Pharmacokinetics of Carbamazepine in Monkeys following Intravenous and Oral Administration

R. H. LEVY ˣ, J. S. LOCKARD, J. R. GREEN, P. FRIEL, and L. MARTIS

Abstract □ The pharmacokinetics of carbamazepine were evaluated in four male rhesus monkeys. A 20-mg/kg dose was administered by intravenous (5-min) infusion and orally (nasal–gastric intubation) in a propylene glycol–ethanol–water solvent. Plasma and urine determinations were performed by GLC. All semilogarithmic intravenous curves exhibited an irregular decay behavior in the first 3-hr period, followed by a linear disappearance phase ($T_{1/2}$ = 1.0–2.4 hr). Urinary excretion measurements confirmed the short elimination half-life and showed that less than 1% of the dose was excreted unchanged. Oral studies also yielded a short elimination half-life (1.0–1.60 hr), which was confirmed by urinary excretion measurements. The oral curves were analyzed pharmacokinetically. The fraction of the dose reaching the systemic circulation ranged between 58 and 87%. Measurable (but insignificant) amounts of drug were found in the feces after intravenous and oral administrations.

Keyphrases □ Carbamazepine—pharmacokinetics, intravenous and oral administrations, monkeys □ Pharmacokinetics—carbamazepine, intravenous and oral administrations, monkeys □ Disposition—carbamazepine, acute intravenous and oral doses, monkeys

Carbamazepine, an iminostilbene derivative, is presently the drug of choice for the treatment of trigeminal neuralgia (1). Similarly to diphenylhydantoin, which is occasionally used for trigeminal neuralgia, it inhibits posttetanic potentiation (2). Following its pharmacological testing, which showed marked anticonvulsant properties in animals (3), it was used in a series of clinical trials as an antiepileptic drug, mostly in Europe and Australia (4–7). Carbamazepine has drawn special attention since several investigators reported that it possesses comparatively less sedative effects than other anticonvulsants and that it produces a "positive psychotropic effect" (8). It is currently under investigation in the United States (9).

Studies on its metabolic fate in animals or humans recently appeared (10, 11), but much less information has been accumulated regarding its pharmacokinetic properties (12, 13). The present studies were undertaken to study the disposition of carbamazepine in the monkey following acute intravenous and oral doses of the drug.

EXPERIMENTAL

Materials—Carbamazepine[1] and cyheptamide[2], the internal standard in the GLC assay, were used. All solvents used were reagent grade.

Subjects—Four male adolescent rhesus monkeys *(Macaca mulatta)*, 3–5 kg, were used. The monkeys were adapted to primate restraining chairs but housed in individual cages between experimental days.

Drug Administration—*Drug Solution and Dosage*—Carbamazepine has a very low water solubility in a relatively wide pH range. Therefore, it was put into solution in a 50% propylene glycol, 20% ethanol, and 30% water mixture at a concentration of 10 mg/ml. Propylene glycol and water without ethanol did not maintain the carbamazepine in solution at room temperature; carbamazepine tended to crystalize out at concentrations less than 20% ethanol. A compatible sterile solution without the drug but with the same percentages of propylene glycol, ethanol, and water was used for flushing the infusion tube.

All experiments (intravenous and oral) were performed at one dose level, 20 mg/kg. This dose (which corresponds to the upper dosing range in patients) was well tolerated by the monkeys and yielded easily quantifiable plasma concentrations.

Procedure—A monkey was placed in a carrying holder at the start of an experimental day (8 am) before the animal's daily feeding period. (The lower extremities, dorsally from the calf to the ankle, were shaved to emphasize the small saphenous veins and branching collaterals.)

In preparation for the drawing of blood samples, a 21-gauge needle of a heparin lock (intermittent infusion set with reseal injection site) was inserted into the small saphenous vein of one leg. The needle, butterfly tab, and junction of the trailing polyethylene tubing were sutured to the skin to secure the infusion set at four points. The animal was then employed in either an intravenous or oral experiment.

Intravenous Experiment—After the heparin lock was positioned and while the monkey was still in the carrying holder, a 21-gauge butterfly needle with tubing was inserted into the small saphenous vein of the other leg for drug infusion.

Unless otherwise indicated, carbamazepine was infused at a constant rate over 5 min, followed immediately by 2 ml of flushing solution. Subsequent to the initial three blood samples, the animal was placed in a primate chair and food and water were made available.

Oral Experiment (Nasal–Gastric)—At least a week intervened between the intravenous and oral experiments. After the heparin lock was secured, the animal was placed in a primate restraining chair and intubated nasally with a polyethylene, 38-cm (15-in.) gastric tube. The drug solution was injected directly into the stomach, followed immediately by 2 ml of flushing solution. The animal was not fed until 4 hr after drug administration; water was allowed *ad libitum*.

Table I—Pharmacokinetic Parameters of Carbamazepine Obtained after Intravenous Administration

Experiment	Animal	Elimination Half-Life, hr	Volume of Distribution, V_d, liter/kg	Total Body Plasma Clearance, liter/hr/kg
P1	M1	1.20	0.883	0.51
P2	M1	1.04	0.885	0.59
P3	M2	1.00	0.722	0.50
P4	M2	1.50	0.959	0.43
P5	M3	2.38	1.580	0.46
P6	M4	1.22	0.968	0.55

[1] Geigy Pharmaceuticals, Ardsley, N.Y.
[2] Ayerst Laboratories, New York, N.Y.

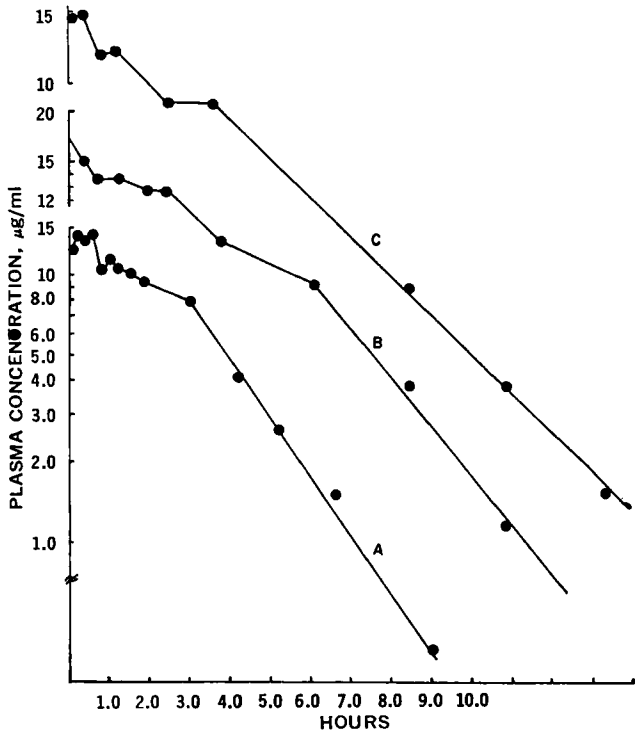


Figure 1—*Plasma concentration–time curves resulting from a 5-min intravenous infusion of carbamazepine (20 mg/kg). Key: A, Monkey M1; B, Monkey M2; and C, Monkey M4.*

Blood and Excreta Samples—Eleven to 14 blood samples were collected over 24 hr in both intravenous and oral experiments. Before obtaining each sample, a needle was inserted into the heparin lock. Then 0.2 ml of heparin was injected into the saphenous vein, and 5–10 drops of blood were allowed to drip until the viscosity and color of the blood were regular.

The blood (1.5–2 ml) was collected in a 3-ml vial containing potassium ethylene diaminetetraacetic acid (EDTA) and potassium sorbate. After each sample, the heparin lock was refilled and the blood was centrifuged (2000 rpm) for approximately 10 min. The plasma was transferred to another container, capped, coded, and frozen at −15° until assayed.

Urine was sampled from 0 to 48 hr and feces from 0 to 96 hr. Urine was collected in screened dropping pans, which prevented contamination by feces. The feces were collected in bags taped to the monkey buttocks bars. The stomach plate of the primate chair had underarm supports, which prevented a monkey from pivoting and contaminating its feces with urine.

The volume of urine at each scheduled collection was recorded. After mixing, a sample of approximately 2 ml was placed in a vial, capped, coded, and frozen at −15°. The gram weight of each scheduled feces collected was recorded, and the total amount was placed in a container, capped, coded, and frozen at −15°.

Plasma, Urine, and Feces Assays—All plasma determinations were made by the GLC procedure of Friel and Green (14) where carbamazepine is assayed directly, using cyheptamide as an internal standard. One milliliter of undiluted urine samples was extracted and chromatographed in the same manner as plasma samples.

Feces were weighed, diluted approximately 1:5 (w/v) with distilled water, and homogenized manually. Then an aliquot (1–15 ml, representing 0.2–4.0 g dry fecal weight) was transferred to a 40-ml glass-stoppered centrifuge tube, and 5 μg of cyheptamide, 2 ml of phosphate buffer (pH 7.2), and 15 ml of chloroform were added. The mixture was shaken manually for 2 min and centrifuged. The chloroform layer was removed and filtered into a centrifuge tube, and the chloroform extract was evaporated to dryness in a water bath at 50° under a gentle stream of air. The remainder of the extraction procedure was identical to that used for plasma and urine samples, *i.e.*, cyclohexane wash, reextraction into chloroform, and evaporation.

Table II—Urinary Excretion of Unchanged Carbamazepine following Intravenous and Oral Administrations

Experiment	Animal	Percent of Dose Excreted in Time Period		
		0–8 hr	0–24 hr	0–48 hr
P1	M1	0.09	0.13	0.59
P3	M2	0.38	0.39	—
P4	M2	0.24	0.45	—
P5	M3	0.17	0.19	—
P6	M4	0.31	0.46	—
P7	M3	0.073	0.083	0.102
P8	M1	Trace	Trace	—
P9	M4	0.46	0.48	0.68
P10	M2	0.12	0.25	—
O1	M1	0.37	0.47	—
O2	M2	0.17	0.17	—
O3	M3	0.06	0.11	—
O4	M4	0.15	0.15	0.15
O5	M2	0.06	0.08	0.09

Calibration curves constructed from spiked monkey plasma, urine, and feces were run with each set of unknown samples. They were linear in the range examined (2–16 μg). Recoveries (relative to aqueous standards) for plasma, urine, and feces were 97, 109, and 101%, respectively. Precision values obtained from a set of duplicate determinations were 4.9, 3.9, and 4.8% for plasma, urine, and feces, respectively.

RESULTS AND DISCUSSION

Kinetics following Intravenous Administration—The plasma concentration–time curves obtained following intravenous administration of carbamazepine to Monkeys M1, M2, and M4 (Experiments P1, P4, and P6, respectively) are shown in Fig. 1. The intravenous curves present several peculiarities. Each semilogarithmic curve is generally composed of two regions: an initial portion with atypical decay followed by a linear portion with an apparent first-order disappearance where a half-life measurement can be made.

Table I gives the pharmacokinetic parameters obtained from all intravenous experiments. The elimination half-lives were obtained from a nonlinear least-squares fit of the terminal monoexponential portion of each curve. For each curve the number of points used in the fit was maximized while the corresponding increase in sum of squares was minimized. The data in Table I show that carbamazepine is rapidly eliminated from the systemic circulation, its biological half-life ranging between 1 and 2.5 hr. These results are in agreement with the values of 55–75 min recently reported for the half-life of carbamazepine in monkeys (13).

The atypical postinjection decay of carbamazepine does not permit an unequivocal assessment of the most appropriate pharmacokinetic model. Total body clearance was computed from the ratio of dose to area under the plasma concentration–time curve (calculated by the trapezoidal rule), and the volume of distribution was derived from the total body clearance and elimination half-life (Table I).

Table II gives the results of urinary excretion measurements performed during all intravenous experiments (P1 and P3–P10). Carbamazepine is probably extensively metabolized since less than 1% of the dose can be recovered unchanged in urine after more than 10 half-lives. These findings concur with a 1% urinary recovery found previously (13).

Table II also shows that in most instances the majority of the total fraction excreted unchanged appears in the first 8 hr, substantiating the rapid elimination found from plasma data. In a few instances (Experiments P4 and P10), the fraction excreted in the first 8 hr is approximately 50% of the total fraction excreted unchanged. The irregularity in frequency of micturition (a collection time interval labeled 0–8 hr can in fact correspond to a much shorter excretion time period) may account for this observation. In Experiments P1 and P9, there appears to be appreciable excretion within the 24–48-hr interval. However, too few 24–48-hr collections were obtained to assess the significance of this finding.

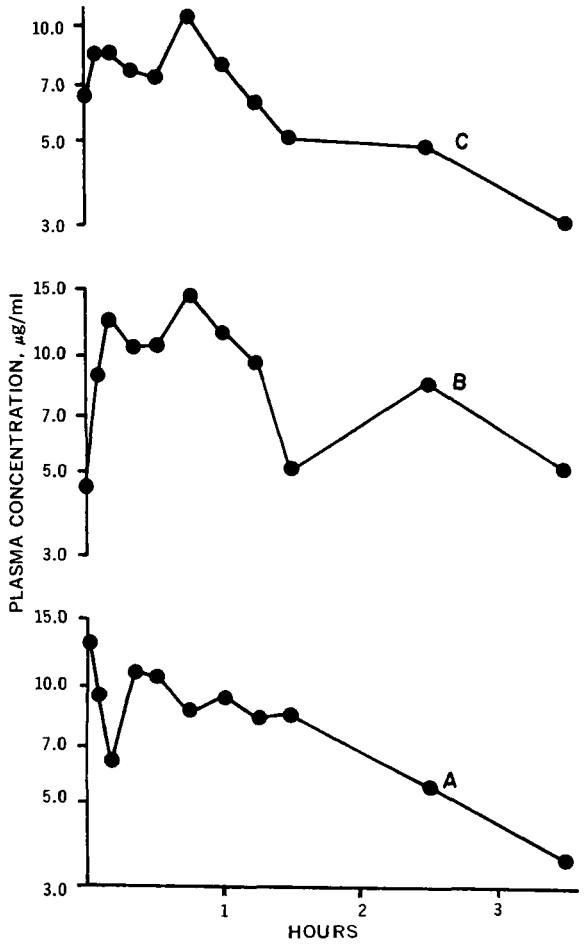


Figure 2—*Plasma levels of carbamazepine resulting from an intravenous infusion of 20 mg/kg in Monkey M2. Key: A, 20-min infusion, solvent of propylene glycol–ethanol–water; B, 5-min infusion, solvent of polyethylene glycol 400–water; and C, 20-min infusion, solvent of polyethylene glycol 400–water.*

The unusual pharmacokinetic behavior of carbamazepine in the first 2 hr after intravenous administration is not explained easily. In view of its very low water solubility, the possibility exists that the plasma level irregularities are related to a phenomenon of drug precipitation in plasma followed by redissolution. Two approaches were used to test this hypothesis. Since the drug was infused over a relatively short time (5 min) in all intravenous experiments shown in Fig. 1, it was decided to infuse the same volume of drug solution over 20 min in Monkey M2 (Experiment P10) for which two intravenous curves were already available (Experiments P3 and P4). Plasma samples as well as urine and feces were collected and assayed. The resulting plasma concentration–time curve is shown in

Table III—Excretion of Carbamazepine in Feces following Intravenous and Oral Dosing

Experiment	Animal	Amount Excreted in Time Period, μg			
		0–8 hr	8–24 hr	24–48 hr	48–96 hr
P1	M1	11.2	Trace	Trace	
P4	M2	16.2	7.8	27.6	
P7	M3	⟶	Trace[a]	4.5	
P9	M4	14.9	<7.0	<7.5	
P10	M2	8.4	8.4	—	
O1	M1	18.5	6.3	Trace	
O3	M3	1.6	1.3	Trace	
O4	M4	⟶	Trace[a]	Trace	
O5	M2	13.8	113.7	7.7	
O6	M1	⟶	⟶	3.8[b]	6.6

[a] 0–24-hr sample. [b] 0–48-hr sample.

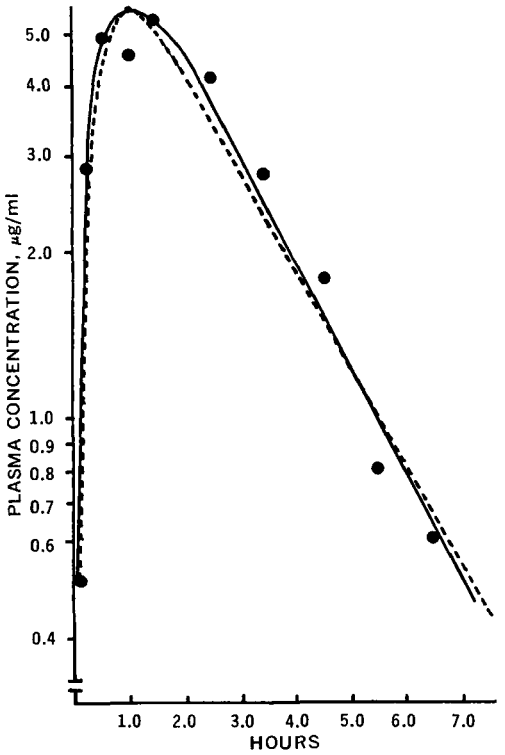


Figure 3—*Plasma concentration–time curve resulting from intragastric intubation of carbamazepine (20 mg/kg) in Monkey M1. Continuous line is the least-squares fit to a biexponential equation (Scheme I); dotted line is the least-squares fit to a triexponential equation (Scheme II).*

Fig. 2 (curve A), where the time scale has been expanded to illustrate the postinjection phenomenon. When this curve is compared to the initial portion of the curves in Fig. 1, it becomes apparent that the pattern is quite similar.

There is still another possibility within this hypothesis of drug precipitation, namely, that the observed phenomenon is related to the solvent mixture used to dissolve carbamazepine. To test this possibility, a new solution of carbamazepine was prepared (at the same concentration) in an equal weight mixture of polyethylene glycol 400 and water. The same dose and volume administered in all the previous intravenous experiments were administered to Monkey M2 over a 5- (Experiment P11) and 20- (Experiment P12) min constant infusion period (Fig. 2, curves B and C, respectively). The pattern observed here is similar to those obtained in Experiments P3, P4, and P10. Therefore, changing both the solvent mixture and the infusion time did not eliminate the postinjection plasma level fluctuations.

Another series of experiments was undertaken to investigate the nature of the plasma level fluctuations in the postinjection period. Feces were collected (three times during 48 hr) during Experiments P1 and P4 and assayed for unchanged drug (Table III). It was found that the first 8-hr samples contained 11 and 16 μg of drug for Experiments P1 and P4, respectively. In the latter experiments, 8 and 28 μg of drug were measured in the following 16- and 24-hr samples, respectively.

To confirm these findings, similar intravenous experiments were

Table IV—Pharmacokinetic Parameters of Carbamazepine Obtained after Oral Administration

Experiment	Animal	One-Compartment Model			Physiological Availability, %
		A	K_a, hr^{-1}	K_e, hr^{-1}	
O1	M1	53	0.764	0.619	58.4
O2	M2	200	0.483	0.455	86.6
O3	M3	200	0.467	0.434	75.2
O4	M4	472	0.582	0.568	65.0

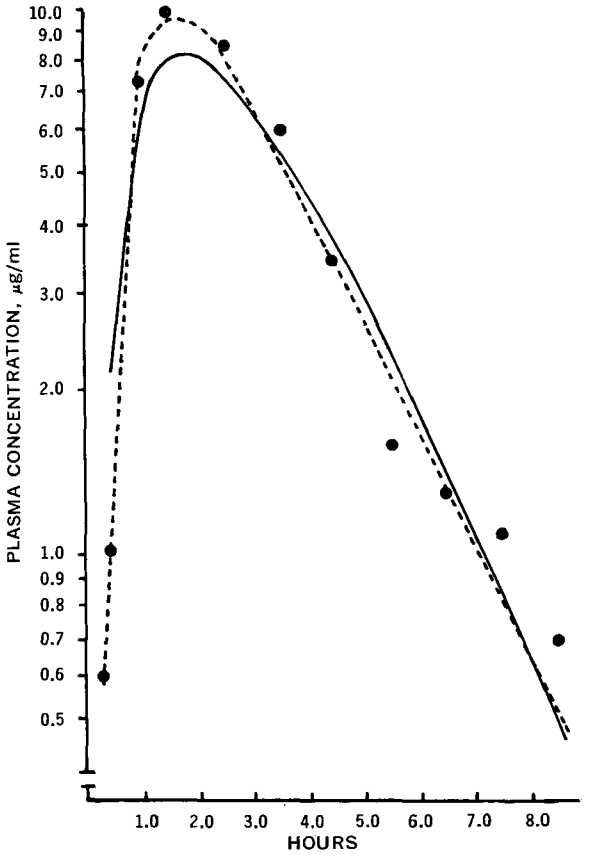
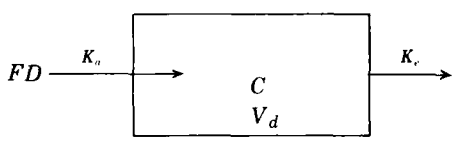


Figure 4—*Plasma concentration–time curve resulting from intragastric intubation of carbamazepine (20 mg/kg) in Monkey M2. Continuous line is the least-squares fit to a biexponential equation (Scheme II); dotted line is the least-squares fit to a triexponential equation (Scheme I).*

performed in each animal and the results show measurable quantities of drug in the feces at various times (Table III). These findings support the hypothesis that carbamazepine undergoes enterohepatic cycling in the monkey under the present experimental conditions. Hence, it is possible that the characteristic fluctuations observed in the 60–90-min period postinjection are caused by reabsorption of the drug after secretion in the bile and emptying in the duodenum. (In addition, food ingestion could influence this phenomenon.)

However, it is not possible to assess the fraction of the dose cycled from the amount of drug measured in the feces, since that amount represents only the portion of the fraction cycled that was not reabsorbed. This phenomenon will be discussed further. Enterohepatic cycling has also been suggested in humans (15).

Kinetics following Oral Administration—Since no information could be gathered from the intravenous data regarding the type of model that describes the pharmacokinetic behavior of carbamazepine in monkeys, it was decided to approach the kinetic analysis of oral data using the one-compartment open model with absorption (Scheme I).

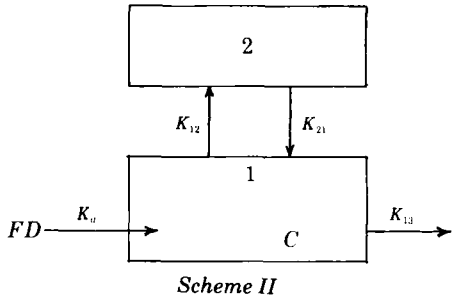


Scheme I

The integrated expression for the one-compartment model is of the form:

$$C = A(e^{-K_e t} - e^{-K_a t}) \qquad \text{(Eq. 1)}$$

where K_a and K_e are the first-order absorption and elimination rate constants, respectively, and A is a function of K_a, K_e, V_d (volume of distribution), and the fraction of the dose absorbed FD. Accordingly, the oral data were fitted to a biexponential equation *with weighting factors of 1 and 1/C,* using a nonlinear least-squares program[3] (16). (Initial estimates of the parameters were obtained using a log-peeling technique.)



Scheme II

Table IV gives the pharmacokinetic parameters obtained for weighted data (reciprocal weighting gave a lower sum of squares, with improved fit at the tail end of the curve). However, these parameters are empirical equation parameters rather than unique model parameters because of the closeness of values of K_a and K_e. Nevertheless, the elimination half-lives calculated from these parameters are in good agreement with those obtained after intravenous administration (Table I). Figures 3–6 show that an adequate fit is obtained for Monkeys M1 and M3 but not for M2 and M4. Hence, a two-compartment model with absorption was also considered (Scheme II)(17).

It was found that in all four instances the data points could be adequately fitted to a triexponential equation of the form:

$$C = \sum_{i=1}^{3} A_i e^{-m_i t} \qquad \text{(Eq. 2)}$$

where A_i is a function of the fraction of the dose absorbed, FD, the volume of distribution of Compartment 1, V_1, and the various first-order rate constants, K_a, K_{12}, K_{21}, K_{13}; and m_i is only a

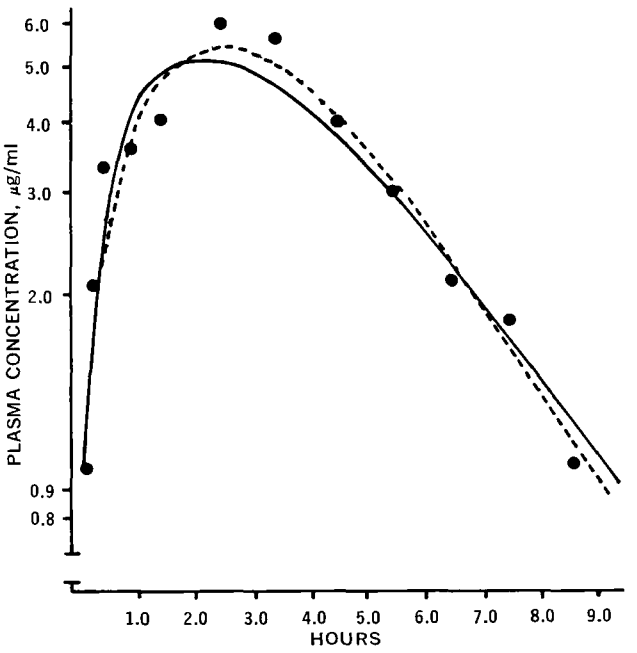


Figure 5—*Plasma concentration–time curve resulting from intragastric intubation of carbamazepine (20 mg/kg) in Monkey M3. Continuous line is the least-squares fit to a biexponential equation (Scheme I); dotted line is the least-squares fit to a triexponential equation (Scheme II).*

[3] On a CDC 6400 computer.

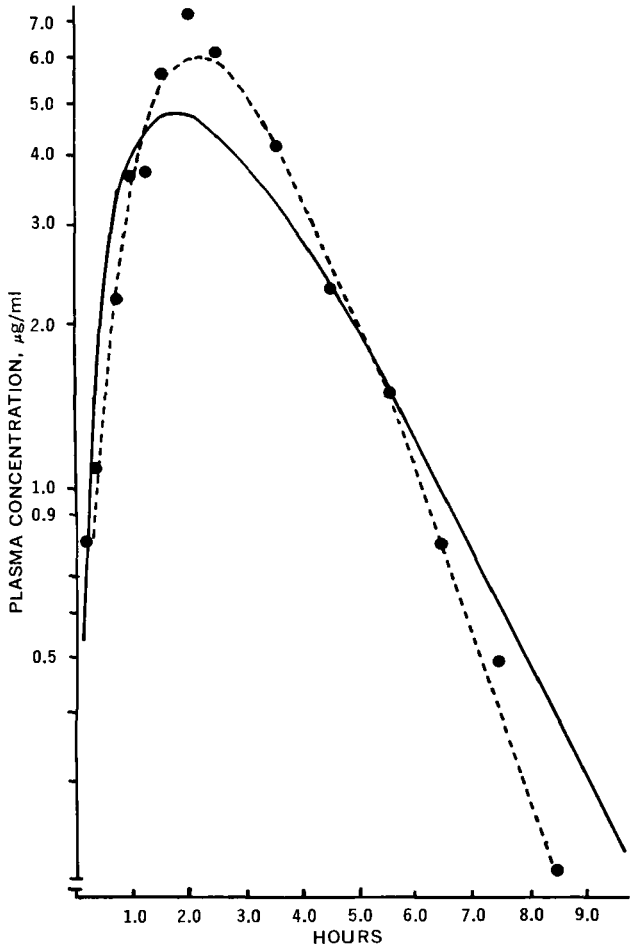


Figure 6—*Plasma concentration–time curve resulting from intragastric intubation of carbamazepine (20 mg/kg) in Monkey M4. Continuous line is the least-squares fit to a biexponential equation (Scheme I); dotted line is the least-squares fit to a triexponential equation (Scheme II).*

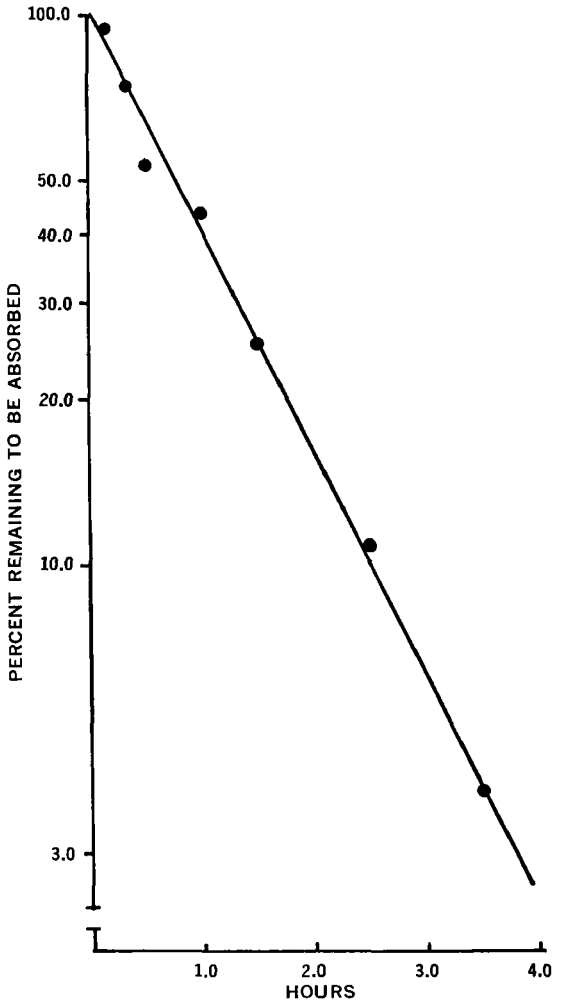


Figure 7—*Semilogarithmic plot of percent drug remaining to be absorbed* versus *time constructed according to Scheme I (Monkey M1).*

function of the first-order rate constants. For Monkeys M2 and M4, the latter fit was more adequate than that previously obtained with Eq. 1.

An attempt was made to calculate the parameters of the two-compartment model from the equation parameters, but it was not possible to obtain reliable model parameters. The parameters α and K_a could not be distinguished from each other because of the lack of prior information from intravenous data; therefore, K_{21}, K_{12}, and K_{13} could not be computed. Also, the parameters A_i and m_i had large standard deviations. Furthermore, the fact that several factors such as initial estimates, weighting function, and number of data points altered drastically the values of the parameters (and their standard deviations) further warned of their unreliability. These and other aspects of the (questionable) reliability of model parameters obtained by nonlinear least-squares fitting procedures were discussed (18), and a more recent review of this subject has also appeared (19).

Wagner–Nelson plots were constructed to gain a better understanding of the absorption process. However, the usual approach of obtaining K_e (from the terminal portion of the oral curve) could not be used in the present case because of the closeness of the absorption and elimination rate constants. Thus, for each animal the elimination rate constant obtained from intravenous data was utilized in the calculation of percent absorbed *versus* time plots. The log percent unabsorbed *versus* time plots yielded straight lines for all four animals (Fig. 7), and the corresponding absorption half-lives (0.52–1.16 hr) were somewhat shorter than those obtained from curve fitting to Eq. 1.

Physiological availability was calculated from the ratio of areas under the plasma concentration–time curves obtained after oral and intravenous administrations. The values obtained (58–87%) indicate incomplete systemic availability for this particular formulation (Table IV). The incomplete bioavailability could be attributed to one or more of several possibilities. Incomplete absorption appears unlikely since concomitant measurements of unabsorbed drug in feces revealed less than 1% of the dose (Table III). The possibility of a "first-pass" effect was considered (20). The fraction of the oral dose appearing in the systemic circulation, θ, was calculated from metabolic clearances and rhesus monkey liver blood flow data (21). The values of θ ranged between 0.87 and 0.85, indicating that this hypothesis could reasonably account for incomplete availability. Also, if non-first-order enterohepatic cycling were present, the method used to measure physiological availability would become invalid. Metabolism of carbamazepine in the gut or in the gut wall would also contribute to incomplete systemic availability. Consequently, a definitive assessment of the physiological availability of carbamazepine in monkey must await further study.

Urinary excretion measurements were also performed during the oral studies (Experiments O1–O5, Table II). The data confirm the findings of the parenteral studies that less than 1% of the dose appears unchanged in urine and that most of it appears in the first 8-hr collection.

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PHARMACEUTICAL ANALYSIS

New Color Reaction for Determination of Bacitracin in Ophthalmic Ointments

J. DOULAKAS

Abstract □ A colorimetric method was developed for the rapid analysis of bacitracin in ophthalmic ointments. The method involves the oxidation of α-aminocarboxylic acid with sodium hypobromite in an alkaline medium and condensation of the resulting aldehyde with phloroglucinol in concentrated hydrochloric acid to yield a pink color which gives an absorbance maximum at 505 nm. The relationship between absorbance and the quantity of bacitracin reacted obeyed Beer's law over the 15–100-μg/ml concentration range studied, and the produced color was stable for several hours. Furthermore, the method, which can be applied directly to the aqueous dissolution sample, gave results comparable to the official microbiological analytical procedure. The standard deviation is equal to ±1.81%.

Keyphrases □ Bacitracin—colorimetric analysis in ophthalmic ointments □ Ophthalmic ointments—colorimetric analysis of bacitracin □ Ointments, ophthalmic—colorimetric analysis of bacitracin □ Colorimetry—analysis, bacitracin in ophthalmic ointments

Although numerous microbiological methods (1–9) have been reported for the determination of bacitracin, the chemical analysis of this antibiotic in dosage forms has been a difficult task. The current widely accepted procedure for the determination of bacitracin employs a microbiological assay. In general, this procedure requires considerably more time than do chemical methods. For this reason, it was thought desirable to develop a chemical assay for the antibiotic in the hope of achieving rapid results with greater accuracy and precision. UV spectrophotometry, because of the very low absorbance of bacitracin, can be used for identification purposes rather than for a quantitative determination (10, 11).

Stretton *et al.* (12) reported the separation of zinc bacitracin, neomycin sulfate, and polymyxin B sulfate by various chromatographic procedures. They worked with the pure antibiotics and determined them colorimetrically, using a ninhydrin reagent, according to Maehr and Schaffner (13), after previous electrophoretic separation.

A simpler and shorter approach seemed to be one which might take advantage of the lability of the aminocarboxylic groups toward oxidation. Since these groups can be oxidized to the aldehyde (14) by sodium hypobromite fairly readily and further condensed with phloroglucinol (15), it was anticipated that this technique could be employed for a quantitative assay of bacitracin.

In the present investigation, it was found that bacitracin could be quantitatively determined by employing a colorimetric technique. The procedure is based upon heating the antibiotic with alkali sodium