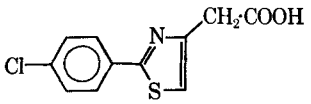
Pharmacokinetics of Fenclozic Acid in Animals and Man

D. S. PLATT

Abstract □ The pharmacokinetic properties of the anti-inflammatory agent, fenclozic acid, have been established in the dog, calf, sheep, and horse after intravenous administration and also in the rat, mouse, guinea pig, monkey, and man after oral dosage. At doses equivalent to those therapeutically effective in the rat, the pharmacokinetics of fenclozic acid are described in all species by the concept of the two-compartment open model. In all species, the compound is restricted mainly within the central compartment, the volume of which is less than 20% of the body weight. Due to serum protein binding, the vascular lymphatic system constitutes a large part of the central compartment volume. The biological half-life of fenclozic acid varies between 3 hr. in the monkey and 118 hr. in the horse; in the guinea pig, rat, dog, and man the half-life is in the range 26–31 hr. There is a direct relationship between the logarithm of the serum concentration and the activity in the adjuvant-induced arthritis test in rats. The pharmacokinetic information obtained has been used in the design of human clinical trials.

Keyphrases □ Fenclozic and ^{14}C-fenclozic acids—pharmacokinetic properties □ Pharmacokinetic parameters—fenclozic acid in animals, humans □ Arthritic subjects—fenclozic acid serum level relation, activity □ UV spectrophotometry—analysis □ TLC—separation, identification

The experimental syndrome of adjuvant-induced arthritis in rats has been used in these laboratories for several years for the detection and evaluation of compounds that may have use in the treatment of rheumatoid arthritis in man (1). Fenclozic acid (I.C.I. 54,450) (Structure I) is one of a series of substituted phenyl-



fenclozic acid [I.C.I. 54,450; 2-(*p*-chlorophenyl)thiazol-4-yl acetic acid]

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thiazolyl acetic acids synthesized in these laboratories which possesses activity in this screening test (2). The pharmacological properties (3) and the metabolism (4) of this compound have been reported. The results of preliminary studies in man appeared recently (5).

In this paper, the pharmacokinetic properties of fenclozic acid in various animal species including man are discussed. The objectives of these serum concentration studies were: (*a*) to compare the properties of fenclozic acid in the different species; (*b*) to define the relationship between serum concentration and activity in the rat arthritis test; and (*c*) to use the information obtained in the design of human clinical trials.

EXPERIMENTAL

Dosing Procedures—Fenclozic acid was administered orally to mice, rats, guinea pigs, and rhesus monkeys by stomach tube as a ball-milled suspension in an inert dispersing fluid. In dogs, fenclozic acid was administered intravenously as either the sodium or *N*-methylglucamine salt dissolved in isotonic saline; for oral dosing, the drug was formulated into plain, uncoated tablets. For intravenous experiments in a calf, sheep, and horse, fenclozic acid was given as a solution of the *N*-methylglucamine salt in isotonic saline; the same solution was used in an oral dose experiment in the calf.

Fenclozic acid was formulated into plain, uncoated tablets or plain capsules for administration to man. In the radioactive experiment reported in this paper, 100 mg. ^{14}C-labeled fenclozic acid (specific activity 0.227 μc./mg.) was filled into a capsule for oral administration.

Analytical Procedure—Fenclozic acid was extracted from serum and assayed by the following method: To 1.0 ml. serum in a stoppered tube were added 1.0 ml. 1.5 *N* HCl and 5 ml. 2,2,4-trimethylpentane containing 5% v/v amyl alcohol. The mixture was shaken for 5–10 min. and centrifuged at low speed to separate the layers. The concentration of fenclozic acid was determined directly by measuring the absorbance of the organic layer at 300 nm. in a conventional spectrophotometer against a reference blank prepared by substituting water for serum in this procedure.

Fenclozic acid is stable in serum for several weeks at 4°. The overall recovery of drug added in known amounts to serum and extracted as described was 75% at initial concentrations in serum ranging from 1 to 500 mcg./ml. The recovery factor is the same for serum from all species, and replicate analyses gave a coefficient of variation not exceeding 5%. A standard solution of fenclozic acid in the organic solvent (1 mcg./ml.) gives an absorbance at 300 nm. of 0.073 in a 1-cm. cell; the response is linear in the range 1–30 mcg./ml.

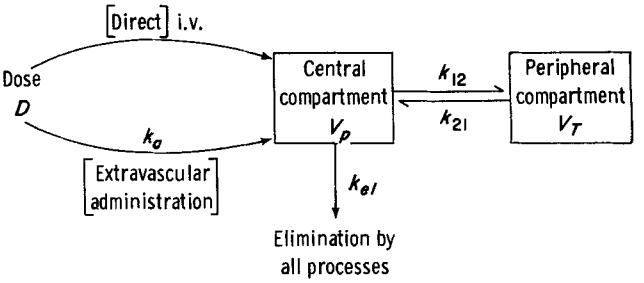
The identity of the extracted material was checked by subjecting aliquots of the organic extract to TLC on fluorescent silica gel plates, using an isopropanol–ammonia–water solvent (20:1:2). Under UV illumination, fenclozic acid and derivatives such as the decarboxylated product or phenyl-ring hydroxylation products quench the background fluorescence; only free fenclozic acid was detected in the organic serum extracts from all species.

Pharmacokinetic Analysis—After intravenous administration, the serum levels of fenclozic acid followed a biexponential decay according to Eq. 1:

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

where Cp is the serum concentration at any time (t) after the dose, α and β are first-order rate constants of the serum level curve, and A and B are zero-time intercepts of the two components of the biexponential curve.

This biexponential decay is implicit to the two-compartment open-model shown in Scheme I. The serum level data of fenclozic acid were subjected, therefore, to a two-compartment pharmacokinetic analysis (6–8) to define both the rate constants of distribu-



Scheme I—The two-compartment open model; k_{12}, k_{21}, *and* k_{el} *are first-order rate constants;* V_p *and* V_T *are the volumes of the two compartments; and* k_a *is a first-order absorption rate constant, when drug is administered by extravascular route.*

Table I—Two-Compartment Analysis of Serum Concentration Data in the Dog, Calf, Sheep, and Horse after Single Intravenous Doses

Constant[b]	Units	Dog (n = 9)[a]	Calf (n = 1)	Sheep (n = 2)	Horse (n = 1)
Dose	mg./kg.	13.7 ± 2.0	10.0	10.0	10.0
A	mcg./ml.	42.9 ± 6.9	36.0	46.5	22.2
B	mcg./ml.	55.8 ± 9.0	100.0	87.0	55.1
Cp^0	mcg./ml.	98.7 ± 13.6	136.0	133.5	77.3
α	hr.$^{-1}$	0.619 ± 0.055	1.925	5.732	0.330
β	hr.$^{-1}$	0.0252 ± 0.002	0.0636	0.152	0.0059
k_{12}	hr.$^{-1}$	0.240 ± 0.022	0.471	1.818	0.091
k_{21}	hr.$^{-1}$	0.361 ± 0.044	1.432	3.832	0.237
k_{el}	hr.$^{-1}$	0.0432 ± 0.004	0.0855	0.235	0.0082
V_p	%[c]	13.9 ± 0.9	7.35	7.60	12.9
V_T	%[c]	9.2 ± 1.1	2.40	3.55	5.0
Vd_{ss}	%[c]	23.1 ± 1.8	9.75	11.15	17.9
$Vd_{ext.}$	%[c]	24.6 ± 2.1	10.00	11.50	18.1
Total area	mcg. hr./ml.	2290 ± 323	1590	600	9400
Half-lives					
$0.693/\beta$	hr.	28 ± 2.5	11	4.5	118
$0.693/k_{el}$	hr.	16 ± 1.2	8	3	84

[a] Values expressed as mean ± *SE*. [b] See *Experimental* section for definition of constants. [c] Volumes expressed as a percentage of total body weight.

tion and elimination and the volumes of distribution *in vivo*. The pharmacokinetic constants, A, B, C_p^0, α, β, k_{12}, k_{21}, k_{el}, V_p, V_T, Vd_{ss}, and $Vd_{ext.}$ have the same meaning as given by Riegelman *et al.* (6, 8). The material balance equations of Riegelman *et al.* (6) were used to derive the fractions of the dose in each compartment at various times after the intravenous dose.

The technique given by Loo and Riegelman (9) was used to calculate the extent of absorption of fenclozic acid into a two-compartment system after oral administration and, where possible, a first-order rate constant for absorption (k_a) was derived. In those cases where intravenously derived constants were not available, serum concentrations were fitted with the aid of a digital computer (GEIS time-shared system), programmed to solve the equations of the two-compartment system with a first-order rate of absorption into the central compartment (10). (Neither iterative digital procedures nor analog facilities were available; the data generated are, therefore, only approximate.)

It was shown (11, 12) that in certain circumstances the kinetics of the two-compartment system can be simplified to those of the one-compartment system when it is required to predict, using single-dose data, the blood levels after multiple oral or intravenous doses of a drug. In general, this simplified technique can be employed when the area (A/α), associated with distribution, is small relative to the total area under the curve. It is a necessary assump-

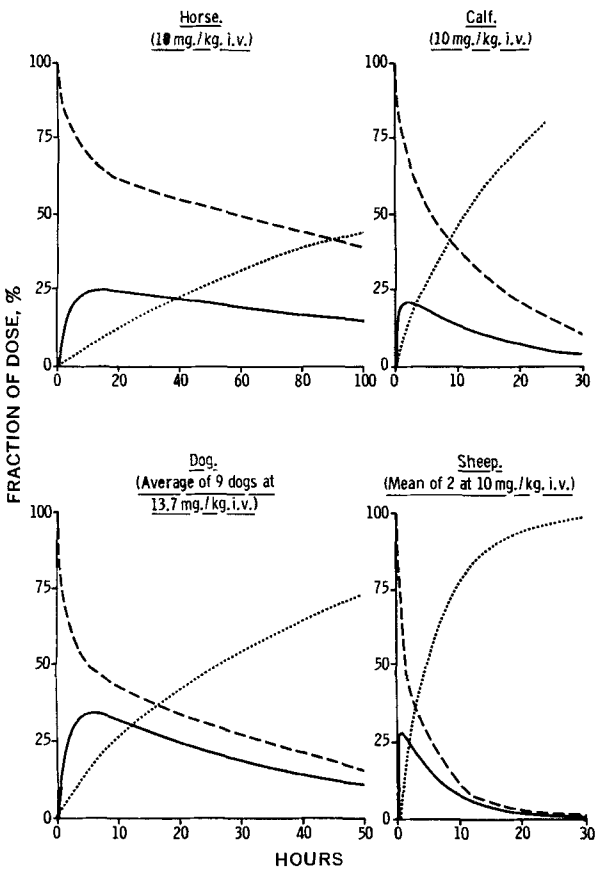


Figure 1—*Distribution and elimination of fenclozic acid in the dog, horse, calf, and sheep following intravenous administration. Curves are generated from the pharmacokinetic constants given in Table I. Key: ..., eliminated; ---, central compartment; and ——, peripheral compartment.*

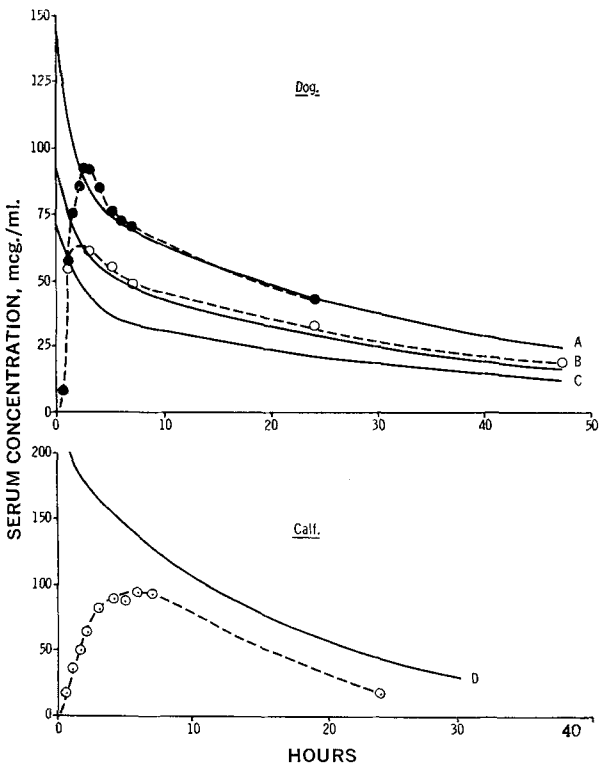


Figure 2—*Comparison of serum concentrations in dogs and a calf after oral or intravenous administration of fenclozic acid. Solid curves are generated from biexponential equations fitting intravenous data. Broken curves are observed concentrations after oral administration. Key: ●---●, dog, 20 mg./kg.* p.o.; *O---O, dog, 10 mg./kg.* p.o.; *and ⊙---⊙, calf, 20 mg./kg.* p.o. *Curve A = dog, 20 mg./kg. i.v. from equation* $Cp = 62.6e^{-0.619t} + 81.4e^{-0.0252t}$. *Curve B = dog, 13.7 mg./kg. i.v. from equation* $Cp = 42.9e^{-0.619t} + 55.8e^{-0.0252t}$. *Curve C = dog, 10 mg./kg. i.v. from equation* $Cp = 31.3e^{-0.619t} + 40.7e^{-0.0252t}$. *Curve D = calf, 20 mg./kg. i.v. from equation* $Cp = 72e^{-1.925t} + 200e^{-0.0636t}$.

Table II—Serum Concentrations of Fenclozic Acid after Single Oral Doses

Hours after Dose	Serum Concentration, mcg./ml.[a] Dog 10-mg./kg. Dose	Dog 20-mg./kg. Dose	Calf, 20-mg./kg. Dose	Rat, 10-mg./kg. Dose	Guinea Pig, 10-mg./kg. Dose	Rhesus Monkey, 10-mg./kg. Dose[b]	Mouse, 10-mg./kg. Dose[c]
0.5		8.4(2)	17.9				
1	54.0 ± 3.4(12)	57.1(2)	36.0	41.5(2)	51.4(2)	53.8 ± 4.3(6)	38.5
1.5		75.4(2)	49.6				
2		85.4(2)	64.0				
2.5		92.3(2)					
3	61.1 ± 1.4(12)	92.4(2)	81.1	53.0(2)	56.4 ± 6.4(4)	37.3 ± 1.9(6)	45.0
4		85.0(2)	89.3				
5	55.3 ± 1.9(12)	76.2(2)	87.5	61.8 ± 1.3(4)	55.7(2)	20.2 ± 2.7(6)	40.0
6		73.2(2)	93.7	56.6 ± 2.0(5)			
7	49.0 ± 1.8(12)	71.0(2)	93.0	66.5(2)	57.8 ± 4.6(4)	13.8 ± 1.2(6)	41.0
24	32.7 ± 1.4(12)	43.0(2)	17.6	37.0 ± 1.5(7)	44.9 ± 3.2(4)		11.5
30					34.4(2)		
48	19.2 ± 1.3(12)		0	20.8 ± 1.9(4)	17.0(2)		
72	14.4 ± 1.3(12)			10.0(1)	10.9(2)		
96				7.5(2)			

[a] When more than two results, mean expressed ±*SE*, with the number of animals in parentheses. [b] See text. [c] Each result from assay of pooled serum sample from five identically treated mice.

tion that each oral dose is completely absorbed. The pharmacokinetic properties of fenclozic acid are in this category. Since serum levels should achieve a steady-state level after multiple administration of a fixed dose at fixed time intervals, the average plateau serum levels after multiple doses to rats, guinea pigs, and dogs were calculated using Eq. 2 (13):

$$C_{av.} = \frac{1.44 \cdot D \cdot t_{1/2}}{Vd_{ext.} \cdot \Delta t} \qquad \text{(Eq. 2)}$$

where $C_{av.}$ is the average plateau serum level over the time interval between doses (Δt), D is dose, $t_{1/2}$ is the biological half-life (0.693/β), and $Vd_{ext.}$ is the biased estimate of the true volume of distribution (8).

When a compound is given by an extravascular route and the terminal, linear segment of the semilogarithmic plot of serum concentration is extrapolated back to zero time, the result obtained by dividing the intercept value into the dose is usually an underestimate of $Vd_{ext.}$ (=dose/V for an intravenous dose) and is a complex of several variables (14). If, however, evidence from a comparison with intravenous data indicates that absorption is both rapid and complete and if α is large relative to β, then the ratio of dose to extrapolated zero-time intercept will give a reasonable estimate of $Vd_{ext.}$. It will be shown later that this approximation can be used at low extravascular doses of fenclozic acid.

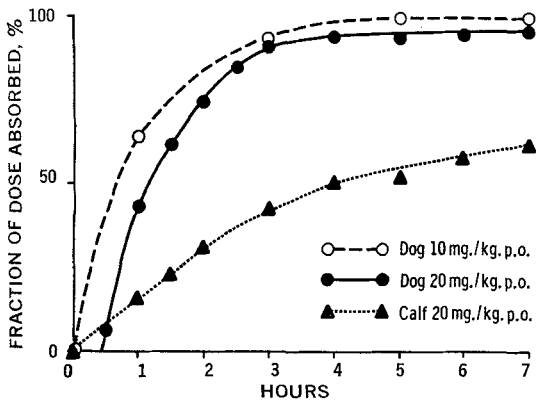


Figure 3—*Extent of absorption of fenclozic acid after oral administration to dogs and a calf. Constants used in the calculations were:* (a) *Dog (10 mg./kg.); total dose, 120 mg.;* k_{12}, *0.240;* k_{21}, *0.361;* k_{el}, *0.0432;* V_p, *1.235 l.** (b) *Dog (20 mg./kg.); total dose, 240 mg.;* k_{12}, *0.240;* k_{21}, *0.361;* k_{el}, *0.0432;* V_p, *1.67 l.** (c) *Calf (20 mg./kg.); total dose, 3320 mg.;* k_{12}, *0.471;* k_{21}, *1.432;* k_{el}, *0.0855;* V_p, *12.2 l. (The* k_{12}, k_{21}, *and* k_{el} *are in hr.*$^{-1}$ *units.)*

**The* V_p *value at the 10-mg./kg. dose level is that calculated from oral dose serum concentration observations (see text). The* V_p *value at the 20-mg./kg. dose level is that observed after intravenous administration (Table I).*

RESULTS

Intravenous Administration—Serum concentrations of fenclozic acid were measured in nine male dogs, one calf, two sheep, and a horse after single, rapid, intravenous injections. The dose ranged from 4 to 20 mg./kg. body weight in dogs; in the remaining species, the dose level was 10 mg./kg.

In every case, the serum levels exhibited a biexponential decay with time, consistent with the kinetics of the two-compartment open model discussed in the *Experimental* section. Biexponential equations to fit the data were derived graphically, using the technique of residuals. The rate constants of distribution and elimination and the volumes of distribution were calculated from these equations; the results are shown in Table I.

The results from the nine dogs were combined since there was a virtually linear relationship between dose and both zero-time concentration (Cp°) and the total area under the serum level–time curve in the dose range tested; a dose of 1 mg./kg. gave a mean Cp° value of 7.2 mcg./ml. and a total area of 167 mcg. hr./ml. The biological half-life (0.693/β), the half-life of elimination (0.693/k_{el}), and the steady-state volume of distribution (Vd_{ss}) were not affected by increasing the dose from 4 to 20 mg./kg. The biological half-life varied between 22 and 41 hr. in the individual dogs (average 28 hr.), whereas the half-life of elimination was in the range of 10–22 hr. (average 16 hr.). The steady-state Vd (Vd_{ss}) varied between 14.5 and 31.3% of body weight (average 23.2%); the biased estimate of total Vd ($Vd_{ext.}$) was on the average only 6% higher than the average Vd_{ss} result.

In the calf, sheep, and horse, as in the dog, the Vd_{ss} of fenclozic acid was low (9.75, 11.15, and 17.9% body weight, respectively); $Vd_{ext.}$ was only marginally higher than Vd_{ss} in all three species. There was an appreciable species variation in biological half-life (11, 4.5, and 118 hr., respectively).

Equilibrium dialysis studies showed that fenclozic acid is extensively bound to serum albumin in all species including man. The low values of Vd_{ss} (Table I) are consistent with a preferential retention of fenclozic acid within the central compartment, which is due, at least in part, to serum protein binding.

The amounts of fenclozic acid in the two body compartments and the amount eliminated from the body by all processes of metabolism and excretion at various times after the dose were calculated for all four species, using the constants given in Table I; the results are shown in Fig. 1. Despite time-scale differences, the pattern of distribution was similar in all four species, the central compartment predominating over the peripheral compartment at all times. The vascular lymphatic system constitutes a large part of the volume of the central compartment.

Oral Administration to Animals—Single oral doses of fenclozic acid were administered to dogs, a calf, mice, rats, guinea pigs, and rhesus monkeys. Twelve dogs (six males and six females) received

Table III—Serum Concentrations of Fenclozic Acid in Rats, Guinea Pigs, and Dogs after Multiple Oral Doses; Comparison of Observed and Calculated Values

Dose, mg./kg.	Serum Concentration (mcg./ml.) ±SE (n)[a]					
	Rat[b]		Guinea Pig[c]		Dog[d]	
	Observed	Calculated[e]	Observed	Calculated[e]	Observed	Calculated[e]
2.5	34 ± 5(3)	29	29 ± 2(3)	29	28 ± 3(4)	22
5	48 ± 1(3)	58	60 ± 3(3)	59		45
10	108 ± 4(10)	116	125 ± 10(3)	118	79 ± 7(8)	90
20	160 ± 14(3)	232	150 ± 4(3)	236	141 (2)	180
40	230 (2)	464	283 (1)	472	213 ± 24(3)	360
50	266 ± 11(15)	580			213 ± 16(6)	450
60					252 ± 34(4)	540
70					288 ± 20(7)	630
75	312 (2)	870				675
80	352 ± 9(4)	928	460 (2)	944	307 ± 19(7)	720
100	378 ± 23(3)	1160				

[a] (n) = number of individual animals tested. [b] Where $t_{1/2}$ = 29 hr., Δt = 24 hr., and $Vd_{ext.}$ = 15.2% body weight (body weight = 200 g.). [c] Where $t_{1/2}$ = 26 hr., Δt = 24 hr., and $Vd_{ext.}$ = 13.2% body weight (body weight = 400 g.). [d] Where $t_{1/2}$ = 30 hr., Δt = 24 hr., and $Vd_{ext.}$ = 20% body weight (body weight = 10 kg.). [e] Calculated using Eq. 2.

a single dose of 10 mg./kg., and two other dogs (one of each sex) had a dose of 20 mg./kg. No sex difference in serum concentrations of fenclozic acid was detected, and the mean levels are shown in Table II. Serum levels were measured in a calf (the same animal was used in the intravenous study) after a dose of 20 mg./kg. (Table II). Male rats, mice, and guinea pigs received oral doses of 10 mg./kg., with the results shown in Table II. Two female rhesus monkeys were examined on three separate occasions; in two experiments the animals received a dose of 5 mg./kg. and in the third experiment, 25 mg./kg. The serum concentrations in these monkeys were linearly related to dose; to simplify comparison with the other species, the observed levels were adjusted to a dose of 10 mg./kg. and the resultant concentrations combined to give the mean results shown in Table II.

Dogs—Experiments involving either intravenous or oral administration of fenclozic acid to dogs were carried out in different groups. Since a linear relationship between dose and serum level was observed in dogs after single intravenous doses of from 4 to 20 mg./kg., it was possible by manipulation of the mean zero-time intercepts *A* and *B* (Table I) to generate intravenous serum level–time curves for doses of 10 and 20 mg./kg. i.v. for comparison with the serum levels observed after equivalent oral doses. At a dose of 20 mg./kg., there was good agreement between generated intravenous data and observed oral data (Fig. 2); but at 10-mg./kg. doses, the oral results were in excess of, but not significantly different from, intravenous levels. In the latter instance, the oral data were virtually identical to the intravenous data for a mean dose of 13.7 mg./kg. Extrapolation of the terminal, linear segment of the semilogarithmic serum concentration plot (oral dose of 10 mg./kg.) to zero time gave an intercept of 55 mcg./ml., indicating a $Vd_{ext.}$ value not in excess of 18% body weight; the approximate mean biological half-life was 30 hr. (range 19–41 hr.). After intravenous administration, the mean $Vd_{ext.}$ was 24.6% body weight and the mean biological half-life was 28 hr. with a similar scatter to that observed after oral dosing. Thus, the oral dose serum levels gave an estimate of $Vd_{ext.}$ lower than that after intravenous doses.

The data in Fig. 2 indicated that absorption of the oral dose was complete in dogs; this was confirmed using the technique of Loo and Riegelman (9) as shown in Fig. 3. Absorption was essentially complete at 5 hr. after either dose level, although there was a delay of about 24 min. at the 20-mg./kg. dose; k_a values of 0.91 and 1.02 hr.$^{-1}$ were calculated, giving absorption half-lives of 0.76 and 0.68 hr. for the 10- and 20-mg./kg. doses, respectively. The calculations were checked by generating serum concentration curves with a digital computer (*Experimental* section), confirming that fenclozic acid was absorbed from dog gut by first-order kinetics into a two-compartment system.

Calf—From the intravenous constants given in Table I, and assuming linearity in the dose range 10–20 mg./kg., a serum level curve for a dose of 20 mg./kg. i.v. was calculated and compared with the oral data at the same dose (Fig. 2). Incomplete absorption from the gut was indicated. The overall extent of absorption, calculated by the technique of Loo and Riegelman (9), was about 70% (Fig. 3), with a first-order rate constant (k_a) of 0.295 hr.$^{-1}$ (absorption half-life, 2.4 hr.). The time required for maximal absorption was in excess of 7 hr., possibly due to the different anatomy of the ruminant alimentary tract.

Rats—The oral dose rat data given in Table II were fitted approximately by a digital computer programmed to solve the equation for first-order absorption into a two-compartment system (10). The following constants were generated: k_{12}, 0.154 hr.$^{-1}$; k_{21}, 0.437 hr.$^{-1}$; k_{el}, 0.033 hr.$^{-1}$; k_a, 0.5 hr.$^{-1}$ (half-life of absorption, 1.4 hr.); Vd_{ss}, 13.5% body weight; V_p, 11% body weight; α, 0.6 hr.$^{-1}$; β, 0.024 hr.$^{-1}$ (biological half-life, 29 hr.); and *B*, 66 mcg./ml. If, however, the terminal, linear segment of a simple semilogarithmic plot of the serum concentrations was extrapolated to zero time, an approximate intercept (*B*) value of 66 mcg./ml. was obtained, giving an approximate $Vd_{ext.}$ value of 15.2% body weight, with a biological half-life of 29 hr., *i.e.*, identical to the computer-generated values. In rats, therefore, reasonable estimates of *B*, β, and $Vd_{ext.}$ were obtained by the simple extrapolation technique. Absorption was complete at 7 hr. after the dose.

Guinea Pigs and Monkeys—Extrapolation of the guinea pig and monkey semilogarithmic serum concentration–time plots to zero time gave intercepts of 76 and 66 mcg./ml., indicating $Vd_{ext.}$ values of the order of 13.2 and 15.2% body weight, respectively. These approximations are in close agreement with the values observed in all other species at equivalent doses. The estimated biological

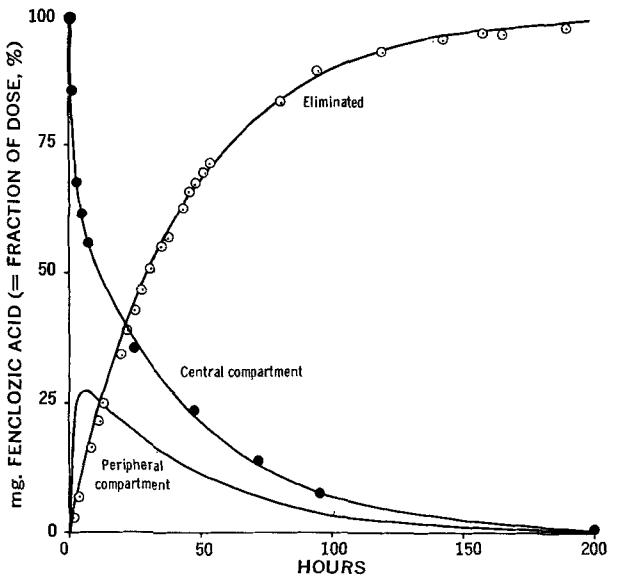


Figure 4—*Distribution and elimination of fenclozic acid in man after an oral dose of 1.43 mg./kg. Solid lines are data generated from the intravenous equation* $Cp = 6e^{-0.49t} + 11e^{-0.0224t}$, *since the observed absorption rate constant* (k_a = 7 hr.$^{-1}$) *was greatly in excess of* α *and* β. *Key:* ⊙, *observed total urinary excretion; and* ●, *observed serum concentration data expressed as a percentage of the zero-time concentration* (Cp° = 17 mcg./ml.).

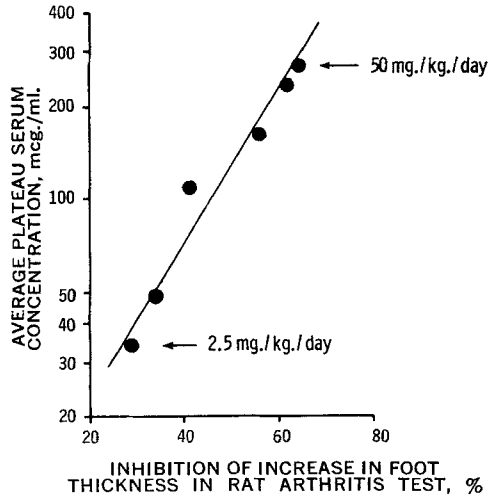


Figure 5—*Relationship of activity on Day 14 in the adjuvant-induced arthritis test in rats and serum concentration.*

half-lives were 26 and 3 hr. for guinea pigs and monkeys, respectively. By analogy with dog data, absorption of the oral dose was probably complete in both species, although the rate appeared to be faster in monkeys and somewhat slower in guinea pigs, the latter being very similar to rats.

Mice—At 10 mg./kg., the observed serum levels were lower than in dogs, rats, and guinea pigs. Accurate biological half-life estimation was not possible from the available data, but the results indicated a value not in excess of 11 hr. and a $Vd_{ext.}$ of not greater than 20% body weight. The shape of the serum level curve indicated a combination of slow absorption with a relatively rapid elimination, rather than incomplete absorption from the gut.

Oral Administration to Man—Serum concentrations of fenclozic acid were determined in human volunteers after single oral doses (in tablet or capsule form) in the range 1.5–6 mg./kg. The results of these studies were reported (5). Extrapolation of semilogarithmic serum level plots to zero time gave an average biological half-life of 25 hr., with an approximate $Vd_{ext.}$ value of 10.6% body weight; *i.e.*, the pharmacokinetic properties in man were similar to those in dogs, guinea pigs, and rats in respect to half-life and similar to all other species in respect to volume distribution.

A patient with rheumatoid arthritis weighing 70 kg. was given a single oral dose of 100 mg. ^{14}C-labeled fenclozic acid. Urine samples were collected for up to 190 hr. after the dose for determination of total radioactivity; 97% of the dosed radioactivity was recovered in the urine. Serum levels of fenclozic acid were determined up to 96 hr. The maximum serum level was observed at 1 hr. after the dose; thereafter the data had the appearance of a typical intravenous dosed serum level curve. A k_a value of about 7 hr.$^{-1}$ was required to allow a fit of the serum level data to be made, with $\alpha = 0.49$ hr.$^{-1}$ and $\beta = 0.0224$ hr.$^{-1}$; *i.e.*, k_a was at least 14-fold greater than α and 300-fold greater than β. [For the other human data (4), k_a values in excess of 2 hr.$^{-1}$ were required to give an approximate fit of the data.] The absorption phase, therefore, in this particular case can be effectively ignored and the data analyzed according to two-compartment intravenous kinetics (6–8).

The serum level data were fitted by the equation: $Cp = 6e^{-0.49t} + 11e^{-0.0224t}$, which on further analysis yielded the following constants: k_{12}, 0.154 hr.$^{-1}$; k_{21}, 0.325 hr.$^{-1}$; k_{el}, 0.0338 hr.$^{-1}$; Vd_{ss}, 12.4% body weight; and $Vd_{ext.}$, 13% body weight. The biological half-life ($0.693/\beta$) was 31 hr.

The amounts of fenclozic acid at various times after the dose in the central and peripheral compartments and also the amounts eliminated were calculated (6); the results are shown in Fig. 4. There was good agreement between observed urinary excretion data and elimination data calculated from the observed serum concentrations, confirming the validity of the two-compartment model in quantitating the fate of fenclozic acid in man and showing that the rate of elimination of human metabolites is dependent upon the rate of formation. The pattern of distribution and elimination in man, as shown in Fig. 4, was similar to that found in other species (Fig. 1).

The observed urinary excretion of total radioactivity confirmed that the oral dose of 100 mg. was completely absorbed, and a similar conclusion was reached following analysis of the serum level data using the technique of Loo and Riegelman (9).

Accumulation in Rat, Guinea Pig, and Dog Serum after Multiple Oral Doses—Serum concentrations of fenclozic acid were measured after 14 oral doses to rats, guinea pigs, and dogs; the results are shown in Table III. These results are compared with the average plateau concentrations, calculated using observed pharmacokinetic properties in Eq. 2. In all three species, there was good agreement between observed and calculated values in the dose range 2.5–10 mg./kg.; but at higher doses the observed values became progressively less than the calculated values. The reason for this discrepancy is not known but may be due in part to incomplete absorption. This factor may be of particular relevance at high doses of fenclozic acid, where ulceration of the gut is a common toxicological finding.

Relationship of Serum Concentrations in Rat to Activity in Adjuvant-Induced Arthritis Test—In the adjuvant-induced arthritis test in rats, compounds are given orally once daily for 14 days, starting on the day before injection of the adjuvant into the footpad. Activity is assessed by measuring the percentage inhibition of the increase in foot thickness of the injected foot on the 13th day following injection of the adjuvant (3).

Gibaldi (16) discussed the relationship between the magnitude of a pharmacological response and the amount of drug in the body and indicated the importance of establishing in pharmacodynamic studies the relationship between therapeutic response and body levels of drug, or some measure of the latter, such as blood concentrations. This relationship tends to be more meaningful than the dose–response relationship since the latter is influenced by changes in the mechanisms of absorption, distribution, and elimination of the drug in question.

The average observed plateau serum concentrations in rats after 14 days of dosing were compared with the observed activities in the rat arthritis test, expressed as percentage inhibitions of the increase in foot thickness (Fig. 5). There was a direct relationship between the logarithm of the serum concentration and therapeutic effectiveness, 50% inhibition occurring at a level of 120 mcg./ml. and 30% at a level of 40 mcg./ml.

DISCUSSION

Serum levels of fenclozic acid followed a biexponential decay in all species given an intravenous dose (dog, calf, sheep, and horse); a similar pattern of distribution and elimination was indicated in rats, guinea pigs, mice, monkeys, and man after oral administration. Application of the kinetic principles of the two-compartment open model (6–8) allowed further analysis of the data. In all species, the volume of the central compartment was small, being generally less than 20% of the animal body weight. At all times after the dose, the amount of fenclozic acid in this central compartment was in excess of that in the peripheral compartment; the vascular lymphatic system constitutes a large fraction of the central compartment since fenclozic acid is bound to serum albumin. The biological half-life varied over a wide range in the species tested, from 3 hr. in the rhesus monkey to 118 hr. in the horse; the dog, rat, guinea pig, and man exhibited similar half-lives (about 30 hr.), although there is considerable interspecies variation in the metabolism of fenclozic acid (4); glutamine conjugation is the major pathway in man (17).

First-order absorption kinetics were indicated in the dog, calf, rat, and man after oral administration either in tablet form, as a salt solution, or as a suspension. In each case the absorption rate constant (k_a) was considerably greater than the elimination rate constant (k_{el}). The most rapid absorption occurred in man (half-life 6–20 min.), followed by dog (about 40 min.), rat (about 80 min.), and calf (140 min.). The oral dose data in guinea pigs suggested a rate similar to that in rats; in monkeys, the rate was probably similar to that in man.

The relationship between distribution and elimination was such that serum levels of fenclozic acid after multiple doses could be adequately predicted using the simplified concept of the one-compartment model. There was good agreement between observed serum levels after multiple doses in rats, dogs, and guinea pigs and levels calculated from the single-dose data, but this was only true

at the lower doses tested; at higher doses, the observed levels were less than expected.

A direct relationship was observed between the logarithm of serum concentration and therapeutic effect in the adjuvant-induced arthritis test in rats. Significant activity in this test (30–50% inhibition) was associated with plateau serum levels in the range 40–120 mcg./ml., given by daily doses of about 5–10 mg./kg. Preliminary volunteer and clinical trials in man were designed on the basis of these observations in the belief that it is more rational to transpose from one animal species to another (in this case, rat to man) on the basis of comparative serum level data (18). Volunteer and patient studies established that doses of 100–200 mg. administered every 12 hr. gave rise to serum levels of fenclozic acid in the approximate range 50–100 mcg./ml. (5). Clinical effectiveness of fenclozic acid (100–200 mg. b.i.d.) was assessed in patients with rheumatoid arthritis in a double-blind crossover trial against aspirin (900 mg. q.i.d.), the results of which (19) lent support to this approach to clinical trial design. The results also allowed evaluation of the rat screening test in terms of human effectiveness.

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Dissociation Constants of Some Isomeric Aminoquinolines: Determination of the Site of Protonation from Shifts in Electronic Absorption Spectra

S. G. SCHULMAN

Abstract □ Dissociation constants of four isomeric aminoquinolines were determined spectrophotometrically and are compared with the same constants determined potentiometrically. The shifts in electronic absorption spectra of the compounds studied, upon protonation, are employed to assign the positions of protonation in the aminoquinolines. It was found that, in neutral and dilute acid solutions, a proton is added to the nitrogen atom in the heterocyclic rings in all compounds studied. Addition of a second proton, to the amino nitrogen, occurs only in strong acid solutions. 2-Aminoquinoline appears to exist in solution predominantly as an imino tautomer, while the other aminoquinolines studied exist predominantly in amino forms.

Keyphrases □ Aminoquinolines, isomeric—dissolution constants □ Dissociation constants—aminoquinolines □ Spectrophotometry, UV, visible—aminoquinolines, dissociation constants □ Potentiometry—aminoquinolines dissociation constants

The states of ionization of pharmacologically active substances and of biologically significant molecules are extremely important in determining the action of drugs. If these molecules have more than one group capable of dissociating or accepting protons in solutions, the sites of ionization may be equally important. Consequently, accurate knowledge of the sites of ionization corresponding to pKa values of drug molecules is vital to the study of chemical structure–biological activity relationships. Of several methods available for the determination of pKa values, potentiometry and spectrophotometry enjoy, by far, the greatest popularity. Potentiometry permits rapid and simple pKa determination but, in the case of polyacidic or polybasic molecules, does not permit simple evaluation of the site of dissociation or protonation unless the functional groups have widely differing characteristic pKa values in monofunctional molecules. In the latter case, the site of protonation can usually be inferred from the pH of the buffer regions of the titration curve. Unequivocal assignment of protonation sites, potentiometrically, can only be made by making derivatives of the molecule of interest in which all acidic or basic sites but one are blocked so that the ionizations can be evaluated one at a time. This is a time-consuming and frequently frustrating experience.