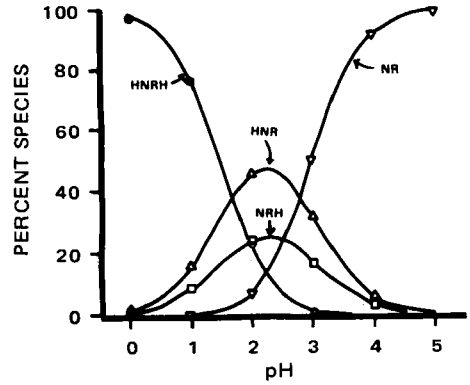

Figure 7—*Species profile for II.*

more zwitterions than unchanged molecules will be present in solution. This can be seen in the species profiles for I and II, for which the ratios of zwitterions to uncharged molecules are 2.33 and 1.864, respectively. The maximum concentration of zwitterionic species occurs at a pH equal to the average of pK_1 and pK_2 or pK_3 and pK_4, *i.e.*:

$$pH = \frac{pK_1 + pK_2}{2} = \frac{pK_3 + pK_4}{2} \qquad \text{(Eq. 19)}$$

For I, the maximum concentration occurs at pH 2.64; for II, it occurs at pH 2.25.

CONCLUSION

The ionization constants calculated for these compounds are reasonable when compared with each other and literature values for similar compounds. The approach used to calculate the individual equilibrium constants involves two methods, one that measures the total influence of all species upon the pH and one that measures only the sum of the concentrations of two species. Although neither method is suitable by itself, their combination enables one to calculate all four constants. Any two methods can be used provided that they are interrelated but not equivalent and one method gives the total influence of all species.

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Pharmacokinetic Studies of Pentylenetetrazol in Dogs

H. W. JUN

Abstract □ Pharmacokinetic profiles of pentylenetetrazol in the dog were studied following rapid intravenous and oral administrations of a convulsant dose (15–20 mg/kg). Plasma level–time curves after a rapid intravenous injection showed biexponential decline, indicating that the disposition of this drug in the dog follows a two-compartment body model. Pharmacokinetic parameters were calculated from the intravenous data. After oral administration of the solution dose, the peak plasma level appeared at about 30 min postdose, indicating that the absorption occurs rapidly. Areas under the oral plasma level–time curves showed that the drug was absorbed completely and that the first-pass metabolism effect was minimal. The ligation studies of the kidney and the liver suggested that the main elimination pathway of this drug was biotransformation in the liver. The average plasma half-life was 1.4 hr. At steady state, the volume of distribution was approximately equivalent to the volume of the total body water.

Keyphrases □ Pentylenetetrazol—pharmacokinetic studies following intravenous and oral administrations, dogs □ Pharmacokinetics—pentylenetetrazol, intravenous and oral administrations, dogs □ Stimulants—pentylenetetrazol, pharmacokinetic studies following intravenous and oral administrations, dogs

Pentylenetetrazol[1] is being used for various clinical purposes in humans and animals. The primary use of this drug in humans is as a central nervous system (CNS) stimulant for the therapeutic management of chronic depression and confusion in mental patients. In animals, this drug is also being used as a CNS stimulant for respiratory failure or collapse during surgical anesthesia.

Although the properties of biological disposition of pentylenetetrazol were studied (1, 2), pharmacokinetic profiles have not been reported. Recently, a sensitive and reproducible GLC determination of this compound in biological fluids was described (3); the method allowed the determination of pharmacokinetic properties of pentylenetetrazol in the dog as reported in this study.

EXPERIMENTAL

Conditions of Dogs—Seven healthy dogs (four beagle and three mongrel breeds), 8–13 kg, were used. Four dogs received a single pentylenetetrazol dose of 15 or 20 mg/kg iv and po on separate occasions. Three dogs were utilized in the liver and kidney ligation studies. When two or more blood level studies were performed on the same dog, at least 2 weeks was allowed between experiments. During the blood level studies, dogs were anesthetized with pentobarbital sodium.

Assay Procedures—Plasma concentrations of pentylenetetrazol were determined by the GLC technique recently developed (3). The

[1] Metrazol, Knoll Pharmaceutical Co., Whippany, N.J.

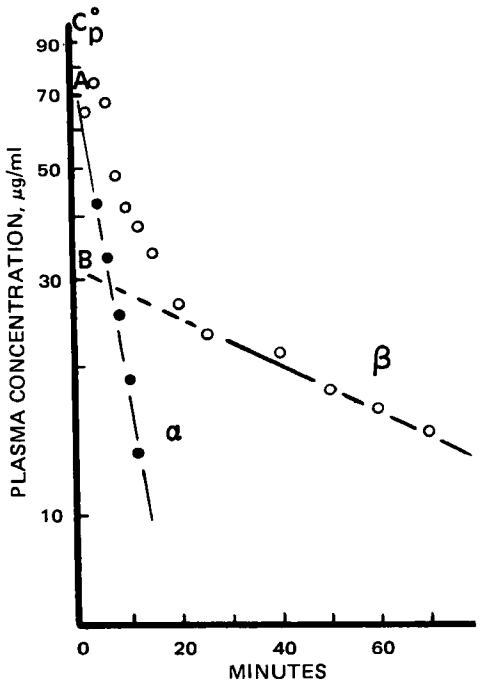


Figure 1—*Plasma concentrations of pentylenetetrazol at different times in a dog (11 kg) after a rapid intravenous injection of 20 mg/kg. Key:* O, *actual plasma concentrations;* ●, *points for the fast disposition phase by the feathering method; and* A *and* B, *extrapolated zero time intercepts of the α- and β-phases, respectively* (C_p^0 = A + B).

sensitivity limit of the assay is 0.5 μg of pentylenetetrazol/ml of plasma using a 2-ml sample. The method is sufficiently sensitive to determine plasma levels of the drug for three biological half-lives after administration of a 15-mg/kg dose.

Drug Administration—*Intravenous Injection*—The pentylenetetrazol dose (15 or 20 mg/kg) dissolved in 3 ml of normal saline was rapidly injected into the blood circulation through the front leg vein.

Oral Route—The oral dose (15 mg/kg) was dissolved in 25 ml of water and administered by stomach tube. Immediately after administration, the tube was flushed with 25 ml of water.

Blood Sampling—After drug administration, blood samples (4–8 ml) were withdrawn at various time intervals with a heparinized disposable syringe through an intravenous catheter inserted into the femoral vein. A three-way stopcock device was used during blood sampling. After each sample was taken, about 4 ml of lactated Ringer's solution was injected into the bloodstream to minimize the blood volume change.

Treatment of Blood Samples—Blood samples were placed in tubes[2] containing edetate sodium and were immediately centrifuged at 2000 rpm for 5 min. Pentylenetetrazol in the plasma portion was assayed by the GLC method (3).

Kidney Ligation Studies—To examine the role of the kidneys in the elimination of pentylenetetrazol in the dog, the following experiments were performed. A 13-kg dog received a rapid intravenous dose (15 mg/kg), and blood samples were withdrawn for 2 hr at various time intervals. After 2 hr (approximately one biological half-life in this dog), the kidneys of the dog were completely ligated; blood samples were withdrawn after ligation for drug analysis.

To ligate the kidneys, a surgical operation was made to place ligating threads around the renal blood vessels prior to drug administration. At the time of the kidney ligation, the threads were tied securely around the renal blood vessels to block the blood supply into the kidneys. Complete ligation was ascertained by the postmortem examination.

Liver Ligation Studies—The effect of liver ligation on the plasma level profiles of pentylenetetrazol was studied in a 12-kg dog. This dog received two intravenous doses of 15 mg/kg at zero time and 140 min after the first dose. The liver ligation was then made 140 min after the second dose by tying off the portal blood vessels with ligating threads placed surgically around the portal blood vessels.

Extreme care was taken to prevent internal bleeding during the liver ligation procedure. Following the first dose, blood samples were withdrawn frequently for 6 hr for analysis. The postmortem examination showed that the liver was completely ligated.

Kidney and Liver Ligation Studies—In this experiment, a 12-kg dog received three successive intravenous doses of pentylenetetrazol (15 mg/kg) at 0, 2, and 4 hr. The kidneys were first ligated 1 hr after the second dose, and the liver was ligated 1.75 hr after the third dose. To monitor the complete plasma level profile of the drug in this dog, blood samples were withdrawn frequently for about 7 hr.

Pharmacokinetic Analysis—The biexponential plasma level profiles of pentylenetetrazol after rapid intravenous injection indicate that the pharmacokinetics of the drug in the dog could be suitably evaluated on the basis of the two-compartment open model (Scheme I). The time course of biexponential decline of plasma levels for this model may be described by:

$$C_p^t = Ae^{-\alpha t} + Be^{-\beta t} \quad \text{(Eq. 1)}$$

$$C_p^0 = A + B \quad \text{(Eq. 2)}$$

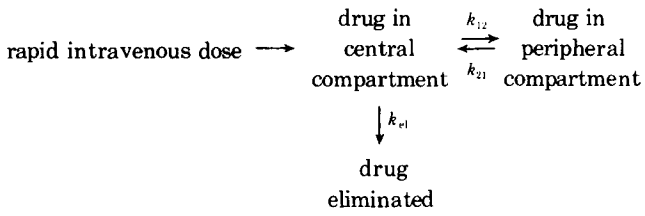
where C_p^t and C_p^0 are the drug plasma concentrations at time t and time zero, respectively; A and B are the extrapolated concentrations at the zero time; and α and β are the first-order hybrid rate constants for the rapid and the slow disposition phases, respectively. The slopes of the log C_p *versus* time and log residual *versus* time plots are $-\beta/2.303$ and $-\alpha/2.303$, respectively. The biological half-life of the drug in a two-compartment open model is determined from the slope of the slow disposition phase. The estimates of the parameters A, B, α, and β are graphically obtained by the feathering method, using plasma data plotted on semilogarithmic scales. These values are used to calculate the model parameters such as k_{12}, k_{21}, and k_{el} by:

$$k_{el} = \frac{C_p^0}{\frac{A}{\alpha} + \frac{B}{\beta}} \quad \text{(Eq. 3)}$$

$$k_{21} = \frac{\alpha\beta}{k_{el}} \quad \text{(Eq. 4)}$$

$$k_{12} = (\alpha + \beta) - (k_{21} + k_{el}) \quad \text{(Eq. 5)}$$

The volume terms, V_p and $(V_d)_{ss}$, are calculated by the method previously described (4).



Scheme I—Two-compartment body model

RESULTS AND DISCUSSION

All plasma level–time curves (Figs. 1–5) of pentylenetetrazol in dogs after rapid intravenous administration exhibited typical biexponential decline. The duration and general shape of the rapid disposition phase were remarkably similar, whereas the slopes of the slow disposition phase varied among subjects (Table I). This result may have been due to more pronounced intersubject variation in the rate of metabolism than in the blood flow rate into tissues and the size of the peripheral compartment for the drug.

The biexponential plasma level profile of this drug indicates that a two-compartment body model can be used to describe adequately the pharmacokinetic properties of pentylenetetrazol in the dog. Several investigators (5, 6) discussed the use of a two-compartment model in pharmacokinetic studies. According to this model, pentylenetetrazol seems to be redistributed rapidly into a peripheral compartment after initial mixing in the central compartment. The slow distribution of this drug into the secondary compartment may be controlled primarily by the partition properties of the drug and different blood flow rates into various body tissues.

[2] Vacutainer, Becton-Dickinson, Rutherford, N.J.

Table I—Pharmacokinetic Parameters of Pentylenetetrazol in Dogs Based on the Two-Compartment Open Model

	Dog 1	Dog 2[a]		Dog 3	Dog 4	Dog 5
Dose, mg/kg	20	15	15	15	15	15
Weight, kg	11	12.5	12.5	9	10	13
A, μg/ml	73	17	24	10	34	23
B, μg/ml	31	19	18	29	28	17
α, hr^{-1}	8.56	6.9	7.92	9.6	12.5	10.2
β, hr^{-1}	0.67	0.54	0.53	0.59	0.784	0.32
Half-life[b], hr	1.03	1.28	1.3	1.17	0.88	2.17
C_p^0, μg/ml	104	36	42	39	62	40
V_p[c], liters	2.12	5.2	4.46	3.46	2.42	4.88
V_p, l/kg	0.19	0.42	0.36	0.39	0.242	0.38
$(V_d)_{ss}$[d], liters	5.1	8.68	8.81	5.1	4.70	10.6
$(V_d)_{ss}$, l/kg	0.46	0.69	0.71	0.59	0.47	0.81
k_{12}, hr^{-1}	4.29	2.6	3.61	3.01	5.61	5.29
k_{21}, hr^{-1}	3.05	3.88	3.7	6.4	6.05	4.53
k_{el}, hr^{-1}	1.89	0.96	1.14	0.78	1.62	0.72

[a] Plasma level data were obtained at two separate occasions in the same dog. [b] $T_{1/2} = 0.693/\beta$. [c] Volume of central compartment, V_p = dose/C_p^0. [d] Volume of distribution at steady state, $(V_d)_{ss} = V_p \times (k_{12} + k_{21})/k_{21}$.

Table I summarizes the pharmacokinetic data obtained after single rapid intravenous injections of pentylenetetrazol in five dogs. The data show that pentylenetetrazol was rapidly eliminated from the body with a mean half-life of 1.4 hr. The range of 0.88–2.2 hr indicates significant intersubject variation. Since pentylenetetrazol is metabolized completely in the dog, the variation in the rate of elimination was primarily due to different enzyme activities in the liver among different subjects. The volumes of the central compartment in these dogs (0.19–0.42 liter/kg) appeared to be approximately equivalent to the extracellular body water of the dog (0.190–0.350 liter/kg) (7), whereas the volumes of distribution at the steady state, $(V_d)_{ss}$ (0.46–0.810 liter/kg), seemed to be consistent with the physiological volume of the total body water of the dog (0.500–0.800 liter/kg) (7). These findings indicate that pentylenetetrazol initially mixes in the extracellular fluid (central compartment), including the blood, and then rapidly redistributes into the extracellular tissue fluid (peripheral compartment). In this model, the highly perfused tissue groups such as the liver and kidney are included in the central compartment.

Figure 1 shows a typical plasma level profile of pentylenetetrazol in Dog 1, 11 kg, after rapid intravenous injection of a 20-mg/kg dose. The profile was plotted on semilogarithmic graph paper with an expanded time scale. Frequent sampling during the first 20 min clearly indicated a biexponential decline of plasma levels. The first two samples at 3 and 5 min postdose showed that complete mixing of the drug in the central compartment occurred very rapidly, whereas the distribution of the drug into the tissue compartment appeared to be completed approximately 20 min after injection. The rapid initial decline in the plasma level indicated that the drug redistributed into the peripheral compartment. After completion of equilibrium in the tissues, the plasma level profile exhibited a linear decline (β-phase). Figure 1 also illustrates the graphical evaluation of the biexponential plasma level profile for the two-compartment open model. The pharmacokinetic parameters of the drug in this dog are listed in Table I.

Figure 2 compares two plasma level profiles in Dog 3, 9 kg, after rapid intravenous and oral administrations of a 15-mg/kg dose. The biexponential decline is shown clearly in the intravenous plasma level profile (curve A). Following oral administration of pentylenetetrazol in aqueous solution, the maximal peak concentration occurred about 30 min after administration (curve B), indicating that this drug is absorbed very rapidly and completely in the GI tract. This finding is in good agreement with the literature information on the absorption of this drug (8). The rapid absorption may be due to unusual physicochemical properties of the drug. Pentylenetetrazol does not include a strong acidic or basic functional group in the molecule and should remain primarily in the unionized form in the GI tract. Solubility also may be a factor for rapid and complete absorption, because this drug is freely soluble in both polar and nonpolar solvents (9).

The areas under the plasma level–time curves were almost the same

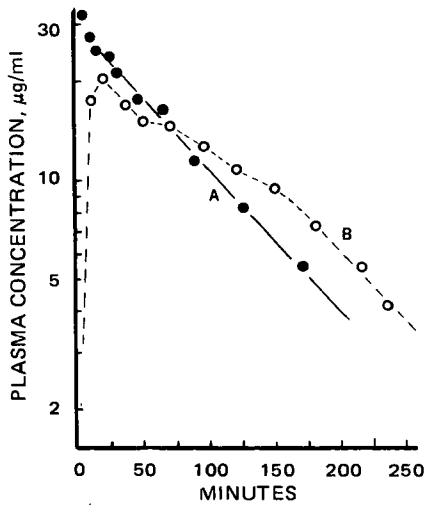


Figure 2—*Plasma concentrations of pentylenetetrazol in a dog (9 kg) after a rapid intravenous injection of 15 mg/kg (●) and oral administration of 15 mg/kg (○).*

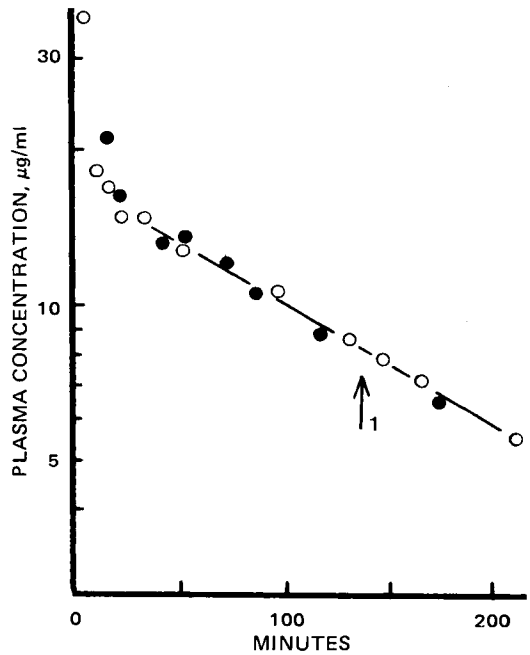


Figure 3—*Plasma concentrations of pentylenetetrazol in a dog (13 kg) after rapid intravenous administrations of 15 mg/kg at two separate times, about 2 weeks apart. Arrow 1 indicates the time at which the kidneys were completely ligated in one experiment (○).*

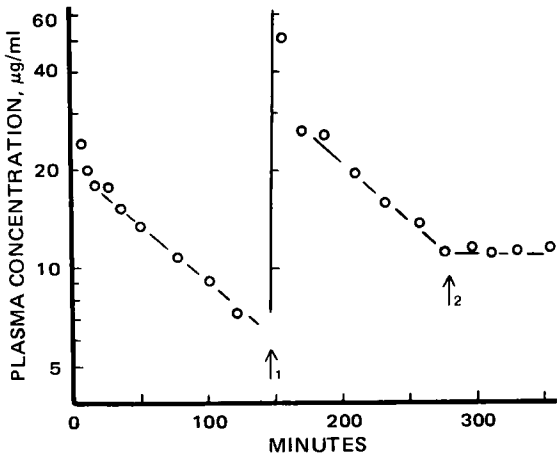


Figure 4—*Plasma concentrations of pentylenetetrazol in a dog (12 kg) after rapid intravenous administration of 15 mg/kg. Arrow 1 indicates the second intravenous dose (15 mg/kg). Arrow 2 shows the time at which the liver was completely ligated.*

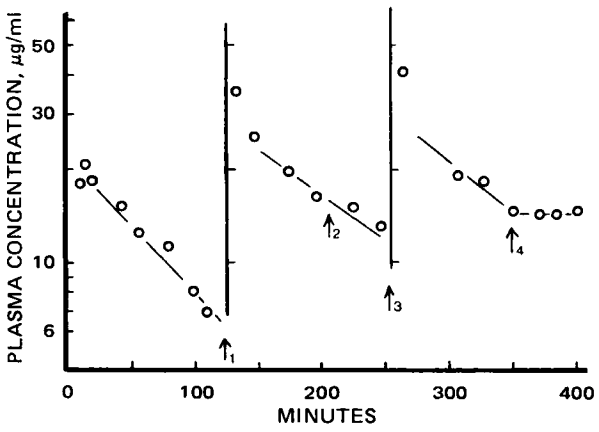


Figure 5—*Plasma concentrations of pentylenetetrazol in a dog (12 kg) after three successive intravenous administrations of equal doses of 15 mg/kg. Key: arrow 1, second intravenous dose; arrow 2, time at which the kidney was ligated; arrow 3, third intravenous dose; and arrow 4, time at which the liver was ligated.*

for the intravenous and oral plasma level–time curves, indicating the complete absorption after oral administration and the minimal first-pass metabolism effect. If the drug was significantly metabolized during the first passage through the liver, a reduced area under the curve would have been seen. The percent ratio of the area under the oral plasma level–time curve to that of the intravenous dose was about 98%. Figure 2 also shows similar biological half-lives of the drug for intravenous and oral routes (1.17 hr).

Figure 3 shows the intravenous plasma level profile of pentylenetetrazol in Dog 5 before and after kidney ligation. The arrow indicates the time of the kidney ligation. After the ligation, the apparent elimination phase remained virtually unchanged, indicating that the kidneys play a minimal role in the elimination of the intact drug. In a similar study, Esplin and Woodbury (1) found that the removal of the kidneys of rats did not affect blood level profiles of pentylenetetrazol. It is clear from this experiment that pentylenetetrazol itself is not excreted in urine in significant amounts, since the kidney ligation does not seem to alter the elimination rate of pentylenetetrazol. In this dog, almost identical plasma level profiles were obtained when an equal dose (15 mg/kg) of pentylenetetrazol was administered at two different times.

Figure 4 illustrates the effect of the liver ligation on the plasma level of pentylenetetrazol. After the first and second intravenous doses, the plasma level declined biexponentially. However, the ligation of the liver, at 2 hr after the second dose, seemed to block the elimination of the drug completely. This result indicates that the liver is probably mainly responsible for the elimination of pentylenetetrazol in the dog. These results are in agreement with the data reported by Esplin and Woodbury (1), who found that hepatectomy of the rat greatly decreased the rate of disappearance of intact pentylenetetrazol. Using rabbits, Tatum and Kozelka (10) showed that liver damage with carbon tetrachloride prevented the elimination of this drug. Therefore, the elimination rate constant for this drug represents the rate of biotransformation in the liver.

Figure 5 further supports the findings of the role of the kidney and liver in the elimination of pentylenetetrazol. In this experiment, when the kidney was first ligated during the second dose interval, no significant effect on the plasma level was observed. However, the liver ligation occurring after the third dose completely stopped the elimination of the drug. Arrows 2 and 4 indicate the times of the kidney and liver ligation, respectively. In this experiment, the dog was alive until the last blood sampling.

In summary, the two-compartment open model adequately describes the pharmacokinetic profiles of pentylenetetrazol in the dog. After oral administration, the drug enters the blood circulation rapidly and completely. The volume of the total body water appears to represent the distribution volume of the drug at the steady state. The elimination of pentylenetetrazol occurs rapidly in the dog. The liver and the kidney ligation studies indicate that the liver is primarily responsible for the elimination. In the clinical use of pentylenetetrazol, it may be important to be aware of the possible effects of administering this drug to patients with severe liver dysfunction. Accumulation of the drug in the body and subsequent toxic effects may occur in these patients.

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