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# Studies on the Metabolism of D- and L-Isomers of 3,4-Dihydroxyphenylalanine (DOPA). III.[1] Absorption, Distribution and Excretion of D- and L-DOPA-$^{14}$C in Rats following Intravenous and Oral Administration[2]

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The excretion and tissue distribution of radioactivity were comparatively investigated between D- and L-isomers of 3,4-dihydroxyphenylalanine(DOPA)-2-$^{14}$C after intravenous and oral administration to rats. Both D- and L-DOPA was eliminated mainly through the urinary route and the initial rate after intravenous administration was faster in the D- than the L-isomer. After oral administration (60 mg/kg), most of radioactivity (85%) from L-DOPA was recovered in the urine, while approximately 50 and 23% from D-DOPA in the urine and feces, respectively, indicating a low absorbability of D-DOPA from the gastrointestinal tract in contrast to an almost complete absorption of L-DOPA. After intravenous administration, the tissue uptake of L-DOPA was significantly higher in most of tissues such as the brain, skeletal muscle, liver, intestine and adrenal. In the pancreas, both isomer showed a high accumulation. After oral administration, the tissue uptake of L-DOPA was significantly decreased and higher than the D-isomer only in the intestine, liver and adrenal, while in all of other tissues including the brain, skeletal muscle and pancreas the D-isomer showed a higher accumulation and a longer retention. This was considered to be due to a considerable metabolism of L-DOPA to dopamine at the peripheral sites as the gastric and intestinal mucosa and liver, while to a gradual absorption and accumulation in the tissues of D-DOPA without being metabolized.

It has been established clinically that[4] 3,4-dihydroxyphenylalanine (DOPA) is an effective agent against Parkinsonism and that[5] the administration of the L-isomer rather than the racemate reduces the side effect significantly with an increased therapeutic effect, suggesting that the D-isomer has only an unbeneficial effect. It was thought to be of particular interest and importance, therefore, to compare the metabolic fate of the D- and L-isomers in animal organism. In the previous papers,[6,1] the distribution of radioactivity was compared between D- and L-DOPA-$^{14}$C by means of whole-body autoradiography after intravenous and oral administration to rats. The results revealed that there are many marked differences in the behaviors of radioactivity between the two isomers and the possible relations to their pharmacological effects were pointed out. In the present paper, the rate and amount of excretion into the urine and feces and the tissue distribution of radioactivity are compared quantitatively by means of liquid scintillation counting between the two isomers, after intravenous and oral administration to rats.

## 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

1) Part II: H. Shindo, N. Miyakoshi, and E. Nakajima, *Chem. Pharm. Bull.* (Tokyo), **20**, 966 (1972).
2) This work was presented at the 3rd Symposium on Drug Metabolism and Action, Fukuoka, November, 1971.
3) Location: *Hiromachi 1-chome, Shinagawa-ku, Tokyo.*
4) G. C. Cotzias, M.H. Van Woert, and L.M. Schiffer, *New Engl. J. Med.*, **276**, 374 (1967).
5) G.C. Cotzias, P.S. Papavasiliou, and R. Gellene, *New Engl. J. Med.*, **280**, 337 (1969).
6) H. Shindo, N. Miyakoshi, and I. Takahashi, *Chem. Pharm. Bull.* (Tokyo), **19**, 2490 (1971).

paper.[6] The specific activity was 24.9 and 26.4 $\mu$Ci/mg for D-and L-DOPA-$^{14}$C, respectively, and the radiochemical purity as radioactive D- and L-isomers was over 97% for the both preparations.

**Animals and Administration**——Male rats of Wistar–Imamichi strain weighing 160±5 g were used in all the experiments. For intravenous injection, the radioactive D- and L-DOPA were diluted with non-radioactive D- and L-DOPA, respectively, to give a constant specific activity of 14.55 $\mu$Ci/mg and dissolved in physiological saline containing 0.001N HCl to a concentration of 3.3 mg/ml. The rats were injected with 0.5 ml of the solution from the tail vein (10 mg/kg). For oral administration, the radioactive compounds were diluted to give a constant specific activity of 2.19 $\mu$Ci/mg and dissolved to a concentration of 9.15 mg/ml. The rats were administered orally with 1 ml of the solution with a stomach tube (57.2 mg/kg).

**Experiments on Excretion**——Thirty six rats were used for the experiments, each group being consisted of three rats. Each rat was placed in an individual metabolic cage and the urine from each group of rats was collected for the period of 30 min, 1, 3, 6, 24, and 96 hr after administration, respectively. For collecting the urine from the group for the period shorter than 6 hr after administration, the animals were sacrificed, the urinary bladder was removed by dissection, the contents drained, the bladder washed and the washings were combined with the urine. The collected urine sample was centrifuged and the supernatant was assayed for radioactivity. The feces were collected for the period of 24 and 96 hr after administration. They were homogenized and extracted with three portions of 100 ml of 4% $HClO_4$ and the supernatant after centrifugation was assayed for radioactivity. The whole carcass was solubilized by warming in about 2 volumes of 30% KOH solution at 80° overnight. After making the solution to 500 ml, 0.5 ml was assayed for radioactivity.

**Experiments on Blood and Tissue Concentration**——After a given time after administration, the rats were sacrificed by bleeding from the carotid artery. The blood was collected into a heparinized test tube and the animal was immediately dissected to remove the brain, heart, lung, liver, kidney, adrenal, pancreas, intestine (duodenum), skeletal muscle (femoral) and skin (abdominal). After weighing, 2 g or the whole of the organs and tissues were homogenized and extracted twice with 4 ml portions of 4% $HClO_4$ in Potter glass homogenizer. The combined extracts were assayed for radioactivity. The adrenal was dissolved in 0.5 ml of 30% KOH solution by warming at 80° for 3 hr. The blood was extracted twice with equal volume of 4% $HClO_4$ and the extract was assayed for radioactivity. The recovery of radioactivity in the extraction procedure was ascertained to be over 98% for blood and all the tissues.

**Radioactivity Measurement**——The urine sample and all the extracts were counted in a Beckman LS-250 liquid scintillation spectrometer, using a counting medium consisted of 8 g PPO, 200 mg dimethyl-POPOP, 200 ml of toluene and 800 ml of 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.

## Result

### Urinary and Fecal Excretion of D- and L-DOPA-$^{14}$C after Intravenous and Oral Administration

In Table I, the urinary and fecal excretion of radioactivity and the residual radioactivity in the body are compared between the two isomers after intravenous injection to rats. The initial rate of the urinary excretion was found to be significantly faster for the D-isomer than the L-isomer and the amount excreted in the urine during the first 1 hr was about 1.5 times higher for the D-isomer than for the L-isomer. During 24 hr period, however, approximately 80% of the dose was excreted into the urine after administration of both D- and L-DOPA, indicating that the excretion is proceeded mainly through the urinary route for the both isomers. In accordance with this, only 2 to 3% of the dose was recovered in the feces from both isomers in 96 hr period. The residual radioactivity in the whole carcass after survival periods of 6, 24 and 96 hr was, however, found to be appreciably higher for the D-isomer than the L-isomer, suggesting a longer retention of radioactive D-DOPA in the tissues. The difference was statistically significant after 24 hr with P value less than 5%.

For the oral administration, a higher dose level of about 60 mg/kg was chosen because of the reason described in the preceding paper.[1] As shown in Table II, the initial rate of urinary excretion of L-DOPA-$^{14}$C was, as expected, much slower than that after the intravenous administration (Table I). In 24 hr period, however, over 85% of the dose was recovered in the urine, while only about 5% in the feces, indicating that the absorption of L-DOPA from the gastro-intestinal tract was almost complete. In contrast, the urinary excretion of D-DOPA-$^{14}$C was found to be limited and only 53% of the dose was recovered in the urine in

TABLE I. Urinary and Fecal Excretion of Radioactivity following Intravenous Administration of L-and D-DOPA-$^{14}$C to Rats(10 mg/kg)

| Hours after administration | %to dose±S.E. ($n$=3) | | | | | |
|---|---|---|---|---|---|---|
| | Urine | | Feces | | Carcass | |
| | L-DOPA | D-DOPA | L-DOPA | D-DOPA | L-DOPA | D-DOPA |
| 0.5 | 32.27±2.57 | 50.01±3.60 | —[a] | — | — | — |
| 1 | 37.10±5.90 | 57.06±3.62 | — | — | — | — |
| 3 | 56.62±5.96 | 54.37±3.90 | — | — | — | — |
| 6 | 68.25±3.77 | 66.56±2.63 | 0.20±0.09 | 0.30±0.22 | 11.87±2.45 | 14.77±1.57 |
| 24 | 76.42±4.87 | 79.88±6.34 | 2.22±1.59 | 1.66±0.46 | 3.62±0.02 | 5.28±0.14 |
| 96 | 88.61±2.94 | 78.59±4.01 | 2.36±0.16 | 2.67±0.67 | 0.72±0.03 | 0.81±0.08 |

a) not determined

TABLE II. Urinary and Fecal Excretion of Radioactivity following Oral Administration of L- and D-DOPA-$^{14}$C to Rats(60 mg/kg)

| Hours after administration | %to dose±S.E. ($n$=3) | | | | | |
|---|---|---|---|---|---|---|
| | Urine | | Feces | | Carcass | |
| | L-DOPA | D-DOPA | L-DOPA | D-DOPA | L-DOPA | D-DOPA |
| 1 | 20.23±1.54 | —[a] | — | — | — | — |
| 3 | 52.46±1.36 | — | — | — | — | — |
| 6 | 71.88±6.08 | 43.68±3.54 | 0.09±0.02 | 0.57±0.52 | 26.42±3.56 | 41.94±1.16 |
| 24 | 87.33±1.96 | 53.37±2.38 | 4.37±1.29 | 22.74±1.62 | 2.72±0.32 | 10.90±1.96 |
| 96 | 88.99±2.51 | — | 4.57±0.44 | — | 0.68±0.08 | — |

a) not determined

24 hr period, while about 23% of the dose in the feces. The residual radioactivity in the whole animal 6 and 24 hr after administration was found to be significantly higher for the D-isomer than for the L-isomer.

**Blood and Tissue Distribution of D- and L-DOPA-$^{14}$C after Intravenous Administration**

As shown in Table III and compared in Fig. 1, significant differences were found between the two isomers in the tissue uptake. The highest concentration of radioactivity was observed already 10 min after injection of L-DOPA-$^{14}$C in most of the tissues, indicating a very fast and high uptake of L-DOPA by the tissues from the blood circulation. Thirty minutes after injection of L-DOPA-$^{14}$C, the concentration of radioactivity was found to be in the order: kidney>pancreas~intestine>liver>adrenal>skin~skeletal muscle>lung>cardiac muscle >brain>blood, while after that of D-DOPA-$^{14}$C in the order: pancreas>kidney≫intestine> skin>lung~liver>adrenal>cardiac muscle~skeletal muscle>blood>brain. Among them, the L-isomer showed a considerably higher concentration than the D-isomer in the liver, adrenal, brain, skeletal muscle and intestine, while the D-isomer an appreciably higher concentration in the pancreas, skin, lung and kidney.

**Blood**——A more rapid decline of the blood concentration was observed for D-DOPA than L-DOPA in the earliest period after injection. Three hours and afterward, however, the both isomers showed a very slow decline, keeping an almost same level.

**Kidney**——Ten minutes after injection, D-DOPA showed a much higher concentration in the kidney than L-DOPA, while the concentration declined very rapidly till 60 min, when the concentration of the L-isomer became higher than the D-isomer. Afterward, the both isomers showed a very slow decline. These behaviors might correspond to a more rapid

decline of the blood concentration of the D-isomer and its more rapid elimination from the blood circulation into the urine.

**Brain**——After injection of L-DOPA-$^{14}$C, the highest concentration of radioactivity was observed already 10 min after injection, indicating a rapid penetration of L-DOPA into the brain, but the concentration declined rather rapidly. After injection of D-DOPA-$^{14}$C, on the other hand, only a very low concentration of radioactivity was detected in the brain, but a broad maximum was shown between 3 and 6 hr after injection, indicating a slight and slow penetration of D-DOPA into the brain. Six hours and afterward, however, the concentration of radioactivity became appreciably higher in the D-isomer than the L-isomer, indicating a retention of D-DOPA and/or its metabolites for a longer period.

TABLE III. Blood and Tissue Concentrations of Radioactivity after Intravenous Administration of L- and D-DOPA-$^{14}$C to Rats (10 mg/kg)

| Tissue | DOPA | $\mu$g/g tissue[a] ± S.E. ($n$=3) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 10 min | 30 min | 60 min | 3 hr | 6 hr | 24 hr | 96 hr |
| Blood[b] | L- | 3.26 ±0.14 | 2.53 ±0.29 | 1.97 ±0.19 | 0.83 ±0.10 | 0.51 ±0.05 | 0.25 ±0.05 | 0.02 ±0.007 |
| | D- | 3.16 ±0.15 | 1.34 ±0.04 | 1.01 ±0.10 | 0.64 ±0.03 | 0.47 ±0.10 | 0.28 ±0.06 | 0.01 ±0.005 |
| Brain | L- | 3.26 ±0.22 | 2.67 ±0.23 | 2.72 ±0.19 | 0.89 ±0.04 | 0.58 ±0.02 | 0.24 ±0.02 | 0.024 ±0.007 |
| | D- | 0.87 ±0.09 | 0.75 ±0.08 | 0.71 ±0.06 | 0.96 ±0.34 | 0.84 ±0.04 | 0.46 ±0.13 | 0.041 ±0.018 |
| Heart | L- | 6.36 ±0.74 | 3.07 ±0.11 | 2.28 ±0.07 | 1.08 ±0.06 | 0.78 ±0.03 | 0.32 ±0.02 | 0.036 ±0.003 |
| | D- | 3.67 ±0.07 | 2.27 ±0.07 | 1.71 ±0.02 | 1.32 ±0.06 | 1.15 ±0.02 | 0.59 ±0.02 | 0.014 ±0.006 |
| Lung | L- | 5.60 ±0.30 | 3.45 ±0.21 | 2.45 ±0.19 | 0.97 ±0.08 | 0.96 ±0.34 | 0.29 ±0.03 | 0.020 ±0.014 |
| | D- | 7.22 ±0.62 | 4.73 ±0.68 | 2.94 ±0.12 | 2.26 ±0.89 | 1.53 ±0.21 | 0.52 ±0.09 | 0.021 ±0.002 |
| Liver | L- | 11.26 ±0.23 | 13.40 ±1.22 | 11.40 ±1.14 | 2.07 ±0.28 | 1.17 ±0.11 | 0.35 ±0.04 | 0.037 ±0.004 |
| | D- | 5.41 ±0.05 | 4.46 ±0.54 | 2.93 ±0.14 | 1.69 ±0.20 | 1.09 ±0.06 | 0.63 ±0.13 | 0.062 ±0.014 |
| Intestine | L- | 34.48 ±1.40 | 24.02 ±2.81 | 14.40 ±0.79 | 2.70 ±0.66 | 1.20 ±0.19 | 0.40 ±0.06 | 0.048 ±0.018 |
| | D- | 11.98 ±1.63 | 8.96 ±1.12 | 5.13 ±0.95 | 3.09 ±1.11 | 1.56 ±0.17 | 0.78 ±0.21 | 0.048 ±0.006 |
| Pancreas | L- | 30.48 ±0.91 | 24.30 ±0.67 | 6.91 ±0.89 | 5.60 ±0.91 | 3.48 ±0.25 | 1.29 ±0.14 | 0.106 ±0.010 |
| | D- | 46.24 ±4.66 | 35.30 ±3.58 | 17.50 ±0.64 | 10.20 ±3.49 | 4.20 ±0.10 | 2.14 ±0.62 | 0.151 ±0.033 |
| Kidney | L- | 41.89 ±3.14 | 32.20 ±4.30 | 18.10 ±2.53 | 5.75 ±0.77 | 4.68 ±0.39 | 2.98 ±0.48 | 0.518 ±0.089 |
| | D- | 96.25 ±2.29 | 31.14 ±3.61 | 10.25 ±0.82 | 5.45 ±0.50 | 4.85 ±0.19 | 5.27 ±0.94 | 0.508 ±0.046 |
| Skeletal muscle | L- | 8.65 ±0.88 | 5.61 ±0.23 | 3.63 ±0.51 | 1.76 ±0.41 | 1.26 ±0.16 | 0.49 ±0.03 | 0.036 ±0.004 |
| | D- | 4.24 ±0.77 | 2.00 ±0.10 | 1.97 ±0.10 | 1.92 ±0.13 | 1.84 ±0.02 | 0.78 ±0.12 | 0.028 ±0.007 |
| Skin | L- | 6.20 ±0.23 | 5.96 ±0.85 | 3.50 ±0.31 | 1.63 ±0.04 | 1.26 ±0.16 | 0.41 ±0.03 | 0.093 ±0.017 |
| | D- | 7.32 ±0.68 | 5.77 ±0.55 | 3.90 ±0.15 | 2.15 ±0.13 | 1.81 ±0.16 | 1.21 ±0.21 | 0.098 ±0.017 |
| Adrenal | L- | 15.31 ±2.25 | 8.83 ±1.30 | 6.72 ±0.81 | 5.46 ±0.57 | 4.82 ±0.80 | 1.98 ±0.15 | 0.98 ±0.007 |
| | D- | 8.07 ±0.18 | 3.69 ±0.39 | 2.42 ±0.30 | 1.91 ±0.38 | 1.44 ±0.05 | 0.88 ±0.02 | 0.54 ±0.09 |

a) Radioactivity was converted to $\mu$g equivalents of DOPA based on the specific activity.
b) $\mu$g/ml

**Adrenal**——A higher concentration was continued after injection of L-DOPA-$^{14}$C than the D-isomer for the whole period investigated.

**Pancreas**——Both D- and L-DOPA-$^{14}$C showed a high uptake by the pancreas, but the concentration was appreciably higher in the D- isomer than the L-isomer for the whole period. The concentration of D-DOPA-$^{14}$C in the pancreas was the highest among all the organs and tissues for a period from 30 min to 3 hr after injection. The concentration of both isomers decreased rapidly till 60 min and thereafter slowly.

**Intestine (duodenum)**——Both isomers showed a high uptake by the intestine, but L-DOPA-$^{14}$C showed a considerably higher concentration than the D-isomer. The concentration of L-DOPA-$^{14}$C 10 min after injection was about 10 times to that of the blood concentration but declined rather rapidly.

**Liver**——After injection of L-DOPA-$^{14}$C, a very high uptake of radioactivity was shown, reaching the maximum sometime around 30 min after injection. After injection of D-DOPA-$^{14}$C, on the contrary, only a slight uptake of radioactivity was detected in the liver. The concentration of L-DOPA-$^{14}$C decreased rapidly to almost the same level as that of the D-isomer 3 hr after injection.

**Skeletal and Cardiac Muscle**——A high uptake of radioactivity by the skeletal muscle was shown only by the L-isomer. For a period of 60 min after injection, L-DOPA-$^{14}$C showed a considerably higher concentration than the D-isomer, but decreased more rapidly than the D-isomer. Three hours and afterward, D-DOPA-$^{14}$C showed an appreciably higher concentration than the L-isomer. The results indicate that L-DOPA penetrates and accumulates rapidly in the skeletal muscle, but eliminated rather rapidly, while D-DOPA shows only a slight and slow penetration and a rather long retention in the tissue. An almost same behavior was noted in the uptake by the cardiac muscle, but the concentrations were much lower than those in the skeletal muscle.

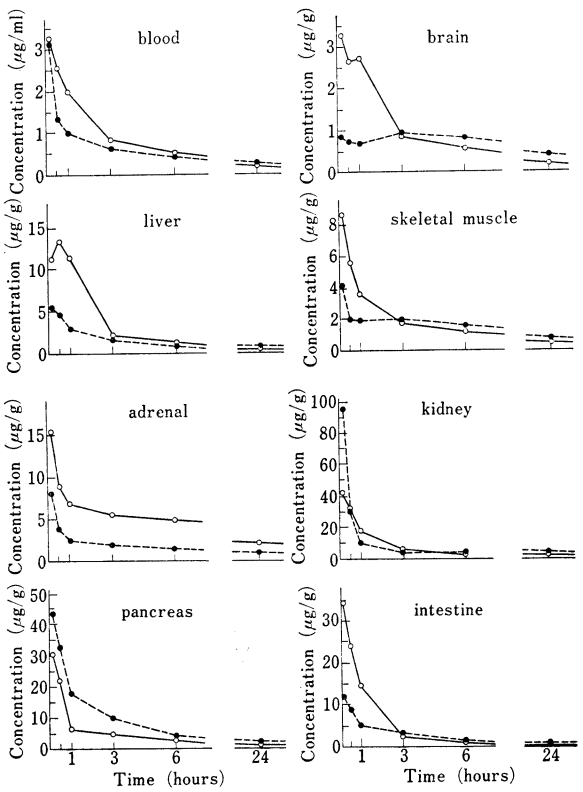


Fig. 1. Blood and Tissue Concentration of Radioactivity after Intravenous Administration of D- and L-DOPA-$^{14}$C (10 mg/kg) to Rats

Each value was expressed as μg equivalent to DOPA and represents the average from 3 experiments.
—○—: L-DOPA; ---●---: D-DOPA

**Skin**——Both D- and L-isomers showed a high uptake of radioactivity by the skin right after injection and declined in almost the same rate. The D-isomer continued an appreciably higher concentration than the L-isomer for the whole period investigated.

**Blood and Tissue Distribution of D- and L-DOPA-$^{14}$C after Oral Administration.**

The blood and tissue concentration of radioactivity after oral administration of 60 mg/kg D- and L-DOPA-$^{14}$C to rats were compared in Table IV and Fig. 2. After administration of

L-DOPA-$^{14}$C, the concentration reached the maximum about 1 hr after administration and was found to be in the following order: intestine≫kidney>liver>pancreas>blood>skin>lung>adrenal>cardiac muscle~skeletal muscle>brain. Thus, the radioactive uptake exceeding the blood level was observed only in the intestine, kidney, liver and pancreas. As can be seen from Fig. 2, generally, the concentration of radioactivity was appreciably higher in the L-DOPA than the D-isomer only in the intestine, liver and adrenal, while in all of the other tissues including the brain the D-isomer showed a higher concentration and a longer retention of radioactivity than the L-isomer. These behaviors are significantly different from those observed after intravenous administration.

The blood concentration was almost the same level for the both isomers 1 hr after administration, but after 6 hr and thereafter the D-isomer maintained an appreciably higher concentration, indicating a considerably longer duration of the blood level after oral administration of D-DOPA. In the kidney, in parallel to the blood level, a higher concentration was continued in the D-isomer as compared to the L-isomer for more than 24 hr.

In the brain, the both isomers showed an almost same level of radioactive uptake 1 hr after administration. Afterward, however, the radioactivity of L-DOPA-$^{14}$C decreased rapidly, while that of the D-isomer appeared to increase further with time to reach the maximum sometime between 3 and 6 hr after administration. Thereafter, the D-isomer showed a long retention of radioactivity and the concentration 6 and 24 hr after administration was approximately two and four times higher to that of the L-isomer, respectively. In the skeletal muscle, the two isomers revealed behaviors which are quite similar to those in the brain. The radioactivity of L-DOPA-$^{14}$C decreased rapidly during 1 and 3 hr after administration, while that of the D-isomer appeared to reach the maximum at a much later time and to keep a high concentration for more than 24 hr.

TABLE IV. Blood and Tissue Concentration of Radioactivity after Oral Administration of L- and D-DOPA-$^{14}$C to Rats (60 mg/kg)

| Tissue | DOPA | μg/g tissue[a] ±S.E. ($n$=3) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 30 min | 60 min | 3 hr | 6 hr | 24 hr | 96 hr |
| Blood[b] | L- | 6.54± 1.03 | 12.06± 0.59 | 5.00± 0.28 | 1.80±0.09 | 0.39±0.08 | 0 |
| | D- | —[c] | 11.97± 0.51 | — | 5.53±0.40 | 2.24±0.61 | — |
| Brain | L- | 1.97± 0.30 | 3.10± 0.71 | 1.18± 0.13 | 1.51±0.12 | 0.61±0.20 | 0 |
| | D- | — | 3.16± 0.59 | — | 3.56±0.21 | 2.59±0.44 | — |
| Heart | L- | 5.67± 0.98 | 6.82± 0.72 | 3.44± 0.30 | 2.73±0.39 | 0.68±0.07 | 0.34±0.09 |
| | D- | — | 11.69± 2.70 | — | 5.54±0.25 | 3.63±1.06 | — |
| Lung | L- | 6.07± 0.99 | 10.70± 1.38 | 4.43± 0.48 | 3.24±0.67 | 0.63±0.03 | 0.09±0.03 |
| | D- | — | 82.85±36.30 | — | 7.55±0.70 | 4.06±0.79 | — |
| Liver | L- | 65.02±17.06 | 81.71± 7.23 | 28.78± 7.42 | 9.37±1.35 | 0.92±0.13 | 0.12±0.01 |
| | D- | — | 17.57± 2.56 | — | 7.62±0.14 | 4.15±0.60 | — |
| Intestine | L- | 305.51±32.06 | 252.83±16.28 | 132.65±36.73 | 11.50±2.73 | 1.48±0.16 | 0.24±0.04 |
| | D- | — | 95.72±59.92 | — | 20.67±4.07 | 4.23±0.59 | — |
| Pancreas | L- | 21.92± 3.69 | 30.18± 1.59 | 7.97± 0.71 | 7.74±0.72 | 2.77±1.00 | 0.53±0.30 |
| | D- | — | 102.85±22.64 | — | 25.96±1.48 | 16.41±3.52 | — |
| Kidney | L- | 60.70± 6.34 | 86.47±12.30 | 35.42± 6.02 | 14.07±1.20 | 5.17±0.68 | 0.25±0.07 |
| | D- | — | 92.09±11.31 | — | 29.93±3.15 | 20.86±0.66 | — |
| Skeletal muscle | L- | 3.90± 0.38 | 6.51± 0.76 | 3.22± 0.29 | 2.47±0.12 | 0.90±0.11 | 0.21±0.04 |
| | D- | — | 7.65± 1.45 | — | 8.03±0.12 | 5.68±0.82 | — |
| Skin | L- | 6.47± 0.89 | 11.64±0.82 | 5.30± 0.30 | 4.82±0.48 | 1.29±0.15 | 0.23±0.02 |
| | D- | — | 23.81±6.25 | — | 10.55±1.23 | 6.34±0.77 | — |
| Adrenal | L- | 6.04± 0.97 | 7.72±1.40 | 5.22± 0.82 | 5.44±0.12 | 5.02±0.48 | 2.00±0.16 |
| | D- | — | 5.60±1.20 | — | 4.05±0.50 | 4.85±0.40 | — |

*a*) Radioactivity was converted to μg equivalents of DOPA based on the specific activity.
*b*) μg/ml
*c*) not determined

In the liver, a considerably higher uptake of radioactivity was shown by L-DOPA-$^{14}$C than the D-isomer, the latter showing only a slight uptake. After 24 hr, however, an appreciably higher concentration appeared to be remained after administration of the D-isomer. The same trend was noted in the concentration of the two isomers in the intestinal tissue.

In the adrenal, an appreciably higher concentration was continued in the L-DOPA than the D-isomer for the whole period. In the pancreas and skin, the D-isomer showed a considerably higher concentration and a longer retention of radioactivity than the L-isomer for the whole period investigated. It is particularly notable that the D-isomer showed a very high uptake in the pancreas, which was the highest in all the tissues investigated, while the L-isomer only a slight uptake.

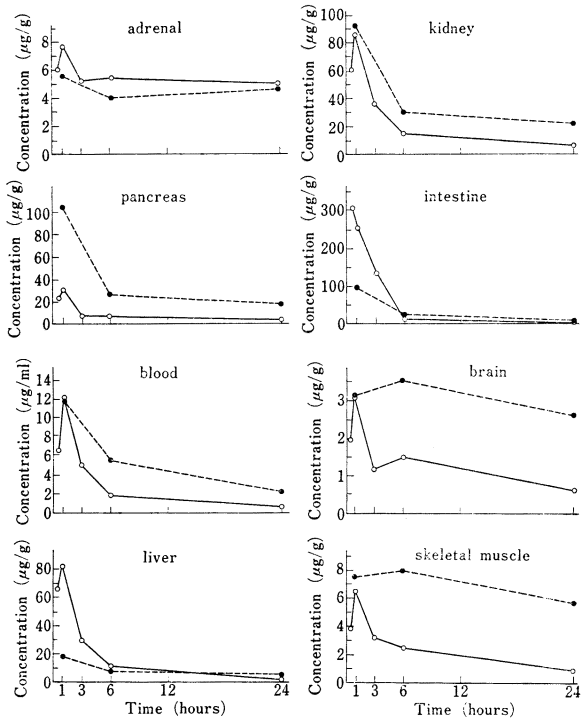


Fig. 2. Blood and Tissue Concentration of Radioactivity after Oral Administration of D- and L-DOPA-$^{14}$C (60 mg/kg) to Rats

Each value was expressed as μg equivalent to DOPA and represents the average from 3 experiments.
—○—: L-DOPA; --●--: D-DOPA

## Discussion

From the present investigations, it was indicated that both D- and L-DOPA are eliminated from the blood circulation mainly through the urinary excretion and that the initial rate of the excretion after intravenous injection is appreciably higher in the D-isomer than the L-isomer. These results are in accordance with the autoradiographic studies[6] which indicated that the elimination of radioactivity from the body was the fastest in dopamine followed by D-DOPA and the slowest in L-DOPA. The uptake of radioactivity by the tissues was, on the other hand, found to be significantly higher in the L-isomer than in the D-isomer in most of the tissues, such as the brain, skeletal muscle, liver, intestine and adrenal, confirming also the results obtained from the autoradiographic studies.[6] Since the distribution of radioactivity observed in the earliest period after intravenous injection is considered to represent mainly the distribution of unchanged DOPA, the observed differences between the two isomers might be primarily due to the differences in the stereospecificity of the transport systems provided in the individual tissue cells. A fast uptake of radioactivity in the brain after injection of L-DOPA-$^{14}$C and its localization in the caudate nucleus was already demonstrated by the autoradiographic technique and the relation to the therapeutic effect against Parkinsonism was discussed.[6] It has been shown from the *in vitro* experiments[7] that only L-isomer of DOPA is penetrated into the brain tissue by an active transport mechanism and an accumulation of L-DOPA in the skeletal muscle is also expected to be due to an active transport mechanism which is specific with respect to the L-antipode. The fact that a very high uptake of radio-

7) H. Yoshida, J. Namba, K. Kaniike, and R. Imaizumi, *Jap. J. Pharmacol.*, 13, 1 (1963).

activity was shown in the pancreas by both D- and L-isomers and the D-isomer showed even a higher concentration than the L-isomer suggests that there might be involved a specified transport system in the pancreas which is not stereospecific at all. A selective accumulation of the L-isomer in the liver and adrenal and its higher accumulation in the intestine are, on the other hand, considered to be due to a stereospecificity in the metabolic system for DOPA, since it is well known that[8] DOPA-decarboxylase is highly specific for the L-isomer. As will be reported in the subsequent paper,[9] in fact, the most of radioactivity accumulated in the liver and intestine 10 min after injection of L-DOPA-$^{14}$C was found to be dopamine glucuronide. That the accumulation of L-DOPA-$^{14}$C in the adrenal is caused from that of catecholamines formed has been demonstrated by the fact that DOPA-1-$^{14}$C showed almost no accumulation of radioactivity in the adrenal.[10] Another observation that, in a later period after injection, an appreciably higher concentration of radioactivity was retained in the tissues such as the brain, skeletal muscle, pancreas and skin after injection of the D-isomer than the L-isomer might be due to a much slower rate of metabolism of D-DOPA than that of L-DOPA. As will be described in the subsequent paper,[9] D-DOPA appears to remain in these tissues mostly in the forms of unchanged DOPA and its 3-O-methylated product.

After oral administration of L-DOPA-$^{14}$C to rats, the radioactivity was found to be almost completely excreted in the urine during 24 hr, indicating that L-DOPA is very easily absorbed from the gastro-intestinal tract, even in a high dose of 60 mg/kg. It has been reported that[11] after oral dose of 500 mg L-DOPA-$^{14}$C to human subjects about 80% of the dose was excreted into the urine during 24 hr. On the contrary, a present finding that after oral administration of D-DOPA-$^{14}$C to rats the excretion of radioactivity into the urine was relatively low (*ca.* 53% of the dose), while a considerable part (*ca.* 23%) was recovered in the feces might indicate that the absorption of D-DOPA from the gastro-intestinal tract is much slower than that of the L-isomer, since the biliary excretion of D-DOPA appears not to occur appreciably because of the fact that the most of radioactivity was recovered in the urine after intravenous administration and that no accumulation of radioactivity was observed in the liver. As will be reported in a subsequent paper,[12] it has been clarified that the absorption rate of L-DOPA from *in situ* ligated loop of rat intestine is much faster than the D-isomer and an active transport mechanism is involved in the absorption of the L-isomer.

It was shown from the autoradiographic study[1] that the brain uptake of radioactivity was extremely low when L-DOPA-$^{14}$C was administered orally instead of intravenously in the dose level of 10 mg/kg, while became evident when the dose was increased over 50 mg/kg. This was interpreted as being due to a considerable metabolism of L-DOPA administered orally in the peripheral tissues such as the gastric and intestinal mucosa and liver. In the present investigations with a dose level of 60 mg/kg, it was shown that the concentration of radioactivity in the brain was the same level in the two isomers 60 min after administration, while the D-isomer maintained a considerably higher concentration than the L-isomer for a period from 1 hr to more than 24 hr, in spite of an appreciably lower absorbability of the D-isomer than the L-isomer. These results might indicate that even when the dose level is as high as 60 mg/kg the extent by which the oral dose of L-DOPA is decarboxylated in the peripheral tissues is still considerably high. In the skeletal muscle, the two isomers showed quite similar behaviors to those in the brain and this can be interpreted in the same way as that in the brain. The uptake of radioactivity by the pancreas can be regarded as a measure of the amount of unchanged DOPA in the circulating blood, since dopamine does not pene-

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9) H. Shindo, T. Komai, K. Tanaka, E. Nakajima, and N. Miyakoshi, *Chem. Pharm. Bull.* (Tokyo), **21**, 826 (1973).
10) H. Tjälve, *Acta Physiol. Scand.*, Suppl. 360 (1971).
11) M.J.T. Peaston and J.R. Bianchine, *Brit. Med. J.*, **1**, 400 (1970).
12) H. Shindo, T. Komai, and K. Kawai, *Chem. Pharm. Bull.* (Tokyo), in press.

trate into the pancreas to any appreciable extent.[6] The present results that a considerably lower uptake of radioactivity was observed in the pancreas after oral administration than that after intravenous injection, in relative to the D-isomer, indicate that the amount of unchanged L-DOPA in the circulating blood is considerably lower when orally administered than that after intravenous administration, in accordance with the above consideration.

An accumulation and a long retention of a high radioactivity in the brain and skeletal muscle after oral administration of D-DOPA-$^{14}$C has been already found[1] by the autoradiographic study, in the dose level of 10 mg/kg. In the present study, this was further demonstrated with a higher dose level of 60 mg/kg and at 24 hr after oral administration the concentration of the D-isomer in these tissues was found to be 4 to 6 times higher than the L-isomer. As was already pointed out and discussed,[1] these facts might give a possible explanation for a clinical finding[5] that the side effects were considerably reduced when L-DOPA was used orally rather than the DL-racemate, that is, for the unbeneficial side effects of D-DOPA. A higher accumulation of D-DOPA in most of the tissues after oral administration than after intravenous administration could be interpreted as being due to its slow rate of absorption from the intestine. As a result of its gradual absorption without being metabolized to any appreciable extent, a certain level of DOPA concentration in the circulating blood might be maintained for a long period and its gradual transfer into the tissues might give rise to a high accumulation of radioactivity. As will be reported in the subsequent paper,[9] the radioactivity in the brain at 60 min after oral administration of D-DOPA-$^{14}$C was found to be composed of unchanged DOPA and 3-O-methyl-DOPA. D-3-O-methyl-DOPA is considered to be very slowly eliminated from the body as well as unchanged D-DOPA, since L-3-O-methyl-DOPA has been reported[13] to be eliminated much more slowly than L-DOPA.

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13) G. Bartholini, I. Kuruma, and A. Pletscher, *Br. J. Pharmcol.*, **40**, 461 (1970).