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Editor's Note: The scope of this article has been limited to a review of the literature in the area of pharmaceutics because reviews of the literature related to other areas of the pharmaceutical sciences are published elsewhere annually.

RESEARCH ARTICLES

Kinetics of Absorption and Excretion of Levodopa in Dogs

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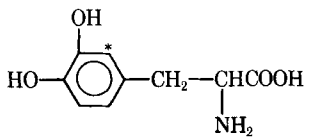
Abstract □ The rate and extent of levodopa absorption and excretion following intravenous and oral administration of 2-^{14}C-levodopa to acute and chronically treated dogs were investigated. Plasma levels of intact levodopa following the intravenous administration declined rapidly during the first 4–6 hr. The elimination-rate constant of intact levodopa from the plasma ranged between 0.73 and 0.99 hr.$^{-1}$, which corresponds to a half-life of approximately 40–60 min. The elimination-rate constant of total plasma ^{14}C ranged between 0.007 and 0.085 hr.$^{-1}$, which corresponds to a half-life of 8–9 hr., indicating that the total ^{14}C was eliminated at one-tenth the rate of intact levodopa. Peak plasma levels of intact levodopa and of total plasma ^{14}C following oral administration were attained 25–30 min. after dosing. Approximately 70–75% of the intravenous and 57–70% of the oral radioactive doses were excreted in urine over a 72-hr. period. Levodopa and dopamine accounted for a very small percentage of the radioactivity in the urine. Approximately 3.0–7.0% of the intravenous or oral radioactive dose was excreted in the feces. The efficiency of absorption of total radioactivity was calculated to range between 83.0 and 92.0%. Analysis of the ratio of intact levodopa levels to total ^{14}C levels indicated that only 22.0–30.0% of the administered dose reached the general circulation as intact levodopa, suggesting that the remainder of the absorbed dose, approximately 60.0%, is biotransformed in the gastrointestinal tract prior to absorption and/or in the liver during its "first passage" to the general circulation.

Keyphrases □ Levodopa, absorption and excretion, dogs—pharmacokinetics □ Pharmacokinetics—labeled levodopa absorption and excretion, dogs □ Absorption kinetics, labeled levodopa—oral, intravenous administration □ Excretion kinetics, labeled levodopa—oral, intravenous administration

Discovery of the presence of dopamine in the striatum and substantia nigral areas of the brain (1, 2) and of its depletion in patients with Parkinson's disease (3, 4) and in experimental animals following nigral lesions (5) provided new therapeutic approaches to Parkinsonism. Among the several means considered for correcting dopamine areas was the administration of the immediate precursor of dopamine, levodopa[1].

Consideration of the need for large doses of levodopa to attain therapeutic efficacy when given orally led to this investigation of the absorption and excretion of levodopa. The study was conducted under two experimental conditions: after the administration of a single dose and following chronic treatment of animals with levodopa. It was expected that the data obtained following the intravenous and oral administration of 2-^{14}C-levodopa (I) to dogs would yield the following information: (*a*) total plasma ^{14}C and intact levodopa levels and corresponding half-lives of elimination, (*b*) rate and extent of absorption from the gastrointestinal tract following oral administration of drug, (*c*) percent recovery of drug in urine, and (*d*) rate and extent of levodopa metabolism.



2-^{14}C-levodopa (*denotes position of radioactive label)
I

MATERIALS AND METHODS

Acute Single-Dose Study—Two beagle dogs, weighing 8–10 kg., were each given a single intravenous and oral 50-mg./kg. dose of 2-^{14}C-levodopa (Lot B-1, specific activity 0.19 μc./mg.). The dose (50 mg./kg.) selected was within the average range of the therapeutic dose administered to humans with Parkinson's disease, and it was

[1] L-3,4-Dihydroxyphenylalanine.

Table I—Doses of ^{14}C-Levodopa as Altered by Emesis

Dog Number and Route of Administration	Total Dose 2-^{14}C-Levodopa, mg.	^{14}C Content of Syringe Washings, mg.	Time of Emesis, min.	^{14}C Content of Emesis Fluid, mg.	Percent Dose Vomited	Actual Dose Received, mg.
Acute Single-Dose Study						
345, i.v.	465.00	25.16	2–6	0.21	0.05	439.63
1810, i.v.	410.15	15.78	2–6	0.15	0.04	394.22
345, p.o.	410.30	—	20	167.17	62.33	154.53
			50	88.60		
1810, p.o.	429.20	—	220	271.82	63.33	157.38
Chronic Multiple-Dose Study						
346, i.v.	481.70	9.08	4	0.04	0.01	472.58
349, i.v.	349.35	16.44	9	0.07	0.02	332.84
346, p.o.	441.00	—	20	2.68	0.61	438.92
349, p.o.	350.40	—	30	1.88	0.54	348.52

200 times below the acute oral toxic dose (LD_{50}) in rats. The intravenous dose was administered as a suspension in 50% propylene glycol, while the oral dose was given to the same animals in a gelatin capsule 2 weeks later.

Following intravenous injection of the drug, the syringe used to administer the dose was rinsed; the radioactive contents of these washings were determined by liquid scintillation counting techniques. Both animals vomited between 2 and 6 min. after dosing. The emesis fluid from each animal was collected, and the radioactive content of each specimen was determined[2] (Table I).

The administration of a single 50-mg./kg. oral dose of 2-^{14}C-levodopa had an emetic effect on both dogs. Dog 345 vomited on two separate occasions after dosing, at 20 and at 50 min. Dog 1810 vomited once, 220 min. after drug administration. The emesis fluid discharged by each animal was collected, and the radioactive content of each specimen was determined (Table I),

Chronic Multiple-Dose Study—Two beagle dogs, weighing 7–8 kg., were each given a single 50-mg./kg. daily oral dose of nonradioactive levodopa (Lot 114096) in a gelatin capsule.

On the 74th day of daily dosing, each of these primed animals received a single 50-mg./kg. oral dose of 2-^{14}C-levodopa, and the ^{14}C-plasma and urinary excretion patterns of the radioactive dose were monitored. As in the case of the single-dose study, both animals vomited, 20 and 30 min., respectively, after dosing. The emesis fluid of each animal was collected, and the radioactive content of each specimen was determined (Table I).

Daily oral dosing (50 mg./kg.) with nonradioactive levodopa was continued to the 87th day. On the 88th day, both animals were given a single 50-mg./kg. i.v. dose of 2-^{14}C-levodopa; once again the ^{14}C-plasma and urinary excretion patterns of the ^{14}C labeled drug were monitored. Both animals vomited, 4 and 9 min., respectively, after dosing. Although the volumes of the emesis fluid discharged were extremely small, the radioactive content of each specimen was determined (Table I).

Both chronically treated animals vomited daily during the first 4 weeks of oral dosing. Subsequently, from the 4th through the 10th week, both the emetic response to daily dosing and the volume of emesis fluid discharged gradually diminished. Inasmuch as emesis occurred infrequently after 10 weeks, the animals appeared to have developed a substantial degree of tolerance to the 50-mg./kg. oral dose of levodopa.

Collection of Specimens—Heparinized blood samples were collected from each animal at 0 hr. (control), at 1, 5, 10, 20, 30, and 45 min., and at 1, 1.5, 2, 3, 4, 6, 8, and 24 hr. after intravenous dosing. After oral administration, the first sample collected was at 10 min., followed by the schedule as indicated after intravenous dosing. All samples were centrifuged at 2000 r.p.m. in the cold (4°), and the ^{14}C content of the separated plasma was determined. Four milliliters of each plasma sample was added to 20.0 ml. of 0.4 *N* perchloric acid; the mixture was shaken for 15 min. on a mechanical shaker, allowed to stand overnight at 0°, and then centrifuged at 2000 r.p.m. for 20 min. at 4°. The perchloric acid extract obtained was decanted, and the radioactive contents of both the extract and residual precipitate were determined.

A 0–24-hr. urine specimen was collected from each animal prior to dosing (control) and from 0–24, 24–48, and 48–72 hr. after dosing. Each sample was collected in the cold at an acid pH (by inserting 1% of 6 *N* HCl in the collection flasks), and the ^{14}C content of each sample was determined.

Fecal specimens were collected from each animal 0–24 hr. prior to dosing (control) and from 0–24, 24–48, and 48–72 hr. after dosing. The samples were homogenized in aqueous acidic medium, and the ^{14}C content of each sample was determined.

Analyses of Specimens—Differential separation and quantitation of intact levodopa in the plasma extracts and in urine were conducted. The catecholamines were separated on alumina by the method of Anton and Sayre (6), while separation of levodopa from dopamine was achieved by the method of Sedvall *et al.* (7). Quantitation of intact levodopa and dopamine followed the spectrofluorometric method of Sourkes and Murphy (8).

RESULTS

Plasma levels of intact levodopa and total plasma ^{14}C (intact levodopa plus biotransformation products), following the administration of a single 50-mg./kg. i.v. dose of the labeled compound to two dogs, are presented in Fig. 1. The corresponding data obtained following chronic treatment are shown in Fig. 2. In both cases, these

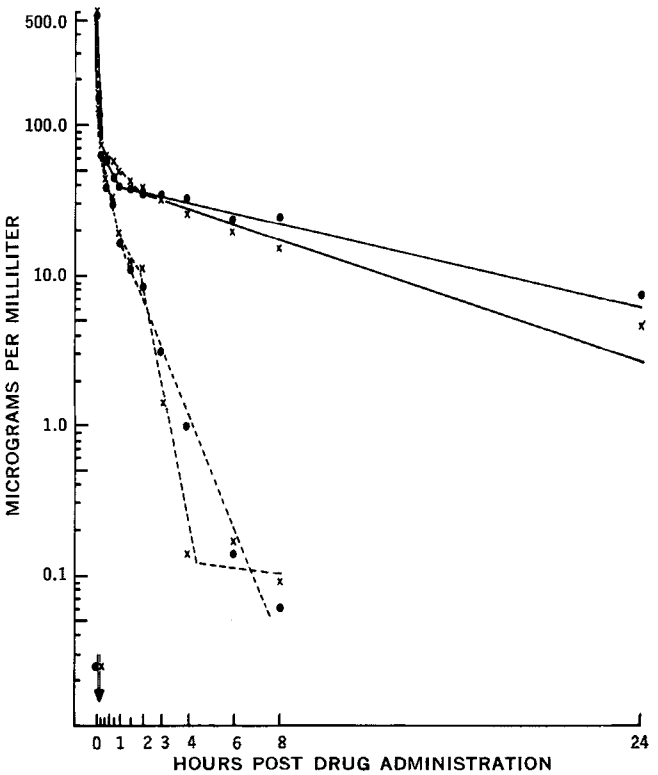


Figure 1—*Total plasma ^{14}C and intact levodopa levels following administration of a single 50-mg./kg. i.v. dose of 2-^{14}C-levodopa to dogs. Key: ●, Dog 345; ×, Dog 1810; —, total plasma ^{14}C; - - -, intact levodopa; and ↓, time of emesis.*

[2] Nuclear-Chicago Mark I scintillation spectrophotometer.

Table II—Percent of Levodopa Dose Excreted in Dog Urine following Administration of a 50-mg./kg. i.v. Dose of 2-^{14}C-Levodopa to Acute and Chronically Treated Animals

Dog Number	Time of Sample Collection, hr.	Percent of Levodopa Dose[a] Excreted			
		^{14}C Excreted	Intact Levodopa[b]	Intact Dopamine[b]	Nonidentified Components
		Acute Single-Dose Study			
345	0–24	66.520	0.040	0.410	66.100
	24–48	4.530	0.002	0.000	4.680
	48–72	2.210	0.001	0.001	2.200
	Total	73.300	0.043	0.411	72.980
1810	0–24	52.680	0.040	0.170	52.460
	24–48	20.710	0.020	0.030	20.660
	8–72	2.760	0.060	0.002	2.700
	Total	76.150	0.120	0.202	65.820
		Chronic Multiple-Dose Study			
346	0–24	56.030	0.026	1.806	56.002
	24–48	14.200	0.012	3.278	14.187
	48–72	1.660	0.101	2.486	1.560
	Total	71.890	0.139	7.570	71.749
349	0–24	65.920	1.617	0.037	64.300
	24–48	4.830	1.122	0.000	3.712
	48–72	0.920	1.286	5.224	—
	Total	71.670	4.025	5.261	—

[a] Dose of ^{14}C-levodopa as altered by emesis. [b] The percentage of these components excreted between 24 and 72 hr. in the multiple-dose study includes residual amounts from the nonradioactive daily doses.

levels fall rapidly during the 1st hr. Thereafter, the rate of decline of the total plasma ^{14}C becomes relatively slower compared to that of the intact levodopa.

During the first 4–6 hr., the plasma levels of intact levodopa fall approximately 5000-fold, after which they remain at a low level for the remainder of the experimental period.

The urinary excretion data presented in Table II show that approximately 75% of the radioactive dose is excreted during a 0–72-hr. period following the intravenous administration of 2-^{14}C-levodopa to the acute and chronically treated animals. Under both conditions, levodopa and dopamine account for a very small percentage of the total radioactive dose. The high percentage of nonidentified components present 24 hr. after dosing and the decrease in total ^{14}C excreted between 24–48 and 48–72 hr. suggest that the biotransformation products persist in the body considerably beyond 72 hr. after drug administration.

Plasma levels of total ^{14}C and of intact levodopa following the oral administration of a single 50-mg./kg. dose of 2-^{14}C-levodopa to two dogs are presented in Fig. 3, while the corresponding data obtained following chronic treatment are shown in Fig. 4. As previously mentioned, both dogs receiving the single dose underwent an emetic response, vomiting approximately 62% of the oral dose. From the total plasma ^{14}C and intact levodopa levels obtained in one of the two dogs, Dog 345 (Fig. 3), it appears that the dose of levodopa available in the gastrointestinal tract after emesis was rapidly absorbed. No detectable plasma ^{14}C levels were obtained in Dog 1810 until 1.5 hr. after oral administration.

From the plasma level data obtained following administration of a 50-mg./kg. oral dose of 2-^{14}C-levodopa to two chronically treated dogs (Fig. 4), it is apparent that the absorption of levodopa from the gastrointestinal tract of both animals is extremely rapid. Peak levels of total plasma ^{14}C and of intact levodopa are obtained 20–30 min

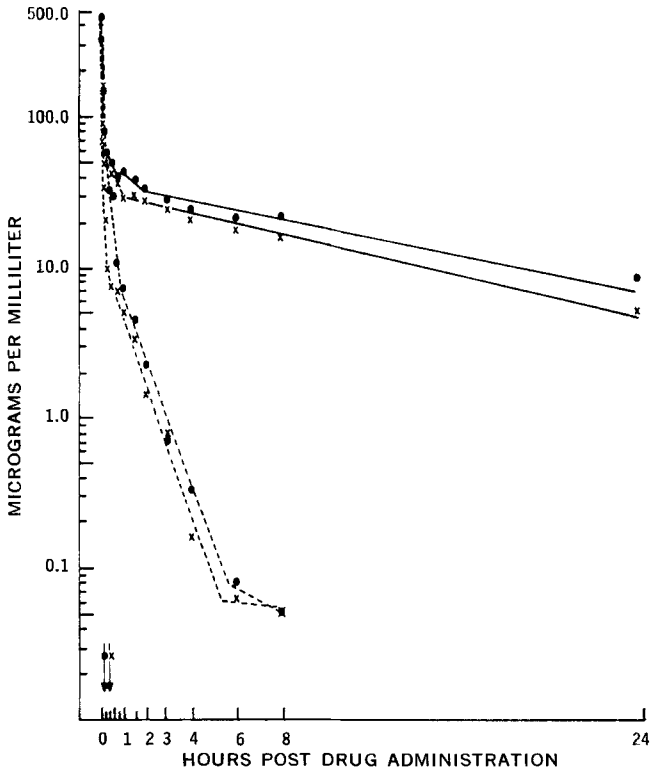


Figure 2—*Total plasma ^{14}C and intact levodopa levels following administration of a 50-mg./kg. i.v. dose of 2-^{14}C-levodopa to chronically treated dogs. Key: ●, Dog 346; ×, Dog 349; —, total plasma ^{14}C; - - -, intact levodopa; and ↓, time of emesis.*

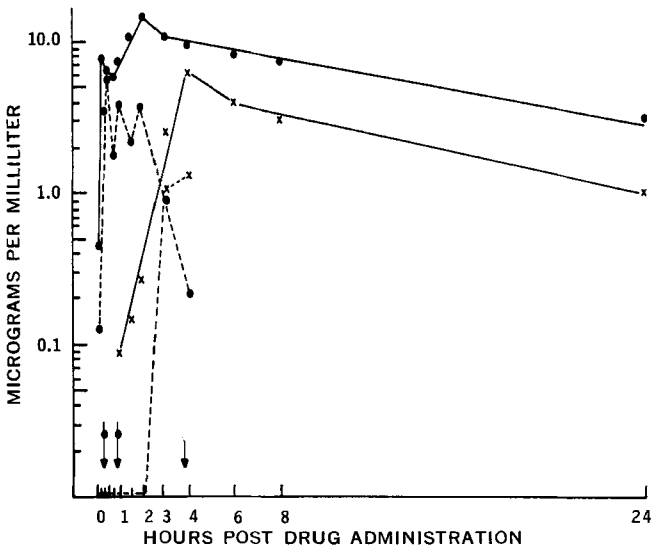


Figure 3—*Total plasma ^{14}C and intact levodopa levels following administration of a single 50-mg./kg. oral dose of 2-^{14}C-levodopa to dogs. Key: ●, Dog 345, ×, Dog 1810; —, total plasma ^{14}C; - - -, intact levodopa; and ↓, time of emesis.*

Table III—Percent of Levodopa Dose Excreted in Dog Urine following Administration of a 50-mg./kg. Oral Dose of 2-^{14}C-Levodopa to Acute and Chronically Treated Animals

Dog Number	Time of Sample Collection, hr.	Percent of Levodopa Dose[a] Excreted			
		^{14}C Excreted	Intact Levodopa[b]	Intact Dopamine[b]	Nonidentified Components
		Acute Single-Dose Study			
345	0–24	45.06	0.021	n.e.	45.036
	24–48	13.27	0.011	n.e.	13.259
	48–72	2.52	0.000	n.e.	2.520
	Total	60.85	0.032	—	60.815
1810	0–24	48.83	1.376	0.464	46.992
	24–48	5.37	0.039	0.030	5.300
	48–72	0.66	0.026	0.000	0.635
	Total	54.86	1.441	0.494	52.927
		Chronic Multiple-Dose Study			
346	0–24	61.19	0.209	1.819	60.984
	24–48	4.73	0.000	2.304	4.730
	48–72	1.60	0.528	2.055	1.069
	Total	67.52	0.737	6.178	66.783
349	0–24	65.39	1.354	0.224	64.039
	24–48	5.92	0.920	0.042	5.002
	48–72	1.21	0.552	6.260	0.661
	Total	72.52	2.826	6.526	69.702

[a] Dose of ^{14}C-levodopa as altered by emesis. [b] The percentage of these components excreted between 24 and 72 hr. in the multiple-dose study includes residual amounts from the nonradioactive daily doses; n.e. = not estimated.

after dosing. In addition, as in the case of the intravenous dose, the rate of decline of intact levodopa is considerably faster than that of the total plasma radioactivity.

The urinary excretion data presented in Table III show that 55–73% of the radioactive dose is excreted over a 0–72-hr. period following oral administration of 2-^{14}C-levodopa to both acute and chronically treated dogs. As was observed following intravenous dosing, intact levodopa and dopamine (measured spectrofluorometrically) account for only a small percentage of the dopamine and its metabolites (total radioactivity) excreted in the urine of these animals.

Table IV shows the amount of total ^{14}C excreted in dog feces following intravenous and oral administration of 2-^{14}C-levodopa to acute and chronically treated animals. No major differences appear to exist in the percent of the ^{14}C dose excreted following intravenous and oral dosing of 2-^{14}C-levodopa.

DISCUSSION

The results obtained following acute and chronic intravenous administration of levodopa to dogs (Figs. 1 and 2) compare favorably with the data of Guldberg and Yates (9) and indicate that intact drug is eliminated from plasma much faster than the total plasma radioactivity. In view of the fact that the difference between the levels of intact levodopa and total plasma ^{14}C is a function of levodopa absorption, tissue distribution, biotransformation, plasma-tissue recycling, and finally elimination, this difference serves as an indication of the presence, relative concentration, and rate of formation of biotransformation products. Some biotransformation products were identified and reported (9–11) to include homovanillic acid, *O*-methyldopa, and catechol acids.

In addition, the ratio of the percent of intact levodopa to the nonidentified components in urine (Table II) is indicative of the almost complete biotransformation of levodopa. These nonidentified components could include phenylacetic acid, phenylpyruvic acid, catecholamines, dihydroxymandelic acid, monophenolic acids, and *O*-methylated derivatives. The suggestion that the biotransformation products persist in the body considerably beyond 72 hr. after drug administration was confirmed by measurements for the presence of residual radioactivity in urine. These measurements showed that approximately 0.1–0.2% of the ^{14}C dose was excreted over a 24-hr. period at 5 days following intravenous administration of the radioactive dose to both acute and chronically treated animals.

Although the plasma data obtained following acute oral administration of levodopa to dogs (Fig. 3) do not lend themselves to interpretation of the absorption characteristics of levodopa from the gastrointestinal tract, the recovery of approximately 57% of the orally administered dose over a 72-hr. period (Table III), as compared to 75% of the intravenous dose (Table II), would indicate at least 76% efficiency of absorption.

As in the case of the intravenously dosed dogs, the ratio of the percentage of nonidentified components to that of intact levodopa in the urine suggests that levodopa undergoes extensive and rapid biotransformation following oral administration.

The plasma level curves following intravenous administration appear to be triexponential. However due to the limited data, only certain pharmacokinetic parameters could be determined (Table V). The elimination-rate constant, β, of intact levodopa from the plasma ranged between 0.73 and 1.02 hr.$^{-1}$ in the four dogs studied. This corresponds to a half-life range of approximately 40–60 min. The elimination-rate constant, β, of total plasma ^{14}C, which includes intact levodopa plus biotransformation products, ranged from 0.077 to 0.085 hr.$^{-1}$, which corresponds to a half-life range of 8–9 hr. Comparison of the intact and total ^{14}C elimination-rate constants indicates that the latter is eliminated approximately 10 times slower than the former. This marked difference in elimination-rate constants emphasizes the need for studying intact levodopa as opposed to total ^{14}C levels in defining the physiological disposition of the drug.

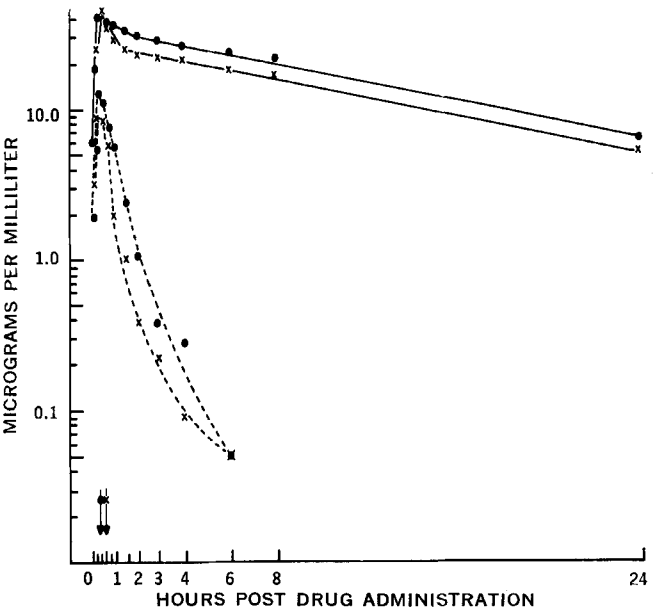


Figure 4—*Total plasma ^{14}C and intact levodopa levels following administration of a 50-mg./kg. oral dose of 2-^{14}C-levodopa to chronically treated dogs. Key: ●, Dog 346; ×, Dog 349; —, total plasma ^{14}C; - - -, intact levodopa; and ↓, time of emesis.*

Table IV—Percent of Levodopa Dose Excreted in Dog Feces following Intravenous and Oral Administration of a 50-mg./kg. Dose of 2-^{14}C-Levodopa to Acute and Chronically Treated Animals

Administration Route	Time of Sample Collection, hr.	Acute Single-Dose Study				Chronic—Multiple-Dose Study			
		Dog 345		Dog 1810		Dog 346		Dog 349	
		mg.	% Dose	mg.	% Dose	mg.	% Dose	mg.	% Dose
Intravenous	0–24	5.76	1.31	n.s.[a]	n.s.	2.96	0.63	8.82	2.65
	24–48	3.22	0.73	9.53	2.42	3.43	0.72	3.78	1.14
	48–72	3.94	0.90	0.72	0.18	0.23	0.05	0.81	0.24
	Total	12.92	2.94	10.25	2.60	6.62	1.40	13.41	4.03
Oral	0–24	0.08	0.05	2.03	1.29	17.09	3.90	23.42	6.72
	24.48	3.96	2.48	0.71	0.45	2.23	0.51	2.70	0.77
	48–72	1.04	0.65	0.16	0.10	0.43	0.10	0.65	0.19
	Total	5.08	3.18	2.90	1.84	19.75	4.51	26.77	7.68

[a] n.s. = no sample defecated.

Table V—Pharmacokinetic Parameters Describing Physiological Disposition of Intravenously and Orally Administered Levodopa in the Dog

	Single Dose		Chronic Dose	
	Dog 345	Dog 1810	Dog 346	Dog 349
Intravenous Administration				
Weight, kg.	9.3	8.2	9.8	7.0
Dose, mg.	440.0	394.0	473.0	333.0
Dose, mg./kg.	47.3	48.0	48.0	47.6
Intact levodopa:				
β, hr.$^{-1}$	0.986	0.948	0.726	1.018
0.693/β, hr. (half-life)	0.70	0.73	0.95	0.68
Percent dose excreted as intact L-dopa in 0–72-hr. urine	0.043	0.12	0.139	4.025
Total ^{14}C:				
β, hr.$^{-1}$	0.077	0.083	0.085	0.079
0.693/β, hr. (half-life)	9.0	8.3	—	8.8
Percent dose excreted in 0–72-hr. feces as total ^{14}C	2.94	2.60	1.40	4.03
Oral Administration				
Dose retained, mg.	159.50	157.40	438.30	348.50
Dose retained, mg./kg.	19.50	19.20	44.70	49.90
Ratio of area under plasma level curve[a] (p.o./i.v.)	0.83	—	0.92	0.92
Percent dose excreted in 0–72-hr. urine as total ^{14}C	60.90	54.90	67.50	72.50
Ratio of dose recovered in 0–72-hr. urine[a] (p.o./i.v.)	0.83	0.72	0.94	1.01
Percent dose excreted in 0–72-hr. feces as total ^{14}C	3.18	1.84	4.51	7.68

[a] Oral area corrected for dose administered.

The route of administration, intravenous or oral, may influence the magnitude of the area under a blood level curve for a rapidly and extensively biotransformed drug. When this occurs, the corresponding areas of intact drug cannot be used directly to assess the extent of drug absorption from the gastrointestinal tract. Comparison of the areas under the total ^{14}C oral and intravenous plasma level curves, however, indicates an efficiency of availability of total ^{14}C from the orally administered dose. This area comparison indicates an efficiency of absorption ranging from 83 to 92% of administered dose (Table V). To assess the proportion of orally administered dose absorbed into the general circulation as intact levodopa, assuming the remainder is degraded in the gastrointestinal tract and/or biotransformed during "first passage" through the liver, it is necessary to compare the ratio of intact levodopa levels to the total ^{14}C levels following each administration. Only the data in two dogs, Dog 345 and Dog 346, one singly dosed and one chronically treated, would lend themselves to this type of an evaluation.

It was estimated that 83% of the administered dose of ^{14}C was absorbed in Dog 345. However, only 22% of the administered dose reached the general circulation as intact levodopa. In Dog 346, 92% of the administered dose of ^{14}C was absorbed, with only 30% of the administered dose reaching the general circulation as intact levodopa. The remainder of the absorbed dose, approximately 60% in both dogs, may have been degraded in the gastrointestinal tract prior to absorption and/or biotransformed in the liver during its "first passage" on its way to the general circulation.

A further measure of the availability of administered ^{14}C following oral administration would be a comparison of the total percent of label (^{14}C) excreted following intravenous and oral administration. These values (Table V) indicated that a range of 72–100% of the administered ^{14}C was absorbed.

In conclusion, the data presented suggest that it is the low availability of intact levodopa following oral administration, coupled with its rapid elimination rate, that results in the need for large and frequent doses to achieve and maintain clinical efficacy.

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Kinetics and Mechanisms of Lactonization of Coumarinic Acids and Hydrolysis of Coumarins II

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Abstract □ The kinetics of lactonization of α-chloro-, α-bromo-, α-phenyl-, α-methyl-, and β-methylcoumarinic acids and the hydrolyses of their respective 3- and 4-substituted coumarins were studied at all pH values and at various temperatures. The order of hydrogen-ion-catalyzed lactonizations is consistent with α-substituted steric effects which destroy the acid-weakening resonance of the conjugated carboxylic system by disrupting the coplanarity of the carboxylic oxygen. The order of reactivity in alkaline hydrolysis is consistent with inductive effects, *i.e.*, accelerated by electron-withdrawing substituents. The order of reactivity for other derived microscopic rate constants is consistent with the model proposed previously to rationalize the apparent discrepancies between the apparent kinetic and spectral pKa′ values. The intramolecular formation of an anionic orthoacid lactonization intermediate by the attack of a phenate anion on the substituted coumarinic acid carboxyl, its subsequent protonation to the orthoacid $H_2C‡$, and its possible loss of a hydroxyl ion to form coumarin are inhibited by electron-withdrawing groups that modify carboxyl carbon electrophilicity. This order of reactivity for these rate constants are as expected for such mechanisms. The ratio of rates of spontaneous dehydration to hydrogen-catalyzed dehydration of the neutral orthoacid lactonization intermediate, $H_2C‡$, to yield coumarin decreases with electron-donating substituents, as expected by the proposed mechanism since hydrogen-ion attack should be inhibited by electron-withdrawing substituents such as halogens.

Keyphrases □ Coumarin hydrolysis—kinetics, mechanism □ Lactonization, coumarinic acids—kinetics, mechanism □ pKa′ values, apparent—coumarins, coumarinic acids □ Substituent effect—coumarinic acid lactonization, coumarin hydrolysis

It was shown (1) that the log k–pH profile for the lactonization of coumarinic acid and the hydrolysis of its lactone, coumarin, and the discrepancy between the spectral (or potentiometric) and apparent kinetic pK_{a_1}' values can be rationalized by a proposed mechanism. This mechanism assumes the intramolecular formation of an orthoacid, $H_2C‡$, which dehydrates both spontaneously and by hydrogen-ion catalysis to give coumarin. The steady-state assumption for $H_2C‡$ permitted the fitting of the log k–pH profile consistent with the analytical pK_{a_1}' value and was consistent with the proposed mechanism.

These present investigations were conducted to determine the pKa′ values, the log k–pH rate profiles, and temperature effects for the hydrolysis of variously 3- and 4-substituted coumarins and for the lactonization of their respective coumaric acids. The purposes were: (*a*) to compare their relative reactivities, (*b*) to compare the extent of the equilibria among all charged forms and the corresponding lactones, and (*c*) to test the proposed mechanism by the expected substituent effects.

EXPERIMENTAL

3-Chlorocoumarin[1] and 3-methylcoumarin[2] were used as received. The 3-phenylcoumarin[3] was recrystallized from dioxane–water and acetone–water mixtures. The preparation of 3-bromocoumarin and 4-methylcoumarin was described previously (1). The reactions were investigated at various pH values between −1 and 13 in hydrochloric acid, phosphate buffer, and sodium hydroxide solutions at temperatures between 8.5 and 50.5° (Tables I–III). All solutions were made up with nitrogen-purged distilled water and, if possible, adjusted to an ionic strength of 0.1 with sodium chloride. The pH at the temperatures of the kinetic runs was read with a Radiometer pH meter and a Sargent combination electrode, or it was calculated (1) from the known activities (2) in strong acid and alkaline solutions. The compositions of the buffer solutions are listed in Tables I–III. Details of specific procedures were the same as those given previously (1).

Lactonization—Generally, 0.05 ml. of about 4×10^{-3} *M* solution of a coumarin that had been completely solvolyzed in 0.01 *M* NaOH was added to 3.0 ml. of the appropriate buffer solution to produce a final concentration of about 6.5×10^{-5} *M*. Only 0.02 ml. of the 4×10^{-3} *M* solution of 3-phenylcoumarin was used due to its low solubility. The stock solutions of solvolyzed 3-bromocoumarin and 3-chlorocoumarin were prepared immediately before the lactonization studies, since small amounts of bromide and chloride ions were detected in the alkaline solutions after several weeks, even when they were stored under refrigeration. The ring closure of the coumarin derivatives was monitored from the change of the UV absorbance in the manner described previously for coumarin (1), and lactonization was complete in the absence of significant concentrations of coumarinate dianion. The wavelengths used for the kinetic studies were 280 nm. for 3-chlorocoumarin and 3- and 4-methylcoumarin; 280 and 295 nm. for 3-bromocoumarin; and 280 and 310 nm. for 3-phenylcoumarin.

The logarithms of the differences in the final absorbance, A_∞, and the absorbance at any time, A, at a specific wavelength were plotted against time. The apparent first-order rate constants (Tables I and II) were determined from the slopes of these plots in accordance with Eq. 1:

$$\log |A_\infty - A| = \frac{-kt}{2.303} + \log |A_\infty - A_0| \qquad \text{(Eq. 1)}$$

Hydrolysis—The hydrolyses of the coumarin derivatives were investigated in the pH region of 11–12.5 (Table III) where complete hydrolysis could be expected. No concentrated stock solution of these substituted coumarins could be prepared because of their low solubilities. These difficulties were circumvented by effecting the ring closure of the respective coumarinic acids in the spectrophoto-

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[3] Pfalz and Bauer Inc., Flushing, N. Y.