[Chem. Pharm. Bull. 20(5) 966—973 (1972)]

UDC 547.581.2.09 : 615.31.033.076.9

Studies on the Metabolism of D- and L-Isomers of 3,4-Dihydroxyphenylalanine (DOPA). II.[1] Autoradiographic Study on the Distribution of ^{14}C-Labeled D- and L-DOPA after Oral Administration in Rats[2]

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(Received October 7, 1971)

The distribution of D- and L-^{14}C-DOPA following oral administration in rats were investigated by means of whole-body autoradiographic technique. With a constant dose level of 10 mg/kg, the distribution patterns of the two isomers were found to be significantly different from those observed after intravenous administration and almost no uptake was shown in the brain and skeletal muscle after oral administration of L-DOPA, while a prominent accumulation after that of the D-isomer. These differences were ascribed to i) a rapid metabolic change of L-DOPA in the peripheral tissues including the gastro-intestinal tract and ii) a much slower absorption of D-DOPA than L-DOPA from intestine. It was further found that the distribution pattern of L-DOPA depends on the amount of oral dose and the brain uptake was increased markedly with increasing the dose level to higher than 50 mg/kg, which is in accord with the effective dose level of L-DOPA clinically applied in the treatment of Parkinsonism. The high accumulation of D-DOPA in the tissues such as the brain and skeletal muscle and its retention for a long period might give a possible explanation for the fact found clinically that the oral use of the DL-racemate rather than the L-isomer causes a severe side effect.

In the preceding paper,[1] the distribution of ^{14}C-DOPA was compared between the D- and L-isomers following intravenous administration in rats and there was found many significant differences in the behavior of radioactivity between the two isomers. The effectiveness and usefulness of DOPA as an agent for Parkinson's disease has been established clinically by the oral dose and it has been found that a large dose of L-DOPA rather than the DL-racemate reduces the side effect significantly and give rise to a pronounced therapeutic effect.[4,5] Thus, it is thought to be of interest and of importance to compare the distribution and fate of D- and L-DOPA after oral administration. In the case of the oral administration, differences between the isomers might also be expected in their transport and metabolism at the gastro-intestinal tract. In the present paper, the distribution of radioactivity was compared between the D- and L-^{14}C-DOPA by means of whole-body autoradiographic technique following the oral administration in rats and a particular interest was focused to the effect of changing the dose level of L-DOPA on the distribution pattern.

Material and Method

Labeled Compounds——D- and L-2-^{14}C–DOPA were prepared by resolving DL-2-^{14}C–DOPA which was purchased from the Radiochemical Center, Amersham, England. The resolution was accomplished by crystallization from water in the presence of a large excess of non-radioactive L- and D-DOPA, as described in the preceding paper.[1] The specific activity was 26.5 and 24.9 μCi/mg for the L- and D-^{14}C-DOPA, respectively, and the radiochemical purity was over 98% for the both compounds.

Autoradiography——Male rats of Wistar–Imamichi strain weighing about 100 to 120 g were used. D- and L-^{14}C-DOPA were dissolved in physiological saline in an appropriate concentration and 0.5 ml of the

1) Part I: H. Shindo, N. Miyakoshi and I. Takahashi, *Chem. Pharm. Bull.* (Tokyo), **19**, 2490 (1971).
2) This work was presented at the 91st Annual Meeting of the Pharmaceutical Society of Japan, Fukuoka, April 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).

solution was administered orally with stomach tube. For a comparative study with a fixed amount of dose, that of 10 mg/kg body weight was used corresponding to the dose level adopted for the study after intravenous injection.[1] For increasing the dose to 50 and 100 mg/kg, the labeled compounds were diluted with non-radioactive L- or D-DOPA in order to give a constant dose of radioactivity (about 25 μCi/rat).

Thirty minutes, 1, 3, 6, 24 and 72 hr after the 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 film and exposed for a constant period of 7 days.

Result

Distribution of D- and L-^{14}C-DOPA after a Constant Dose of 10 mg/kg

It was found generally that the distribution pattern of radioactivity after oral administration was significantly different from that observed after intravenous administration of the same dose,[1] particularly in the case of radioactive L-DOPA.

Thirty minutes after oral administration of 10 mg/kg L-^{14}C-DOPA, the highest radioactivity was still observed in the gastro-intestinal contents, but a high concentration was observed in the kidney and urinary bladder followed by the liver, indicating that L-DOPA is well absorbed from the gastro-intestinal tract. Relatively high blood level of radioactivity was observed. A high radioactivity was found to be localized in the adrenal medulla, the cortex being devoid of radioactivity. Only a concentration comparable to the blood level was observed in the pancreas and no radioactive uptake in the brain, both of which are the organs where L-DOPA accumulated in a high concentration after intravenous administration.[1]

After administration of D-^{14}C-DOPA, the highest radioactivity was observed in the kidney and urinary bladder as well as the gastro-intestinal contents. A high concentration was observed in the pancreas, which was considerably higher than the L-isomer. Only a concentration comparable to the blood level was observed in the liver as well as the lung and also in the adrenal. No radioactive uptake was detected in the brain.

One hour after administration of L-^{14}C-DOPA (Fig. 1-A), the concentration of radioactivity in the blood and tissues appear to reach the maximum levels. The highest concentration was still observed in the gastro-intestinal contents, kidney and urinary bladder, but the concentration in the liver was increased markedly. A high accumulation of radioactivity was shown in the adrenal medulla, while only a concentration lower than the blood level in the pancreas. An accumulation of radioactivity was observed in the skin including the hair follicles and in the intestinal mucosa. Only a very low concentration of radioactivity was detected in the brain, a slightly higher concentration in the caudate nucleus. In the skeletal muscle, only a low concentration of radioactivity was distributed and the pattern suggested that the distribution was not in the muscle fibers, but mostly in the extracellular spaces, in contrast to the observation that[1] the radioactivity appears to be accumulated in the muscle fibers after intravenous administration.

One hour after administration of D-^{14}C-DOPA (Fig. 1-B), the blood concentration was approximately the same level to that of the L-isomer, while the concentration in the liver never exceeded the blood level. On the other hand, a high accumulation of radioactivity was shown in the pancreas, the concentration being considerably higher than that of the L-isomer. A very low concentration of radioactivity was distributed over the whole brain and only a concentration comparable to the blood level in the adrenal.

Three hours after administration of L-^{14}C-DOPA the radioactivity was found to be eliminated from the body to a large extent and a high concentration was observed only in the intestinal contents and urinary bladder, followed by the adrenal medulla and renal medulla, as shown in Fig. 2. The concentration in the liver was decreased to a very low level and

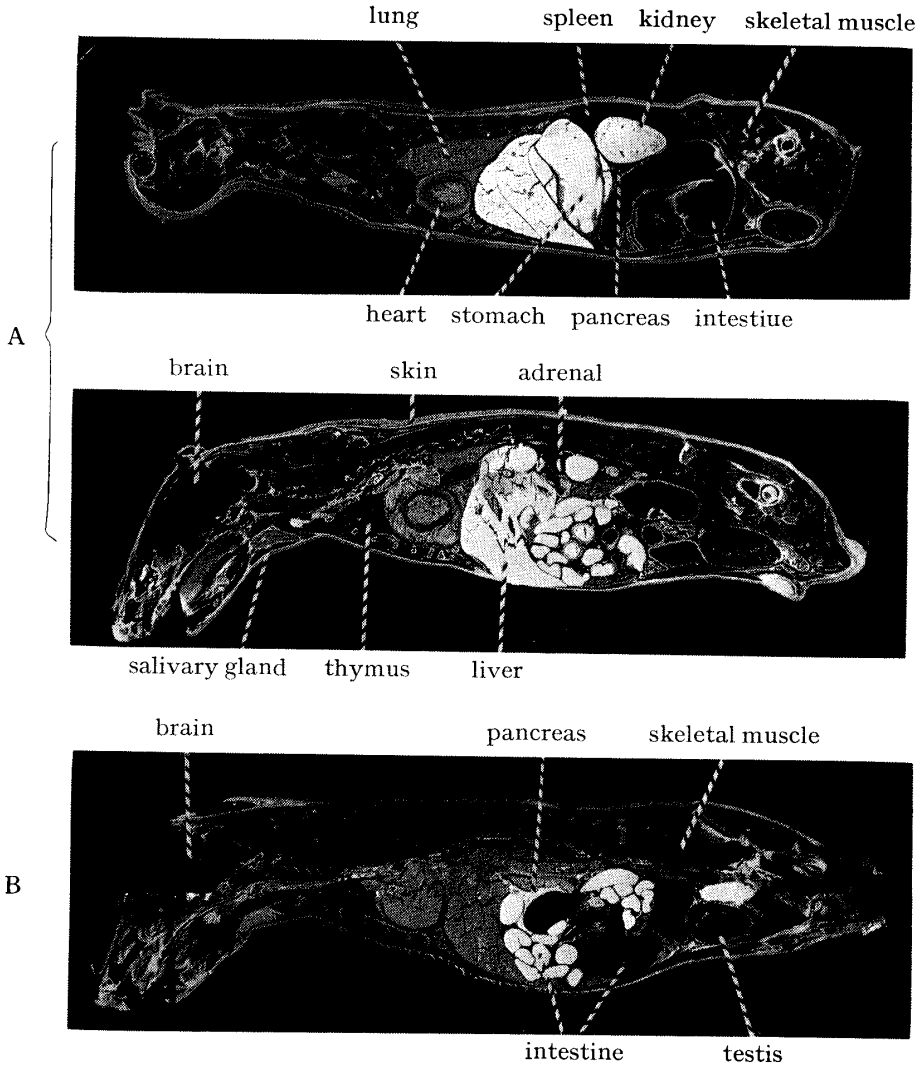

Fig. 1. Autoradiograms from Male Rats 1 hr after Oral Administration of L-(A) and D-^{14}C-DOPA (B) (10 mg/kg)

only an appreciable level of radioactivity was remained in the pancreas. Only an extremely low concentration was detected in the brain.

Three hours after administration of D-^{14}C-DOPA, in contrast to the L-isomer, the concentration of radioactivity in the tissues appears to reach the maximum about this time after administration. Thus, generally the tissue concentrations of radioactivity become much higher in the D-isomer than in the L-isomer, as can be seen from Fig. 2. The blood concentration was also appreciably higher than the L-isomer. The concentration in the liver did not exceed the blood level, while an accumulation of a high radioactivity was continued to be observed in the pancreas. A uniform distribution of an appreciable concentration was observed in the skeletal muscle and the pattern suggested that the radioactivity was distributed not only in the extracellular spaces, but also in the muscle fibers. No accumulation exceeding the blood level was observed in the adrenal, while some uptake which exceeded the blood level in the thymus and spleen. In the brain also an appreciable concentration of radioactivity was found to be distributed through the whole brain, a higher concentration being located in the grey matter, as shown in Fig. 2-C.

Six hours after administration of L-^{14}C-DOPA, the concentration of radioactivity showed a further decline and an appreciable radioactivity was detected only in the intestinal contents, adrenal medulla, renal medulla and pancreas. After administration of the D-isomer, on the other hand, a very high concentration was observed in the renal medulla and pancreas

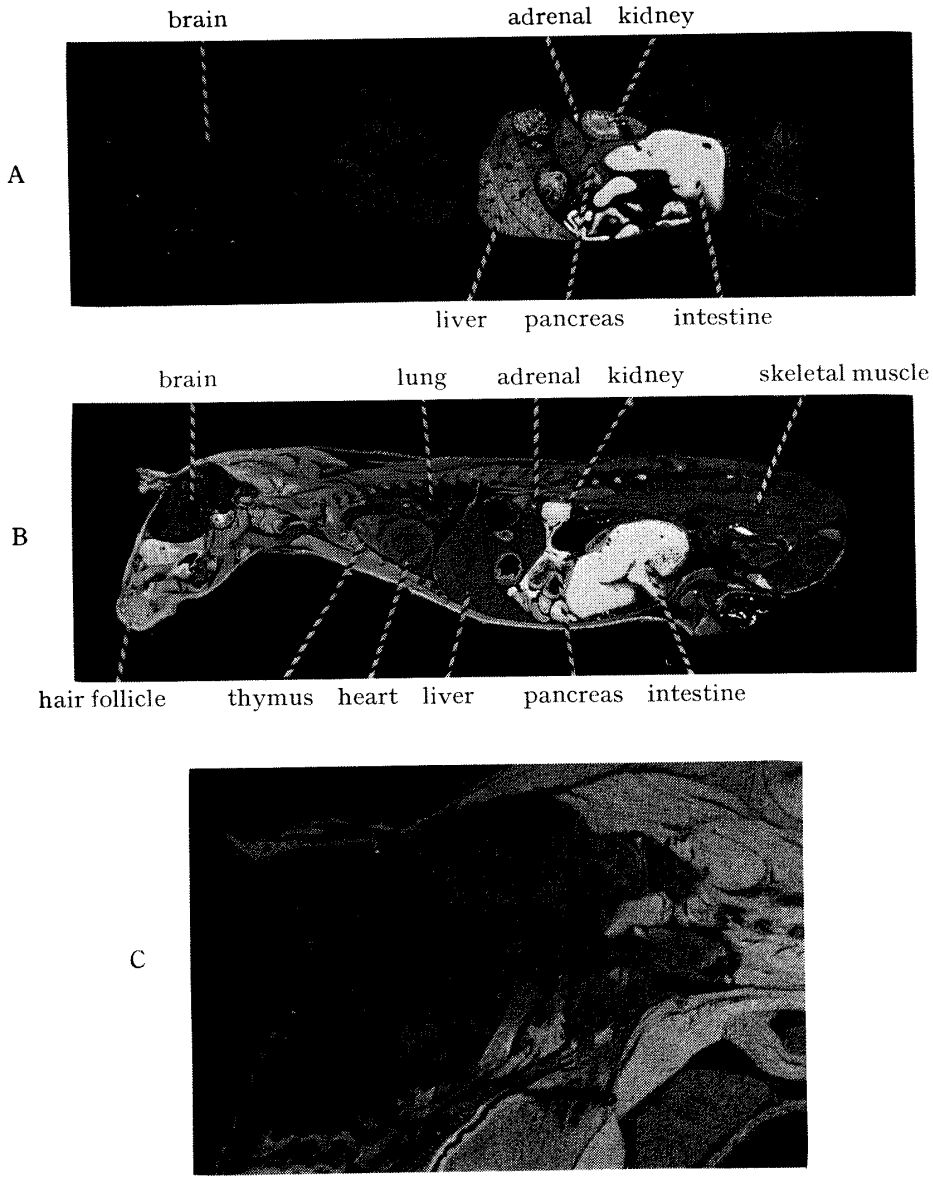

Fig. 2. Autoradiograms and Its Enlargement from Male Rats 3 hr after Oral Administration (10 mg/kg) of L- (A) and D-^{14}C-DOPA (B,C)

as well as in the intestinal contents and a prominent radioactivity was continued to be retained in the skeletal muscle and brain, as shown in Fig. 3. These results indicate that D-DOPA shows a considerably higher uptake and a longer retention in the body tissues than the L-isomer after oral administration, in contrast to the previous finding[1] that L-DOPA showed a much higher uptake in the tissues than the D-isomer after intravenous injection.

After 24 hr, the most of radioactivity of L-DOPA was disappeared from the body with only retention of some radioactivity in the renal medulla and adrenal medulla (Fig. 4-A). After administration of the D-isomer, the radioactivity showed a much longer retention and an appreciable radioactivity was still observed in the pancreas, renal medulla, retina, hair follicles, skeletal muscle, brain and testis (Fig. 4-B). After 72 hr, the most of radioactivity from D-DOPA also disappeared from the body, an appreciable radioactivity being retained only in the renal medulla.

Changes of the Distribution Pattern of L-^{14}C-DOPA depending upon the Amount of Dose

The observed differences in the distribution pattern of L-DOPA between after different route of administration is considered to be due to a metabolic change of L-DOPA at the peripheral organs such as the stomach mucosa, intestine and liver after its oral

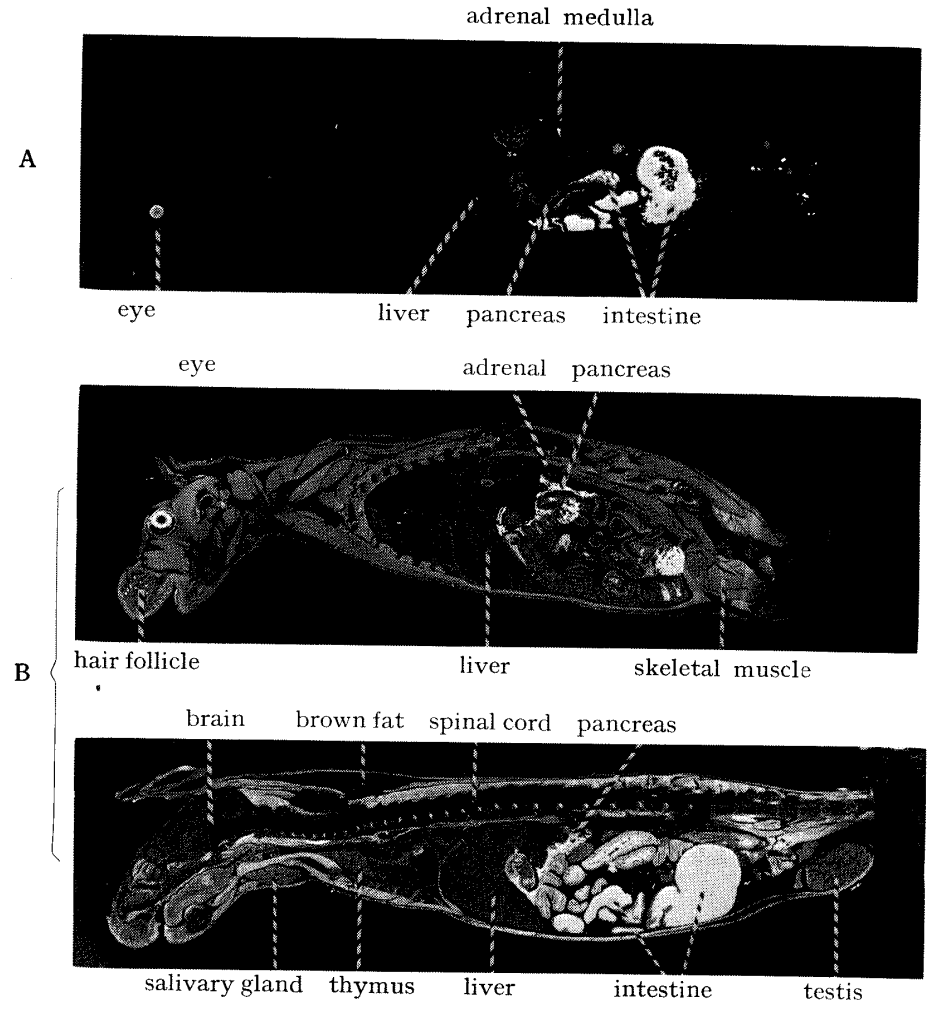

Fig. 3. Autoradiograms from Male Rats 6 hr after Oral Adminsitration of L- (A) and D-^{14}C-DOPA (B) (10 mg/kg)

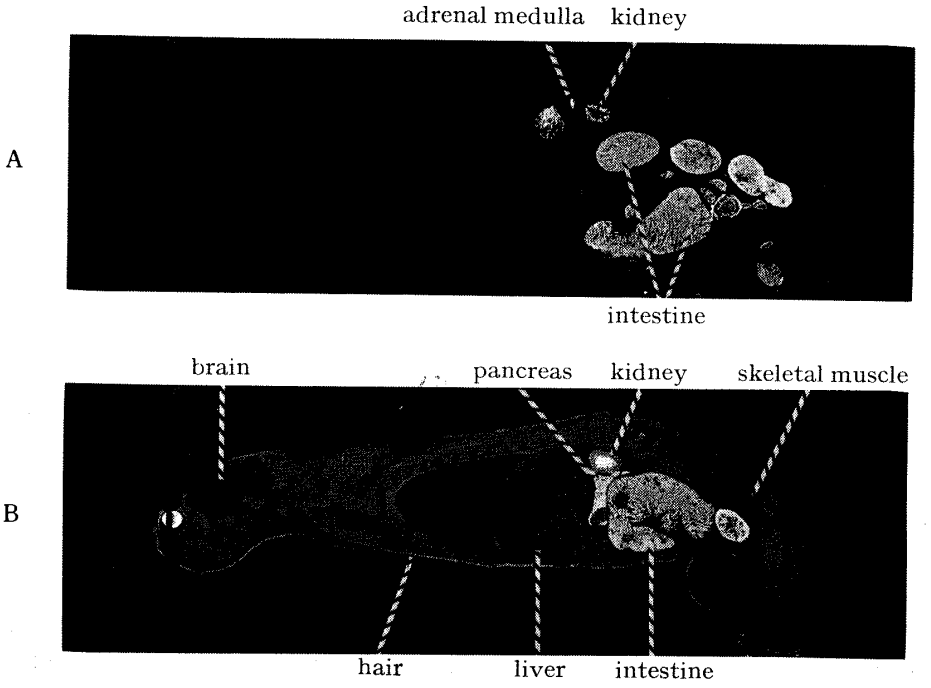

Fig. 4. Autoradiograms from Male Rats 24 hr after Oral Administration of L-(A) and D-^{14}C-DOPA (B) (10 mg/kg)

administration. It might be expected, therefore, that the distribution pattern of radioactivity after oral administration of L-^{14}C-DOPA could change depending upon the amount of dose. Autoradiograms 1 hr after oral administration of 2, 10, 50 and 100 mg/kg L-^{14}C-DOPA in rats were thus compared. As the results, as examplified in Fig. 5, progressive changes were found to be observed in the distribution pattern of radioactivity with increasing the dose level.

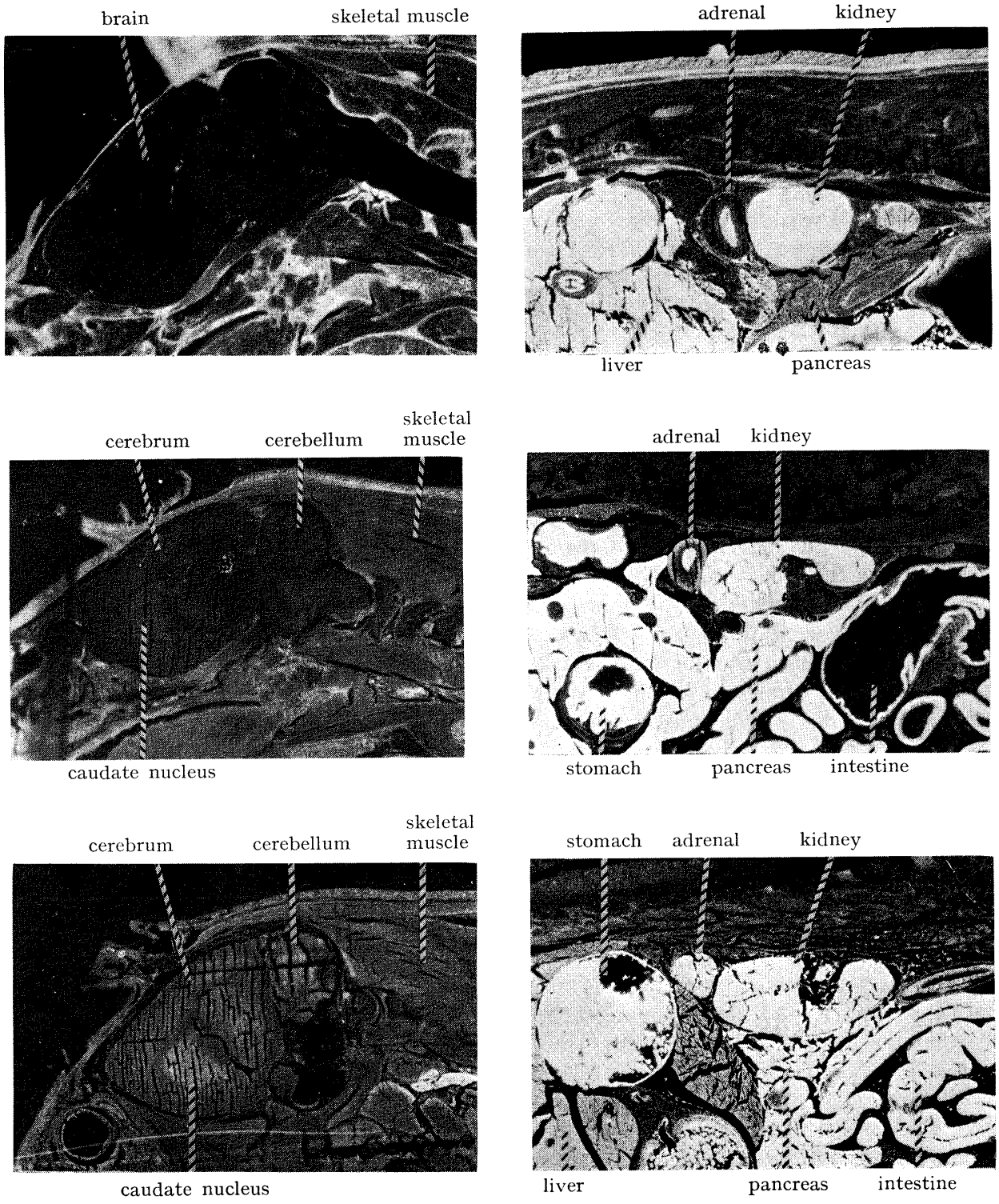

Fig. 5. Enlargements of Autoradiograms from Mice 1 hr after Oral Administration of a Various Dose of L-^{14}C-DOPA

A: 10 mg/kg, B: 50 mg/kg, C: 100 mg/kg

i) The uptake of radioactivity by the brain was increased significantly with increasing the amount of dose. No radioactivity was detected in the brain after oral dose of 2 mg/kg and only a very low radioactive uptake after that of 10 mg/kg. After oral dose of 50 and 100 mg/kg L-^{14}C-DOPA, on the other hand, an appreciable uptake of radioactivity was shown in the brain and a specific localization in the caudate nucleus became evident (Fig. 5).

ii) Almost no accumulation of radioactivity was observed in the pancreas after oral dose of 2 mg/kg, while the concentration was significantly increased with increasing the dose. A high accumulation of radioactivity was observed in the pancreas with a dose level higher than 50 mg/kg.

iii) In the adrenal, an accumulation of radioactivity was observed only in the medulla after a low dose of 2 or 10 mg/kg, while the concentration in the outer layer of the cortex was increased with increasing the dose over 50 mg/kg (Fig. 5).

iv) The uptake of radioactivity by the skeletal muscle was also increased with increasing the dose level. After oral dose of 2 or 10 mg/kg L-^{14}C-DOPA, the concentration in the skeletal muscle was very low and the distribution appeared to be mostly restricted in the extracellular spaces. After oral dose of higher than 50 mg/kg, on the other hand, a high accumulation of radioactivity was observed in the skeletal muscle and the pattern indicated an accumulation of radioactivity in the muscle fibers.

Discussion

In the preceding paper,[1] it was reported that a prominent radioactive uptake was shown by the brain, particularly by the caudate nucleus, as early as 1 min after intravenous injection of D-^{14}C-DOPA and the localization in the caudate nucleus was prominent till 6 hr after injection, while after that of the D-isomer only a slow and very low distribution of radioactivity was observed over the whole brain. In the present investigation, however, it was found that after oral administration of 10 mg/kg L-^{14}C-DOPA almost no uptake of radioactivity was shown in the brain, while after that of the same dose of the D-isomer a prominent radioactivity was accumulated in the brain, although the rate was slow and no localization was observed in the caudate nucleus. It was another present finding that the uptake of radioactivity by the brain was increased markedly with increasing the amount of oral dose of L-^{14}C-DOPA and after oral administration of a dose higher than 50 mg/kg body weight a prominent uptake of radioactivity was observed in the caudate nucleus.

These results might be interpreted as being due to the fact that L-DOPA, when orally administered in a relatively low dose, is decarboxylated to dopamine to a considerable extent at the peripheral sites of localizing dopa-decarboxylase, such as the stomach mucosa,[6] intestinal tissue and liver,[7] resulting in a relatively low concentration of DOPA in the circulating blood. It was already demonstrated[1] that dopamine cannot pass through the blood brain barrier and is not accumulated in both the pancreas and skeletal muscle. It was, therefore, considered[1] that the radioactivity accumulated in these organs after intravenous injection of L-^{14}C-DOPA might be unchanged DOPA, at least in the earliest period. In accordance with these considerations, no accumulation of radioactivity was observed in both the pancreas and skeletal muscle after oral dose of less than 10 mg/kg and it was notable that the distribution pattern was similar to that observed[1] after intravenous administration of ^{14}C-dopamine rather than that of L-^{14}C-DOPA. With increasing the dose level, on the other hand, the uptake of radioactivity in both the pancreas and skeletal muscle became evident. This latter fact is considered to be due to a saturation of the peripheral dopa-decarboxylase with a large amount of the substrate and an increased level of unchanged DOPA in the circulating blood. From our *in vitro* studies as will be described in the subse-

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quent paper,[8] it has been clarified that L-DOPA is decarboxylated in the intestinal tissue to a considerable extent, while the D-isomer is not metabolized to any appreciable extent and, further, that the extent is depressed significantly with increasing the concentration of L-DOPA in the medium. Thus, the dose level of about 50 mg/kg which corresponds to approximately 2.5 g per body in human subjects appears to be the level of occurring a saturation of the peripheral decarboxylation reaction with the substrate to an appreciable extent. This is in good accord with the clinical finding by Cotzias, *et al.*[4,5] that a therapeutic effect against Parkinsonism became evident when the oral dose of L-DOPA was increased gradually to such a large amount as several grams per day.

It has been reported from *in vitro* studies[9] that only L-DOPA is transferred into the brain tissues by an active transport mechanism, while D-DOPA has been considered not to be able to pass through the blood brain barrier.[10] It has been pointed out from our previous studies[1] that D-DOPA appears to be also transferred slowly into the brain, probably through a passive diffusion process. In the present studies, it was further demonstrated that D-DOPA administered orally is gradually accumulated in the brain and a much higher concentration was achieved as compared to that after the intravenous administration. This might be interpreted as being caused from a much longer duration of D-DOPA concentration in the circulating blood after oral administration than that after intravenous administration. In fact, as will be reported in the subsequent paper,[8] it has been clarified that L-DOPA is absorbed from rat intestine in a much faster rate than D-DOPA and an active transport mechanism is involved only in the absorption of the L-isomer. It might be probable, therefore, that D-DOPA is absorbed gradually from the intestine without being metabolized to any extent, resulting in a continuance of a certain level of DOPA concentration in the circulating blood and a gradual transfer and accumulation in the brain.

It might be said more generally that the tissue accumulation is much higher and continued for a much longer period after oral administration of D-DOPA than after that of the same dose of L-DOPA. It has been further clarified in this laboratory[11] that after oral administration of D-^{14}C-DOPA no dopamine and its metabolites is detected in the brain, but the accumulated radioactivity is mostly due to DOPA and its 3-O-methylated product, indicating that D-DOPA has no therapeutic effect against Parkinsonism. Therefore, provided that there is no interaction in the processes of the absorption and metabolism between the two isomers, it might be expected that when DL-DOPA is administered orally the L-isomer is metabolized rapidly in the peripheral tissues to a considerable extent and eliminated from the body without any effective accumulation in the brain, while unbeneficial D-DOPA which is possible to cause some undesirable toxic effect is accumulated in the brain and skeletal muscle. This might give a possible explanation for the clinical finding by Cotzias, *et al.*[5] that when L-DOPA was used orally rather than the DL-racemate the side effect was significantly reduced with an increase in the therapeutic effect on Parkinson's disease.

Acknowledgement The authors express their deep gratitudes to Dr. G. Sunagawa, director of this laboratories, and to Dr. K. Tanabe for their kind encouragement. Thanks are also due to Mr. T. Kurano and Y. Saito of Sankyo Chemical Industries for the preparation oi labeled compounds.

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