Influence of Route of Administration on Physiological Availability of Levodopa in Dogs

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Abstract □ The physiological basis for the reduced levodopa bioavailability following oral administration was investigated. Four dogs received single 25-mg/kg doses of ^{14}C-levodopa on three separate occasions in a crossover fashion *via* hepatoportal catheter, intravenous, and oral administrations. Plasma and urine specimens were analyzed for intact levodopa and total radioactivity. The ratios of areas under the plasma concentration–time curves following hepatoportal and intravenous administrations were close to unity, and the shapes of the curves were virtually identical. Following oral administration, however, significant reductions in the areas under the plasma concentration–time curves were observed. These data indicate that the physiologically impaired bioavailability of orally administered levodopa occurs almost exclusively as a result of metabolic degradation within the GI lumen and/or gut wall.

Keyphrases □ Levodopa—bioavailability, effect of route of administration, oral, intravenous, and hepatoportal catheter compared, dogs □ Bioavailability—levodopa, effect of route of administration, dogs □ Administration route—levodopa, oral, intravenous, and hepatoportal catheter compared, bioavailability, dogs

For many drugs, quantitative differences in pharmacological response and/or toxicity were observed when identical drug doses were administered by oral *versus* parenteral routes. An example is the antiarrhythmic agent lidocaine (1), which was therapeutically less active and produced more nausea when administered orally than when administered intravenously to dogs.

Such differences in therapeutic activity may frequently be understood by investigation of the physiological, biochemical, and pharmacokinetic characteristics of drug absorption. Information about these parameters may contribute to the pharmacological and toxicological profile of a drug by allowing the investigator to understand the basis of the observed differences in activity. In addition, the findings may suggest a means of defining or overcoming potential problem areas in drug development.

BACKGROUND

Bioavailability is determined most frequently by comparison of blood level–time curve shapes and areas following oral and intravenous administrations. In instances where bioavailability is incomplete, the ratio of oral to intravenous blood level curve areas is less than unity. This result may arise for a variety of reasons, which may be physicochemical and/or physiological in nature, including poor dissolution of the drug in the GI fluids, poor permeability of the drug across the GI mucosa, gut wall metabolism, metabolism by GI bacteria, enzymatic metabolism in the luminal contents, and first-pass liver metabolism.

Levodopa is an example of a drug exhibiting reduced oral bioavailability. Since the initial report of its isolation from *Vicia faba* beans by Guggenheim (2), levodopa has been the subject of numerous investigations. Interestingly, Guggenheim administered the compound to himself and was the first to note the emetic properties. Subsequent to the discovery of levodopa decarboxylase (2), levodopa and dopamine were proposed as intermediates in the biosynthesis of norepinephrine and epinephrine from L-tyrosine (Scheme I).

Dopamine was first identified as a normal brain constituent in 1957 (2). It was determined upon autopsy that patients suffering from the incapacitating effects of parkinsonism exhibited depleted dopamine levels in certain areas of the brain (3). Cotzias (4) became involved with levodopa as a result of his work with Chilean miners suffering from chronic manganese poisoning. He observed a similarity in the clinical manifestations of manganese poisoning and parkinsonism and began fruitful studies on parkinsonian brain abnormalities and their control.

Clinical treatment of parkinsonism involves the administration of levodopa, since dopamine itself cannot cross the blood–brain barrier (5). Once across the blood–brain barrier, levodopa can be biotransformed to dopamine and exert its effect. Satisfactory absorption of orally administered levodopa from the GI tract is an obvious prerequisite for therapeutic efficacy. Considerable interpatient variability in plasma levodopa concentrations has been observed (6) and may be indicative of variability in bioavailability.

Prior to systemic absorption of levodopa, the GI milieu may reduce significantly the amount of levodopa available (7). Biotransformation products subsequently may alter gastric secretion and motility; one such group of products, the phenylcarboxylic acids, may contribute to the drug's nauseating effect (7). In rats, the presence of an active transport absorption system (passive diffusion playing only a minor role) makes levodopa unique, since this process may be inhibited by a lack of molecular oxygen and the presence of other amino acids.

The existence of a similar levodopa absorption pattern in humans could explain some problems associated with the variability in absorption and bioavailability of the various dosage forms (8). Granerus *et al.* (9) calculated that only about 26% or less of the ingested dose reaches sytemic circulation and suggested that the remainder was decarboxylated in the intestines. However, these studies did not distinguish between possible first-pass liver and intestinal metabolism.

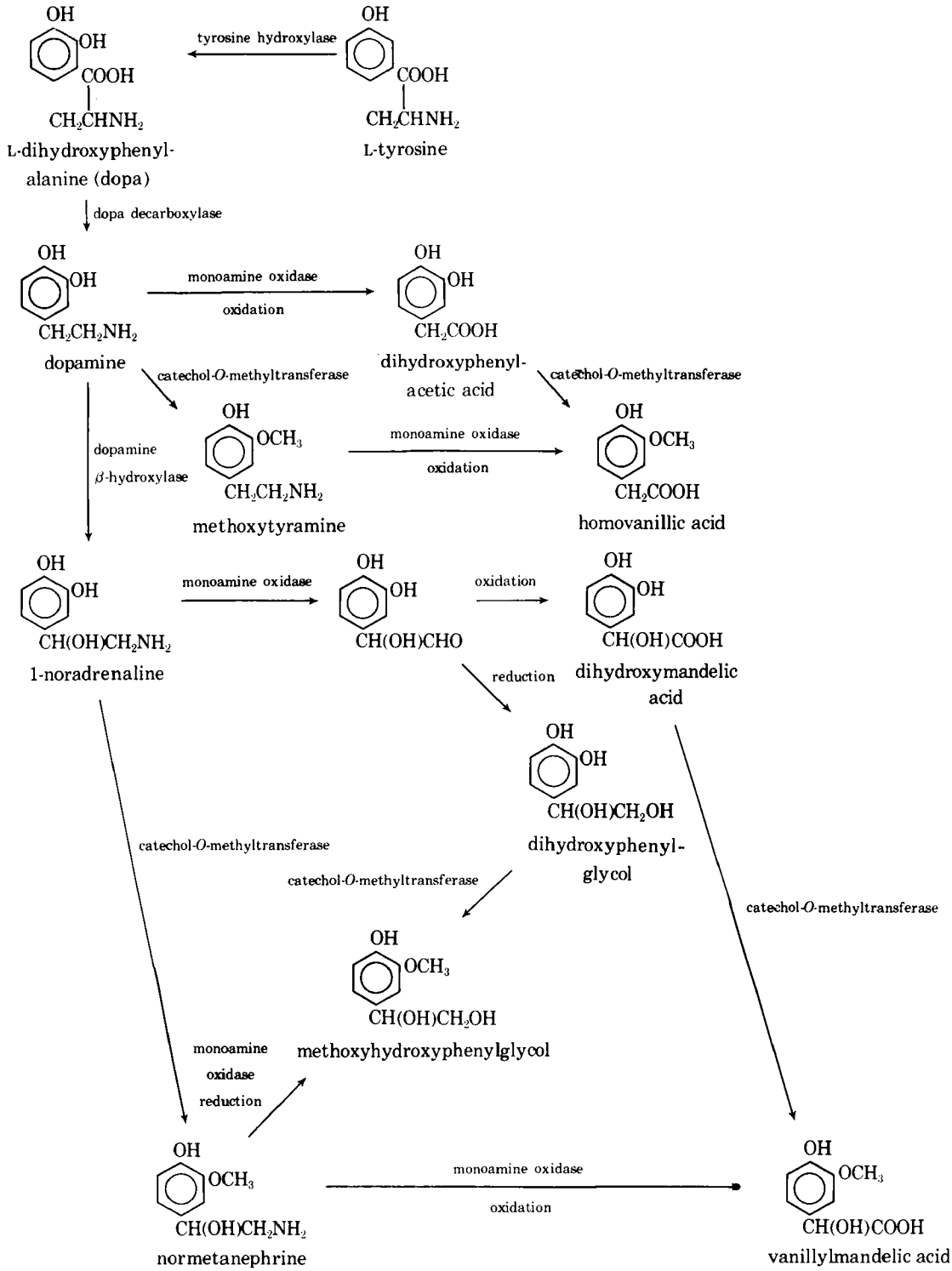
Coutinho *et al.* (10) administered radiolabeled levodopa to dogs and reported higher intact plasma levels and corresponding areas under the plasma concentration–time curves following intravenous administration as compared to oral administration of equivalent doses. Their data indicated that approximately 70–75% of the intravenous and 57–70% of the orally administered radioactivity were excreted in the urine over 72 hr, with levodopa and dopamine accounting for a small percentage of the excretion products. Approximately 3.0–7.0% of the intravenous or oral doses was excreted in the feces.

The efficiency of absorption of total radioactivity ranged between 83.0 and 92.0% of the administered dose. However, when calculated by area analysis, only 22.0–30.0% of the administered oral dose reached the systemic circulation as intact levodopa. These findings suggested that the remainder of the absorbed dose, approximately 60.0%, was biotransformed in the GI tract prior to absorption and/or in the liver during its first pass prior to reaching the general circulation.

The present investigation with levodopa was undertaken to elucidate the mechanism(s) responsible for the reduced oral levodopa bioavailability. Plasma level profiles of levodopa in each dog were compared subsequent to intravenous, hepatoportal, and oral administrations. Comparisons within the same animals permitted determination of the relative contributions of GI and first-pass liver metabolism to the observed reduced levodopa bioavailability.

EXPERIMENTAL

Hepatoportal Cannulation—Four beagle dogs, 10–14.2 kg, were anesthetized with 25–30-mg/kg iv doses of pentobarbital sodium. An incision was made on the left lateral portion of the body, and the spleen was exteriorized. An area of approximately 3 cm of a small tributary of the splenic vein adjacent to the spleen was cleared of



Scheme I—Metabolic pathway of levodopa

surrounding tissue. The vein was tied distally with "0" size silk, and a small incision was made in the vein through which a catheter[1] was introduced.

The catheter used for chronic intravascular implantation consisted of tubing, 0.147 cm o.d. and 0.086 cm i.d.[2], inserted over a 19-gauge, 2.54-cm disposable needle[3] to which a one-way stopcock[4] was attached. The catheter was extended into the hepatic portal vein so that its tip was brought just to the liver. The spleen was returned to its normal position, the free end of the catheter was exteriorized through a stab wound, and the incision closed.

The hepatoportal vein catheter was reintroduced into the stab wound just under the skin and brought through the loose subcutaneous tissue on the left lateral side and exteriorized in the area of the middle of the rib cage. The catheter was protected from damage, by the animal, by securing it in a pocket of a jacket[5]. The dogs were allowed to recover for approximately 1 week. A radiopaque solution,

[1] Coated with Teflon (du Pont).
[2] Teflon tubing, Alpha Wire Co., Elizabeth, N.J.
[3] B. D. 5686, Rutherford, N.J.
[4] B. D. 3154, Rutherford, N.J.
[5] Alice B. King Co., Los Angeles, Calif.

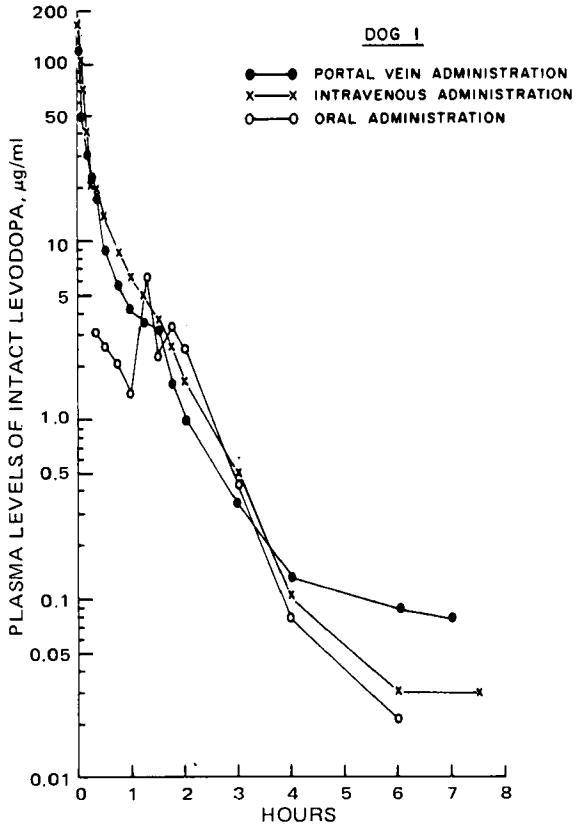


Figure 1—*Intact levodopa plasma levels following intravenous, hepatoportal, and oral administrations of 25-mg/kg doses of levodopa to Dog 1.*

diatrizoate sodium[6], was used for a subsequent X-ray to ensure that the catheter was in its proper position prior to initiating the studies.

The hepatoportal catheter allowed for the rapid administration of drug into the hepatic portal vein, assuring 100% passage of the dose through the liver prior to entering the systemic circulation and completely bypassing the GI tract.

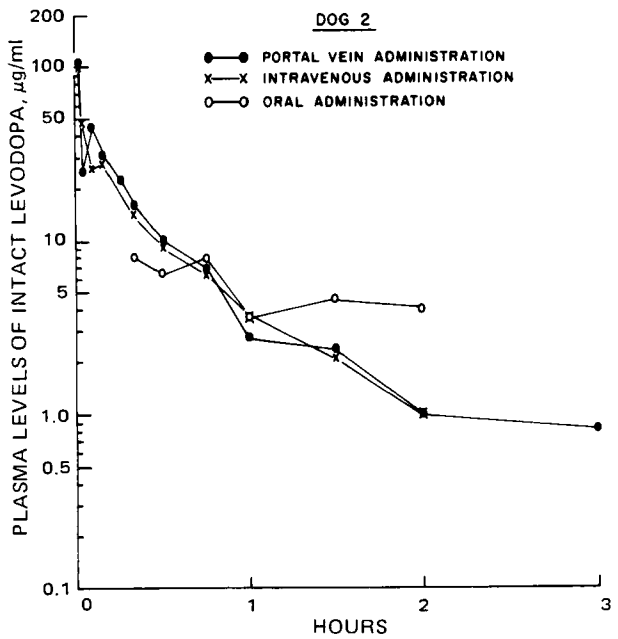


Figure 2—*Intact levodopa plasma levels following intravenous, hepatoportal, and oral administrations of 25-mg/kg doses of levodopa to Dog 2.*

[6] Hypaque, Winthrop Laboratories, New York, N.Y.

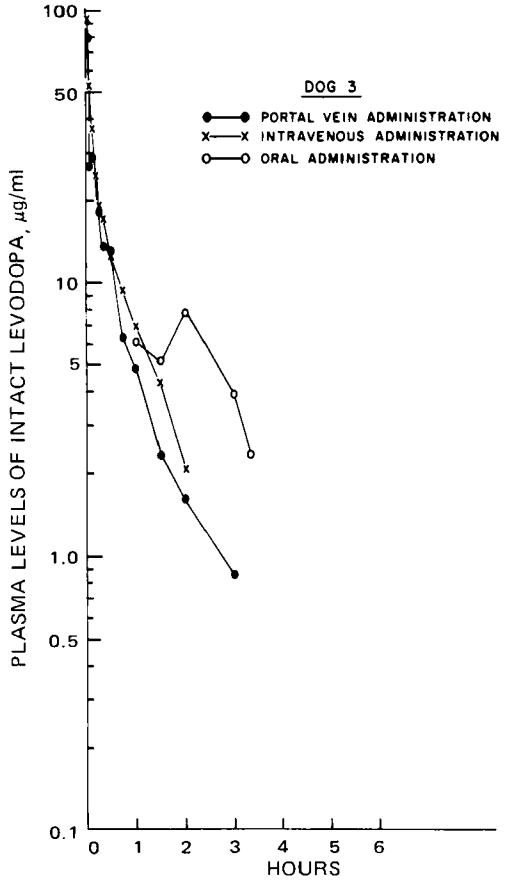


Figure 3—*Intact levodopa plasma levels following intravenous, hepatoportal, and oral administrations of 25-mg/kg doses of levodopa to Dog 3.*

Study Design—Four unanesthetized, fasted dogs (24 hr) with previously implanted hepatic portal vein catheters were used in a three-way crossover experiment. A minimum of 2 weeks elapsed between drug administrations. Levodopa was dissolved immediately prior to administration in 11 ml of 0.27 *N* hydrochloric acid.

In the first of the three administrations, 25-mg/kg doses of 2-^{14}C-levodopa (specific activity of 0.3 μCi/mg) were administered as a bolus into the hepatic portal vein. In the second administration, the 25-mg/kg doses of ^{14}C-levodopa were administered intravenously as a bolus to each dog *via* a catheter in the jugular vein. The third administration to each dog consisted of 25-mg/kg doses of solid ^{14}C-levodopa (in hand-packed gelatin capsules) administered orally.

Ten-milliliter blood specimens were obtained by venipuncture at the time intervals indicated in Figs. 1–4, with the first specimen obtained 1 min following intravenous and hepatoportal administrations and 10 min following oral administration. The blood was immediately transferred to tubes containing 0.2 ml of heparin (10,000 units/ml) and 10 mg of metabisulfite. The specimens were spun down in a refrigerated centrifuge, and the plasma was separated, frozen, and subsequently analyzed for intact levodopa and total radioactivity. The total volume of urine voided was collected at 24-hr intervals from 24 hr prior to dosing to 96 hr postdosing, and total carbon-14 radioactivity levels were determined.

Intravenous and hepatoportal administrations resulted in emesis 3–5 min postadministration. Oral administration resulted in emesis 20–32 min postadministration. In all cases, emesis fluid was collected and the radioactivity content was determined. Radioactivity was not detectable in the emesis fluid following either parenteral route of administration. Recoverable radioactivity of the emesis fluid following oral administration was determined, and the doses were corrected as indicated in Table I.

Analytical Procedure (11)—Heparinized blood specimens, with 10 mg of metabisulfite added to stabilize the levodopa at the time of collection, were chilled immediately in an ice bath. The plasma was

Table I—Pharmacokinetic Parameters of Levodopa in the Dog

	Dog 1			Dog 2			Dog 3			Dog 4		
	Intravenous	Hepatoportal	Oral	Intravenous	Hepatoportal	Oral	Intravenous	Hepatoportal	Oral	Intravenous	Hepatoportal	Oral
Levodopa dose, mg	300	300	300	256	265	269	335	335	368	212	250	250
Dose retained, mg	300	300	267	256	265	82	335	355	234	212	250	250
Dog weight, kg	12.0	12.0	12.0	10.2	10.6	10.8	13.4	14.2	14.7	10.0	10.0	10.0
Dose, mg/kg	25	25	22.3	25	25	7.5	25	25	15.9	21.2	25	25
A, μg/ml	161	87.3	—	156	65.6	—	78.4	72.7	—	132	110.2	—
B, μg/ml	26.3	11.2	—	24.2	4.5	—	23.9	21.7	—	19.4	26.1	—
α, hr^{-1}	14.58	7.44	—	42.30	4.64	—	17.46	17.50	—	19.50	16.60	—
β, hr^{-1}	1.37	1.09	1.21	1.66	0.63	—	1.21	1.42	0.60	1.24	1.63	1.09
$0.693/\beta$, hr	0.51	0.64	0.57	0.42	1.10	—	0.57	0.49	—	0.69	0.43	0.64
k_{12}, hr^{-1}	6.54	—	—	27.06	—	—	9.42	—	—	10.38	—	—
k_{21}, hr^{-1}	3.23	—	—	7.14	—	—	5.00	—	—	3.57	—	—
k_{10}, hr^{-1}	6.18	—	—	9.90	—	—	4.21	—	—	6.78	—	—
V_1, ml	1605	—	—	1412	—	—	3274	—	—	1397	—	—
Area under plasma level curve, μg/ml/hr	29.7	22.5	7.2[a]	18.4	19.6	10.0[a]	22.2	19.3	16.5[a]	22.3	23.3	5.2
Ratio areas compared to iv	—	0.76	0.24	—	1.06	0.54	—	0.84	0.74	—	1.04	0.22
Total carbon-14 excreted in urine, 0–96 hr, % of dose	75	121	62	65	76	88	89	100	116	73	89	94
Ratio carbon-14 excreted in urine compared to iv	—	1.6	0.83	—	1.2	1.4	—	1.1	1.3	—	1.2	1.3

[a] Area corrected for dose equivalent of 25 mg/kg.

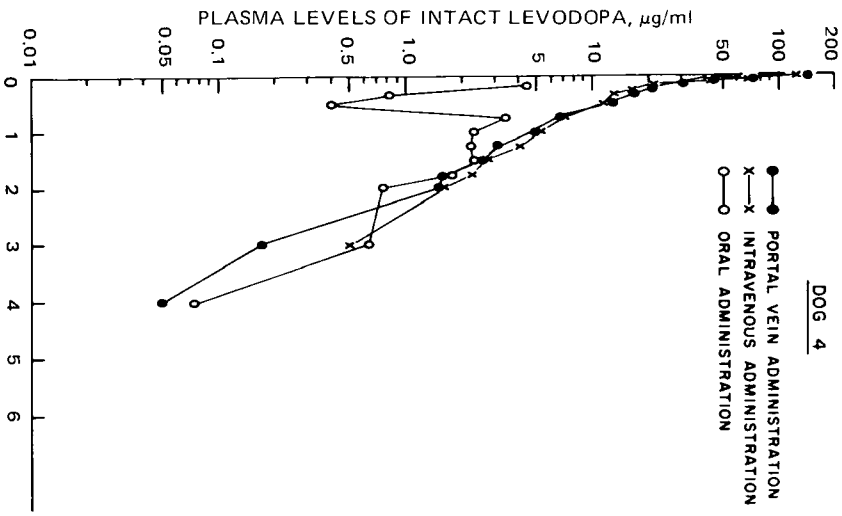


Figure 4—*Intact levodopa plasma levels following intravenous, hepatoportal, and oral administrations of 25-mg/kg doses of levodopa to Dog 4.*

separated in a refrigerated centrifuge. A 0.5-ml aliquot of plasma or a 0.2-ml aliquot of urine was added to 10 ml of scintillation solution[7] and counted for total radioactivity in a scintillation counter[8].

A second aliquot of 0.2 ml of plasma was added to 1.0 ml of a 20-mg/ml solution of edetic acid. One milliliter of water and 2 drops of a 2% solution of naphthoresorcinol in ethyl alcohol were added, and the specimen was mixed. Subsequently, 0.1 ml of 5 *N* sodium hydroxide was added, and the mixture was allowed to stand at room temperature for 5 min. The aqueous layer was washed once with 1 ml of 1-butanol. One milliliter of the aqueous layer was saturated with 0.5 g of sodium chloride and then acidified with 0.1 ml of 6 *N* hydrochloric acid. The levodopa derivative was extracted with 2 ml of ethylhexanol, with a mean (*SE*) recovery of 88.4 ± 1.9%, and determined spectrofluorometrically with excitation at 440 nm and emission at 470 nm.

RESULTS

Intact plasma levodopa levels in the four dogs are presented in Figs. 1–4 following all three routes of administration. Visual inspection of the intravenous and hepatoportal intact plasma level curves for each dog (Figs. 1–4) indicate that their overall disposition profiles were virtually identical. The areas under the plasma level–time curves were calculated using the trapezoidal rule. The (hepatoportal–intravenous) ratio of the areas under the plasma level curves ranged from 0.76 to 1.06, with a mean of 0.93. This ratio closely approximates unity and suggests that the liver did not substantially contribute to the reduced physiological availability of orally administered levodopa.

The corresponding (oral–intravenous) ratio of areas under the plasma level curves ranged from 0.23 to 0.74, with a mean of 0.44. This decreased area under the oral plasma level curves for unchanged levodopa when compared with the corresponding values obtained following the hepatoportal and intravenous administrations indicates

[7] Aquasol, New England Nuclear.
[8] Packard Tri-Carb model 3380.

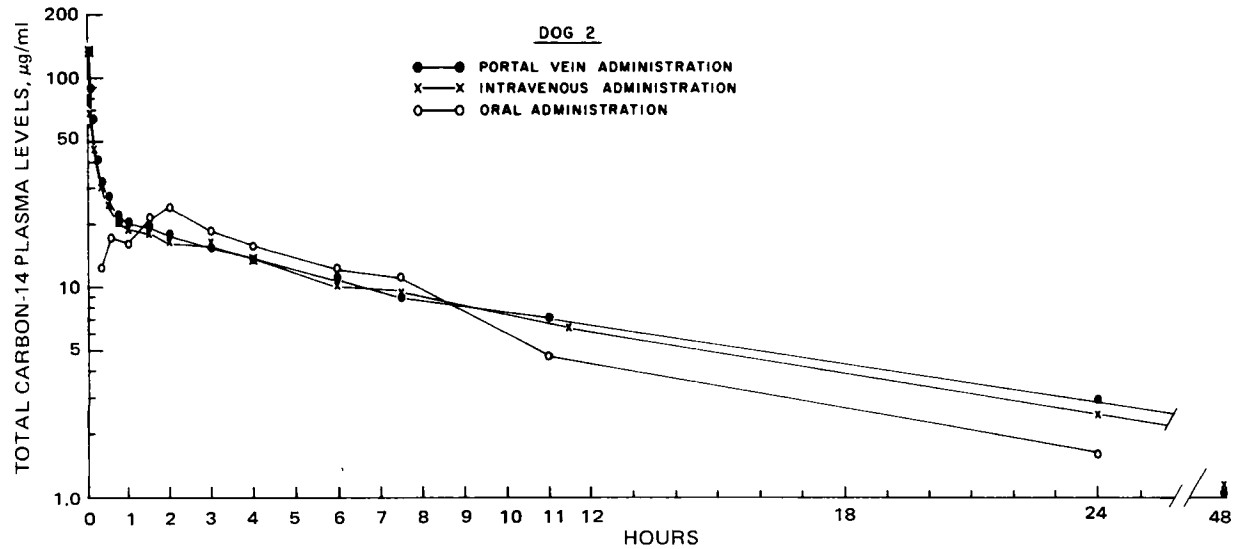


Figure 5—*Total carbon-14 plasma levels following intravenous, hepatoportal, and oral administrations of 25-mg/kg doses of levodopa to Dog 2.*

that the organ responsible for the reduced bioavailability of orally administered levodopa is the GI tract and not the liver.

The relatively complete recovery of administered total carbon-14 radioactivity in the urine following all three routes of administration (Table I) suggests complete absorption of the administered radioactive dose. Complete oral absorption of the administered dose is further confirmed by the comparable areas under the plasma level curves of total carbon-14 levels following all three routes of administration, as exemplified in Fig. 5 for one dog.

Following rapid intravenous injection of levodopa in the four dogs, plasma concentration–time curves could be described satisfactorily by a biexponential equation (12):

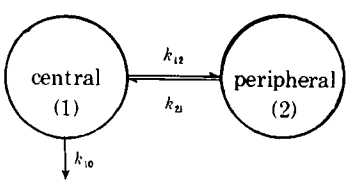
$$Cp = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{(Eq. 1)}$$

where t is time in minutes, Cp is plasma concentration (micrograms per milliliter) at time t; α and β are macroscopic rate constants having units of hours^{-1}, and A and B are coefficients having units of micrograms per milliliter. Initial estimates of the parameters A, B, α, and β were determined by appropriate graphical analysis (12). Initially, it was assumed that the plasma concentration–time data were consistent with the two-compartment open model (12) shown in Scheme II, where Compartments 1 and 2 are the central and peripheral compartments, respectively; k_{12}, k_{21}, and k_{10} are first-order microscopic rate constants (hours^{-1}) representing distribution and elimination processes; Cp is the concentration of drug (micrograms per milliliter) in Compartment 1 at time t (hours); and V_1 is the volume (milliliters) of Compartment 1.

The biexponential equation was fitted to the observed data using the NONLIN program (13), which uses an adaptation of Hartley's modification of the Gauss–Newton method for the fitting of nonlinear regression functions by least squares. Graphical estimates of A, B, α, and β were converted (12) to estimates of k_{12}, k_{21}, k_{10}, and V_1. These latter parameters were used as initial estimates for the program and were directly iterated. A weighting factor of $1/(Cp)^2$ was used in the analysis. Final estimates of the four parameters were converted to the parameters A, B, α, and β. Table I shows the results of these computer analyses. Visual inspection of the observed points about the fitted curves indicated a satisfactory randomness of scatter. Percent coefficients of variation (14) of the parameters k_{12}, k_{21}, k_{10}, and V_1 averaged 23.1, 12.3, 8.31, and 15.8%, respectively.

The question arises, however, as to the appropriateness of this model. Numerous *in vitro* studies indicated that extracts prepared from various animal tissues (15) are capable of decarboxylating levodopa. The wide variety of tissues capable of metabolizing levodopa alludes to the possibility that aromatic L-amino acid decarboxylase activity exists to varying degrees in virtually all regions of the body. Therefore, a more appropriate pharmacokinetic model might contain an irreversible elimination–metabolism step from both the central and peripheral compartments. However, an explicit solution to the parameters of such a model does not exist, except in the special situation where information on metabolite disposition is available (16).

Alternatively, if one assumes or calculates the ratio of elimination rate constants from the peripheral and central compartments, the model parameters may be solved (17, 18). Unfortunately, the present body of information with respect to the relative importance of the various dog tissues in the decarboxylation of levodopa is insufficient to permit even a rough approximation of this ratio. Consequently, the model-independent pharmacokinetic parameters A, B, α, and β may be considered to hold the greatest significance at this time.



Scheme II—Two-compartment open model

DISCUSSION

The data in this study indicate that an orally administered dose of levodopa will be absorbed completely in terms of total radioactivity administered. However, the bioavailability of intact levodopa to the general circulation is reduced by a factor of at least one-half when administered orally. The ratios of areas under the intact levodopa plasma concentration–time curves following the hepatoportal vein administration and intravenous administration approximated unity, and the shapes of these curves were virtually identical within the same dog.

This observation indicates that the first pass of levodopa through the liver does not appreciably contribute to the reduced bioavailability observed following oral administration. The corresponding areas under the intact levodopa plasma concentration–time curves following oral administration were reduced significantly. These observations confirm that the physiologically impaired bioavailability of orally administered levodopa occurs virtually exclusively in the GI tract.

Rivera-Calimlim *et al.* (19) investigated the *in vitro* metabolism of ^{14}C-levodopa by incubating the drug with rat gastric and intestinal everted sacs and separating the drug and metabolites from tissue and serosal and mucosal fluids. These investigators demonstrated that extensive metabolism occurred and was associated with high levels of metabolites in both gastric and intestinal tissues and fluids. This metabolism occurred as a result of the decarboxylase activity in these tissues. The investigators also were able to show a significant inhibition of metabolism when ^{14}C-levodopa was incubated with tissues from rats pretreated with the decarboxylase inhibitor α-methylhydrazine, confirming that the decarboxylase enzymes were promoting metabolism.

Taubin and Landsberg (20) showed that, following intraperitoneal and intravenous administrations of levodopa to the rat, the duodenum

and ileum formed dopamine and *O*-methylated metabolites in greater amounts than in other tissues; they suggested that the rat gut has specific mechanisms for the uptake and metabolism of levodopa. Further studies (21) showed that, following intravenous administration to the rat, the total radioactivity levels in the duodenum exceeded by 10-fold the levels observed in the plasma, heart, or stomach and that the duodenal *O*-methylation reaction was predominantly localized in the mucosa.

All these findings are consistent with the conclusion reported herein: that the GI tract is virtually exclusively responsible for reduced physiological bioavailability of levodopa following oral administration. These findings are also consistent with other studies (22) indicating that coadministration of decarboxylase inhibitors can produce higher plasma levels following oral administration. However, the higher plasma levels probably also are due to inhibition of decarboxylase enzymes throughout other parts of the body. Since the therapeutic utility of levodopa depends upon adequate absorption of intact drug into the systemic circulation, the high doses needed for efficacy probably are required, in large part, because of the extensive biotransformation of levodopa in the GI tract.

Bianchine *et al.* (23) studied the absorption of levodopa in human subjects with uncomplicated parkinsonism following the oral administration of 500 mg of ^{14}C-levodopa. Negligible radioactivity was recovered in both the stools and expired air of these patients. Over 80% of the radioactivity was recovered in the urine, indicating virtually complete oral absorption of the administered radioactivity in humans. The investigators further showed that, by minimizing the duration of exposure of levodopa to the gastric mucosa, they could increase serum levodopa levels. Patients with slow gastric emptying exhibited the lowest serum drug levels, whereas patients receiving the levodopa *via* a direct duodenal infusion exhibited the highest levels. Rivera-Calimlim *et al.* (24) showed that higher serum levodopa levels occurred at earlier times in gastrectomized patients and that the lowest serum levels were observed in one patient who had retained the drug in the stomach for 7 hr. These clinical observations are indicative of GI metabolism as the basis for the reduced oral bioavailability of levodopa.

Clinical investigators administering the decarboxylase inhibitor benserazide [*N*-*dl*-seryl-*N*-(1,2,3-trihydrobenzyl)hydrazine] concomitantly with levodopa demonstrated increased serum levodopa levels (25–28). This procedure allowed for the reduction of the levodopa dose in a group of 20 parkinsonian patients (27) and resulted in fewer side effects. Benserazide does not pass the blood–brain barrier (28, 29) but inhibits extracerebral decarboxylase activity, allowing for greater systemic and brain levels of intact levodopa and a reduction in dose.

In conclusion, the data presented here indicate that substantially more intact levodopa reaches the systemic circulation following the intravenous and hepatoportal parenteral routes of administration than *via* oral administration. Such findings confirm that the physiologically impaired bioavailability of orally administered levodopa occurs virtually exclusively in the GI tract. The overall absorption characteristics of levodopa appear to be similar in dogs and humans, suggesting that in humans the physiologically impaired and variable absorption of orally administered levodopa results from its metabolism in the GI tract.

REFERENCES

(1) R. N. Boyes, H. J. Adams, and B. R. Duce, *J. Pharmacol. Exp. Ther.*, **174**, 1(1970).

(2) A. Carlsson, *Acta Neurol. Scand.*, **48**, Suppl. 51, 11(1972).

(3) R. M. Brogden, T. M. Speight, and G. S. Avery, *Drug*, **2**, 262(1971).

(4) G. C. Cotzias, *Hosp. Pract.*, **4**, 35(1969).

(5) W. Birkmayer, W. Danielcyk, E. Newmayer, and P. Rieder, *J. Neural Transm.*, **34**, 133(1973).

(6) S. Bergman, G. Curzon, J. Friedel, R. B. Godwin-Austen, C. D. Mardsen, and J. D. Parkes, *Br. J. Clin. Pharmacol.*, **1**, 417(1974)

(7) L. Rivera-Calimlim, C. A. Dujovne, J. P. Morgan, L. Lasagna, and J. R. Bianchine, *Eur. J. Clin. Invest.*, **1**, 313(1971).

(8) D. N. Wade, D. T. Mearrick, and J. L. Morris, *Nature*, **242**, 463(1973).

(9) A. Granerus, R. Jagenburg, and A. Svanborg, *Arch. Pharmacol.*, **280**, 429(1973).

(10) C. B. Coutinho, H. E. Spiegel, S. A. Kaplan, M. Yu, R. Christian, J. J. Carbone, J. Symington, J. A. Cheripko, M. Lewis, A. Touchin, and T. Crews, *J. Pharm. Sci.*, **60**, 1014(1971).

(11) R. Montalbo, W. A. Diel, and A. G. Glazko, *Clin. Res.*, **20**, 411(1972).

(12) S. Riegelman, J. C. K. Loo, and M. Rowland, *J. Pharm. Sci.*, **57**, 117(1968).

(13) C. M. Metzler, G. L. Elfring, and A. J. McEwen, "A Users Manual for NONLIN and Associated Programs," The Upjohn Co., Kalamazoo, MI 49001, Apr. 1974.

(14) H. G. Boxenbaum, S. Riegelman, and R. M. Elashoff, *J. Pharmacokinet. Biopharm.*, **2**, 123(1974).

(15) W. Lovenberg, H. Weissbach, and S. Udenfriend, *J. Biol. Chem.*, **237**, 89(1962).

(16) M. Rowland, L. Z. Benet, and S. Riegelman, *J. Pharm. Sci.*, **59**, 364(1970).

(17) T. Suzuki and Y. Saitoh, *Chem. Pharm. Bull.*, **21**, 1458(1973).

(18) D. Lalka, W. J. Jusko, and T. J. Bardos, *J. Pharm. Sci.*, **64**, 230(1975).

(19) L. Rivera-Calimlim, J. P. Morgan, C. A. Dujovne, J. R. Bianchine, and L. Lasagna, *Biochem. Pharmacol.*, **20**, 3051(1971).

(20) H. L. Taubin and L. Landsberg, *Clin. Res.*, **20**, 872(1972).

(21) L. Landsberg, M. Berardino, and P. Silva, *ibid.*, **22**, 321A(1974).

(22) D. S. Tandon and L. Rivera-Calimlim, *Fed. Proc.*, **33**, 498(1974).

(23) J. R. Bianchine, L. Rivera-Calimlim, J. P. Morgan, C. A. Dujovne, and L. Lasagna, *Ann. N.Y. Acad. Sci.*, **179**, 126(1971).

(24) L. Rivera-Calimlim, J. P. Morgan, C. A. Dujovne, J. R. Bianchine, and L. Lasagna, *J. Clin. Invest.*, **49**, 79a(1970).

(25) A. Pletscher and G. Bartholini, *Clin. Res. Ther.*, **12**, 344(1971).

(26) E. M. Miller and L. Wiener, *Neurology*, **5**, 482(1974).

(27) A. Barbeau, L. Gillo-Joffroy, and H. Mars, *Clin. Res. Ther.*, **12**, 353(1972).

(28) W. Birkmayer and E. Neumayer, *Z. Neurol.*, **202**, 257 (1972).

(29) G. Bartholini, W. P. Burkard, A. Pletscher, and H. M. Bates, *Nature*, **25**, 852(1967).

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