma sample; the sensitivity can be increased by using larger plasma samples. The method was applied successfully to the determination of plasma nefopam levels in humans in pharmacokinetic studies at therapeutic doses.

Keyphrases □ Nefopam—GLC analysis in plasma □ GLC—analysis, nefopam in plasma □ Analgesics—nefopam, GLC analysis in plasma

Nefopam[1], 3,4,5,6-tetrahydro-5-methyl-1-phenyl-1*H*-2,5-benzoxazocine hydrochloride (I), is a member of a new class of analgesics with a unique heterocyclic structure. It was originally synthesized by Klohs *et al.* (1) and was introduced as an analgesic drug in 1975 in Mexico and in 1976 in the Federal Republic of Germany. It is currently in the late stages of clinical testing in the United States, and a number of studies concerning the analgesic properties of I were reported[2] (2–5). *In vitro* metabolic data were published (6).

A prerequisite for *in vivo* metabolic and pharmacokinetic studies is a sensitive and specific assay. Since the recommended therapeutic dose of this basic drug for humans is relatively low, the plasma I concentrations are in the low nanogram per milliliter range. This report describes a sensitive and specific GLC method for the accurate determination of small amounts of I in human plasma. The method has been used to follow plasma pharmacokinetics of I. Another similar GLC method recently was published by a group collaborating with this laboratory (7).



I

EXPERIMENTAL

Reagents—Ether was freshly distilled each day; all other reagents were analytical reagent grade. A *d,l*-mixture of I was used, and the ^{14}C-I (^{14}C-label in the 1- and 6-positions of the oxazocine ring; radiochemical purity >99%) employed for recovery studies was also the racemate. The internal standard (orphenadrine, II) and I were dissolved in methanol. Aqueous solutions of 0.1 *N* HCl and 0.1 *N* NaOH were prepared in distilled water. Carbon disulfide was diluted with methanol to give a final concentration of 2% methanol.

Blank Plasma—Human plasma was obtained from volunteers who had fasted overnight and had not been on any medication for the previous week.

Apparatus—GLC was carried out on a chromatograph[3] with a hydrogen flame-ionization detector. Glass columns (182 cm × 2 mm i.d.) were rinsed with methanol and acetone and dried. They were packed with 3% 100–120-mesh cyclohexanedimethyl succinate on Gas Chrom Q[4]. The column oven was operated at 221° while the injection port and detector were operated at 225 and 260°, respectively. The accuracy and sensitivity of the assay were enhanced markedly by use of a digital electronic integrator[5] for calculating the relative detector response.

For the maximal detector response of I, the flow rates were set as follows: helium carrier gas, 40 ml/min; hydrogen, 40 ml/min; and air, 250 ml/min. The recorder chart speed was 1.25 cm/min. The integrator was operated under the following conditions: noise suppression, 2; recorder presentation, 20; slope sensitivity, 0.03 (up slope and down slope); baseline reset, 0; area threshold, 100; front shoulder, off; and rear shoulder, 1000 mv.

Standard Solutions—The internal standard solution contained 10

[1] Riker Laboratories, St. Paul, Minn.
[2] In Ref. 2, blood level data should read nanogram instead of microgram.
[3] Fisher Victoreen model 4400, equipped with a Varactor electrometer.
[4] Applied Science Laboratories, State College, Pa.
[5] Hewlett-Packard model 3370 B.

0022-3549/78/1200-1720$01.00/0