(7) L. Malspeis, M. S. Bathala, T. M. Ludden, H. B. Bhat, S. G. Frank, T. D. Sokoloski, B. E. Morrison, and R. H. Reuning, *Res. Commun. Chem. Pathol. Pharmacol.*, **12**, 43 (1975).
(8) K. A. Connors and K. S. Albert, *J. Pharm. Sci.*, **62**, 845 (1973).
(9) J. G. Wagner, "Fundamentals of Clinical Pharmacokinetics," Drug Intelligence Publications, Hamilton, Ill., 1975, p. 83.
(10) T. M. Ludden, M. S. Bathala, L. Malspeis, M. A. Drum, and R. H. Reuning, *Drug Metab. Dispos.*, **6**, 321 (1978).
(11) C. M. Metzler, NONLIN, Technical Report 7292/68/7292/005, The Upjohn Co., Kalamazoo, Mich., 1969.
(12) W. G. Kramer, R. P. Lewis, T. C. Cobb, W. F. Forester, Jr., J. A. Visconti, L. A. Wanke, H. G. Boxenbaum, and R. H. Reuning, *J. Pharmacokinet. Biopharm.*, **2**, 299 (1974).
(13) C. F. Poole, in "Handbook of Derivatives for Chromatography," K. Blau and G. S. King, Eds., Heyden, London, England, 1977, pp. 160–163.
(14) E. J. Cone, B. A. Phelps, and C. W. Gorodetzky, *J. Pharm. Sci.*, **66**, 1709 (1977).
(15) "Biology Data Book," vol. III, 2nd ed., P. L. Altman and D. S. Dittmer, Eds., Federation of American Societies for Experimental Biology, Bethesda, Md., 1974, pp. 1702–1705.
(16) H. E. Dayton and C. E. Inturrisi, *Drug Metab. Dispos.*, **4**, 474 (1976).
(17) M. J. Kogan, K. Verebey, and S. J. Mulé, *Res. Commun. Chem. Pathol. Pharmacol.*, **18**, 29 (1977).
(18) D. P. Vaughan, *J. Pharm. Pharmacol.*, **27**, 458 (1975).

ACKNOWLEDGMENTS

Supported by Contract HSM 42-73-182 (Ohio State), Grant DA-00473 (Ohio State), and Contract HSM-72-72-167 (Warner-Lambert/Parke-Davis) from the National Institute on Drug Abuse, U.S. Department of Health, Education, and Welfare, Rockville, Md.

The authors thank Dr. E. H. Fairchild for the mass spectrum.

Pharmacokinetic Description of Drug Interactions by Enzyme Induction: Carbamazepine–Clonazepam in Monkeys

ALLEN A. LAI * and RENÉ H. LEVY ˣ

Received April 24, 1978, from the *Departments of Pharmaceutical Sciences and Neurological Surgery, Schools of Pharmacy and Medicine, University of Washington, Seattle, WA 98195.* Accepted for publication September 6, 1978. *Present address: Clinical Research Department, Medical Division, Burroughs Wellcome Co., Research Triangle Park, NC 27709.

Abstract □ The applicability of a pharmacokinetic model for drug interactions by enzyme induction was tested in rhesus monkeys using a design in which both the inducer (carbamazepine) and the induced agent (clonazepam) were infused chronically. Two types of studies were conducted. Studies I and II examined the kinetic behavior of plasma clonazepam levels during induction and postinduction, respectively. The addition of carbamazepine (Study I) caused the preinduction clonazepam steady-state level to decrease exponentially to a lower (induced) steady state after lag times of 14.0–60.5 hr, and the removal of carbamazepine (Study II) caused induced clonazepam steady-state levels to climb exponentially to a higher steady state after lag times of 34.0–81.0 hr. The extent of induction ranged between 23 and 54%. The time course of clonazepam levels was described in terms of a one-compartment induction model with zero-order input and a metabolic clearance that increased (Study I) or decreased (Study II) exponentially with time. In both studies, induced clonazepam half-lives (3.7–7.7 hr) were significantly shorter ($p < 0.0005$) than control values (5.2–12.2 hr). Apparent enzyme turnover half-lives were shorter in Study II (2.7–19.3 hr) than in Study I (6.9–66.4 hr). A two- to threefold increase in urinary excretion of D-glucaric acid during carbamazepine administration provided additional evidence that the present interaction was due to enzyme induction.

Keyphrases □ Interactions—carbamazepine and clonazepam in monkeys by enzyme induction, pharmacokinetic description □ Carbamazepine—interaction with clonazepam by enzyme induction, pharmacokinetic description, monkeys □ Clonazepam—interaction with carbamazepine by enzyme induction, pharmacokinetic description, monkeys □ Enzyme induction—pharmacokinetic description of carbamazepine–clonazepam interaction, monkeys □ Pharmacokinetics—description of carbamazepine–clonazepam interaction by enzyme induction, monkeys □ Models—drug interactions by enzyme induction, carbamazepine–clonazepam, monkeys

Many drugs possess enzyme-inducing properties and thereby stimulate their own metabolism (autoinduction) and/or that of other drugs (heteroinduction). In humans and animals, experimental evidence for enzyme induction is often associated with decreases in plasma concentrations of the induced agent. Recently, a pharmacokinetic theory was proposed to describe the kinetics of plasma drug levels during auto- and heteroinduction (1). This theory proposes that the metabolic clearance of the induced species increases exponentially during induction. The time course of this increase in clearance is governed by the apparent turnover half-life of the induced metabolic enzyme(s).

The simplest and most critical experimental validation of this theory entails administration of both an inducer and an induced agent by constant rate intravenous infusion. Preliminary studies with this design were performed in monkeys using carbamazepine as the inducer and valproic acid and ethosuximide as the induced agents (2, 3). Following addition of carbamazepine, plasma levels of these two drugs decreased in an exponential fashion to a lower steady state, as expected.

The present study, in addition to investigating shifts of steady-state levels during induction, focused on determining control and induced half-lives of clonazepam. Furthermore, the hypothesis that removal of an inducer causes a return of metabolic clearance from an induced state to the basal state was tested by following increases in plasma clonazepam levels upon removal of carbamazepine.

EXPERIMENTAL

Animal Preparation—Five healthy male rhesus (*Macaca mulatta*) monkeys, 4.0–5.6 kg, were chair adapted for 1 month prior to surgical implantation of catheters at two different sites. The jugular and femoral veins were catheterized for drug infusion and blood sampling, respectively. Each monkey was maintained in a three-level restraining chair during individual studies and was given cage rest at appropriate intervals. Patency of catheters was assured by a slow, continuous saline infusion (1 ml/hr). The daily diet consisted of fresh fruit (bananas, oranges, and apples) and monkey chow.

0022-3549/79/0400-0416$01.00/0

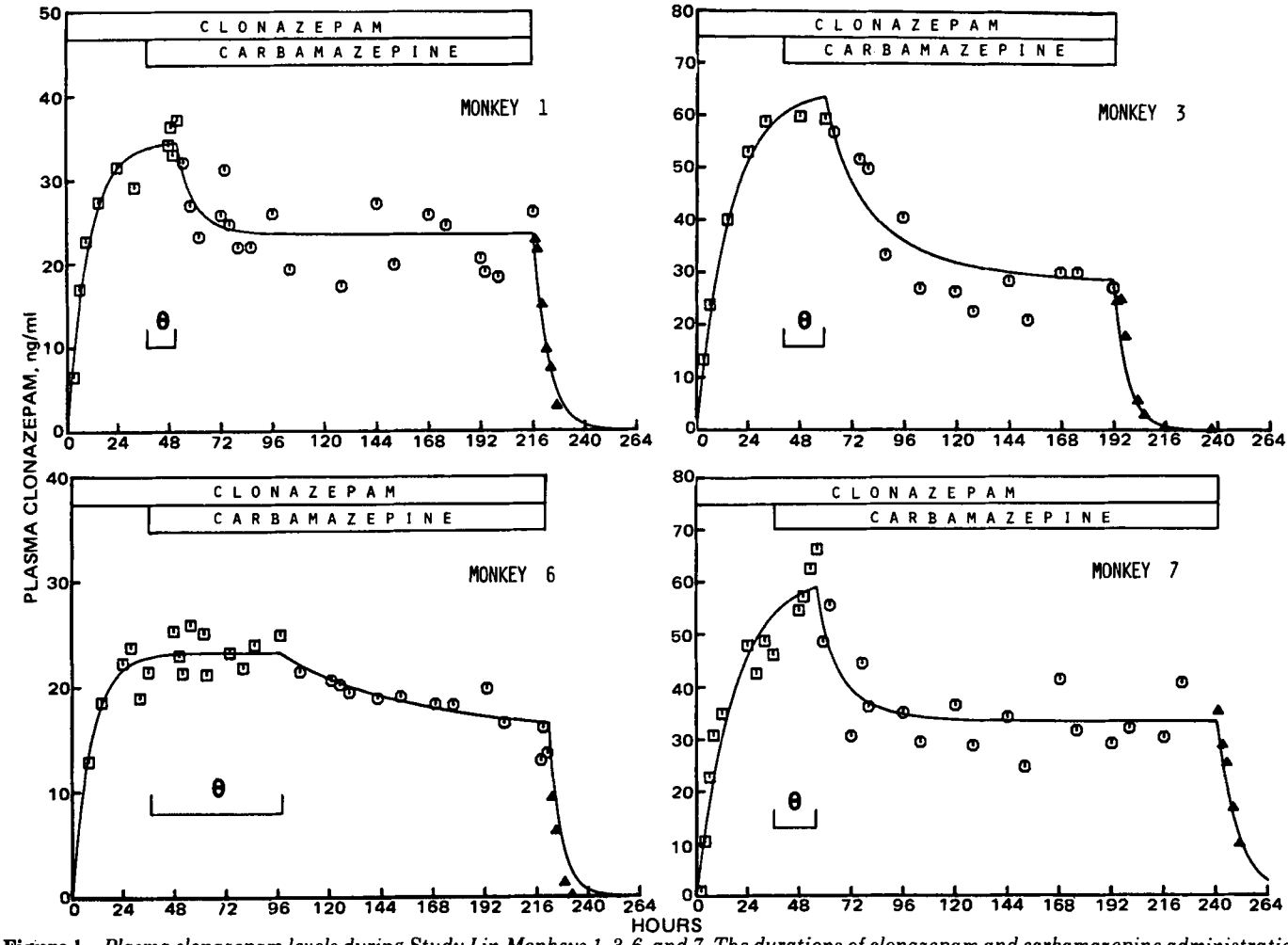


Figure 1—*Plasma clonazepam levels during Study I in Monkeys 1, 3, 6, and 7. The durations of clonazepam and carbamazepine administration are illustrated at the top of individual plots. The continuous lines were simulated with parameters obtained from curve fitting of experimental data to Eqs. 3, 4, and 6.*

Protocol and Analytical Procedures—All intravenous solutions were polyethylene glycol 400–water mixtures (60:40). Three types of intravenous solutions were prepared: Solution A, clonazepam only (80–130 μg/ml); Solution B, both clonazepam and carbamazepine (80–130 μg/ml and 10 mg/ml, respectively); and Solution C, carbamazepine only (10 mg/ml).

Two types of studies were conducted. In Study I, clonazepam was infused in four monkeys (Monkeys 1, 3, 6, and 7) at a constant rate for 8–9 days to achieve and maintain steady-state levels; carbamazepine was added to the infusion after 2 days. (Solution A was used for Days 1–2 and Solution B for Days 3–9.) In Study II, carbamazepine was infused in four monkeys (Monkeys 3, 4, 6, and 7) from Days 1 to 6; clonazepam was infused from Days 4 to 12. (Solution C was used for Days 1–3, Solution B for Days 4–6, and Solution A for Days 7–12.) In both studies, 30–34 blood samples (1.2 ml each) were collected. The plasma of these samples was separated and frozen until assay. Four to six 24-hr urine samples also were collected during each study.

Plasma levels of clonazepam were measured by the electron-capture GLC method of de Silva and Bekersky (4), and those of carbamazepine were measured by the GLC–chemical-ionization mass spectrometric method of Trager *et al.* (5). Urinary excretion of D-glucaric acid was determined using the spectrophotometric procedure of Ishidate *et al.* (6).

Data Analysis—Experimental plasma concentration–time data were fitted to appropriate pharmacokinetic model equations using the BMDX85 nonlinear least-squares program. All data points were assigned equal weights. A one-tailed paired t-test was used in all statistical comparisons.

Table I—Daily Urinary Excretion of D-Glucaric Acid from Monkeys during Carbamazepine–Clonazepam Interaction Studies

Monkey	Control Period, mg/day	Clonazepam Infusion Period[a], mg/day	Induction Period[b], mg/day
6[c]	30.8	34.1	70.7
7[c]	16.9	13.3	23.3
3[d]	18.1	16.0	45.3
4[d]	18.3	23.2	50.5
7[d]	9.3	11.7	25.5

[a] Animals during this period received only clonazepam. [b] Animals during this period received both clonazepam and carbamazepine. [c] Study I. [d] Study II.

RESULTS AND DISCUSSION

Plasma Clonazepam Levels during Studies I and II—Figure 1 shows experimental plasma clonazepam levels as a function of time in Monkeys 1, 3, 6, and 7 during Study I. The entire time course can be divided into three phases (I-A, I-B, and I-C), as illustrated by the three different symbols representing the experimental data points. In Phase I-A, plasma clonazepam concentrations increased exponentially and steady-state plasma levels (preinduction steady state, C_0) were essentially achieved within 36–48 hr. In Phase I-B, the addition of carbamazepine caused clonazepam levels to decrease to lower steady-state levels (induced steady state, C_∞).

Lag times (θ = 14.0, 17.5, 60.5, and 19.0 hr in Monkeys 1, 3, 6, and 7, respectively) were observed between the addition of carbamazepine and the start of the decrease of clonazepam levels. Upon termination of both clonazepam and carbamazepine infusions, clonazepam levels decayed to less than 5% of C_∞ within 24–36 hr (Phase I-C). The scatter in exper-

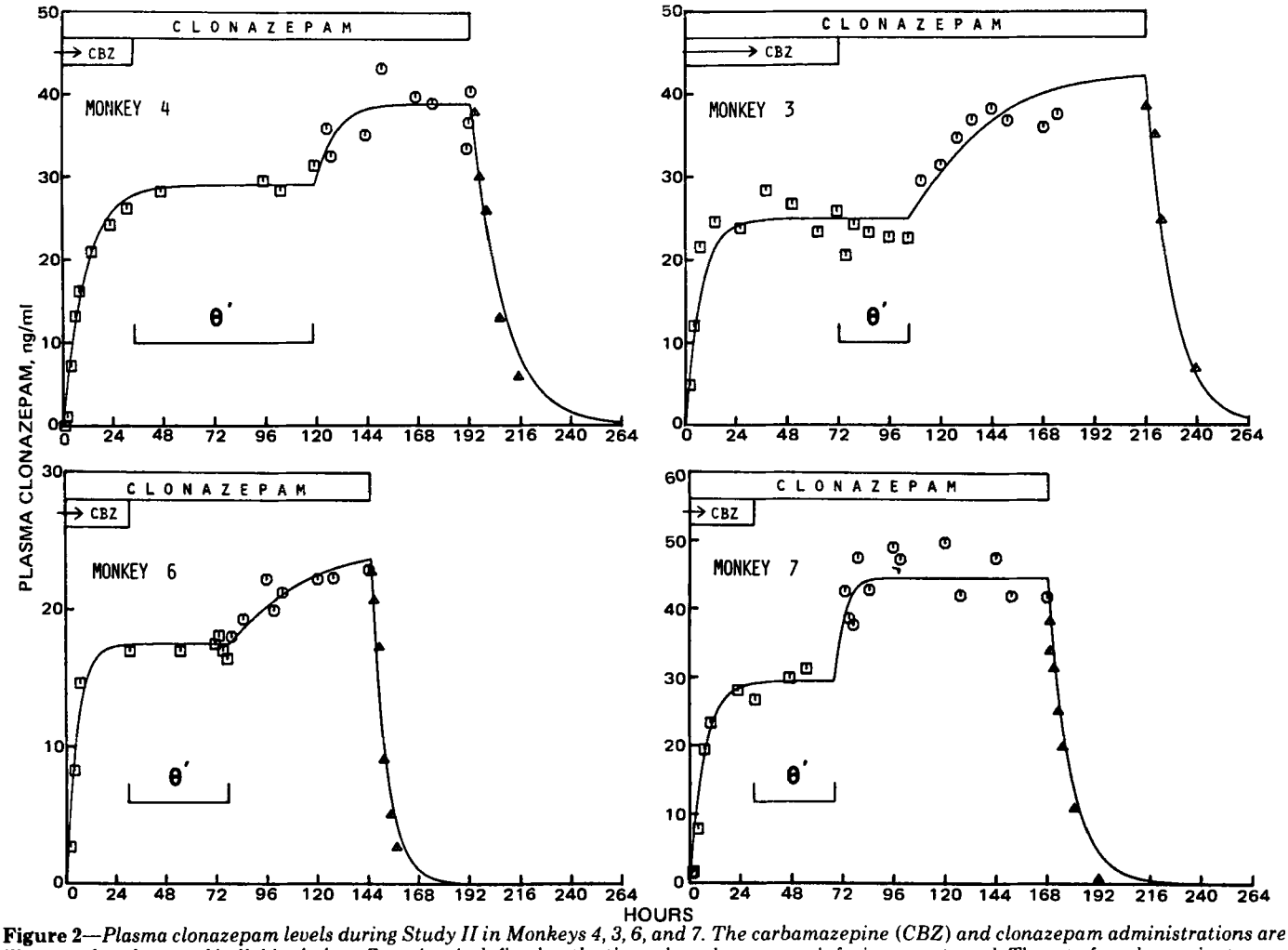


Figure 2—*Plasma clonazepam levels during Study II in Monkeys 4, 3, 6, and 7. The carbamazepine (CBZ) and clonazepam administrations are illustrated at the top of individual plots. Zero time is defined as the time when clonazepam infusion was started. Three to four days prior to zero time, animals were started on the carbamazepine infusion (indicated by an arrow starting prior to zero time in the carbamazepine bar). The continuous lines were simulated with parameters obtained from curve fitting of experimental data to Eqs. 7–9.*

imental data points during both steady states (C_0 and C_∞) was probably due to diurnal oscillations (7).

Figure 2 shows experimental clonazepam levels as a function of time in Monkeys 4, 3, 6, and 7 during Study II. The time course of this study can also be divided into three phases. As noted earlier, animals were pretreated with an infusion of carbamazepine (for 3–4 days) prior to initiation of clonazepam administration (at $t = 0$). Carbamazepine administration was continued during clonazepam infusion for 33.5, 72.0, 31.0, and 31.0 hr in Monkeys 4, 3, 6, and 7, respectively.

In Phase II-A, plasma clonazepam levels increased exponentially and reached an induced steady state, C_∞, within 24 hr in all monkeys. Upon removal of carbamazepine and after variable lag times (θ' = 81.0, 34.0, 47.0, and 37.0 hr in Monkeys 4, 3, 6, and 7, respectively), clonazepam levels increased to a higher postinduction steady state, C_0' (Phase II-B). Clonazepam infusion was terminated (at 215.5, 193.0, 144.5, and 168.0 hr in Monkeys 4, 3, 6, and 7, respectively), and experimental plasma levels declined to less than 5% of C_0' within 48 hr (Phase II-C).

Urinary Excretion of D-Glucaric Acid—Control urinary excretion of D-glucaric acid in four monkeys (Monkeys 3, 4, 6, and 7) ranged from 9.3 to 30.8 mg/day with a mean (±*SD*) of 20.1 ± 7.5 mg/day (Table I). Values obtained during intravenous infusion of clonazepam *alone* were not significantly different, ranging from 11.7 to 34.1 mg/day with a mean (±*SD*) of 21.5 ± 9.5 mg/day. However, urinary excretion of D-glucaric acid increased two- to threefold when carbamazepine was added to the infusion ($p < 0.005$). The values obtained during this induction period ranged from 23.3 to 70.7 mg/day with a mean (±*SD*) of 47.7 ± 19.1 mg/day.

Plasma Levels of Carbamazepine—Since most plasma samples were needed for the determination of clonazepam levels, it was not possible to document the entire time course of carbamazepine autoinduction (8–16) during the present interaction study. However, occasional plasma samples assayed for carbamazepine concentrations showed that the preinduction steady-state levels (1.22–2.84 μg/ml) were significantly higher ($p > 0.0005$) than the induced steady-state levels (0.79–1.97 μg/ml).

Pharmacokinetic Modeling—In the analysis of experimental data, the induction theory recently developed by Levy *et al.* (1) was applied. This theory describes body levels of an induced agent during induction and postinduction in terms of changes in total body clearance. The time course of these changes is governed by an induction rate constant, K_I or K_I'. In single-enzyme induction, K_I and K_I' represent the degradation rate constants of the induced enzyme in the presence and absence of the inducing agent, respectively. In multienzyme induction, K_I or K_I' becomes a hybrid of the degradation rate constant of all induced enzymes. Upon induction, total body clearance, $Q(T)$, of the induced agent increases exponentially from Q to Q' according to (1)[1]:

$$Q(T) = Q' - (Q' - Q) \exp(-K_I t) \qquad \text{(Eq. 1)}$$

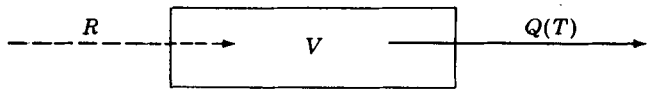
Conversely, in the postinduction period, $Q(T)$ decreases from Q' to Q as given by (1):

$$Q(T) = Q - (Q - Q') \exp(-K_I' t) \qquad \text{(Eq. 2)}$$

The distinction between K_I and K_I' allows for the possibility that the inducer may cause induction not only by increasing the enzyme synthesis rate but also by decreasing the degradation rate constant from K_I' to K_I.

[1] A derivation of this equation will be published and is available upon request.

Changes in plasma clonazepam levels during Studies I and II can be described in terms of the pharmacokinetic model shown in Scheme I:



Scheme I—Model I

where V is a single-compartment volume of distribution for clonazepam, R is the zero-order infusion rate, and $Q(T)$ is as defined in Eq. 1 (Study I) or Eq. 2 (Study II).

During Phase I-A, clonazepam levels at any time, t, simply follow the one-compartment infusion equation with constant clearance, Q:

$$C = \frac{R}{Q}(1 - e^{-Qt/V}) \qquad \text{(Eq. 3)}$$

During Phase I-B, the decrease in clonazepam levels is given by the following previously reported (1) equation[2]:

$$C = \frac{R}{Q^{t-\tau_1}} + \frac{R}{Q}\exp\left\{\frac{-Q'}{V}(t-\tau_1) + \frac{Q'-Q}{VK_1}[1-\exp(-K_I(t-\tau_1))]\right\}\exp\left(\frac{-Q\tau_1}{V}\right) \qquad \text{(Eq. 4)}$$

where τ_1 is the time from commencement of Study I to the decrease in clonazepam "steady-state" levels (inclusive of lag time θ), $Q^{t-\tau_1}$ is given in Eq. 1 where t is replaced by $t - \tau_1$, and other terms are as previously defined. If induction commences after plasma clonazepam levels have achieved C_0, the last term in Eq. 4 approaches zero and Eq. 4 can be approximated by its first term:

$$C = \frac{R}{Q^{t-\tau_1}} \qquad \tau_1 \geq 5V \ln 2/Q \qquad \text{(Eq. 5)}$$

During Phase I-C, the decrease in clonazepam levels from C_∞ is simply described by:

$$C = \frac{R}{Q'}e^{-Q'(t-\tau_2)/V} \qquad \text{(Eq. 6)}$$

where τ_2 is the time at which clonazepam infusion is stopped.

Conversely, plasma clonazepam levels during Phases II-A, II-B, and II-C can be described by the following three equations, respectively:

$$C = \frac{R}{Q'}(1 - e^{-Q't/V}) \qquad \text{(Eq. 7)}$$

$$C = \frac{R}{Q^{t-\tau_3}} + \frac{R}{Q'}\exp\left\{\frac{-Q}{V}(t-\tau_3) + \frac{Q-Q'}{VK_I'}[1-\exp(-K_I'(t-\tau_3))]\right\}\exp\left(\frac{-Q'\tau_3}{V}\right) \qquad \text{(Eq. 8)}$$

$$C = \frac{R}{Q}e^{-Q(t-\tau_4)/V} \qquad \text{(Eq. 9)}$$

where τ_3 is the time from the start of clonazepam infusion to the increase in clonazepam steady-state levels inclusive of the lag time θ', $Q^{t-\tau_3}$ is as given in Eq. 2 where t is replaced by $t - \tau_3$, and τ_4 is the time at which clonazepam infusion is stopped. A simple and accurate approximation of Eq. 8 (analogous to Eq. 5) is:

$$C = \frac{R}{Q^{t-\tau_3}} \qquad \tau_3 \geq 5V \ln 2/Q' \qquad \text{(Eq. 10)}$$

A computer subroutine was developed for use in conjunction with the BMDX85 nonlinear least-squares regression analysis program to determine the four pharmacokinetic parameters (Q, Q', K_I or K_I', and V) of Model I. This subroutine yields a single set of best values for the four parameters by fitting experimental data points from the three phases of each study simultaneously to appropriate model equations. This subroutine was tested using generated data without and with 5–10% random error.

Curve fitting of generated data without error yielded the input values. For data with 5 and 10% random error, the program yielded values for Q, Q', and V that deviated from input values by 6–8%. However, the values obtained for K_I (or K_I') deviated more, by as much as 20–25% in a few cases. This behavior of K_I (or K_I') can be attributed in part to the fact that its value is determined only from data in Phase I-B (or Phase II-B) of the interaction study. Also, the value of K_I (or K_I') can be easily affected by slight changes in the value of Q' (or Q).

Analysis of Experimental Data—The approach used in the present study was to fit experimental data from Studies I and II to Eqs. 3, 4, and 6 and Eqs. 7–9, respectively (Method A) as well as to Eqs. 3, 5, and 6 and Eqs. 7, 10, and 9, respectively (Method B). The continuous lines in Figs. 1 and 2 represent the results of curve fitting with Method A. A measure of the fit of experimental data to model equations can be given by the correlation coefficient. The mean correlation coefficient ($\pm SD$) for Method A was 0.938 ± 0.030 (Table II); for Method B, it was 0.900 ± 0.116. Thus, the induction theory proposed by Levy *et al.* (1) adequately describes the present interaction between clonazepam and carbamazepine.

A comparison of Methods A and B in terms of the four parameters (Q, Q', K_I or K_I', and V) shows negligible differences, indicating that Eqs. 5 and 10 are accurate approximations of Eqs. 4 and 8 under the present experimental conditions. A summary of pharmacokinetic parameters determined with Method A, along with values for θ and θ' (obtained directly from clonazepam level *versus* time plots), are given in Table II. For ease of interpretation, control and induced elimination half-lives of clonazepam are listed. Similarly, apparent turnover half-lives of induced enzyme(s) [ratio between ln 2 and K_I (or K_I')] are shown in place of induction rate constants.

In Study I, the mean value ($\pm SD$) for the control (preinduction) half-life of clonazepam was 9.0 ± 2.4 hr; for the volume of distribution, it was 8.2 ± 2.1 liters/kg. These parameters were comparable but slightly larger than those reported previously (7). The mean induced half-life of clonazepam ($\pm SD$) was shorter (4.9 ± 1.1 hr) than the control value. Statistical comparison showed that the difference was significant ($p \leq 0.005$). Apparent turnover half-lives of the induced enzyme(s) were 6.9, 27.2, 66.4, and 11.4 hr for Monkeys 1, 3, 6, and 7, respectively. The extent of induction, f_i, as measured by the fractional decrease in C_0 was calculated using:

$$f_i = \frac{C_0 - C_\infty}{C_0} \qquad \text{(Eq. 11)}$$

Values for f_i ranged from 0.32 to 0.54.

Table II—Pharmacokinetic Parameters Determined from Clonazepam–Carbamazepine Interaction Studies in Monkeys

Monkey	Volume of Distribution, liters/kg	Control Half-Life of Clonazepam, hr	Induced Half-Life of Clonazepam, hr	Apparent Turnover Half-Life of Induced Enzyme(s), hr	Extent of Induction	Lag Time, hr	Correlation Coefficient from Curve Fitting
			Study I				
1[a]	8.5 (12)[b]	7.0[c] (14)	4.7 (11)	6.9 (53)	0.23	14.0[d]	0.924
3	10.8 (13)	10.5[c] (17)	4.6 (13)	27.2 (29)	0.54	17.5[d]	0.993
6	7.7 (12)	6.2[c] (13)	4.0 (11)	66.4 (36)	0.37	60.5[d]	0.936
7	5.8 (12)	12.2[c] (17)	6.6 (12)	11.4 (38)	0.45	19.0[d]	0.953
			Study II				
4	9.6 (14)	8.4[e] (15)	5.0 (12)	18.2 (25)	0.31	81.0[f]	0.964
3	11.2 (9)	10.2[e] (11)	7.7 (8)	5.7 (54)	0.25	34.0[f]	0.923
6	8.1 (10)	5.2[e] (10)	3.7 (8)	19.3 (33)	0.28	47.0[f]	0.909
7	7.9 (10)	7.3[e] (12)	4.8 (9)	2.7 (35)	0.34	37.0[f]	0.905

[a] Replaced by Monkey 4 in Study II. [b] Numbers in parentheses are the standard deviations in percent. [c] Preinduction half-life. [d] Value for θ. [e] Postinduction half-life. [f] Value for θ'.

For Study II, the mean value ($\pm SD$) for the control (postinduction) half-life of clonazepam was 7.8 ± 2.1 hr; for the volume of distribution, it was 9.2 ± 1.5 liters/kg. These two parameters were in agreement with those found in Study I and comparable to those reported earlier (7). The mean induced half-life of clonazepam was 5.2 ± 1.7 hr. This parameter was significantly shorter ($p \leq 0.005$) than the control value. The extent of induction, f_i, was of the same order of magnitude as that of Study I. Values for f_i ranged from 0.25 to 0.41.

Apparent turnover half-lives of induced enzymes in Study II ranged from only 2.7 to 19.3 hr as compared to 6.9–66.4 hr in Study I. There was also a difference in lag time values between Studies I and II. Values for θ' (Study II) were longer than corresponding θ values (Study I). These differences (in apparent turnover half-lives of induced enzymes and lag times) will be discussed further.

Mechanistic Considerations—The observed reduction in plasma clonazepam levels and half-lives was attributed to enzyme induction with carbamazepine as the inducing agent. The two- to threefold increase in urinary D-glucaric acid excretion supports this contention. Several major anticonvulsants (phenytoin and phenobarbital) that are potent enzyme inducers increase urinary excretion of D-glucaric acid in humans (17, 18). Although the exact mechanism whereby both the drug-metabolizing system and the glucuronic acid pathway are stimulated remains unknown, urinary excretion of D-glucaric acid is commonly used as an index of induction (19, 20).

The long lag times (both θ and θ') are also consistent with an interaction due to enzyme induction. Recent investigations of the molecular mechanism of enzyme induction have accumulated sufficient information to allow a detailed description of events leading to enzyme induction (21–23). The facts that the effect of carbamazepine (reduction in plasma clonazepam levels) was not elicited until 14.0–60.5 hr after its addition to the infusion and that its effect did not dissipate until 34.0–81.0 hr after withdrawal fit within the time frame of the sequence of molecular events leading to increased protein synthesis.

The observed decreases in plasma clonazepam levels during carbamazepine induction are also compatible with a number of reports indicating that carbamazepine is able to induce the metabolism of a variety of drugs (2, 3, 24, 25). In rats, repeated oral doses of carbamazepine enhanced cytochrome P-450 and NADPH cytochrome C-reductase activity (26).

Two alternative explanations for the present interaction between clonazepam and carbamazepine were considered: (*a*) displacement of clonazepam from its plasma protein binding sites by carbamazepine with a resulting modification of the pharmacokinetics of the former, and (*b*) enzyme activation by carbamazepine with enhanced activity in the metabolism of clonazepam. A recent study (27) on the pharmacokinetics of tolbutamide displacement by sulfadimethoxine from protein binding sites in sheep showed that the lag time for sulfadimethoxine to cause a change in plasma tolbutamide concentration was essentially nil. The long lag times (θ and θ') observed in the present study exclude an interaction due to competitive binding. Furthermore, *in vitro* equilibrium dialysis experiments showed that carbamazepine did not displace clonazepam from its protein binding sites (28). To date, the literature contains little or no information on the pharmacokinetic implications of enzyme activation. However, it is unlikely that carbamazepine continues enzyme activation 27.0–74.0 hr[2] after elimination (Study II).

In the present study, values of K_I obtained in Study I were three to five times less than values of K'_I determined in Study II for the three monkeys (Monkeys 3, 6, and 7) in which both interaction studies were conducted. Therefore, enzyme turnover during Study I was apparently three to five times slower than during Study II. Such behavior was most unusual and warranted further explanation.

In studies of mechanism of induction (29) designed to distinguish between increased enzyme synthesis and decreased enzyme breakdown[3], the half-life of the initial shift from the basal to induced enzyme level is the degradation half-life of the enzyme in the presence of the inducer. The half-life of return to the basal enzyme level after the inducer is withdrawn is the degradation half-life of the enzyme in the absence of the inducer.

Such an approach was used to analyze the mechanism whereby continuous administration of phenobarbital to rats increased the amount of liver microsomal barbiturate-oxidizing enzyme (30). The half-life of the upward shift in enzyme level was 2.2 days, and that of the downward shift was 2.6 days. Thus, enzyme turnover was essentially unaffected by phenobarbital, and the main cause of the increase in enzyme level was an increase in its synthesis rate. If similar reasoning is applied to the data of Studies I and II, it is suggested that carbamazepine and/or its metabolites acted by causing an increased enzyme synthesis rate as well as by stabilizing some enzymes[4].

Therapeutic Implications—Clonazepam is a new anticonvulsant and, therefore, is often added to therapeutic regimens of other anticonvulsants. Thus, a thorough understanding of potential interactions between clonazepam and these agents is essential. Knop *et al.* (31) reported that the clearances of clonazepam in four epileptic patients also receiving other antiepileptic drugs (phenytoin, phenobarbital, carbamazepine, *etc.*) were considerably larger than the values in healthy subjects. Another report documented the interaction between clonazepam and phenytoin (32). More recently, Bekersky *et al.* (33) found that phenobarbital increased the total plasma clearance of clonazepam by 102% in dogs.

The results of the present studies prompted an investigation of the interaction between carbamazepine and clonazepam in normal volunteers. It was found that steady-state clonazepam levels decreased by 19–37% in seven subjects following addition of a daily dose of carbamazepine (28).

REFERENCES

(1) R. H. Levy, A. A. Lai, and M. S. Dumain, *J. Pharm. Sci.*, **68**, 398 (1979).

(2) P. H. Corbisier and R. H. Levy, *APhA/Academy of Pharmaceutical Sciences Abstracts*, **7** (1), 163 (1977).

(3) *Ibid.*, **7** (2), 134 (1977).

(4) J. A. F. de Silva and I. Bekersky, *J. Chromatogr.*, **99**, 447 (1974).

(5) W. F. Trager, R. H. Levy, I. H. Patel, and J. M. Neal, *Anal. Lett.*, **B11** (2), 119 (1978).

(6) M. Ishidate, M. Matsui, and M. Okada, *Anal. Chem.*, **11**, 176 (1965).

(7) A. A. Lai and R. H. Levy, *J. Pharm. Sci.*, **67**, 295 (1978).

(8) P. L. Morselli, P. Biandrate, A. Frigerio, and S. Garattini, *Eur. J. Clin. Invest.*, **2**, 297 (1972).

(9) M. Eichelbaum, K. Ikbom, L. Bertilsson, V. A. Ringberger, and A. Rane, *Eur. J. Clin. Pharmacol.*, **8**, 337 (1975).

(10) P. L. Morselli, in "Advances in Neurology," vol. 11, J. K. Penry and D. D. Daly, Eds., Raven, New York, N.Y., 1975, pp. 279–294.

(11) P. L. Morselli, M. Gerna, D. DeMaio, G. Zanda, F. Viani, and S. Garattini, in "Clinical Pharmacology of Anti-Epileptic Drugs," H. Schneider, D. Janz, C. Gardner-Thorpe, H. Meinardi, and A. L. Sherwin, Eds., Springer-Verlag, New York, N.Y., 1975, pp. 166–180.

(12) W. H. Pitlick and R. H. Levy, *J. Pharm. Sci.*, **65**, 462 (1976).

(13) A. P. Gerardin, F. V. Abadie, J. A. Campestrini, and W. Theobald, *J. Pharmacokinet. Biopharm.*, **4**, 521 (1976).

(14) P. L. Morselli, L. Bussi, and M. Gerna, in "Epileptic Seizures—Behavior—Pain," W. Birkmayer, Ed., Hans Huber, Bern, Switzerland, 1976, pp. 141–150.

(15) J. W. Faigle, S. Brechbuhler, K. F. Feldmann, and W. J. Richter, in *ibid.*, pp. 159–165.

(16) W. H. Pitlick and R. H. Levy, *J. Pharm. Sci.*, **66**, 647 (1977).

(17) A. G. Hildebrandt, I. Roots, M. Speck, K. Saalfrank, and H. Kewitz, *Eur. J. Clin. Pharmacol.*, **8**, 327 (1975).

(18) A. Richens and G. W. Houghton, in "Clinical Pharmacology of Anti-Epileptic Drugs," H. Schneider, D. Janz, C. Gardner-Thorpe, H. Meinardi, and A. L. Sherwin, Eds., Springer-Verlag, New York, N.Y., 1975, pp. 89–110.

(19) E. M. Aarts, *Biochem. Pharmacol.*, **14**, 359 (1965).

(20) J. Hunter, J. D. Maxwell, M. Carrella, and D. A. Stewart, *Lancet*, **1**, 572 (1971).

(21) T. D. Gelehrter, *N. Engl. J. Med.*, **294**, 522 (1976).

(22) *Ibid.*, **294**, 589 (1976).

(23) *Ibid.*, **294**, 646 (1976).

(24) J. Hansen, K. Siersbaek-Nielsen, and L. Skovsted, *Clin. Pharmacol. Ther.*, **12**, 539 (1971).

(25) O. Penttila, P. J. Neuvonen, K. Aho, and R. Lehtovaara, *Br. Med. J.*, **2**, 470 (1974).

(26) J. W. Faigle, S. Brechbuhler, K. F. Feldmann, and W. J. Richter, in "Epileptic Seizures—Behavior—Pain," W. Birkmayer, Ed., Hans Huber, Bern, Switzerland, 1976, pp. 127–140.

(27) J. Thiessen and M. Rowland, *J. Pharm. Sci.*, **66**, 1063 (1977).

(28) A. A. Lai, R. H. Levy, and R. E. Cutler, *Clin. Pharmacol. Ther.*,

[2] Obtained by subtracting five to seven elimination half-lives of carbamazepine (16) from θ'.

[3] Assuming that enzyme activation has been ruled out.

[4] Inhibition of degradation of hepatic enzymes.

23, 117 (1978).
(29) H. L. Segal and Y. S. Kim, *Proc. Natl. Acad. Sci. USA*, **50**, 912 (1963).
(30) I. M. Arias and A. deLeon, *Mol. Pharmacol.*, **3**, 216 (1967).
(31) H. J. Knop, E. Van der Kleijn, and L. C. Edmunds, in "Clinical Pharmacology of Anti-Epileptic Drugs," H. Schneider, D. Janz, C. Gardner-Thorpe, H. Meinardi, and A. L. Sherwin, Eds., Springer-Verlag, New York, N.Y., 1975, pp. 247–260.
(32) O. Sjo, E. F. Hvidberg, J. Naestoft, and M. Lund, *Eur. J. Clin. Pharmacol.*, **8**, 249 (1975).
(33) I. Bekersky, A. C. Maggio, V. Mattaliano, Jr., H. G. Boxenbaum, D. E. Maynard, P. D. Cohn, and S. A. Kaplan, *J. Pharmacokinet. Biopharm.*, **5**, 507 (1977).

ACKNOWLEDGMENTS

Presented at the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, New York meeting, May 1977.

Supported in part by NIH Research Contract N01-NS-1-2282 and Research Grant NS-4053 awarded by the National Institute of Neurological and Communicative Disorders and Stroke, U.S. Public Health Service.

The authors thank Dr. William Trager for helpful discussion and James Cook, Douglas Ng, Raina Hanson, John Hokanson, Kenneth Lee, Jennifer Andrews, and John Neal for technical assistance. They also thank Hoffmann-La Roche for the generous supply of clonazepam and nitrazepam.

Analysis of Fludrocortisone Acetate and Its Solid Dosage Forms by High-Performance Liquid Chromatography

THERESE M. AST and HAMED M. ABDOU ˣ

Received May 25, 1978, from *E. R. Squibb & Sons, Inc., New Brunswick, NJ 08902.* Accepted for publication September 8, 1978.

Abstract □ A newly developed reversed-phase high-performance liquid chromatographic assay and test method for determining content uniformity are described for fludrocortisone acetate. The method is stability indicating and separates most known degradation products and impurities. In addition, the method is simple, sensitive, accurate, and relatively free of interferences. The coefficient of variation for multiple weight assays is between 0.3 and 1.8%.

Keyphrases □ Fludrocortisone acetate—and solid dosage forms, high-performance liquid chromatographic analysis □ High-performance liquid chromatography—analysis, fludrocortisone acetate and solid dosage forms □ Steroids—fludrocortisone acetate and solid dosage forms, high-performance liquid chromatographic analysis

Fludrocortisone acetate (9-fluoro-11β,17,21-trihydroxypregn-4-ene-3,20-dione 21-acetate) (I) is a powerful synthetic adrenocortical steroid. As an unsaturated 3-keto steroid, it may be analyzed by the isoniazid method (1) or with 4-aminoantipyridine hydrochloride reagent (2). The latter is used in the USP procedure for content uniformity. As a 17α-ketol steroid, fludrocortisone acetate is analyzed by the blue tetrazolium method (1), the official USP assay. A colorimetric assay in fermentation broths (3) and an automated colorimetric method for assaying single tablets of fludrocortisone acetate (4) also were reported.

Since none of these methods is selective for fludrocortisone acetate, it was the purpose of this study to develop a specific, stability-indicating, high-performance liquid chromatographic (HPLC) assay that would circumvent any excipient interferences. The HPLC method described here is capable of separating fludrocortisone acetate from its known degradation products and possible impurities. Norethindrone was used as an internal standard. The HPLC data were in excellent agreement with results obtained by the isoniazid assay. The method is simple, selective, and highly reliable.

EXPERIMENTAL

Reagents and Materials—Fludrocortisone acetate and norethindrone USP reference standards and acetonitrile[1] (UV grade and distilled in glass) were used. The mobile phase was 42 ± 2% acetonitrile in water.

The internal standard solution was prepared by dissolving norethindrone in acetonitrile to concentrations of 65 μg/ml for assay and 10 μg/ml for content uniformity analysis.

Standard Solutions and Calibration Curves—Fludrocortisone acetate solutions were prepared to contain 50, 70, 100, and 120 μg of fludrocortisone acetate/ml for assay and 15, 20 and 25 μg/ml for content uniformity analysis.

Sample Preparation—*Assay*—A portion of ground tablets equivalent to 2.5 mg of fludrocortisone acetate was transferred to a 50-ml low actinic centrifuge tube, and 5 ml of distilled water was added by pipet. After the suspension was mixed[2] for 1 min, 20 ml of internal standard solution was added, and the resulting mixture was transferred to a mechanical shaker for 40 min. The tablet extract was then centrifuged, and the clear liquid was injected into the HPLC system. The ratio of the peak heights of fludrocortisone acetate to the internal standard was determined, and the quantity of fludrocortisone acetate in the sample was calculated using the assay calibration curve.

Content Uniformity Analysis—One tablet (containing 0.1 mg of fludrocortisone acetate) was transferred to a 10-ml low actinic centrifuge tube, and 1 ml of distilled water was added. A vortex mixer[2] was used to effect complete disintegration, and then 4 ml of the internal standard solution was added. The mixture was then treated as in the assay.

Apparatus and Operating Conditions—The liquid chromatograph consisted of a reciprocating pump[3], a 20-ml loop injector[4], and a UV detector[3] equipped with a 254-nm filter. The analytical column was 30-cm × 4-mm i.d. stainless steel, packed with porous siliceous microbeads to which a stationary phase of octadecyltrichlorosilane was chemically bonded[3]. A flow rate of 1.8–2.0 ml/min, maintained at about 800–1000 psi, was used at ambient temperature. The output of the detector was displayed on a recorder having a full-scale range of 10 mv[5].

System Suitability Test—The standard preparation, 100 μg/ml, was chromatographed five or six times, and the peak response was measured as the ratio of resulting peak heights of fludrocortisone acetate to the internal standard. The relative standard deviation was less than 2%. The resolution factor between fludrocortisone acetate and the internal standard was more than three. For a particular column, resolution may be increased by decreasing the amount of acetonitrile in the mobile phase.

Placebo Analysis—A placebo of the sample commercial tablets used in this study was prepared and extracted in parallel with the sample. The placebo chromatogram was checked for interfering peaks near fludrocortisone acetate or norethindrone.

Recovery Studies—The efficiency of the recovery was checked by

[1] Burdick & Jackson Laboratories, Muskegon, Mich.

[2] Vortex-Genie mixer, Scientific Industries, Springfield, Mass.
[3] μBondapak-C_{18}, Waters Associates, Milford, Mass.
[4] Chromatronix HPSV-20, Spectra-Physics, Santa Clara, Calif.
[5] Model 410, Pharmacia Fine Chemicals, Piscataway, N.J.

0022-3549/79/0400-0421$01.00/0