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Studies on the Metabolism of D- and L-Isomers of 3,4-Dihydroxyphenylalanine (DOPA). V.[1] Mechanism of Intestinal Absorption of D- and L-DOPA-^{14}C in Rats[2]

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The rate and mechanism of intestinal absorption of D- and L-isomers of 3,4-dihydroxyphenylalanine (DOPA) were comparatively investigated in rats by means of *in situ* ligated loop and *in vitro* tissue accumulation techniques. L-DOPA was found to be very easily and much more rapidly absorbed from the ligated loop of rat intestine than the D-isomer and the absorption was proved to follow saturation kinetics and to be inhibited competitively by L-alanine and L-phenylalanine, while not by D-phenylalanine and D-DOPA. The *in vitro* tissue uptake of L-DOPA-^{14}C revealed an accumulation of radioactivity against the concentration gradient, which was depressed by metabolic inhibitors as DNP and sodium azide. L-DOPA was extensively metabolized in the intestinal tissue to dopamine, its glucuronide and DOPAC, while the D-isomer was not metabolized to any appreciable extent. The rate of absorption and the tissue uptake of L-DOPA-^{14}C was, however, not affected by inhibition of DOPA-decarboxylase. A saturability of the intestinal DOPA-decarboxylase activity with the substrate was demonstrated by everted sac method with increasing L-DOPA concentration in the incubation medium. From these results, it was concluded that L-DOPA, but not D-DOPA, is absorbed from rat intestine by an active transport mechanism, which proceeds independently from the decarboxylation process. These differences between the two isomers were also discussed in relation to those in their distribution patterns after oral administration to rats.

In the previous papers of this series,[4–6] the distributions of radioactivity were compared between D- and L-isomers of ^{14}C-labeled 3,4-dihydroxyphenylalanine (DOPA) following intravenous and oral administration to rats. The results revealed marked differences in the fate of the two isomers and suggested that there are high specificities in the transport and metabolic systems with respect to the optical isomers of DOPA. As reported in the preceding paper,[1] in fact, comparative investigations on the urinary and tissue metabolites confirmed a presence of significant differences in the metabolic fate of DOPA between the optical isomers. As to the absorption of DOPA, it was observed that[6] after oral administration of D- and L-DOPA-^{14}C to rats over 85% of the dose was excreted in the urine for the L-isomer, while only 53% of the D-isomer appeared in the urine with a significant fecal excretion (about 23%), suggesting that L-DOPA is quite easily absorbed from the gastro-intestinal tract, while the absorption of the D-isomer is much lower. In the present investigation, the intestinal absorption of D- and L-DOPA-^{14}C was studied comparatively by means of *in situ* ligated loop of rat small intestine and the tissue accumulation method. Subsequently an active transport mechanism was elucidated for the absorption of the L-isomer.

1) Part IV: H. Shindo, T. Komai, K. Tanaka, E. Nakajima, and N. Miyakoshi, *Chem. Pharm. Bull.* (Tokyo), **21**, 826 (1973).
2) This work was presented in part at the 3rd Symposium on Drug Metabolism and Action, Fukuoka, November, 1971.
3) Location: *Hiromachi 1-chome, Shinagawa-ku, Tokyo.*
4) H. Shindo, N. Miyakoshi, and I. Takahashi, *Chem. Pharm. Bull.* (Tokyo), **19**, 2490 (1971).
5) H. Shindo, N. Miyakoshi, and E. Nakajima, *Chem. Pharm. Bull.* (Tokyo), **20**, 966 (1972).
6) H. Shindo, E. Nakajima, K. Kawai, N. Miyakoshi, and K. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **21**, 817 (1973).

Material and Method

Labeled Compounds——D- and L-DOPA-2-^{14}C were prepared by the optical resolution of DL-DOPA-2-^{14}C which was purchased from the Radiochemical Center, Amersham, England, as described in the previous paper.[4] The specific activity was 24.9 and 26.4 μCi/mg for D- and L-DOPA-^{14}C, respectively, and the radiochemical purity as radioactive D- and L-isomers was over 97% for both preparations. Dopamine-2-^{14}C (25.0 μCi/mg) was purchased from the Radiochemical Center.

4-Bromo-3-hydroxybenzyloxyamine dihydrogen phosphate (NSD 1055), used as a DOPA-decarboxylase inhibitor, was prepared in this laboratories. All of other reagents and standard samples used are the commercial products.

Experiments on Absorption from Rat *in Situ* Acute Loop of Intestine——Male rats of Wistar–Imamichi strain weighing about 180 g were used after fasting for 16 hr. After anesthetizing with ether, the intestine was exteriorized through a central mid-line incision and an acute loop of about 8 cm long was prepared from the upper part of the jejunum by ligature of both ends. The solution of 1 mg D- or L-DOPA-^{14}C in 0.5 ml physiological saline was injected into the lumen of the loop with a syringe, the loop returned and incision satured. After a given time, the loop was removed and the liquid contents were drained off five times, each with 2 ml ice-cold saline. It was ascertained that the last washing contained no radioactivity.

The combined washings were centrifuged after adding an equal volume of 7% $HClO_4$. The residue was reextracted with 4% $HClO_4$ and the combined supernatant was assayed for radioactivity. The tissue was homogenized in 4 to 5 volumes of 4% $HClO_4$ with Potter Ervehjem glass homogenizer and centrifuged. The residue was reextracted and the combined supernatant was assayed for radioactivity. The recovery of radioactivity by the extraction procedure was ascertained to be over 98% by determining the radioactivity of the final residue after solubilizing with NaOH.

The amount of the drug taken into the blood stream from the loop was then obtained by subtracting the residual amount in the lumen and that retained in the tissue from the dose and was referred as the amount truely absorbed.

The pH of the extracts thus obtained were adjusted to 5.0—5.5 with 2N KOH and after allowing to stand at 0° overnight the precipitates were removed by filtration. The solution was concentrated to dryness under reduced pressure and the residue dissolved in a small amount of 50% 10^{-3}N HCl–ethanol. The metabolites were separated by cellulose thin-layer chromatography (E. Merck, F_{254}, 0.1 mm thickness) using the solvent system of butanol: acetic acid: water (4: 1: 1). The radioactive spots were detected by autoradiography and quantitatively transferred into the counting vials by scratching carefully with a spatula and the radioactivity was counted after shaking in 15 ml liquid scintillator.

Experiments on Absorption from Rat Everted Sac of Intestine——The upper small intestine was removed from rats to a length of about 15 cm. After cutting into two 7 cm sections, the intestine was everted carefully by means of a stainless steel rod according to Wiseman's method.[7] A sac was prepared by ligature of both ends after filling with 0.5 ml Krebs–Henseleit bicarbonate buffer of pH 7.1 as the serosal fluid. The sacs were incubated in 15 ml of the buffer solution containing various concentrations of L-DOPA-^{14}C at 37° under a constant gassing with a mixture of 95% O_2 and 5% CO_2. After 30 min, the sacs were removed, washed thoroughly by shaking in about 100 ml cold saline and blotted with a filter paper. One end was cut to drain off the contents, which were spotted on the thin-layer plate to separate the metabolites. The tissue was washed again in cold saline and the radioactive metabolites in the tissue were extracted and separated in the same way as described above.

Experiments on Absorption by Intestinal Tissue Segments——The upper small intestine was removed from the rat, everted with a stainless steel rod and cut into rings of 3—5 mm length. About 600 mg of the everted intestinal rings were selected at random and placed into a 100 ml Erlenmeyer flask with 15 ml Krebs–Henseleit bicarbonate buffer (pH 7.1) containing 0.3% glucose and, in some cases, an appropriate amount of inhibitor. After preincubation for 10 min at 37° under gassing with 95% O_2–5% CO_2, L-DOPA-^{14}C was added at a concentration of 0.2 mg/ml and the incubation was continued for 30 or 60 min. The segments were then removed from the medium, washed four times each with 20 ml ice-cold saline, blotted with a filter paper and weighed. The radioactive substances were extracted from the segments in the same way as described above and assayed for radioactivity. The extracellular and intracellular spaces were determined in the control experiment using inulin-^{14}C as described previously,[8] and the tissue accumulation of L-DOPA-^{14}C was expressed as an intracellular to extracellular concentration ratio of radioactivity, C_{ICF}/C_{ECF}.

Radioactive Measurements——The radioactivity was counted in a Beckman LS-250 liquid scintillation spectrometer using a counting medium consisted of 8 g PPO, 200 mg dimethyl-POPOP, 200 ml toluene and 800 ml dioxane. The counting efficiencies were determined by ^{137}Cs external standard method and the counts were converted to disintegration per minutes (dpm) with Olivetti Programma 101 computor.

7) T.H. Wilson and G. Wiseman, *J. Physiol.*, 123, 116 (1954).
8) T. Komai and H. Shindo, *J. Vitaminol.*, 18, 55 (1972).

Result

Absorption of D- and L-DOPA from *in Situ* Rat Intestine

The time course of the absorption of radioactive D- and L-DOPA and dopamine from an acute loop of rat small intestine are shown in Table I and Fig. 1. Ten and 30 min after administration of L-DOPA-^{14}C, approximately 55 and 94% of the dose has disappeared from the lumen and approximately 41 and 83% was transferred into the blood stream, respectively, indicating that L-DOPA is absorbed quite readily from the small intestine. On the contrary, after administration of the D-isomer only approximately 22 and 55% of the dose has disappeared from the lumen and approximately 9 and 29% transferred into the blood stream after 10 and 30 min, respectively, indicating that the rate of absorption of D-DOPA from the intestine is much lower than that of the L-isomer. The absorption of dopamine was found to be slower than that of D-DOPA (Table I). When the amounts absorbed in the first 10 min are compared, the results indicated that the absorption rate of D-DOPA and dopamine was about 1/3 and 1/6 that of L-DOPA, respectively.

Table I. Absorption of D- and L-DOPA-^{14}C and Dopamine-^{14}C from Ligated Loop of Rat Small Intestine (Dose: 1.0 mg/loop)

Drug	min after administration	$n^{a)}$	% to dose		
			Residual amount in lumen	Retained in tissue	Absorbed into blood
L-DOPA	5	3	69.65	12.20	18.15
	10	3	43.65	15.02	41.33
	30	4	5.84	11.11	83.05
	60	2	1.85	7.39	90.76
D-DOPA	5	3	87.65	9.35	3.40
	10	6	78.50	12.98	8.53
	30	4	45.45	25.87	28.68
	60	5	27.78	23.19	49.63
Dopamine	10	3	88.98	7.45	3.57
	30	3	79.25	9.32	11.43

Each value represents the mean of 2 to 6 experiments.
a) number of experiments

Since D- and L-DOPA are expected to have a very close similarity in their physico-chemical nature, the above result may suggest that there is involved some specific transport mechanism in the absorption of the L-isomer rather than a simple diffusion process. The absorption of different doses of D- and L-DOPA-^{14}C from the loop of intestine were, therefore, investigated. As shown in Table II, the absorption rate was found to be decreased with increasing the amount of dose in both D- and L-DOPA, but the extent was much more significant with the L-isomer. The Lineweaver–Burk reciprocal plots of L-DOPA absorption in the first 10 min, as shown in Fig. 2, indicated a typical saturation kinetics, one of the characteristics for an active transport mechanism, which gave $V_{max}=0.91$ μmole/min and $K_t=20$ mmoles.

The effect of 2 mole ratio of various amino acids on the absorption of L-DOPA-^{14}C is shown in Table III. The results indicated that both L-alanine and L-phenylalanine showed a significant inhibition on the absorption of L-DOPA, while D-phenylalanine as well as D-DOPA did not show any appreciable inhibition. When the absorption of a various amounts of L-DOPA-^{14}C was examined in the presence of a constant amount (20 mM) of L-phenylalanine, the Lineweaver–Burk plots revealed a straight line intersecting the ordinate at the same point as that of the line representing the absorption of L-DOPA alone, as shown in Fig. 2; this in-

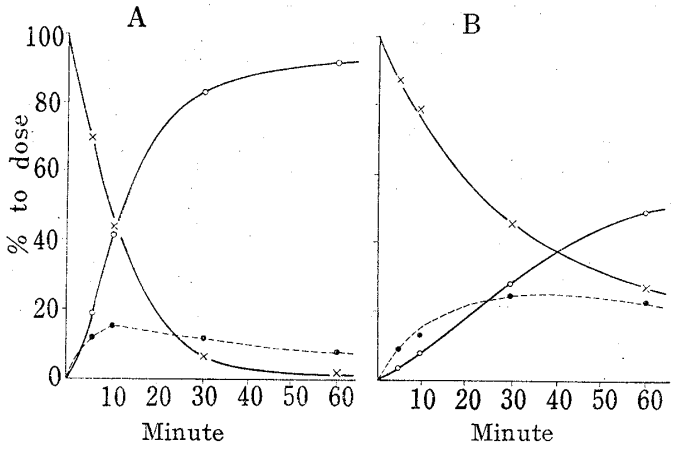

Fig. 1. Time Course of the Absorption of L- (A) and D-DOPA (B) from Rat Ligated Loop of Small Intestine (dose: 1.0 mg/loop)

—○—: absorption into blood stream
---●---: retained in the tissue
—×—: residual amount in the lumen
Each point represents the mean from two to six experiments.

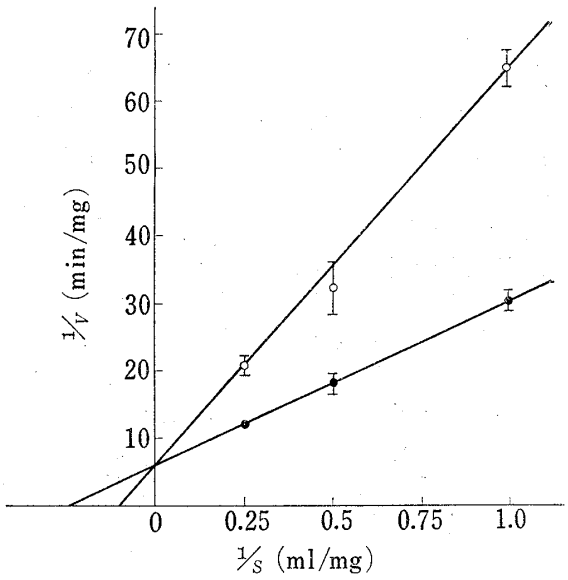

Fig. 2. Lineweaver-Burk Plots of the Absorption of L-DOPA with and without L-Phenylalanine

——○——: L-DOPA in the presence of L-phenylalanine
——●——: L-DOPA alone
Solution (0.5 ml) of different amount of L-DOPA-^{14}C with or without 20 mM L-phenylalanine was injected into ligated loop of rat small intestine and the amount absorbed was determined from the residual activity in the lumen after 10 min. Each value represents the mean of three experiments.

TABLE II. Absorption of Various Doses of D- and L-DOPA from Ligated Loop of Rat Small Intestine (10 min after Administration)

Dose (mg/loop)	% to dose					
	Residual amount in lumen		Retained in tissue		Absorption into blood	
	L-DOPA	D-DOPA	L-DOPA	D-DOPA	L-DOPA	D-DOPA
0.1	16.22	74.39	38.63	15.27	45.15	10.34
0.5	33.81	81.24	22.29	13.80	43.90	4.96
1.0	43.65	78.50	15.02	12.98	41.33	8.53
2.0	58.58	86.86	11.96	10.13	29.46	3.02
3.0	68.30	87.98	10.83	9.79	20.88	3.91

Each value represents the mean from three experiments.

dicated that the inhibition of L-phenylalanine on the absorption of L-DOPA is competitive in nature. All of these results suggest that L-DOPA is absorbed from rat intestine actively through the neutral amino acid transport system.[9]

In Vitro Transport of L-DOPA into Rat Intestinal Tissue

When the intestinal tissue segments were incubated with 0.2 mg/ml L-DOPA-^{14}C, the intracellular to extracellular concentration ratio of radioactivity easily exceeded unity and reached 1.87 and 2.88 after 30 and 60 min, respectively. The effect of some metabolic inhibitors on the tissue uptake of L-DOPA-^{14}C are shown in Table IV. 2,4-Dinitrophenol and

9) R.P. Spencer, *Am. J. Clin. Nutr.*, 22, 292 (1969).

Table III. Interference with L-DOPA Absorption by L-Phenylalanine and Other Amino Acids

Amino acid added[a]	Absorption of L-DOPA[b]	%-inhibition
None	55.68	—
L-Alanine	35.97	35.4
L-Phenylalanine	32.41	41.8
D-Phenylalanine	56.53	−1.5
D-DOPA	55.40	0.5

a) 0.5 ml of 20 mM solution
b) Amount absorbed from the lumen during 10 min after administration of 1.0 mg (0.005 mmole) L-DOPA-^{14}C into the loop (% to dose).

sodium azide showed a significant inhibition at concentrations of 10^{-4} and 10^{-2}M, respectively. Ouabain which inhibits the active transport of cations exhibited an appreciable inhibition at the concentration of 10^{-4}M. These results are again consistent with the assumption that L-DOPA is transported into the intestinal epithelial cells by an active transport mechanism.

Table IV. Effect of Metabolic Inhibitors on L-DOPA Uptake by the Intestinal Tissue Segments

Inhibitor	Concentration (M)	C_{ICF}/C_{ECF}[a]	%-inhibition
None	—	1.87	—
2,4-Dinitrophenol	10^{-4}	0.96	48.34
Sodium azide	10^{-2}	0.80	57.22
Ouabain	10^{-4}	1.33	28.88

Each value represents the mean from five experiments.
a) Intracellular to extracellular concentration ratio of radioactivity after 30 min incubation of the intestinal segments in the buffer containing 0.2 mg/ml L-DOPA-^{14}C with or without inhibitor.

Metabolism of D- and L-DOPA in the Intestinal Tissue

In order to study the possible metabolism of DOPA at the intestinal tissue, the radioactive substances were extracted from the intestinal tissue 5 min after administration of D- and L-DOPA-^{14}C into the ligated loop and separated by thin-layer chromatography. The results are shown in Table V. After administration of D-DOPA-^{14}C, most of radioactivity retained in the tissue was found to be unchanged DOPA, indicating that D-DOPA is not metabolized in the intestinal tissue to any appreciable extent and thus might be transferred into the blood stream as unchanged DOPA. Analysis of the tissue extracts after 30 min also gave the same result and no radioactivity could be detected at the positions corresponding to dopamine and its metabolites. After administration of L-DOPA-^{14}C, as can be seen from Table V, only about 27% of the tissue radioactivity was detected as unchanged DOPA, while about 43, 16 and 6% as dopamine, dopamine glucuronide and DOPAC, respectively. Thus, dopamine and its metabolites accounted for about 65% of the total radioactivity, indicating that L-DOPA is very rapidly decarboxylated to dopamine in the intestinal tissue and thus might be transferred into the blood stream largely as dopamine. The radioactive spot at *Rf* 0.05 was identified as dopamine glucuronide by the incubation with β-glucuronidase followed by thin-layer chromatography. Thin-layer chromatography of the extracts from the lumen 5 min after administration revealed that the most of the residual radioactivity in the lumen was unchanged DOPA with both D- and L-DOPA.

In order to anticipate the relative amount absorbed as unchanged DOPA and the effect of increasing the dose level, the everted sacs of rat small intestine were incubated in the medium containing various concentrations of L-DOPA-^{14}C and the radioactive substances transferred

Table V. Metabolism of D- and L-DOPA-^{14}C in the Intestinal Tissue during the Absorption

Standard	*Rf* value[a]	% to total radioactivity	
		D-DOPA	L-DOPA
	0	9.3	9.0
Dopamine-glucuronide[b]	0.05	—[c]	16.0
DOPA	0.16	90.7	26.6
Noradrenaline	0.29	—	—
Dopamine	0.41	—	42.9
3-O-Methyldopamine	0.51	—	—
3,4-Dihydroxyphenylacetic acid (DOPAC)	0.78	—	5.5
3-Methoxy-4-hydroxy phenylacetic acid (HVA)	0.87	—	—

One mg D- and L-DOPA-^{14}C was injected into the ligated loop of rat small intestine and the radioactive substances were extracted from the intestinal tissue after 5 min.
a) Cellulose-TLC, solvent system; butanol: acetic acid: water (4:1:1).
b) identified as described in the text
c) Radioactivity was not detected.

into the serosal fluid as well as those retained in the tissue were analyzed. The results, as shown in Table VI, indicated that the percentage of unchanged DOPA in both the tissue and the serosal fluid increased markedly with increasing the L-DOPA concentration in the medium and that the percentage of unchanged DOPA was always appreciably higher in the serosal fluid than that retained in the tissue.

Table VI. Relation between the Metabolism of L-DOPA in the Everted Sac Intestine and the Concentration of L-DOPA-^{14}C in the Medium

	DOPA concn. (mg/ml)	% to total radioactivity				
		DOPA	Dopamine	Dopamine-glucuronide	DOPAC	HVA
Serosal fluid	0.1	8.8	55.3	8.3	19.3	4.8
	0.2	16.0	49.0	11.8	9.2	5.0
	0.5	21.8	64.1	2.6	2.8	4.3
	1.0	60.5	30.7	1.5	0.9	2.4
	2.0	73.9	18.2	1.8	0.6	2.1
	4.0	80.2	12.3	—[a]	2.4	2.4
Tissue	0.1	2.9	51.9	16.2	8.9	—
	0.2	6.7	77.9	7.5	3.0	—
	0.5	20.5	71.7	3.5	0.9	—
	1.0	27.0	53.3	5.8	1.9	—
	2.0	45.3	36.0	6.9	3.1	—
	4.0	60.1	33.9	—	—	—

Everted sac of rat small intestine was incubated in the medium containing various concentration of L-DOPA-^{14}C for 30 min at 37° and the serosal fluid and the tissue extracts were analysed for radioactive substances.
a) No significant amount was detected.

Effect of DOPA-Decarboxylase Inhibitor on the Intestinal Absorption of L-DOPA

In order to examine if the decarboxylation process bears some connection with the observed rapid absorption of L-DOPA, the rate of absorption was reexamined with rats wherein dopa-decarboxylase inhibitor was administered prior to the experiments. It was confirmed by the separate experiments that the DOPA-decarboxylase activity of $9000 \times g$ supernatant from the rat intestine was inhibited almost completely (86 to 90% of the control) when 100 mg/kg NSD-1055[10] had been administered intraperitoneally 30 to 60 min prior to the isolation of tissue.

10) R.J. Levine and A. Sjoerdsma, *J. Pharmacol. Exptl. Therap.*, **146**, 42 (1964).

Thus, the absorption of L-DOPA-^{14}C was determined with the ligated loop of rat jejunum of which 100 mg/kg NSD-1055 was administered 60 min prior to the experiment. The results indicated that, as shown in Table VII, the amount absorbed from the lumen during the first 10 min was almost the same as that in the control rats, although a slight but an appreciable decrease was noted in the amount retained in the tissue and, correspondingly, a slight increase in that transferred into the blood stream in the treated rats. Furthermore, when the intestinal tissue segments isolated from rat administered with the inhibitor were incubated with L-DOPA-^{14}C, the intra- to extra-cellular concentration ratio of radioactivity exceeded the unity and reached 1.74 after 30 min, which is almost the same as that reached by the control tissue (1.87). When 10^{-4}M 2,4-dinitrophenol was added in the incubation medium, this accumulation was depressed markedly to give the ratio of 0.63, below the unity, in the same way as that observed in the control tissue. These results suggest that the rate of absorption might not be affected by the inhibition of decarboxylation process and thus the active transport process of L-DOPA through the epithelial cell membrane might proceed independently from the decarboxylation process.

Table VII. Effect of DOPA-decarboxylase Inhibition on the Absorption of L-DOPA from Intestine

Treatment	Ligated loop		Tissue segments C_{ICF}/C_{ECF}[b]
	Absorption from lumen (%)[a]	Retained in tissue (%)[a]	
None	60.62±2.42	16.14±1.92	1.87
NSD-1055 (100 mg/kg)	63.03±3.43	11.77±1.25	1.74
NSD-1055+2,4-DNP (10^{-4}M)	—		0.63

Each value represents the mean from three experiments.
a) The amount absorbed duriug 10 min after injection of 1 mg L-DOPA-^{14}C into the lumen of the loop.
b) Intra- to extracellular concentration ratio of radioactivity after 30 min incubation with 0.2 mg/ml L-DOPA-^{14}C.

Discussion

It has been well established that the most of natural L-α-amino acids and some synthetic L-α-amino acid analogues are transported from the intestine by an active transport mechanism.[11] In the studies using ligated loop of rat intestine, Young, *et al.*[12] found that L-α-methyl-DOPA was absorbed at a greater rate than the D-isomer and that the L-isomer inhibited the absorption of L-histidine, whereas the D-isomer did not, suggesting that it was absorbed from the intestine by a mechanism other than a simple diffusion. On the other hand, however, Wass, *et al.*[13] reported more recently that neither L-α-methyl-DOPA nor L-DOPA was proved to be transported against a concentration gradient using everted sacs and everted segments of rat small intestine. In our previous investigation[6] on the urinary and fecal excretion of L- and D-DOPA-^{14}C following intravenous and oral administration to rats, it was indicated that L-DOPA appears to be absorbed from the intestine much more easily than the D-isomer. In the present investigations, the intestinal absorption of D- and L-isomers of radioactive DOPA were comparatively examined using both *in situ* ligated loop technique and *in vitro* tissue accumulation method. The obtained results that i) L-DOPA was absorbed much more rapidly than the D-isomer from *in situ* ligated loop of rat jejunum, ii) the dose–absorption relationship of L-DOPA followed a saturation kinetics, iii) the absorption was inhibited by L-alanine and

11) G. Wiseman, "Absorption from the intestine," Academic Press, New York, 1964 p. 54.
12) J.A. Young and K.D.G. Edwards, *Am. J. Physiol.*, **210**, 1130 (1966).
13) M. Wass and D.F. Evered, *Life Sci.*, **10**, 1005 (1971).

L-phenylalanine competitively, while not by D-phenylalanine and D-DOPA, and iv) the radioactive L-DOPA was accumulated in the tissue segments *in vitro* and the accumulation was depressed by metabolic inhibitors as 2,4-DNP and sodium azide, are all in good accordance with the assumption that L-DOPA, but not D-DOPA, is absorbed from rat small intestine actively through a carrier–mediated transport system for neutral amino acids.[9,14] The finding that the absorption and the accumulation were not affected by DOPA-decarboxylase inhibition and the accumulation was depressed by DNP in the same way as that in the control tissue might suggest that the active transport process through the epithelial cell membranes proceeds independently from the decarboxylation process. Relative affinities of substituted L-phenylalanines toward the transport carrier for neutral amino acids have been investigated by Hajjar, *et al.*[15] using rat intestine and a correlation was elucidated to be present between the inhibition constant and the electronic effects of the substituents. Although L-DOPA was not examined in their study, it is reasonable to regard L-DOPA as an additional member of the series.

An extensive decarboxylation of L-DOPA in the intestinal tissue has been elucidated in the rat gut *in vitro* by Rivera-Calimlim, *et al.*[16] and it has been reported in human subjects that[17] orally administered L-DOPA undergoes decarboxylation before reaching the general circulation. In accordance with these results, it was found in the present study that D-DOPA does not nudergo any metabolic change in the intestinal tissue, while L-DOPA undergoes an extensive metabolism to give dopamine and its glucuronide and DOPAC. The finding that after incubation of rat everted sacs with L-DOPA the percentage of unchanged DOPA transferred into the serosal fluid was significantly higher than that retained in the tissue might suggest that the rate of transfer of unchanged DOPA into the blood stream is larger than that of dopamine. Another finding that the percentage of unchanged DOPA transferred into the serosal fluid was increased markedly with increasing the concentration of L-DOPA-^{14}C in the incubation medium might be interpreted as that the DOPA-decarboxylase activity in the intestinal tissue is saturable with the substrate and the concentration of 1.0 to 2.0 mg/ml medium is that wherein the activity is saturated enough to give a transfer of largely unchanged DOPA into the serosal compartment. These results agree well with the clinical finding that[18] a therapeutic effect against Parkinsonism became evident when the oral dose of L-DOPA was increased to a relatively large amount such as several grams per day and with our previous finding[5] that the distribution pattern of radioactivity after oral administration of L-DOPA-^{14}C to rats depends on the amount of the dose and the brain uptake was increased markedly by increasing the dose level to more than 50 mg/kg.

The present finding that D-DOPA is absorbed gradually from the small intestine without being metabolized to any appreciable extent might explain our previous finding[5] that after oral administration of D-DOPA-^{14}C to rats a much higher accumulation and a longer retention of radioactivity in the organs and tissues were observed than those after intravenous administration, since gradual absorption may result over a long duration of a certain level of unchanged DOPA in the blood circulation and a gradual transfer and accumulation in the organs and tissues.

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14) E.C.C. Lin, H. Hagihira, and T.H. Wilson, *Am. J. Physiol.*, **202**, 919 (1962).
15) J.J. Hajjar and P.F. Curran, *J. Gen. Physiol.*, **56**, 673 (1970).
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