[Chem. Pharm. Bull. 25(8)2001—2012(1977)]

UDC 547.565'484.2.04 : 542.98

Metabolic Fate of 3,4-Dihydroxyphenylpyruvic Acid(DHPP) in Rats. II.[1] *In Vivo* Evaluation of DHPP as a Possible New Precursor of Brain Dopamine

Toshihiko Maeda, Nobuhiro Miyakoshi, and Hideyo Shindo

Central Research Laboratories, Sankyo Co., Ltd.[2]

(Received December 9, 1976)

Metabolic fate of 3,4-dihydroxyphenylpyruvic acid-2-^{14}C (DHPP-2-^{14}C) in rats was investigated and a rapid conversion of DHPP to L-3,4-dihydroxyphenylalanine (L-DOPA) was demonstrated *in vivo*. After intravenous administration, a high uptake of the radioactivity was observed in the adrenal medulla, caudate nucleus, pancreas, hair follicle and renal medulla, in a similar way as L-DOPA-2-^{14}C. DHPP itself appears not to pass through the blood-brain barrier. After oral administration, very little uptake of the radioactivity was observed in the brain even at high doses (10, 50 and 100 mg/kg). Most radioactivity was recovered into the urine (about 44.0% and 85.4% after oral and intravenous administration, respectively) but respiratory excretion of the radioactivity was also observed (28.01 and 6.30% after oral and intravenous administration, respectively). In order to evaluate the efficacy of DHPP as oral precursor of brain dopamine, the brain and other tissue uptake of the radioactivity were compared between DHPP-2-^{14}C and L-DOPA-3-^{14}C after oral administration at various dosages. The results revealed only extremely low uptake of radioactivity in the brain after DHPP administration as compared to L-DOPA. This was found to be mainly due to an extremely slower absorption of DHPP than L-DOPA from intestine.

Keywords——3,4-dihydroxyphenylpyruvic acid; L-3,4-dihydroxyphenylalanine; dopamine; metabolism; whole-body autoradiography; absorption; distribution; excretion; rat intestine; rat brain

In the previous paper,[1] it was shown that 3,4-dihydroxyphenylpyruvic acid (DHPP) is easily transformed specifically to L-3,4-dihydroxyphenylalanine (L-DOPA) in rat tissue homogenates *in vitro*. Therefore, it is expected that DHPP can act as L-DOPA precursor *in vivo*. In particular, the finding that in the intestinal homogenates DHPP was easily transformed to L-DOPA, while almost no dopamine was formed, provided a promising aspect to the possible use of DHPP as a new brain dopamine precursor with less peripheral decarboxylation than oral L-DOPA. In the present investigations, a series of experiments has been carried out to evaluate this possibility *in vivo* following oral and intravenous administration of DHPP-2-^{14}C to rats. The results, however, lead to a conclusion that DHPP could not be used as a therapeutic agent against parkinsonism which is able to surpass L-DOPA. Several reasons for this estimation along with the metabolic characteristics of DHPP were described and discussed in this paper.

Material and Method

Material——DHPP-2-^{14}C was prepared as described in the previous paper.[1] The specific activity was 11.56 μCi/mg and the radiochemical purity was ascertained to be over 95% by cellulose thin-layer chromatography using the solvent system: *n*-butanol–methanol–1 N formic acid (6:2:1). L-DOPA-3-^{14}C was purchased from the Radiochemical Center, Amersham, England. The specific activity was 46 μCi/mg. L-DOPA, L-3-methoxy-4-hydroxyphenylalanine (3-O-methyl-DOPA), dopamine, 3-O-methyldopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxyphenylacetic acid (HVA) were all the

1) Part I: T. Maeda and H. Shindo, *Chem. Pharm. Bull.* (Tokyo), **25**, 1992 (1977).
2) Location: *Hiromachi 1-chome, Shinagawa-ku, Tokyo.*

commercial products. Non-radioactive DHPP was prepared by the hydrolysis of azlactone intermediate prepared from acetylglycine and protocatechualdehyde.[3]

Whole-body Autoradiography——Male rats of Wistar-Imamichi strain weighing about 120 g were used. DHPP-2-^{14}C was dissolved in physiological saline and administered orally (10, 50 and 100 mg/kg) and intravenously (10 mg/kg) at a constant radioactive dose of 14.5 μCi/animal. One hr after oral administration and 1, 10 and 30 min, 1 and 24 hr after intravenous administration, the rats were slightly anesthetized with ether and sacrificed by immersion in a mixture of hexane and solid carbon dioxide at about −70°. After a frozen animal was embedded on a microtome stage with aqueous carboxymethylcellulose gel, sagittal 50 μ sections were cut with a heavy microtome (Yamato Type 1111) in a freezing room and dried at −10°. The dried sections were brought to contact with Sakura Type N X-ray films and exposed for 7 days.

Experiments on Urinary, Fecal and Respiratory Excretion——DHPP-2-^{14}C was administered to rats orally and intravenously at the dose of 10 mg/kg (13.9 μCi/animal). Each rat was housed in a metabolic cage permitting the separate collection of urine and feces periodically. Urine was collected in a conical flask added with 2 ml of 1 N hydrochloric acid, for the first 6 hr and every 24 hr thereafter. After 3 days, the rats were sacrificed and each carcass was solubilized in 400 ml of 30% NaOH solution by warming at 80°. The urine sample (0.5 ml), after adjustment of the volume, was pipetted into a counting vial, added with toluene-dioxane liquid scintillator (8 g PPO, 200 mg dimethyl POPOP, 200 ml toluene and 800 ml dioxane) and measured the radioactivity in a Packard Model 3380 liquid scintillation spectrometer. The fecal samples were softened with water by standing overnight and homogenized with Polytron (Kinematica Co., Ltd., Switzerland). After diluting it to a constant volume, an aliquot was added with toluene-dioxane liquid scintillator to be measured the radioactivity. The carcass lysate (0.5 ml), after adjustment of the volume, was added with toluene-dioxane liquid scintillator to be measured the radioactivity.

The excretion of radioactivity into the respiratory air was measured continuously with Triton 955B high sensitivity β-ray gas monitor (Johnston Laboratories, Inc., Cockeysville, Maryland, U.S.A.). DHPP-2-^{14}C was administered intravenously or orally to rats at the dose of 10 mg/kg. Each rat was placed in a plastic cage with 800 ml volume and the respiratory air was introduced into the ion chamber by an air flow of 2 l/min. The radioactive concentration in the air was continuously determined automatically and recorded graphically. The air left the monitor was captured by serial three traps of each 200 ml of 10% NaOH solution. The total amount (T μCi) excreted was calculated by integrating the recorded curve against time according to the following equation:

$$T = \int_0^t CF\,dt \qquad (t = 24 \text{ hr})$$

where C is the concentration of radioactivity on the chart (μCi/m^3) and F is the flow rate of air (m^3/min). The efficiency of the measurement was found to be 92.5% by introducing a known amount of $^{14}CO_2$ gas liberated from $Ba^{14}CO_3$.

Two-dimensional Paper-chromatographic and Electrophoretic Separation of Urine and Tissue Metabolites——Rats were administered with DHPP-2-^{14}C and sacrificed after 30 min. The liver, kidney, brain, pancreas, heart and small intestine were excised and homogenized in ice-cold 4% perchloric acid. After centrifugation at 2000 rpm, the supernatants were neutralized with 2 N KOH solution under ice cooling. After being allowed to stand overnight, the precipitated potassium perchlorate was separated by centrifuging at 2000 rpm. The supernatants were lyophyllized and redissolved in a small amount of water.

An aliquot (10—50 μl) of the urine sample or the tissue extracts was spotted on a paper (40×40 cm, Toyo Roshi No. 50) and developed with the solvent system: *n*-butanol–acetic acid–water (4: 1: 1).[4] The authentic samples were also spotted and developed concomitantly. After developing about 20 cm, the chromatogram was once dried and wetted with 0.01 M potassium phosphate buffer (pH 6.5). Subsequently, paper electrophoresis was carried out in a transverse direction by applying with 500 volt for 1 hr. The chromatograms were exposed on Sakura Type N X-ray films for 7 days to obtain the autoradiograms. By referring to the spots of authentic samples which were visualized by spraying an equivolume mixture of 1% ferric chloride and 1% potassium ferricyanide,[5] the radioactive spots of the metabolites were cut off into the counting vials, added with 1 ml of 70% ethanol and 10 ml of toluene-dioxane liquid scintillator to be measured the radioactivity.

Determination of the Brain and Other Tissue Uptake of Radioactivity after Oral Administration of DHPP-2-^{14}C and L-DOPA-3-^{14}C——Rats were administered with DHPP-2-^{14}C or L-DOPA-3-^{14}C orally in the dose of 5, 10, 20, 50, 75 and 100 mg/kg at a constant radioactive dose. After 1 hr, the animals were sacrificed by exanguination and the blood was collected in heparinized tubes. An aliquot (0.2 ml) of the blood samples was absorbed by a piece of cellulose pad and oxidized in an automatic sample oxidizer (Packard Tri-Carb

3) C.R. Harington and S.S. Randall, *Biochem. J.*, **25**, 1028 (1931); J. Harley-Mason and W.R. Waterfield, *Tetrahedron*, **19**, 65 (1963).
4) H. Shindo, T. Komai, K. Tanaka, E. Nakajima and N. Miyakoshi, *Chem. Pharm. Bull.* (Tokyo), **21**, 826 (1973).
5) G.M. Barton, R.S. Evans and J.A.F. Gardner, *Nature* (London), **170**, 249 (1952).

Model 306) to be measured the radioactivity. The brain, liver, kidney, lung, pancreas, spleen, heart, gastrointestinal tracts and testis were excised immediately and homogenized with Polytron in ice-cold 4% perchloric acid. After an adequate volume adjustment, 0.5 ml of the homogenates were solubilized with 2 ml of NCS tissue solubilizer (Nuclear Chicago Corp.), added with 15 ml of toluene liquid scintillator (8 g PPO, 200 mg dimethylPOPOP and 1000 ml toluene) to be measured the radioactivity. The radioactive concentrations were converted to μg equivalents of DHPP and DOPA on a basis of the specific activity, respectively.

Experiments on Absorption from Rat *in Situ* Acute Loop of Intestine——Male rats weighing about 120 g were used after fasting for 16 hr. After anesthetizing with ether, the rats were incised through abdominal mid-line and an acute loop of about 8 cm length was prepared from the upper, middle and lower part of intestine by ligating the both ends. The gastric loop was prepared by ligating the pyloric and cardiac ends. DHPP-2-^{14}C in physiological saline was injected into the lumen of the loop by a syringe in the dose of 1 mg/0.5 ml/loop. After 10, 30, 60 and 120 min, the loop was excised and the liquid contents in the lumen were washed out five times with ice-cold physiological saline. The combined washings were centrifuged at 9000 ***g*** and an aliquot of the supernatant was measured the radioactivity. The tissue of the loop was homogenized in 5 fold volumes of ice-cold 4% perchloric acid with Potter-Ervehjem glass homogenizer and centrifuged at 9000 ***g***. After re-extraction of the precipitates, the combined supernatant was measured of the radioactivity. The amount of DHPP-2-^{14}C transferred from the loop into the portal blood was calculated by subtracting the residual amount in the lumen and the amount retained in the tissue from the administered dose.

Result

Whole-body Autoradiography of DHPP-2-^{14}C after Intravenous and Oral Administration to Rats

The autoradiograms after intravenous administration of DHPP-2-^{14}C are represented in Fig. 1. After 1 min, the highest uptake of radioactivity was shown in the kidney cortex which exceeded the blood level and a moderate uptake was observed in the liver with a spotted pattern of radioactivity in the hepatic vein. Appreciable amount of radioactivity was shown in the adrenal medulla and hair follicles, but there was little radioactivity in the brain. After 10 min, the blood radioactivity declined considerably and the highest uptake was shown in the kidney, being extended from the cortex to the medulla and the urinary bladder, indicating a very rapid excretion of radioactivity through the urinary route probably corresponding to a rapid metabolism of DHPP to dopamine *via* L-DOPA and an active secretion of dopamine into the urine. A high uptake was observed in the liver, adrenal medulla, pancreas, hair follicles and intestinal mucosa. No appreciable uptake was detected in the brain, indicating that DHPP cannot pass easily through the blood brain barrier. Thirty minutes to 1 hr after administration, the highest uptake was observed in the liver, followed by the kidney, adrenal medulla, pancreas, hair follicles and intestinal contents. In the kidney, the highest concentration was located in the medulla rather than the cortex. An appreciable radioactivity was observed in the brain by this time and activity was recognized to be localized in the caudate nucleus. The distribution pattern of radioactivity is thus similar to that after administration of L-DOPA with respect to the characteristic distribution of radioactivity in the caudate nucleus, adrenal medulla, pancreas, hair follicles, renal medulla and intestinal mucosa,[6] demonstrating the transformation of DHPP-2-^{14}C to L-DOPA-2-^{14}C *in vivo*. After 24 hr a high radioactivity remained only in the adrenal medulla and renal medulla, in consistent with the retention of L-DOPA-2-^{14}C.[6] It has been observed in the autoradiography after oral administration of L-DOPA-2-^{14}C that the brain uptake of radioactivity increases significantly with increasing the oral dose.[6] Therefore, in order to evaluate the effectiveness of DHPP as the brain dopamine precursor, autoradiography was carried out after oral administration of DHPP-2-^{14}C wherein the oral dose was increased gradually (10, 50 and 100 mg/kg). However, only an extremely low uptake of radioactivity was observed in the brain at any

6) H. Shindo, N. Miyakoshi and I. Takahashi, *Chem. Pharm. Bull.* (Tokyo), **19**, 2490 (1971); H. Shindo. N. Miyakoshi and E. Nakajima, *Chem. Pharm. Bull.* (Tokyo), **20**, 966 (1972).

dose level of DHPP-2-^{14}C, even at a high oral dose of 100 mg/kg. As shown in Fig. 2, the blood concentration of radioactivity was extremely low 1 hr after oral administration and there were no significant differences in the distribution pattern of radioactivity among three different oral dosages. A high radioactivity continues only in the gastric and intestinal contents and a moderate level of radioactivity was distributed only in the liver, kidney, adrenal medulla, pancreas and hair follicles. The uptake in other organs including the brain was as a low degree as the blood level. The excretion pattern of radioactivity through the urine

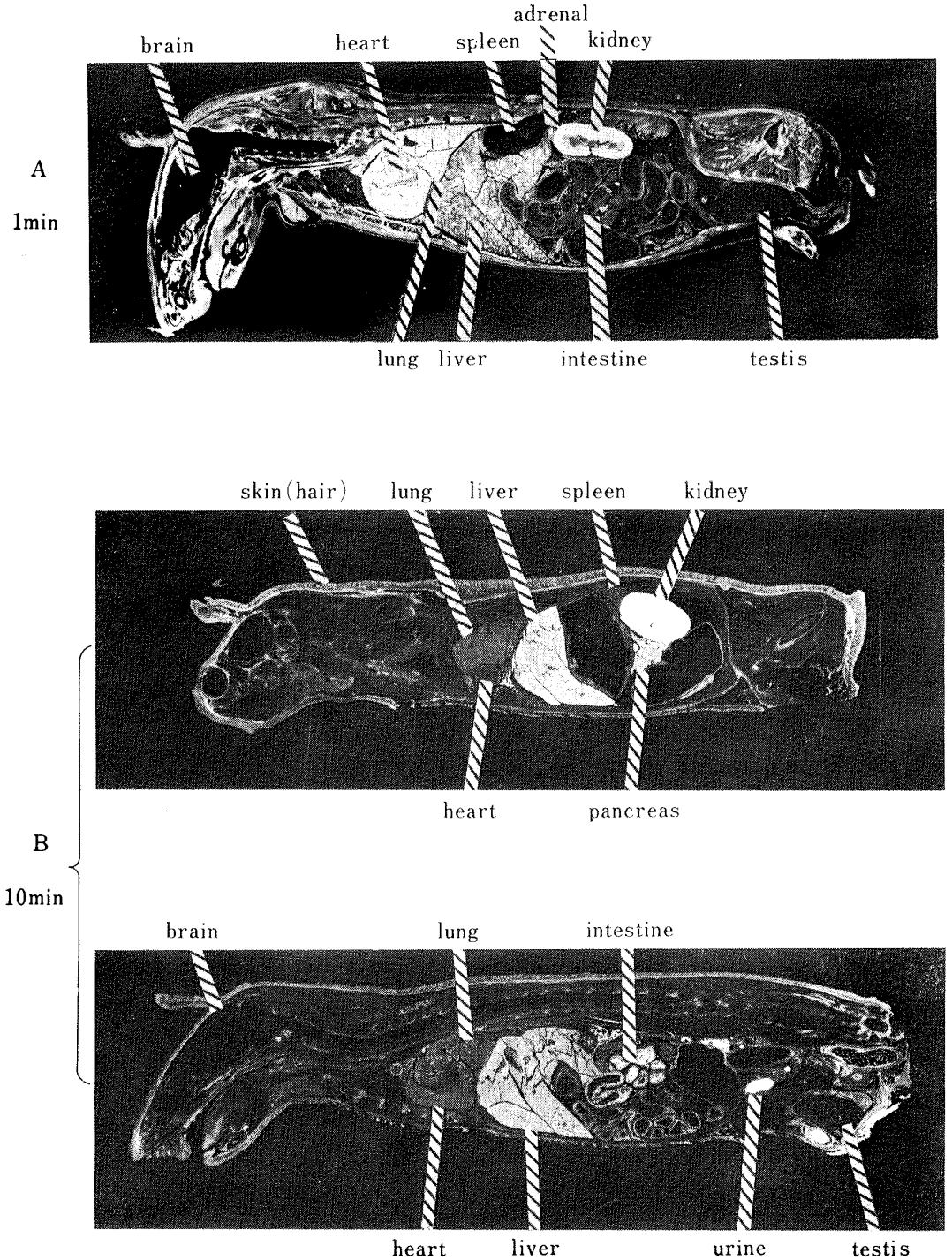

Fig. 1–A,B. Autoradiograms after Intravenous Administration of DHPP-2-^{14}C to Rats (10 mg/kg). A: 1 min, B: 10 min

was detected appreciably, but the high retention of radioactivity in the gastrointestinal tract indicates a slow and gradual absorption of DHPP from the intestine.

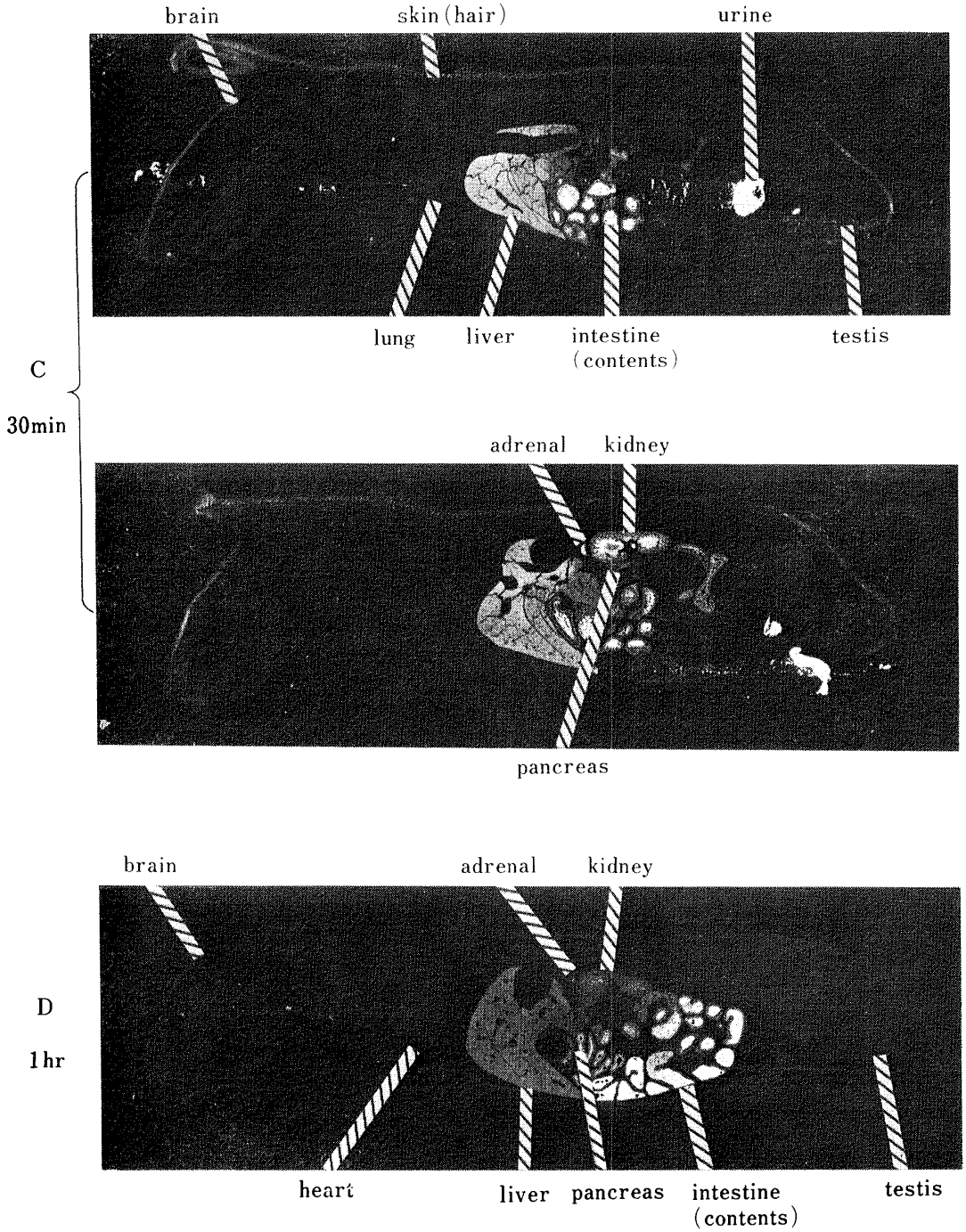

Fig. 1-C,D. Autoradiograms after Intravenous Administration of DHPP-2-^{14}C to Rats (10 mg/kg), C: 30 min, D: 1 hr

Urinary, Fecal and Respiratory Excretion of Radioactivity after Intravenous and Oral Administration of DHPP-2-^{14}C to Rats

The time course of urinary and fecal excretion of radioactivity after intravenous and oral administration of DHPP-2-^{14}C (10 mg/kg) to rats are shown in Fig. 3. Most of the urinary excretion was completed before 24 hr after the both routes of administration, but the urinary excretion after oral administration was found to be significantly lower than that after

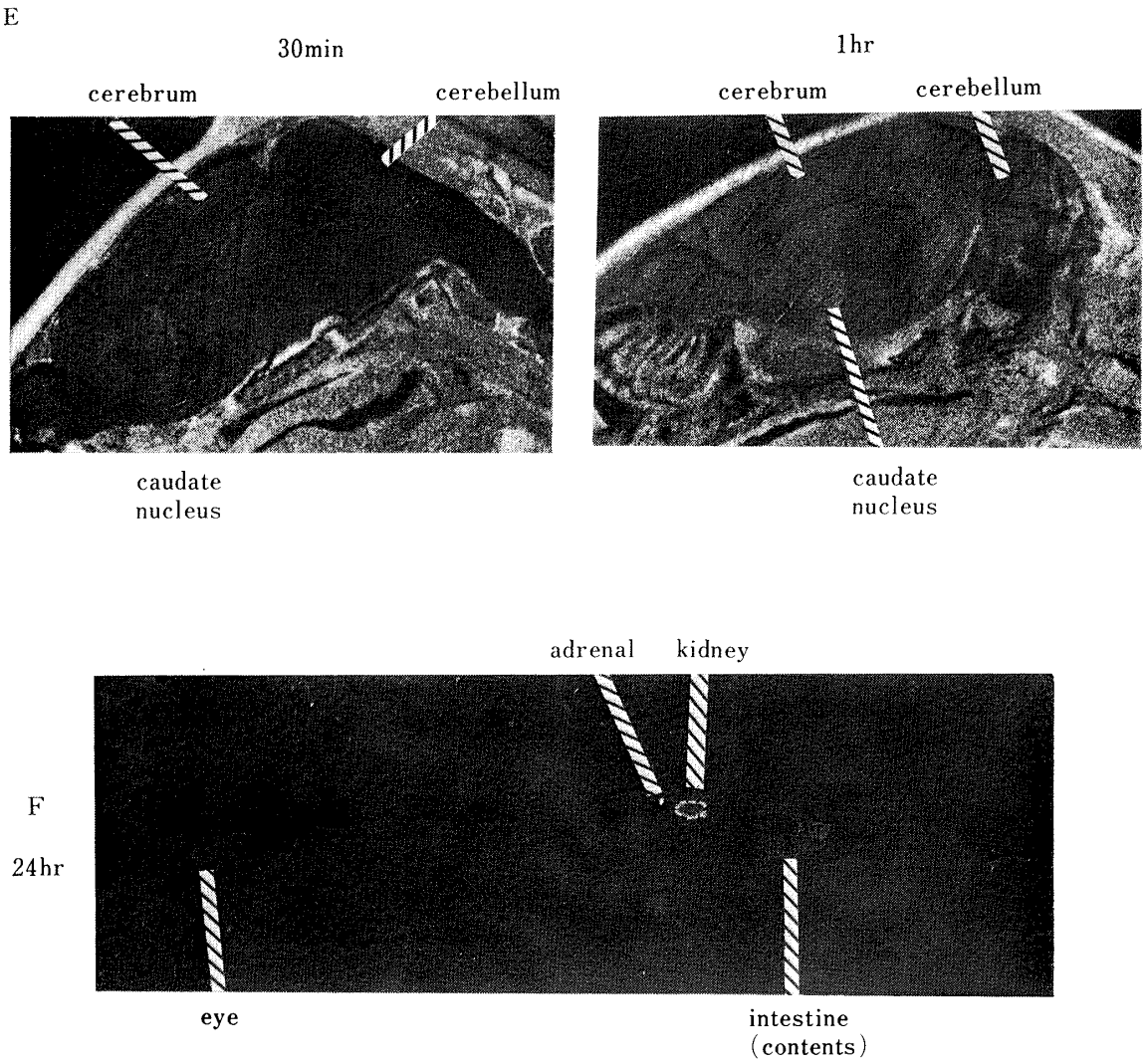

Fig. 1–E,F. Autoradiograms after Intravenous Administration of DHPP–2–^{14}C to Rats (10 mg/kg), E: enlargement of C and D, F: 24 hr

intravenous administration, suggesting that the absorption of DHPP from the intestine is rather limited. During 72 hr after oral administration, about 44 and 11.7% of the dose was excreted in the urine and feces, respectively, as compared to the respective values of about 85.4 and 2.8% after intravenous administration. In the whole carcass, however, only 0.4 and 0.1% of the administered dose was recovered, the total recovery of the radioactivity being an unusually low value of 56.1% after oral administration as compared to 88.3% after intravenous administration.

Thus, the respiratory excretion of radioactivity as $^{14}CO_2$ was tested *in vivo*. After oral administration of DHPP-2-^{14}C (10 mg/kg), it was found that a considerable radioactivity appeared in the respiratory air with a lag time of 2 to 3 hr after administration, as shown in Fig. 4. The cummulative amount of excretion reached to about 28% of the administered dose. Following intravenous administration, also an appreciable radioactivity appeared in the respiratory air, but, as compared in Fig. 4, the excretion started soon after the administration without any lag time and the total excretion was only about 1/5 to that after oral administration, about 6% of the dose. The total recovery of radioactivity including the respiratory $^{14}CO_2$ during 72 hr period after administration was summarized in Table I.

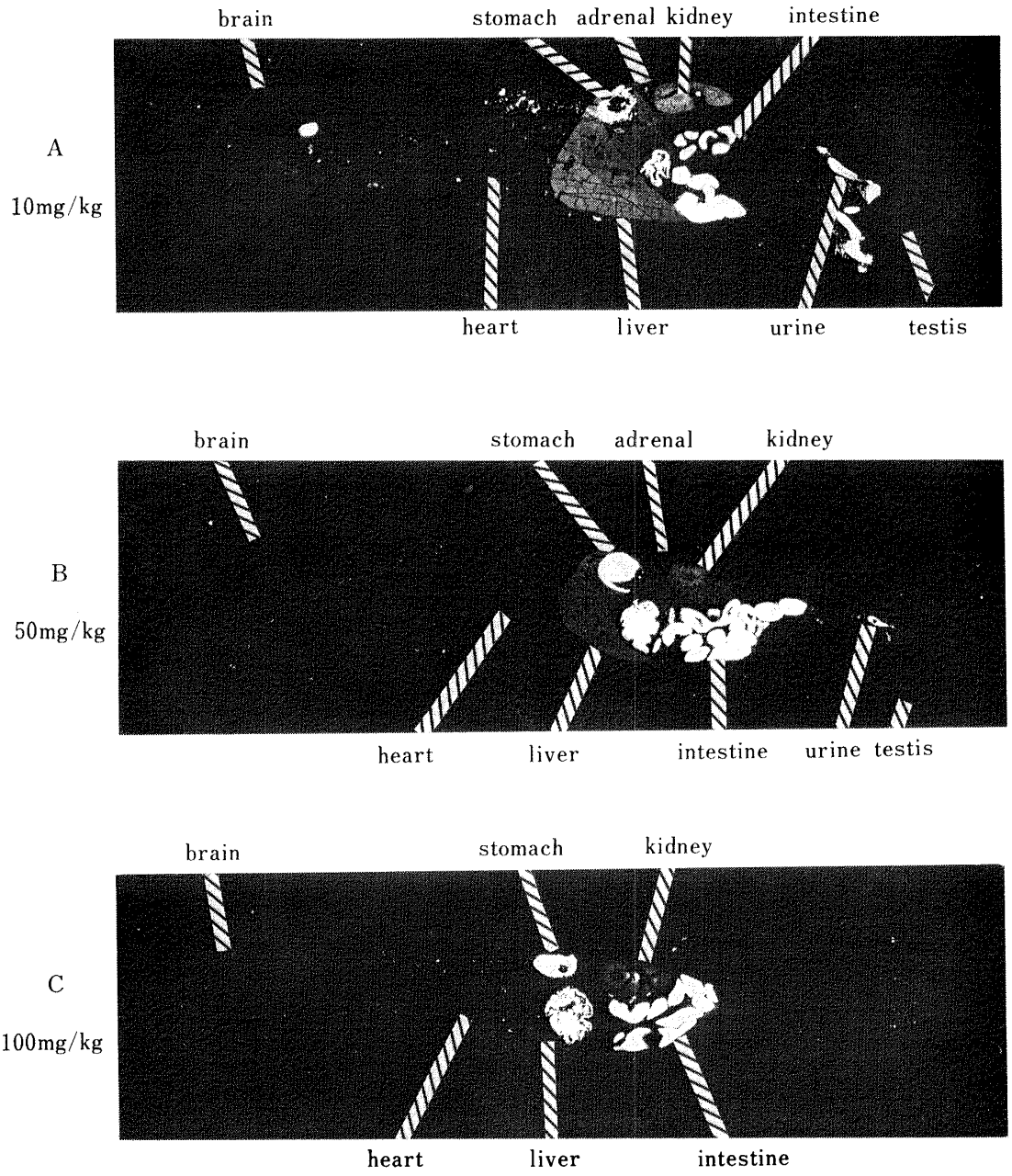

Fig. 2. Autoradiograms 1 hr after Oral Administration of DHPP-2-^{14}C to Rats at Various Dosages (A: 10 mg/kg, B: 50 mg/kg, C: 100 mg/kg)

Urinary Metabolites of DHPP-2-^{14}C after Intravenous and Oral Administration to Rats

The urinary metabolites after oral and intravenous administration of DHPP-2-^{14}C to rats were separated by two-dimensional PPC-PEP method, as represented in Fig. 5. Unchanged DHPP and DOPA were detected only as a minor portion, while dopamine, its conjugate, HVA and DOPAC were detected as the main metabolites, demonstrating that a ready conversion of DHPP to L-DOPA and its subsequent metabolites occurrs *in vivo*. The relative percentage of DHPP and its metabolites in the urine are shown in Table II. Three major metabolites of L-DOPA, that is: dopamine, its conjugate and HVA[4] accounted for the most part of the total urinary radioactivity, about 68 and 77% after oral and intravenous administration, respectively.

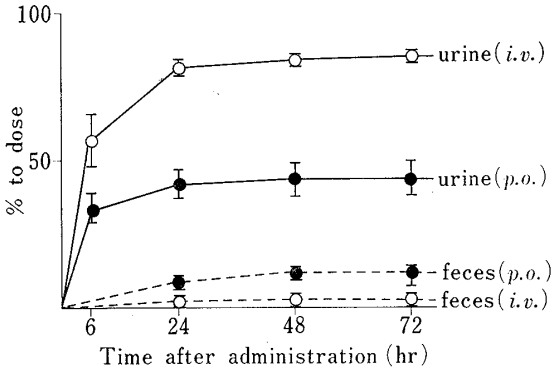

Fig. 3. Urinary and Fecal Excretion of Radioactivity after Oral and Intravenous Administration of DHPP-2-^{14}C to Rats

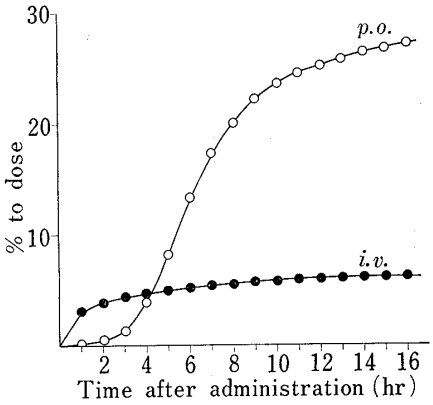

Fig. 4. Excretion of Radioactivity into Expiration after Oral and Intravenous Administration of DHPP-2-^{14}C (10 mg/kg, $n=2$)

Table I. Excretion of Radioactivity after Oral and Intravenous Administration of DHPP-2-^{14}C to Rats (10 mg/kg, $n=3$, 72 hr)

	% to dose	
	p.o.	*i.v.*
Urine	43.97±5.48	85.44±1.22
Feces	11.68±2.41	2.79±0.26
Carcass	0.42±0.25	0.11±0.02
Expiration[a)]	28.01	6.30
Total	84.08	94.64

a) $n=2$.

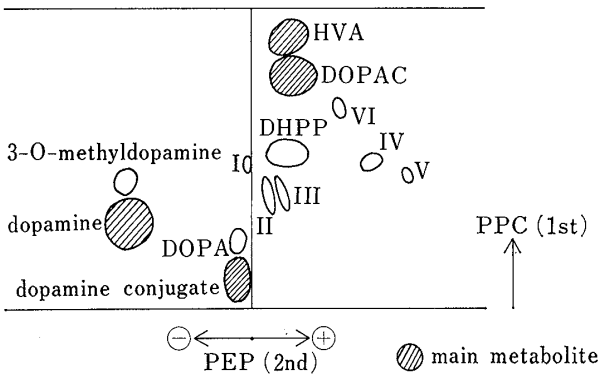

Fig. 5. Autoradiogram of Urinary Metabolites of DHPP-2-^{14}C after Oral and Intravenous Administration to Rar Rats

Table II. Urinary Metabolites after Oral and Intravenous Administration of DHPP-2-^{14}C to Rats (10 mg/kg, $n=3$, 24 hr)

Metabolite	% to radioactivity	
	p.o.	*i.v.*
L-DOPA	0.66±0.03	0.98±0.06
DHPP	3.06±0.59	4.20±0.66
Dopamine	23.77±4.04	58.77±0.85
Dopamine conjugate	23.16±1.89	7.63±0.37
3-O-methyldopamine	4.03±0.51	2.57±0.35
DOPAC	5.88±0.57	6.70±0.81
HVA	21.13±3.45	10.32±0.67
Unknown I	0.50±0.09	0.57±0.23
Unknown II	4.84±1.27	4.19±0.67
Unknown III	4.89±0.30	1.69±0.23
Unknown IV	7.07±1.61	1.68±0.42
Unknown V	1.00±0.56	0.69±0.32
Unknown VI[a)]	—	—

a) Detected only in tissue extracts.

It was noted, however, that when DHPP-2-^{14}C was administered intravenously dopamine was excreted into the urine mostly as the free form, accounting for about 60% of the total urinary radioactivity. On the contrary, when administered orally, dopamine was excreted to a much larger extent as the conjugate form (Table II). This difference is similar to that observed between intravenous and oral administration of L-DOPA-2-^{14}C[4] and thus suggest that the metabolic conversion of DHPP to L-DOPA in the kidney is highly participating after intravenous administration, while that in the liver and, probably, in the intestine is also participating after oral administration.[7]

Metabolites in several tissues 30 min after intravenous administration of DHPP-2-^{14}C were separated similarly and as shown in Table III, the metabolites derived from L-DOPA were detected in all the tissues. The main metabolite was dopamine conjugate in the liver and intestine, and 3-O-methyl DOPA in other tissues. HVA and DOPAC were also detected in all the tissues. These results were well accordant with that after administration of L-DOPA-2-^{14}C.[4] Dopamine was detected in the brain, indicating that the exogeneous DHPP can be the precursor of brain dopamine. However, the content was extremely low (3.06%) as compared to that after intravenous administration of L-DOPA-2-^{14}C (15.6%).

Table III. Tissue Metabolites 30 min after Intravenous Administration of DHPP–2–^{14}C to Rats (10 mg/kg)

Metabolite	% to radioactivity					
	Brain	Heart	Intestine	Kidney	Liver	Pancreas
L-DOPA	4.08	7.75	4.28	2.96	3.05	10.67
3-O-Methyl DOPA	33.54	25.36	13.26	3.06	2.99	50.05
Dopamine	3.06	1.05	1.09	8.75	0.37	2.31
Dopamine conjugate	—	7.09	61.87	6.84	79.90	4.26
3-O-Methyldopamine	0.84	—	—	9.87	0.22	0.96
DOPAC	6.00	4.65	5.18	5.25	1.29	7.75
HVA	10.55	19.86	11.51	30.12	1.07	23.19
DHPP	—	4.31	2.80	—	—	—
Unknown I	—	—	—	—	—	—
Unknown II	—	—	—	8.70	0.44	—
Unknown III	—	0.97	—	16.95	5.21	—
Unknown IV	—	—	—	—	—	—
Unknown V	8.70	28.20	—	7.51	2.07	—
Unknown VI	32.06	—	—	—	2.60	—

In order to evaluate DHPP as the brain dopamine precursor more quantitatively, the concentration of radioactivity in the blood, brain and other tissues was compared between L-DOPA-3-^{14}C and DHPP-2-^{14}C after oral administration of their various dosages to rats. The results are shown in Fig. 6 and Table IV, expressed as μg equivalents of L-DOPA and DHPP, respectively. After oral administration of DHPP-2-^{14}C the radioactive uptake by the brain was found to be the same degree as that after administration of L-DOPA-3-^{14}C at the low dosages (5 and 10 mg/kg) and increased linearly with increasing the amount of dose. After administration of L-DOPA-3-^{14}C, the brain uptake of radioactivity showed a marked increase with increasing the amount of dose over 20 to 50 mg/kg, in consistent with the previous autoradiographic observation.[6] It must be concluded therefore that DHPP cannot surpass L-DOPA in the amount of the brain uptake after oral administration at any dose amount.

7) L. Landsberg, M. Berardino and P. Silva, *Clin. Res.*, **22**, 688A (1974).

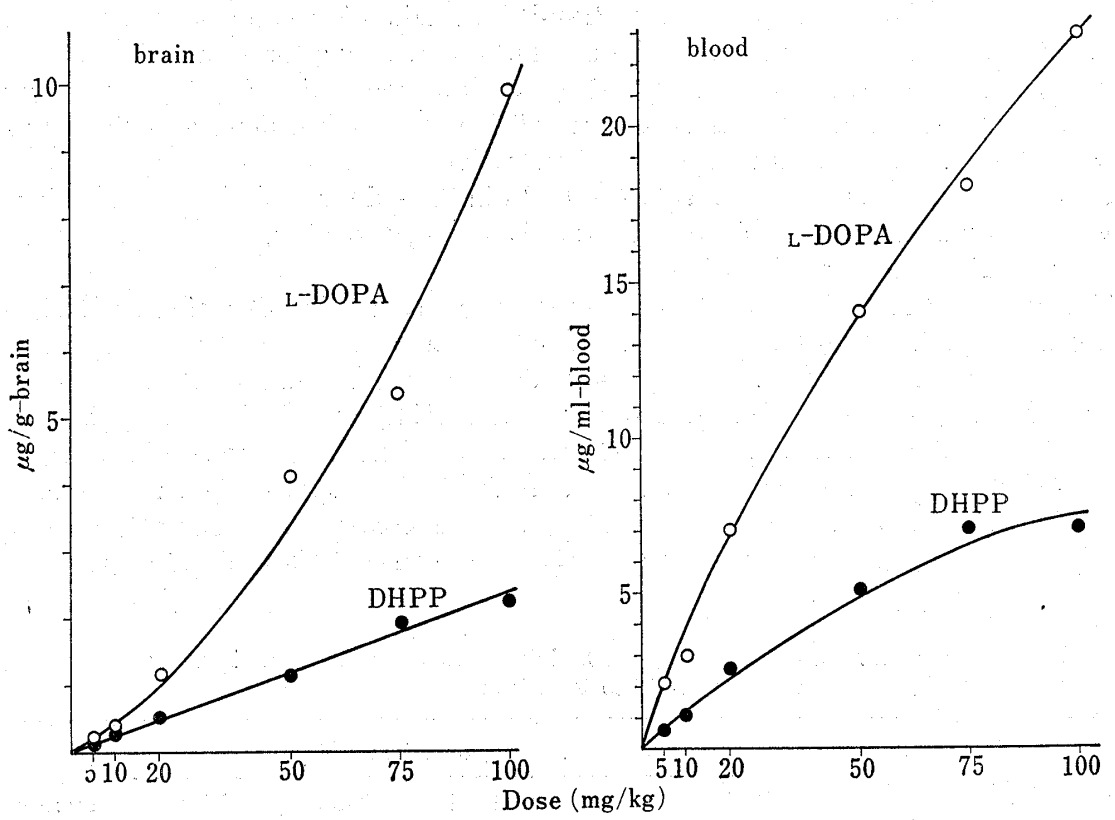

Fig. 6. Brain and Blood Concentration of Radioactivity 1 hr after Oral Administration of L-DOPA-3-^{14}C and DHPP-2-^{14}C to Rats

Table IV. Concentration of Radioactivity 1 hr after Oral Administration of L-DOPA-3-^{14}C and DHPP-2-^{14}C to Rats at Various Dosages

Tissue	Dose (mg/kg)											
	5		10		20		50		75		100	
	L-DOPA	DHPP	L-DOPA	DHPP	L-DOPA	DHPP	L-DOPA	DHPP	L-DOPA	DHPP	L-DOPA	DHPP
Blood	2.17	0.63	3.02	1.22	6.85	2.66	14.12	5.26	18.37	7.07	23.20	6.98
Brain	0.22	0.18	0.36	0.26	1.17	0.53	4.11	1.12	5.34	1.95	9.90	2.23
Heart	1.87	0.40	1.76	2.92	5.07	5.39	9.76	2.12	13.86	5.54	20.43	19.93
Gastrointestinal tracts	37.57	97.22	126.69	291.57	217.97	444.27	557.43	1056.65	996.55	1635.76	1320.10	2117.65
Kidney	9.52	2.12	13.00	4.23	26.65	12.53	60.90	22.54	90.70	36.82	89.86	50.29
Liver	10.32	2.57	24.26	5.24	50.46	12.70	179.73	22.87	221.23	36.03	303.28	34.42
Lung	1.88	1.07	3.17	3.25	8.93	2.89	13.37	4.83	16.46	5.24	24.03	15.40
Pancreas	2.43	1.12	2.97	2.69	12.31	4.87	49.88	8.56	96.26	16.37	140.35	64.34
Spleen	1.03	0.50	2.18	0.91	9.43	8.64	12.56	3.79	18.19	3.64	31.32	49.96
Testicle	1.62	0.41	0.97	1.13	3.02	0.99	6.70	2.45	8.58	3.43	14.67	3.30

μg equivalent-L-DOPA or DHPP/g-tissue or ml-blood.

The blood level of radioactivity after administration of DHPP-2-^{14}C was shown to be much lower than that after administration of L-DOPA-3-^{14}C (Fig. 6). In all other organs and tissues examined, the uptake of radioactivity was also much lower in DHPP-2-^{14}C than L-DOPA-3-^{14}C, as shown in Table IV. It was noted also that the radioactivity in the gastro intestinal tracts was about two fold higher in DHPP-2-^{14}C than L-DOPA-3-^{14}C, suggesting that the absorption of DHPP from intestine occurrs much more slowly than L-DOPA.

Absorption of DHPP-2-^{14}C from *in Situ* Rat Intestine

In order to see the rate of intestinal absorption of DHPP, the absorption profile of DHPP-2-^{14}C from *in situ* acute loop of rat intestine was examined in comparison with L-DOPA-3-^{14}C. The results revealed that, as shown in Fig. 7, the absorption of DHPP from the small intestine is proceeded much more slowly than L-DOPA. After 60 min, only about 30% of DHPP-2-^{14}C administered had disappeared from the lumen and only less than 9% of the dose was transferred into the portal blood stream. On the contrary, the absorption of L-DOPA was almost completely finished by the same time: over 95% of the dose had disappeared from the lumen and about 90% of the dose was estimated to be transferred into the portal blood stream. It was also revealed that there was no significant difference among the absorption rate of DHPP from the upper, middle and lower parts of small intestine and the absorption from the stomach was slower (Table V).

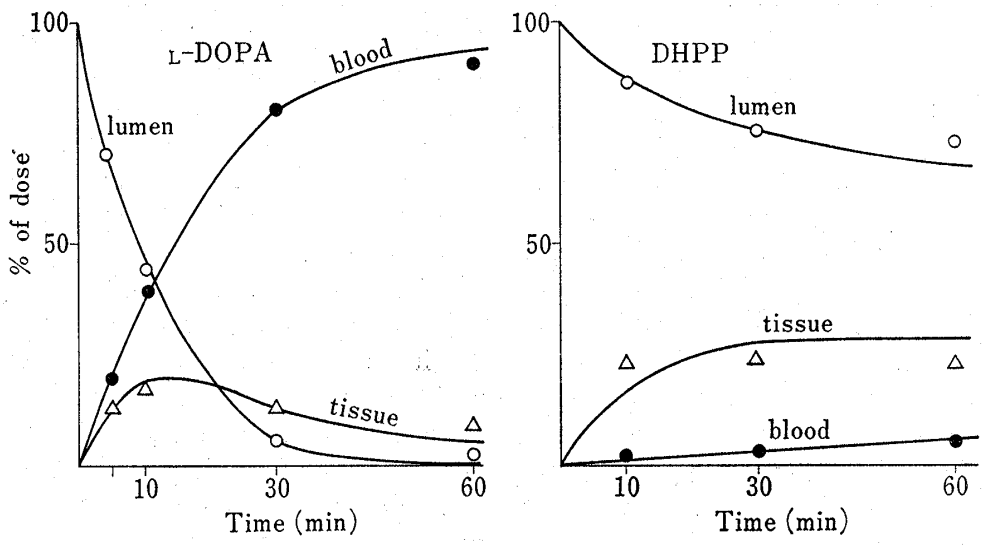

Fig. 7. Absorption of DHPP and L-DOPA from Acute Loop of Rat Small Intestine (1 mg/8 cm-loop)

TABLE V. Absorption of DHPP from Acute Loop of Various Site of Gastrointestinal Tracts (1 hr, 1 mg/8 cm-loop)

		% to dose		
		Disappearence from lumen	Accumulation in loop tissue	Transference into blood stream
Stomach		25.92± 2.34	10.53±2.41	15.40±2.55
Intestine	Upper	30.45±10.5	22.45±7.79	8.28±4.51
	Middle	30.69± 2.54	15.89±2.45	14.80±2.20
	Lower	36.49± 4.49	8.01±1.52	28.48±4.73

Discussion

In the present investigations, it was found that the most of radioactivity in the urine and tissue after administration of DHPP-2-^{14}C to rats were explained by the metabolites derived from L-DOPA, that is: dopamine and its conjugate, 3-O-methyldopamine, 3-O-methyl DOPA, DOPAC and HVA. The distribution characteristics of radioactivity was also shown to be very similar to that after administration of L-DOPA-2-^{14}C and a high uptake was observed in the adrenal medulla, caudate nucleus of the brain, pancreas, renal medulla and hair follicles. From these results, it might be concluded that DHPP is readily converted to

L-DOPA *in vivo* as well as *in vitro*, accepting an amino group from endogenous amino donors. The fact that dopamine and its metabolites were detected in the brain after intravenous administration of DHPP-2-^{14}C indicates that DHPP can be actually a precursor of dopamine in the central nervous system. Because DHPP was proved not to pass easily through the blood-brain barrier, these metabolites must be derived from L-DOPA incorporated into the brain after being formed from DHPP peripherally.

It was expected from *in vitro* studies that the oral use of DHPP instead of L-DOPA must be advantageous with respect to a much less decarboxylation in the intestine and liver, resulting in a higher availability of L-DOPA in the general circulation.[1] From the present evaluation *in vivo* in comparison with L-DOPA, however, it must be concluded that the oral use of DHPP as a precursor of brain dopamine cannot surpass that of L-DOPA, because of the following reasons. The first is that the absorption of DHPP from intestine is much slower than L-DOPA. The brain uptake of radioactivity after oral administration of DHPP-2-^{14}C was found to be considerably lower than that after administration of L-DOPA-3-^{14}C for the whole dose ranges tested (5 to 100 mg/kg). This is considered to be due to a slow absorption of DHPP from intestine and a slow supply of DHPP and/or L-DOPA to the liver will not be able to overcome the decarboxylation of L-DOPA, resulting in a substantially low concentration of L-DOPA in the general circulation. It has been clarified that L-DOPA is absorbed from intestine by an active transport mechanism, sharing the transport system for neutral amino acid.[8] On the contrary, a slow rate of DHPP absorption may be explained by the transport through a passive diffusion mechanism. The second finding is that an appreciable amount of DHPP-2-^{14}C administered was metabolized to $^{14}CO_2$ after administration to rats and a metabolic pathway other than that through L-DOPA must be also involved. The excretion was found to be larger after oral administration (28%) than after intravenous administration (6%), suggesting a first pass effect in the liver. In fact, as described in the previous paper,[1] among main organs, the highest activity of degrading DHPP-2-^{14}C to $^{14}CO_2$ was found to be in the liver *in vitro*.[1] The third reason is that DHPP itself cannot pass through the blood-brain barrier. By means of whole-body autoradiography after intravenous administration of DHPP-2-^{14}C, only a faint radioactive uptake was detected in the brain. *In vitro*, DHPP was found to be easily transformed to L-DOPA in the brain homogenates.[1]

Among these factors, the main reason responsible for the failure as an oral precursor of brain dopamine is considered to be a very slow rate of absorption of DHPP from the intestine. Esterification of DHPP to increase the absorbability is now being attempted in this laboratories. Apart from the parkinsonism therapy, however, it is expected that DHPP might reduce the blood concentration of phenylalanine which is known to be unusually high in phenylketonurea,[9] because DHPP is transformed to L-DOPA accepting an amino group from endogeneous amino donors among which phenylalanine showed the highest activity.[1]

Acknowledgement The authors express their deep gratitudes to Prof. S. Tsurufuji of Tohoku University for his valuable discussions and to Dr. K. Arima, the director of this laboratories, for his kind encouragements. The authors are also grateful to Mr. T. Kurano and Mr. Y. Fujima of Sankyo Chemical Industries and Mr. T. Hashimoto of this laboratories for their kind and careful advices during the course of the preparation of the labeled compound. The authors must express appreciation to Mr. K. Kawai and Miss K. Tanaka of this laboratory for their collaboration.

8) H. Shindo, T. Komai and K. Kawai, *Chem. Pharm. Bull.* (Tokyo), **21**, 2031 (1973).
9) G.A. Jervis, R.J. Block, D. Bolling and E. Kanze, *J. Biol. Chem.*, **134**, 105 (1940).