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Metabolic Fate of 3,4-Dihydroxyphenylpyruvic Acid(DHPP) in Rats. I. Specific Transformation of DHPP to L-3,4-Dihydroxyphenylalanine *in Vitro*

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3,4-Dihydroxyphenylpyruvic acid-2-^{14}C (DHPP-2-^{14}C) was prepared from glycine-2-^{14}C and the specific formation of L-3,4-dihydroxyphenylalanine (L-DOPA) from DHPP was shown *in vitro*. The metabolism of L-DOPA-3-^{14}C was also investigated *in vitro* for comparison. In rat brain and small intestinal homogenates, L-DOPA was formed gradually as the main metabolite from DHPP-2-^{14}C but dopamine and its metabolites were very slight amount. On the contrary, dopamine was detected dominantly from L-DOPA-3-^{14}C in small intestinal homogenates. In the liver and kidney homogenates, DHPP was metabolized extensively and disappeared immediately with concomitant rapid formation of L-DOPA. Dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) were detected subsequently as the main metabolite. The same extensive metabolism of L-DOPA-3-^{14}C was also shown in the liver homogenates. Formation of L-DOPA from DHPP was identified by thin-layer chromatography and reverse isotope dilution method. L-Phenylalanine, L-tyrosine, L-tryptophan and L-glutamate were revealed to be effective amino donors to DHPP. The aromatic L-amino acids were particularly effective but D-phenylalanine showed little activity. The liberation of $^{14}CO_2$ from DHPP-2-^{14}C was also recognized *in vitro*. The $^{14}CO_2$ liberating activity from DHPP-2-^{14}C located in 105000 *g* supernatant soluble fraction in the liver and kidney wherein the activity in the liver was about 4 fold higher than that in the kidney. The activity was inhibited by sodium azide, sodium diethyldithiocarbamate, *p*-hydroxyphenylpyruvate and phenylpyruvate but not influenced by SKF 525A, carbon monoxide, EDTA, α,α'-dipyridyl and pyruvate. Methylene blue, dichlorphenolindophenol and ascorbate activated about 2—3 fold.

Keywords——3,4-dihydroxyphenylpyruvic acid; L-3,4-dihydroxyphenylalanine; dopamine; metabolism; rat liver; rat kidney; rat brain; rat intestine

It has been well established[2)] that L-3,4-dihydroxyphenylalanine (L-DOPA) is a very effective agent against parkinsonism, as a dopamine precursor in brain. Because of its extensive metabolism in the peripheral tissues, a massive dose of L-DOPA is required in the oral use clinically and there are inevitable side effects which are believed to be caused from dopamine formed in the peripheral tissues such as the liver and intestine.[3)]

In our previous paper,[4)] it was demonstrated that the D-isomer of DOPA is metabolized to dopamine in rat kidney and further postulated that D-DOPA is deaminated to 3,4-dihydroxyphenylpyruvic acid (DHPP) by D-amino acid oxidase and DHPP is further transformed to L-DOPA by transaminase. L-DOPA thus formed appears to be decarboxylated to dopamine immediately, the latter being excreted into the urine possibly by an active transport

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mechanism.[5] The transformation of D-DOPA to L-DOPA *via* DHPP occurrs almost exclusively in the kidney by the fact that there is no appreciable D-amino acid oxidase activity in any other tissue except the kidney. Unlike L-DOPA, D-DOPA is not effective in the parkinsonism therapy along with its toxic side effect.[6]

The metabolic intermediate, DHPP, on the other hand, is expected to be convertible to L-DOPA *in vivo* easily by transaminase which occurrs in many organs including the intestine and liver. Furthermore, it has been known that DHPP is not a substrate of DOPA decarboxylase, but has an inhibitory effect on the enzyme.[7] DHPP is, therefore, expected as a new dopamine precursor which has less side effects than L-DOPA when administered orally. In the present investigations, DHPP-2-^{14}C was prepared starting from glycine-2-^{14}C and the metabolic fate in rats was investigated. In this paper, a specific transformation of DHPP to L-DOPA was demonstrated *in vitro* by the tissue homogenates.

Material and Method

Material——DHPP-2-^{14}C was prepared in the following way.[8] Twenty mCi of glycine-2-^{14}C (44 mCi/mmol, Daiichi Pure Chemical Co., Ltd.) was dissolved in 3 ml water with 66 mg of non-radioactive glycine. After adding with 3 ml acetic anhydride, the mixture was stirred for 2 hr at room temperature. The reaction mixture containing N-acetylglycine-2-^{14}C was then lyophillized and added with 202 mg protocatechualdehyde and 110 mg anhydrous sodium acetate. The solid mixture was added with 5 ml acetic anhydride and heated at 90—100° for 7.5 hr. After cooling, 100 ml of benzene was added and the reddish brown solution was loaded on a silica gel column (2×40 cm) suspended in benzene. Azlactone intermediate was first eluted as a sharp radioactive peak with the solvent system: benzene–ethylacetate (3:1). This fraction was further purified on the silica gel column and evaporated to dryness *in vacuo*. After adding with 5 ml of 1 N hydrochloric acid, the solution was heated under a nitrogen gas atmosphere at 95° for 1.5 hr. The pale yellow hydrolysates were transferred into a tube with ground glass stopper and the volume was made up to 20 ml with water. DHPP-2-^{14}C was extracted by shaking with each 20 ml portion of ethylacetate three times and the combined extracts were evaporated to dryness *in vacuo*. The reddish brown residue was added with 300 mg non-radioactive DHPP and dissolved in a minimal volume of hot water. After treating the hot solution with active charcoal, it was filtered and allowed to stand overnight in a refrigerator. DHPP-2-^{14}C (140 mg) was obtained as a white powder which was further purified by recrystallization from acetone–benzene. After treating the acetone solution of DHPP-2-^{14}C with active charcoal, which was removed by filtration, benzene were added dropwise until white turbidity appeared. The white crystals of pure DHPP-2-^{14}C (97 mg) were obtained after standing the solution overnight. The specific activity was 11.56 μCi/mg (2.27 mCi/mmol) 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). It was proved that the labeled position localizes only at the carbonyl carbon from the fact that no radioactive CO_2 was generated by oxidative decarboxylation with 3% hydrogen peroxide. L-DOPA-3-^{14}C was purchased from the Radiochemical Center, Amersham, England. The specific radioactivity was 46 μCi/mg (9.2 mCi/mmol).

L-3,4-Dihydroxyphenylalanine (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 commercial products. Non-radioactive DHPP was prepared in the same way as described above from acetylglycine and protocatechualdehyde. 4-Bromo-3-hydroxybenzyloxyamine hydrochloride (NSD 1055)[9] used as DOPA decarboxylase inhibitor was prepared in this laboratories.

Experiments on the Metabolism of DHPP-2-^{14}C with Rat Tissue Homogenates——Male rats of Wistar-Imamichi strain weighing 150—200 g were sacrificed by decapitation and the liver, kidney, brain and the upper part of small intestine were excised and minced on crashed ice. The tissues were then added with 4 fold volumes of 0.05 M potassium phosphate buffer (pH 7.0) and homogenized with Polytron (Kinematica Co., Ltd., Switzerland). The centrifuge tubes containing 1 ml of 20% tissue homogenates and 0.1 ml of the potassium phosphate buffer were preincubated at 37° for 5 min and then 0.1 ml of DHPP-2-^{14}C solution was

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added (1 mM). After the incubation was continued for 10, 20, 30, 45 and 60 min, the reaction was terminated by adding with 0.5 ml of 4 N sulfuric acid. The tubes were added with the equivalent volume of saturated barium hydroxide solution to be neutralized and centrifuged at 2000 rpm for 10 min. The supernatant (10 μl) was spotted on a cellulose thin-layer plate (Merck F_{254}, 0.1 mm thickness) and developed with the solvent system: *n*-butanol–methanol–1 N formic acid (6: 2: 1). The authentic samples were spotted and developed at the same time. The chromatograms were exposed on the industrial X-ray films (Sakura Type N) for two weeks. The radioactive spots were scraped into the counting vials, added successively with 1 ml of 70% ethanol and 10 ml of toluene–dioxane liquid scintillator (8 g of PPO, 200 mg of dimethyl POPOP, 200 ml of toluene and 800 ml of dioxane) and measured the radioactivity in a Packard Model 3380 liquid scintillation spectrometer.

Identification of L-DOPA by Reverse Isotope Dilution Method——The specific formation of L-DOPA from DHPP-2-^{14}C was identified by the reverse isotope dilution method. The supernatants (2 ml) obtained after incubation of DHPP-2-^{14}C with the liver, kidney, brain and intestinal homogenates as described above were added with 250 mg of non-radioactive L-DOPA and warmed to dissolve completely. After the solution was allowed to stand overnight in a refrigerator, the white precipitates of radioactive DOPA were collected and the specific radioactivity of the crystal was determined. The recrystallization was repeated from a minimal amount of hot water until a constant specific radioactivity was obtained. The content of radioactive L-DOPA (W_x mg) and the percentage (X%) in the original sample (R dpm) were calculated from the mean specific radioactivity of the crystals (S dpm/mg), the amount of non-radioactive L-DOPA added (Wmg =250 mg) and the specific radioactivity of DHPP-2-^{14}C (S_0 dpm/mg) according to the following equations.

$$W_x = WS/(S_0 - S)$$

$$X = 100S(W + W_x)/R$$

Determination of Transaminase Activity in the Liver with Various Amino Donors——The liver homogenates (20%) were centrifuged at 9000 ***g*** in a refrigerating centrifuge and the supernatant was dialyzed against 0.05 M potassium phosphate buffer (pH 7.0) overnight. Two ml of the dialyzed supernatant in a centrifuge tube was added with 0.1 ml of NSD-1055 (0.1 mM), 0.1 ml of dithiothreitol (1 mM) and 0.1 ml of pyridoxal-5′-phosphate (0.1 mM) and then preincubated at 37° for 5 min. One ml of DHPP (2.7 mM) and 0.5 ml of the solution of various amino acid (10 mM), as an amino donor, were added and plugged tightly with gum stopper after the tube was filled with nitrogen gas. After the incubation was continued for 60 min, the gum stopper was removed and the tube was heated in boiling water for 2 min and centrifuged at 2000 rpm. The supernatants were passed through Dowex-1 anion exchange column (Cl form, 0.7×2 cm). L-DOPA was eluted with 2 ml of 1 M KCl solution completely. After the elution was continued further with distilled water until the volume of the eluate reached 10 ml, 2 ml of the eluate was assayed for L-DOPA content with Nair's method as described in the previous paper.[4,10]

Measurement of $^{14}CO_2$ Liberation from DHPP-2-^{14}C *in Vitro*——A filter paper (2×8 cm, Toyo Roshi No. 51A) wetted with 0.2 ml of ethanolamine was placed in a counting vial.[11] Centrifuge tubes containing 1 ml of the tissue homogenates (liver, intestine, kidney and brain) were preincubated at 37° for 5 min and 0.1 ml solution (1 mM) of DHPP-2-^{14}C was added. The vials were then connected to the top of the incubation tubes with a thick gum tube. After the incubation was continued for 30 min, the reaction was terminated by injection of 0.5 ml 4 N sulfuric acid through the gum tube wall. After the incubation was continued for another 30 min to complete the absorption of radioactive CO_2 by ethanolamine, the vials were removed and added successively with 1 ml of methanol and 10 ml of toluene–dioxane liquid scintillation fluid to be measured the radioactivity.

In order to observe the subcellular localization of the activity, 20% homogenates of the liver were first centrifuged at 9000 ***g*** for 20 min. The precipitates were suspended in the original volume of potassium phosphate buffer. The 9000 ***g*** supernatant was then centrifuged at 105000 ***g*** for 1 hr and the supernatant fraction was removed. The pellet was suspended in the original volume of the buffer (microsomal fraction). All these fractions were assayed for the activity by the above method.

Result

Metabolism of DHPP-2-^{14}C in Rat Tissue Homogenates

In Fig. 1, 2 and 3, the time course of the formation of the metabolites from DHPP-2-^{14}C (1 mM) was shown when incubated with 20% homogenates of rat small intestine, liver, kidney and brain. In the former two cases, those from L-DOPA-3-^{14}C were also shown for comparison. As can be seen from the figures, DHPP was found to be easily metabolized in all these tissues, but the rate of metabolism was the slowest in the intestine, while the fastest in the liver.

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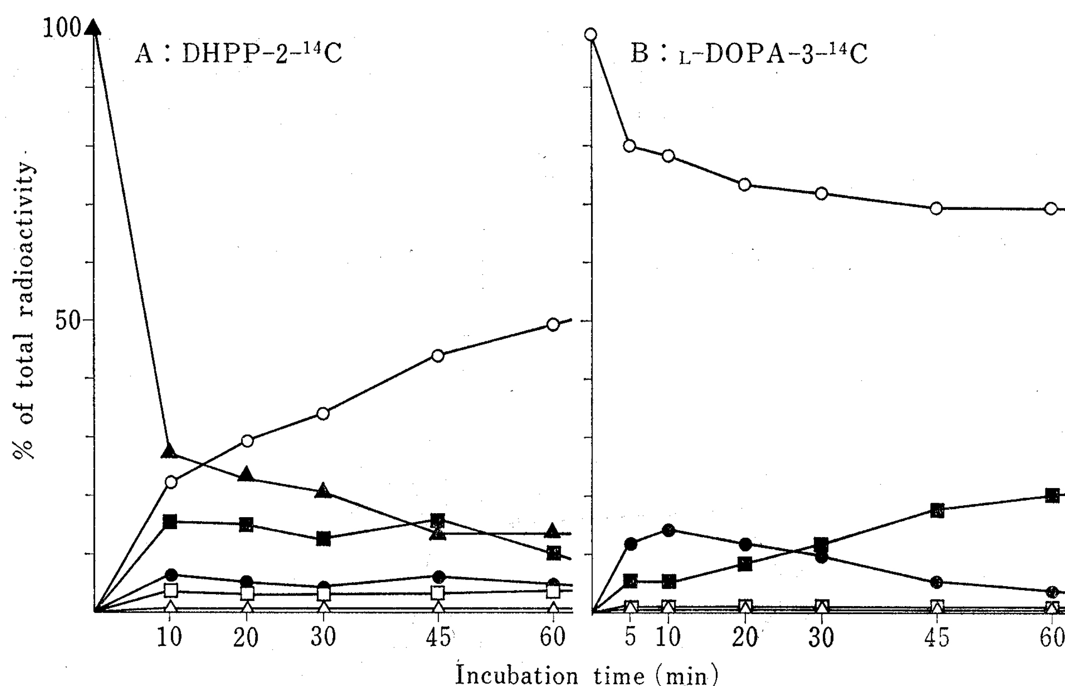

Fig. 1. Time Course of Metabolites Formation from DHPP-2-^{14}C (A) and L-DOPA-3-^{14}C (B) with Rat Small Intestine Homogenate

—○—: L-DOPA; —●—: dopamine; —△—: HVA; —▲—: DHPP; —□—: DOPAC;
—■—: spot at origin (TLC).
DHPP-2-^{14}C: 1mM (A). L-DOPA-3-^{14}C: 1 mM (B).

With the homogenates of small intestine, DHPP disappeared gradually, while DOPA was recognized as the main metabolite as early as 10 min after incubation and increased with the time. After the incubation for 30 and 60 min, about 21 and 14% of the total radioactivity remained as unchanged DHPP, respectively, while about 35 and 50% was detected as DOPA, respectively. As described later, DOPA formed from DHPP was shown to be exclusively the L-isomer by the reverse isotope dilution method. The formation of dopamine and its metabolites was, however, very low and was only less than 6% of the tatol radioactivity for the whole incubation period. This is considered to be due to an inhibitory effect of DHPP on DOPA decarboxylase and, in fact, the extent of suffering from decarboxylation was considerably lower than that observed when L-DOPA was incubated with the homogenates, as compared in Fig. 1.

With the liver homogenates, on the contrary, the disappearence of DHPP was very rapid. As early as 10 min after incubation, only about 5% of the total radioactivity was found to be unchanged DHPP and after 20 min radioactive DHPP was scarcely detected on the thin-layer chromatogram. Correspondingly, the rate of L-DOPA formation was very rapid and reached the maximum after incubation for 10 min and disappeared completely after 60 min. On the other hand, dopamine was found to increase rapidly and became the main metabolite after 30 min, indicating a rapid decarboxylation in the liver. DOPAC, the oxidative metabolite of dopamine, also tended to increase gradually. A more rapid decarboxylation of L-DOPA than DHPP in the liver was demonstrated by the incubation of L-DOPA-3-^{14}C with the liver homogenates, as compared in Fig. 2.

With the brain homogenates, as shown in Fig. 3-A, the metabolic pattern of DHPP was similar to that with the intestinal homogenates, except that the disappearence of DHPP appears to be more rapid. Concomitantly with the disappearence of DHPP, L-DOPA appeared and increased rapidly as the main metabolite, while dopamine and its metabolites accounted for only less than 6% of the total radioactivity for the whole period of incubation. With the kidney homogenates (Fig. 3-B), the metabolism of DHPP occurred very rapidly just

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as in the case of the liver homogenates. L-DOPA appeared first as the main metabolite and then dopamine was formed concomitantly with the decreaseof L-DOPA, indicating an occurrence of rapid decarboxylation in the kidney as well as in the liver. DOPAC also tended to increase gradually with time.

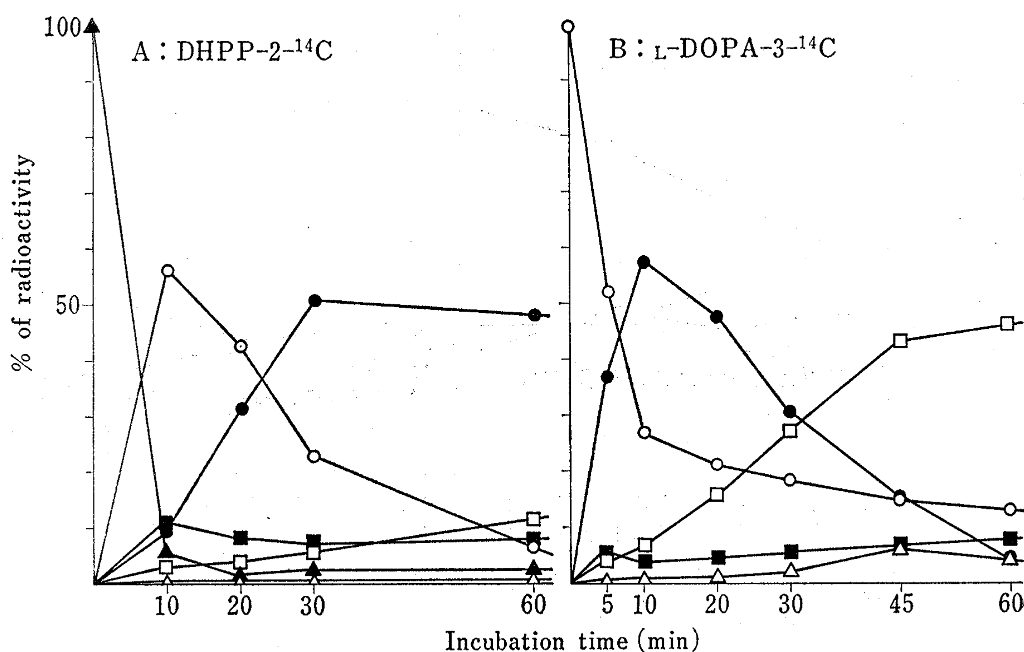

Fig. 2. Time Course of Metabolites Formation from DHPP-2-^{14}C (A) and L-DOPA-3-^{14}C (B) with Rat Liver Homogenate

—○—: L-DOPA; —●—: dopamine; —△—: HVA; —▲—: DHPP; —□—: DOPAC; —■—: spot at origin (TLC).
DHPP-2-^{14}C: 1 mM (A). L-DOPA-3-^{14}C: 1 mM (B).

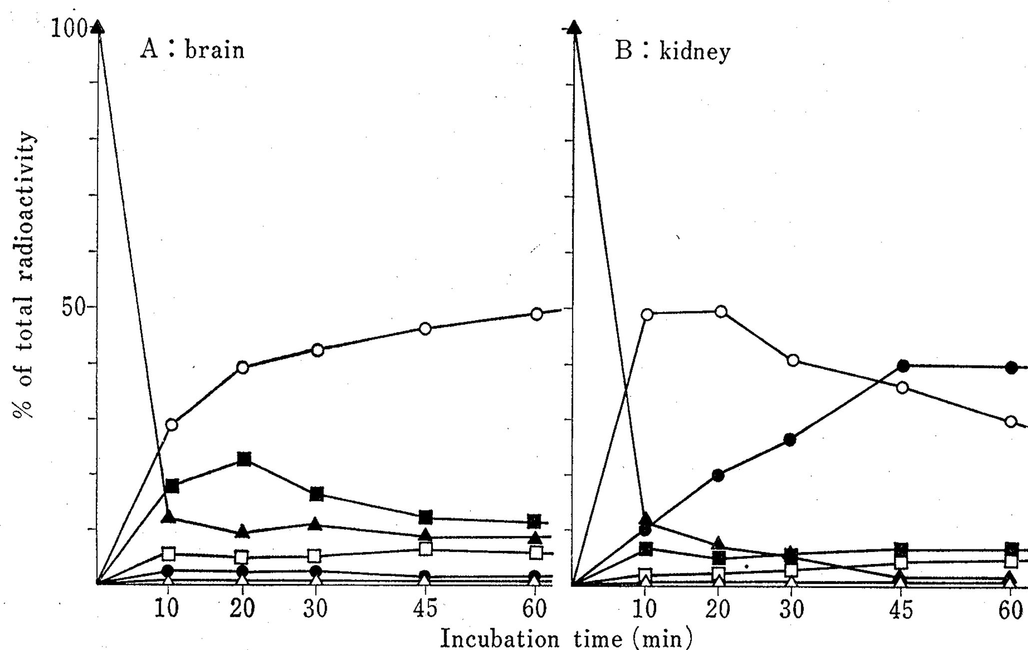

Fig. 3. Time Course of Metabolites Formation from DHPP-2-^{14}C with Rat Brain (A) and Kidney (B) Homogenates

—○—: L-DOPA; —●—: dopamine; —△—: HVA; —▲—: DHPP; —□—: DOPAC; —■—: spot at origin (TLC).
DHPP-2-^{14}C: 1 mM.

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The formation of L-isomer of DOPA from DHPP was further ascertained by means of reverse isotope dilution method. Samples were selected as those expected to contain the highest concentration of L-DOPA, after incubation with liver, kidney, intestine and brain homogenates. A known amount of non-radioactive L-DOPA was added to each incubation mixture and, after being dissolved completely by heating, the crystals of radioactive L-DOPA could be isolated. A constant specific radioactivity was obtained after the repeated recrystallization in all cases and the content of L-DOPA was calculated as shown in Table I. There was a good coincidence between the content of L-DOPA calculated from this method and that obtained from the radioassay of thin-layer chromatograms, demonstrating the optically specific transformation of DHPP to L-DOPA.

TABLE I. Identification of L-DOPA by Reverse Isotope Dilution Method

Tissue	DPM of each crystal (dpm/mg L-DOPA)				L-DOPA content (%)	
	1st.	2nd.	3rd.	Average	TLC method	This method
Brain	1381.1	1387.9	1327.3	1365.5	48.47	49.83
Liver	1759.8	1757.7	1692.7	1736.7	56.46	54.32
Kidney	1287.2	1184.8	1198.2	1223.4	48.42	43.24
Intestine	1677.9	1721.7	1707.8	1702.4	49.82	47.14

Following incubation mixture was determined of L-DOPA. Brain: 60 min in Fig. 3-A, liver: 10 min in Fig. 2-A, kidney: 10 min in Fig. 3-B, intestine: 60 min in Fig. 1-A.

Donors of Amino Group for DHPP to form L-DOPA

From the foregoing results, it was revealed that DHPP is readily converted to L-DOPA accepting an amino group from the donors which exist endogeneously. Thus, various amino acids were examined of their ability as the donor of amino group to DHPP, using dialyzed 9000 *g* supernatant fraction of rat liver homogenates. As shown in Fig. 4, L-glutamate, L-tryptophan and L-phenylalanine, particularly the latter two, were found to be a very effective amino donor at the concentration of 10 mM. Contrary to L-phenylalanine, D-phenylalanine showed little activity as an amino donor, demonstrating again a high specificity for L-isomer in the transamination reaction. Amino donating ability of tyrosine was examined at the concentration of 1 mM in a separate experiment, because of its low solubility. It was revealed that tyrosine also acts as an effective amino donor (Fig. 5). Relative effectiveness of these amino acids as amino donor to DHPP was almost the same to that observed previously with the kidney homogenates[4] and it might be concluded that aromatic L-amino acids such as L-phenylalanine present endogeneously can play a role as amino donor in the transformation of DHPP *in vivo* in the liver and intestine.

Liberation of $^{14}CO_2$ from DHPP-2-^{14}C *in Vitro*, a Possible Alternative Metabolic Pathway

As will be reported in the subsequent paper,[12] when DHPP-2-^{14}C was administered orally to rats it was found that an appreciable amount of the dose was excreted into the respiratory air as $^{14}CO_2$, suggesting a participation of an alternative metabolic pathway of DHPP. This must be different from the metabolism through L-DOPA, since only 1-position of DOPA can be decarboxylated to form dopamine and its metabolites. Thus, 20% homogenates of rat liver, kidney, brain and small intestine were examined for their $^{14}CO_2$ generation from DHPP-2-^{14}C *in vitro*. As shown in Fig. 6, an appreciable liberation of radioactive CO_2 was found with the liver and kidney homogenates, while the brain and small intestine had almost no activity. The cecal contents which was also examined for the activity as 20% suspension showed no activity, indicating that the intestinal bacterial

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flora does not play any important role in the *in vivo* generation of $^{14}CO_2$. Therefore, the main organs responsible for the *in vivo* generation of $^{14}CO_2$ from DHPP-2-^{14}C must be the liver and kidney wherein the liver has about four times higher activity than that of the kidney. The potassium phosphate buffer as a control was completely devoid of the activity, indicating that the reaction proceeds under an enzymatic catalysis.

In order to know some property of the enzyme responsible for $^{14}CO_2$ generation from DHPP-2-^{14}C effect of several inhibitors (1 mM) were examined in the liver homogenates. As

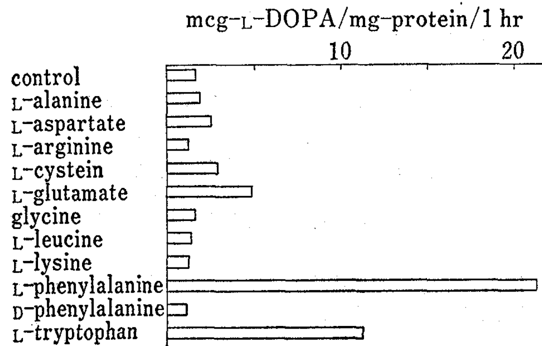

Fig. 4. Amino Donors to DHPP

DHPP, 2.7 mM; amino acid, 10 mM; dithiothreitol, 1 mM; pyridoxal-5′-phosphate, 0.1 mM; NSD-1055 (DOPA decarboxylase inhibitor), 0.1 mM. Incubation period: 1 hr.

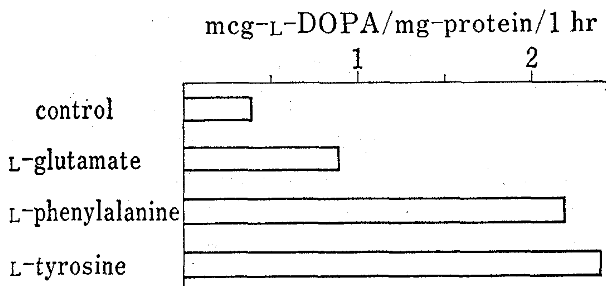

Fig. 5. Amino Donors to DHPP

DHPP, 2.7 mM; amino acid, 1 mM; dithiothreitol, 1 mM; pyridoxal-5′-phosphate, 0.1 mM; NSD-1055 (DOPA decarboxylase inhibitor), 0.1 mM. Incubation period: 1 hr.

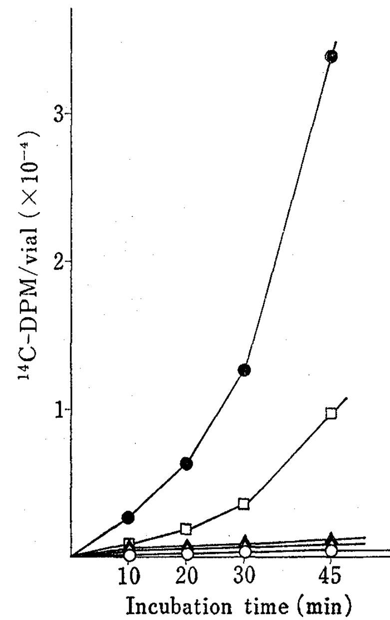

Fig. 6. Liberation of $^{14}CO_2$ from DHPP-2-^{14}C with Rat Tissue Homogenates

DHPP-2-^{14}C, 1 mM. Each 20 % homogenate of rat tissues and 20 % suspension of cecal contents were used.
—●—: liver; —□—: kidney; —▲—: cecal contents; —△—: intestine; —○—: brain.

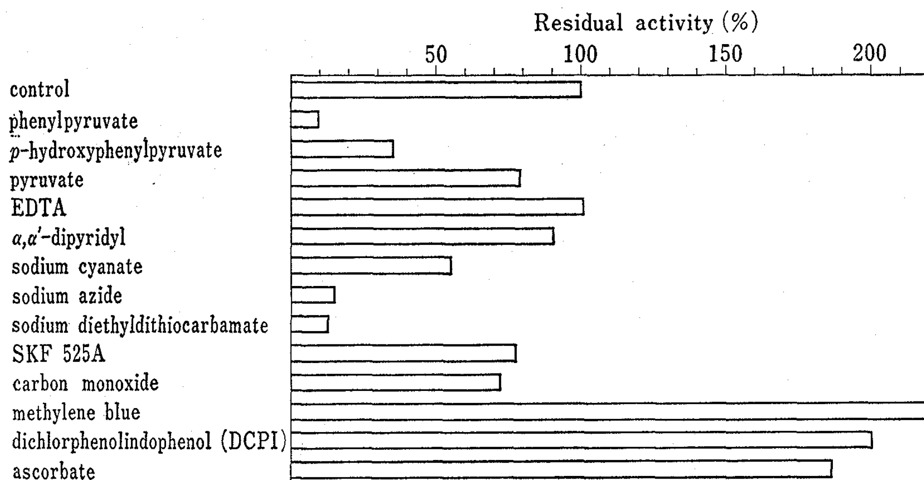

Fig. 7. Effects of Various Inhibitors and Activators on $^{14}CO_2$ Liberation from DHPP-2-^{14}C with Rat Liver Homogenate

DHPP-2-^{14}C, 1 mM; each inhibitors, 1 mM. Incubation period: 1 hr.

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shown in Fig. 7, phenylpyruvate and *p*-hydroxyphenylpyruvate exhibited a strong inhibition, whereas pyruvate did not show any appreciable inhibition, suggesting that the enzyme is specific to aromatic α-keto acids. EDTA and α,α'-dipyridyl exhibited almost no inhibition, while sodium cyanide inhibited moderately (−50%) and sodium azide and sodium diethyldithiocarbamate strongly (85—90%). These results suggest that the participating enzyme contains a heavy metal like other oxidases.[13] Dichlorphenolindophenol (DCPI), methylene blue and ascorbic acid stimulated the activity about 2 fold.

After fractionation of the liver homogenates, it was found that the activity localized almost exclusively in the 105000 ***g*** supernatant fraction, as shown in Table II, while no appreciable activity was recognized in the microsomal fraction. In accordance with this result, SKF 525A and carbon monoxide, which inhibit the microsomal oxigenating enzymes strongly, did not show any appreciable inhibition (Fig. 7).

Table II. Subcellular Localization of the $^{14}CO_2$ Liberating Activity from DHPP-2-^{14}C in Rat Liver

Preparation	DPM/vial[a]	% of control
Total homogenate (Control)	34995	100.00
9000 ***g*** supernatant fraction	24369	69.63
9000 ***g*** precipitate fraction	1267	3.62
Microsomal fraction	254	0.73
105000 ***g*** supernatant fraction	13230	37.80
105000 ***g*** supernatant fraction+DCPI	107914	308.37

a) Each figure is a mean of duplicated experiments.
DHPP-2-^{14}C, 1 mM. Incubation period: 1 hr.

Discussion

The present result *in vitro* that DHPP was rapidly converted to L-DOPA with the intestinal homogenates, while the formation of dopamine and its metabolites was very low may suggest that DHPP would be gradually but readily converted to L-DOPA in the small intestine when administered orally, but the latter would not be readily decarboxylated in the intestinal tissue during its absorption. This is considered to be due to the inhibitory effect of DHPP on DOPA decarboxylase and expected to be advantageous as oral precursor of brain dopamine, since L-DOPA is rapidly decarboxylated in the intestinal tissue during the absorption.[14]

In the liver homogenates, on the contrary, DHPP was converted not only to L-DOPA but also to dopamine and its metabolites in almost the same rate as that when L-DOPA was used as the substrate. This may be due to the fact that the disappearence rate of DHPP is so fast that no inhibitory effect of DHPP on DOPA decarboxylase is exerted.

The result that DHPP was gradually transformed to L-DOPA in the brain homogenates may suggest that when DHPP was transported into the brain from the blood circulation as unchanged drug, it is also transformed to L-DOPA gradually in the brain tissue.

All these results provide a very promissing aspect for the use of DHPP as an oral precursor of brain dopamine which is superior than the oral use of L-DOPA with respect to the lesser decarboxylation at the peripheral organs.

In the transformation of DHPP to L-DOPA, L-phenylalanine, L-tyrosine, L-tryptophan and L-glutamate were found to be effective amino donor; the aromatic L-amino acids were

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particularly active. The fact that D-phenylalanine exhibited little activity as an amino donor again confirms the optically specific transamination reaction. The relative effectiveness of these amino acids was well in accord with that observed in the kidney homogenates as described before[4] and, therefore, it seems that the transaminase which catalyzes the L-DOPA formation from DHPP is the same enzyme in the liver and kidney.

On the other hand, an appreciable generation of $^{14}CO_2$ was also recognized in the liver homogenates, suggesting that a metabolic pathway of DHPP wherein the carbon atom at the 2-position is degraded. Although the mechanism is not clear, a pathway other than that leading to L-DOPA must be involved, since the labeled carbon is located at the 2-position in contrast to that at 1-position which is degraded from L-DOPA by the decarboxylation reaction. The following properties were found concerning the enzyme participates in the liberation of $^{14}CO_2$ from DHPP-2-^{14}C. i) The activity of the enzyme was the highest in the liver followed by the kidney wherein the activity was about 1/4 of that in the liver. Almost no activity was observed in the intestine and brain as well as in the bacterial flora. ii) SKF 525A and carbon monoxide, the potent inhibitor of the microsomal drug oxidizing enzymes, failed to inhibit the enzyme. In fact, the enzyme located not in the microsomal fraction, but in the 105000 ***g*** supernatant fraction. iii) The activity was inhibited by the metal chelating reagents, and thus the enzyme must contain a heavy metal in an active center of the enzyme. iv) Redox reagent such as DCPI increased the activity two to three fold. Such stimulation was often reported with oxidases which contain a heavy metal. v) *p*-Hydroxyphenylpyruvate and phenylpyruvate inhibited the liberation of $^{14}CO_2$, but pyruvate did not inhibit. This indicates that the enzyme seems to be specific to aromatic phenylpyruvates.

Two plausible pathways could be postulated. The first possible mechanism is the degradation of the aromatic ring after formation of 2,4,5-trihydroxyphenylacetate. It is well known that tyrosine is first metabolized to *p*-hydroxyphenylpyruvate which is finally oxidized to CO_2. It has been reported by Fellman, *et al.*[15] that DHPP is oxidized to 2,4,5-trihydroxyphenylacetate with *p*-hydroxyphenylpyruvate hydroxylase which catalyzes the hydroxylation of *p*-hydroxyphenylpyruvate to homogentisate. Therefore, it is possible that 2,4,5-trihydroxyphenylacetate further suffers aromatic ring fission and degradation to CO_2 by the metabolic system of tyrosine.

As another possible mechanism, a direct cleavage of pyruvic acid chain from aromatic ring of DHPP-2-^{14}C and the following degradation of pyruvic side chain to $^{14}CO_2$ will also be postulated. Further studies on the precise mechanism is now under investigation in this laboratories.

From the present results *in vitro*, DHPP was expected to act as L-DOPA *in vivo* as a precursor of dopamine in the brain. Furthermore, DHPP appears to be more advantageous than L-DOPA when it is administered orally, because the decarboxylation in the intestinal tissue of L-DOPA formed from DHPP is predicted to be low on account of the inhibitory effect of DHPP on DOPA decarboxylase. *In vivo* and the clinical importance of DHPP must depend on the rate of intestinal absorption, the availability of endogenous amino donors to DHPP and participation of the degradation to CO_2 *in vivo*, which will be evaluated *in vivo* in the subsequent paper.

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