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

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The distribution of D- and L-^{14}C-DOPA and ^{14}C-dopamine were investigated by means of whole-body autoradiographic technique following intravenous injection in rats. Significant differences were found in the distribution pattern of radioactivity between the two isomers, as summarized in the following: i) a rapid and marked uptake of L-DOPA by the brain and the localization in the caudate nucleus, in contrast to a slow and a very low distribution of D-DOPA over the whole brain, ii) an extremely high uptake and a long retention of L-DOPA in the adrenal medulla, iii) a much higher concentration of L-DOPA in the skeletal muscle and liver than D-DOPA, and an appreciably faster initial rate of elimination of D-DOPA into the urine, and iv) an accumulation and a long retention of both isomers in the pancreas, renal medulla and hair follicles, with an appreciably higher concentration in the D-isomer. ^{14}C-Dopamine did not show any radioactivity in the brain, confirming that it cannot pass through the blood-brain barrier. These results were discussed in relation to the possible differences in their metabolism and transport and to the therapeutic effect of L-DOPA against Parkinsonism.

3,4-Dihydroxyphenyl-alanine (DOPA) has been long established to be a precursor of catecholamines[3] and recently was found to be an effective agent for Parkinsonism clinically.[4] It was further found that the administration of the L-isomer rather than DL-racemate reduces the side effects significantly with an increased therapeutic effect on Parkinsonism.[5]

The distribution of DOPA in mice after intravenous injection has been investigated by Rosell, *et al.*[6] and that in gerbils by Rossum, *et al.*[7] by whole-body autoradiographic technique, but both of these studies were performed using DL-racemate labeled with ^{14}C. It is therefore of interest and of importance to compare the distribution and the behavior in the body between the optical isomers of which one of them is biologically active.

In the present paper, ^{14}C-labeled D- and L-DOPA were intravenously administered into rats and their distributions were compared at various survival periods by whole-body autoradiographic technique. The distribution of ^{14}C-dopamine, which has been considered to be responsible for the pharmacological action of DOPA in the brain, was also investigated for comparison.

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[8] by crystallization from water in the presence of a large excess of non-radioactive L- and D-DOPA. The radio-

1) Presented at the 91st Annual Meeting of the Pharmaceutical Society of Japan, Fukuoka, April 1971.
2) Location: *Hiromachi 1-chome, Shinagawa-ku, Tokyo.*
3) M. Sandler and C.R.J. Ruthven, *Progress in Medicinal Chem.*, **6**, 200 (1969).
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) S. Rosell, G. Sedvall and S. Ullberg, *Biochem. Pharmacol.*, **12**, 265 (1963).
7) J.M. Van Rossum, G.C.B. Wijffels and N.V.M. Rijntjes, *Europ. J. Pharmacol.*, **7**, 337 (1969).
8) Achieved by Mr. T. Kurano and Y. Saito of the Research and Development Department, Sankyo Chemical Industries, Ltd.

active DL-DOPA with specific activity of 52 mCi/mmole (261 μCi/mg) was dissolved in hot water containing a small amount of $NaHSO_3$ and the solution was added with non-radioactive L-DOPA so that the amount of the L-isomer in the solution becomes 10 times larger than that of the D-isomer. After being dissolved completely by heating and concentrated *in vacuo*, the precipitates were filtered and recrystallized from water repeatedly until the specific activity reached a constant value of 26.5 μCi/mg (5.22 mCi/mmole). The mother liquid after separation of L-^{14}C-DOPA was then added with non-radioactive D-DOPA and the radioactive D-DOPA was crystallized in the same way as L-DOPA. Recrystallizations gave a constant specific activity of 24.9 μCi/mg (4.91 mCi/mmole). The predicted specific activity for the pure L-^{14}C-DOPA to be obtained was 26.1 μCi/mg and the radiochemical purity of the preparation could be calculated to be over 98%, the radiochemical contamination of D-DOPA being less than 2%. The radiochemical purity of the D-^{14}C-DOPA preparation must be even higher than that of the L-preparation. Dopamine-2-^{14}C with specific activity of 55 mCi/mmole (290 μCi/mg) was purchased from the Radiochemical Center.

Autoradiography——Male rats of Wistar-Imamichi strain weighing about 100 g were used. D- and L-^{14}C-DOPA and ^{14}C-dopamine, which was diluted with non-radioactive dopamine to reduce the specific activity to 25 μCi/mg, were dissolved in physiological saline and were injected intravenously into rats from the tail vein in the dose of 10 mg/kg (about 25 μCi/rat). One, 10 and 30 min, 1, 3, 6, 24 and 72 hr after injection, 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 carboxy methyl cellulose 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

One minute after intravenous injection of L-^{14}C-DOPA (Fig. 1), the highest uptake of radioactivity was shown by the kidney, gastric and intestinal mucosa and adrenal, followed by the pancreas. An appreciable uptake was already observed in the brain and a higher concentration was found to be localized in the caudate nucleus, thalamus and cerebellar cortex. A high uptake was also noted in the pituitary, choroid plexus and trigeminal nerve. In the adrenal, a high concentration was observed in the medulla and zona fasciculata, while a lower concentration in the region between them, the zona reticularis (Fig. 3-A). The skeletal and cardiac muscles showed a considerable radioactive uptake. Relatively low concentration of radioactivity was shown in the liver as a spotted appearence and a concentration comparable to the blood level in the lung. In the spleen, some uptake was observed only in the white pulp (Fig. 3-A).

After injection of D-^{14}C-DOPA, a much lower uptake of radioactivity was shown generally by most of the organs and tissues as compared to the L-isomer. One minute after injection (Fig. 1), the highest uptake of radioactivity was shown by the kidney, followed by the pancreas. A high radioactivity was also shown in the gastric and intestinal mucosa and in the adrenal, but the concentrations appear to be considerably lower than the L-isomer (Fig. 3-B). Almost no uptake of radioactivity was observed in the brain, except the pituitary and choroid plexus. In the skeletal and cardiac muscles, only a low concentration of radioactivity was distributed and the pattern suggested that the distribution was not in the muscle fibers, but only in the extracellular spaces. The blood level and the concentration in the lung, on the other hand, appeared to be appreciably higher than the L-isomer.

Ten minutes after injection of L-^{14}C-DOPA (Fig. 2), the blood level was declined significantly and the highest radioactivity was shown in the kidney followed by the adrenal and pancreas. The concentration in the lung was also declined, while that in the liver was increased considerably. In the adrenal, the high radioactivity was still remained in both the medulla and the outer layer of the cortex. In the brain, a localization of high radioactivity became more pronounced in the caudate nucleus, cerebellar cortex, thalamus and grey matter of the spinal cord. A high accumulation of radioactivity was observed in the skeletal muscle and skin including hair, while relatively low concentration in the cardiac muscle. In the

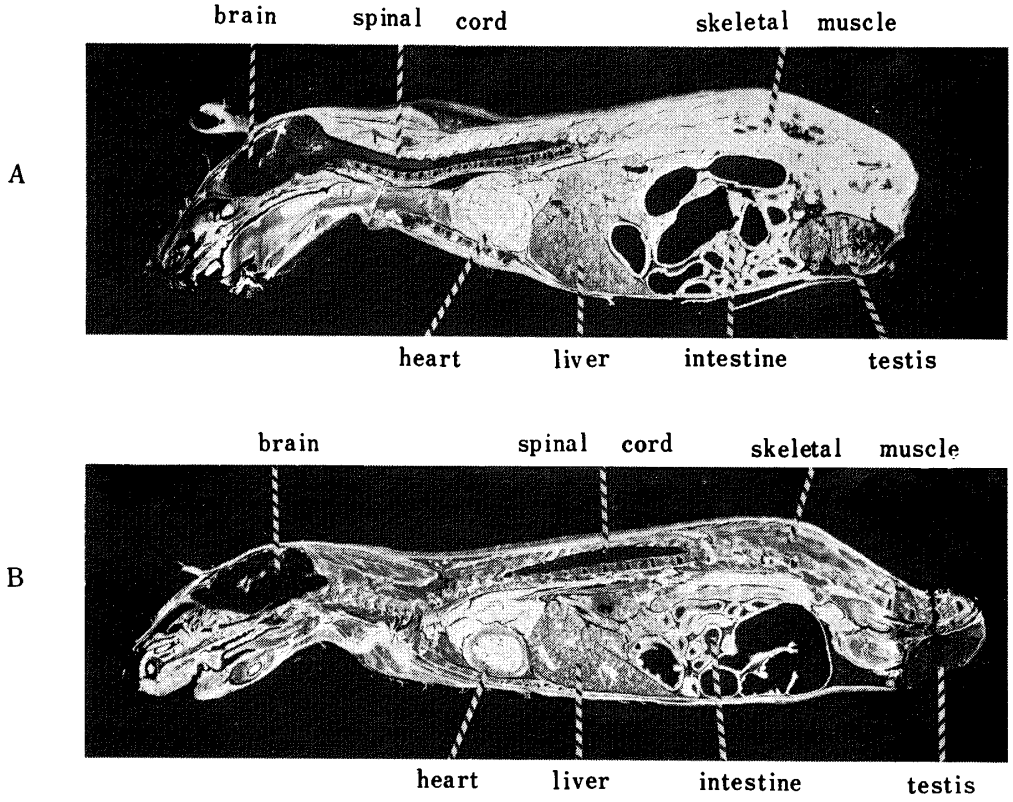

Fig. 1. Autoradiograms from Rats 1 min after Intravenous Injection of L- (A) and D- ^{14}C-DOPA (B)

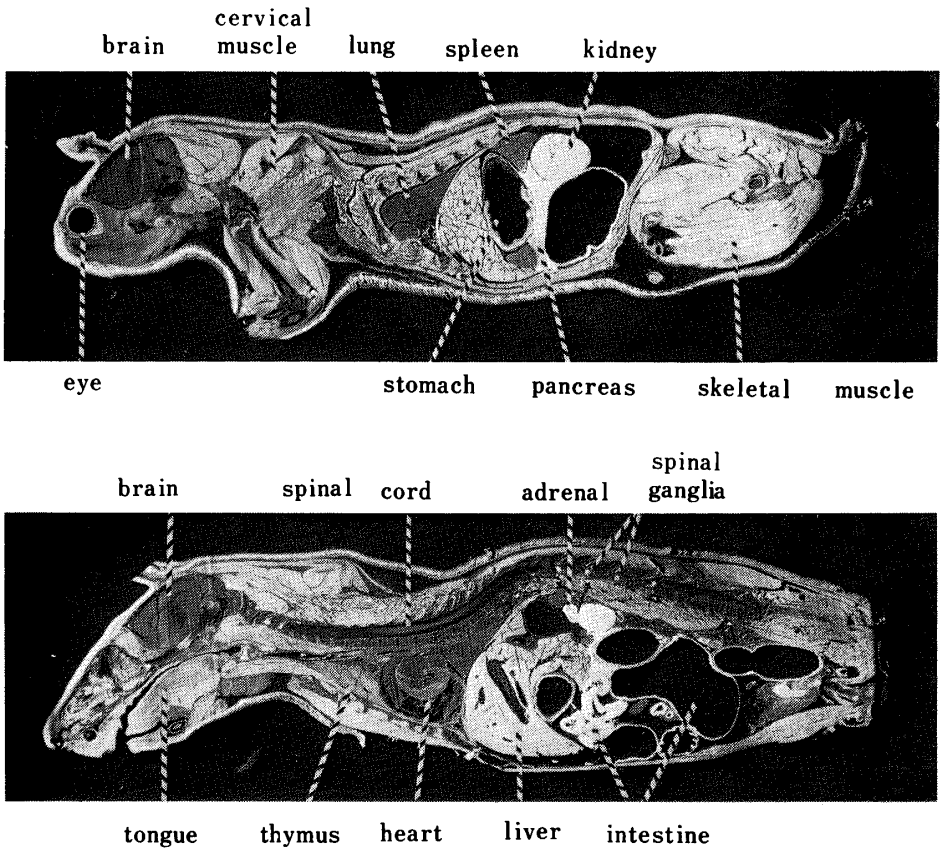

Fig. 2. Autoradiograms from Rat 10 min after Intravenous Injection of L-^{14}C-DOPA

gastric mucosa, the highest concentration was observed in the submucosa (Fig. 3-C). A high radioactivity was noted in the eye, probably in the retina, and spinal ganglia.

After injection of the D-isomer, the blood level was significantly higher than the L-isomer and the highest radioactivity was shown in the kidney and pancreas. The concentration in the lung was comparable to the blood level, while uptake by the liver was considerably lower than the L-isomer. Thus the concentration in the liver was higher than that in the lung for the L-isomer, while the reverse was for the D-isomer. In the brain, a uniform distribution of a very low radioactivity was observed, suggesting a slight and slow penetration of D-DOPA into the brain. In the skeletal muscle, a distribution pattern in the extracellular spaces was still predominant. A high accumulation of radioactivity was noted in the skin and hair.

Thirty minutes after injection of L-^{14}C-DOPA, the localization of radioactivity in the different regions of the brain became the most evident and the highest concentration was observed in the caudate nucleus followed by the thalamus, cerebellar cortex and brain-stem, as shown in Fig. 3-D. The highest concentration in the body was shown in the pancreas and

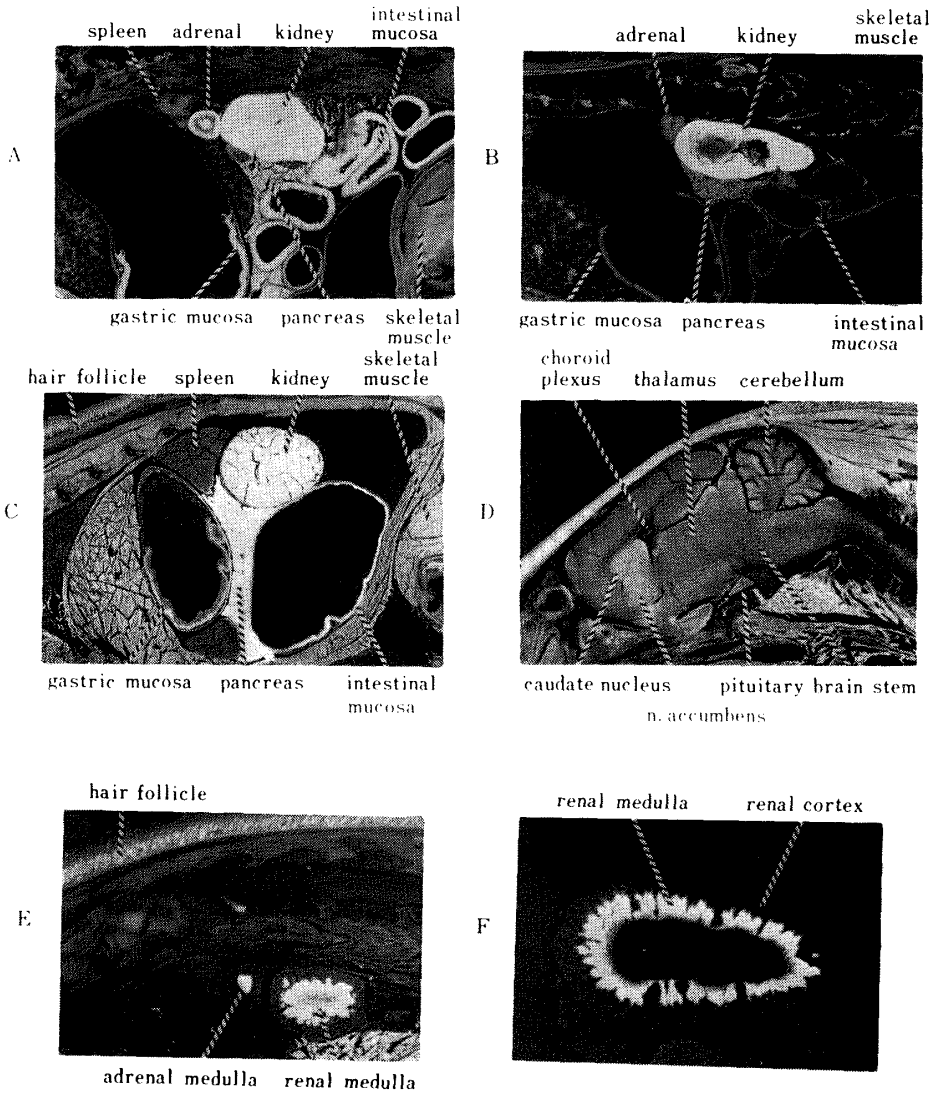

Fig. 3. Enlargements of the Autoradiograms from Rats 1 min (A), 10 min (C), 30 min (D) and 6 hr (E) after Intravenous Injection of L-^{14}C-DOPA and 1 min (B) and 24 hr (F) after That of D-^{14}C-DOPA

adrenal medulla followed by the liver, intestinal mucose and kidney, as shown in Fig. 4. The concentration in the liver was the highest at this time of the survival period, which was significantly higher than the D-isomer. The concentration in the adrenal cortex was found to be significantly decreased, a high accumulation being retained only in the medulla. A high concentration was also continued in the skeletal muscle, skin, hair and spinal ganglia. Some radioactivity was observed in the intestinal contents, suggesting some excretion of radioactivity through the biliary or pancreatic secretion.

Thirty minutes after injection of D-^{14}C-DOPA, the blood level was significantly decreased and, as compared in Fig. 4, the concentration of radioactivity in most of the tissues was significantly lower than that of the L-isomer, indicating that the rate of elimination of radioactivity from the body, mainly into the urine, is considerably faster for the D-isomer than the L-isomer. A high radioactivity was still shown in the pancreas, kidney and intestinal mucosa. A high accumulation of radioactivity was observed in the skin, where most of the radioactivity

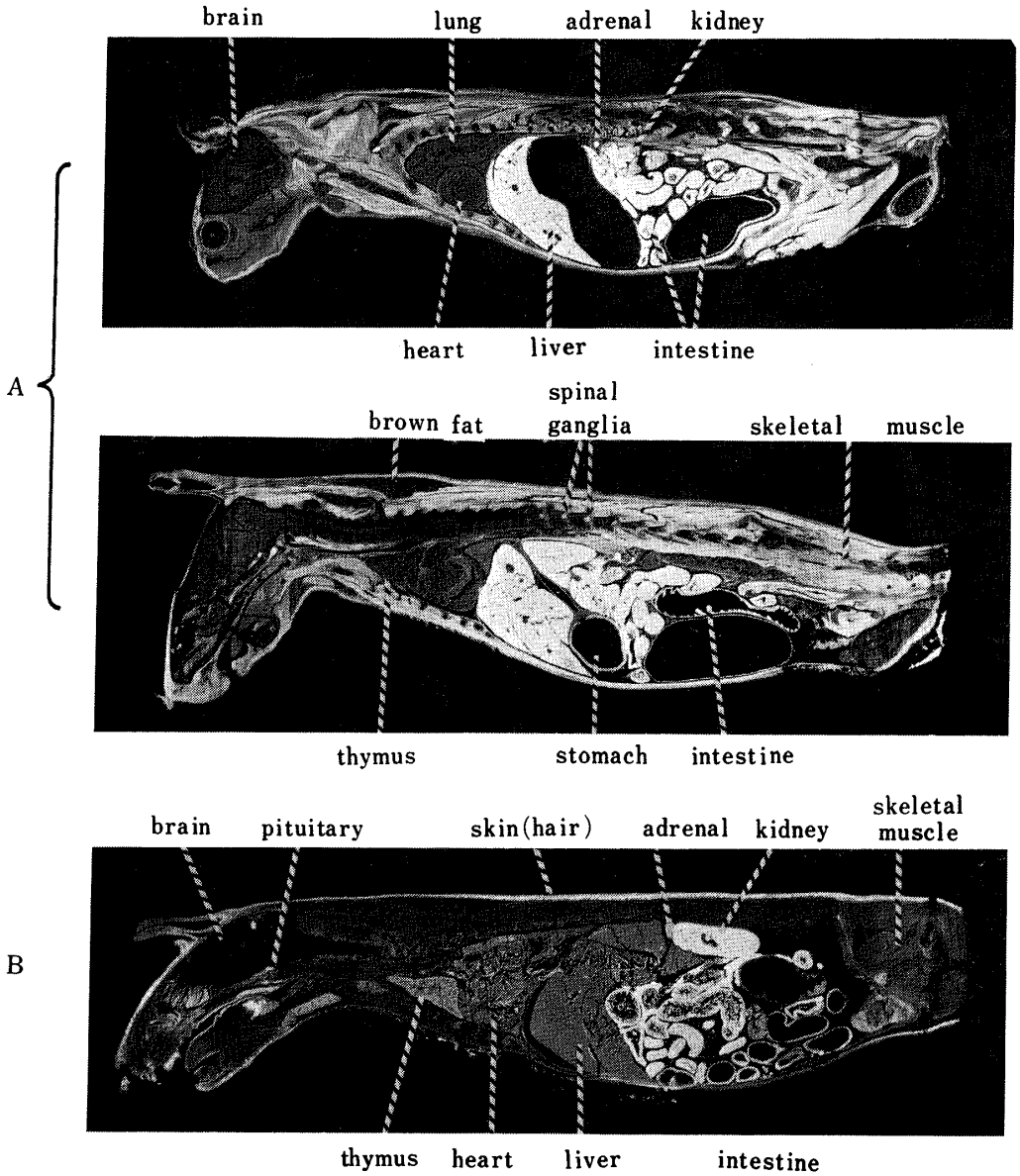

Fig. 4. Autoradiograms from Rats 30 min after Intravenous Injection of L- (A) and D-^{14}C-DOPA (B)

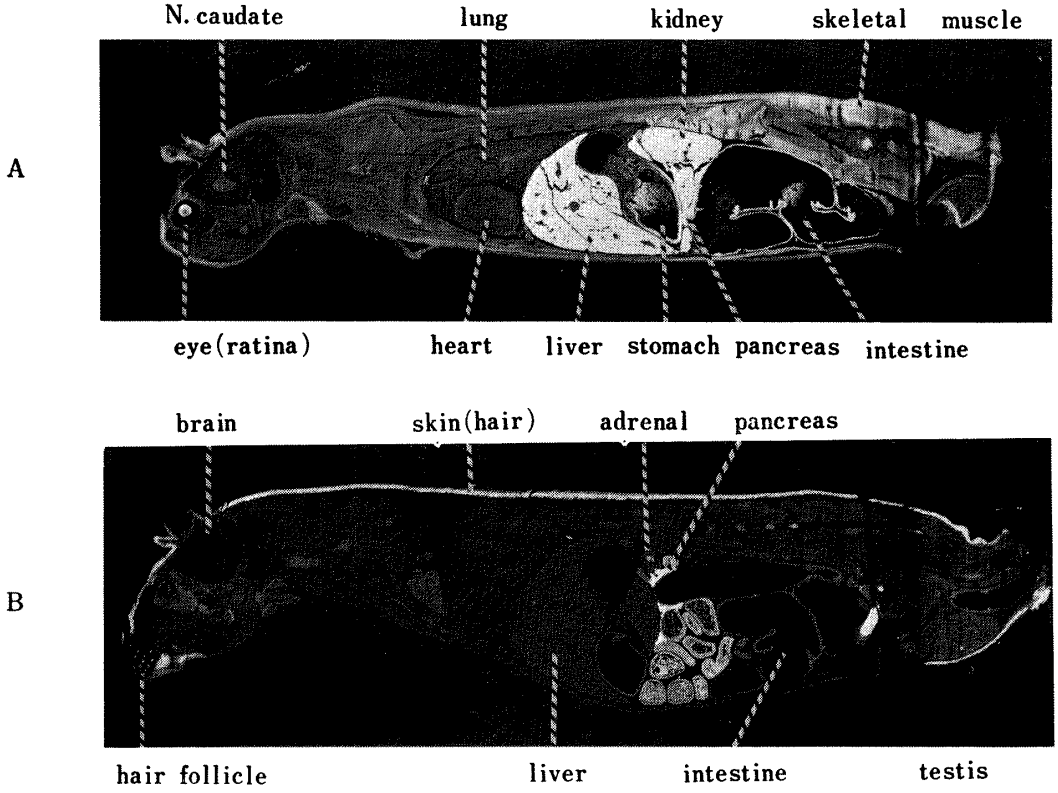

Fig. 5. Autoradiograms from Rats 1 hr after Intravenous Injection of L- (A) and D-^{14}C-DOPA (B)

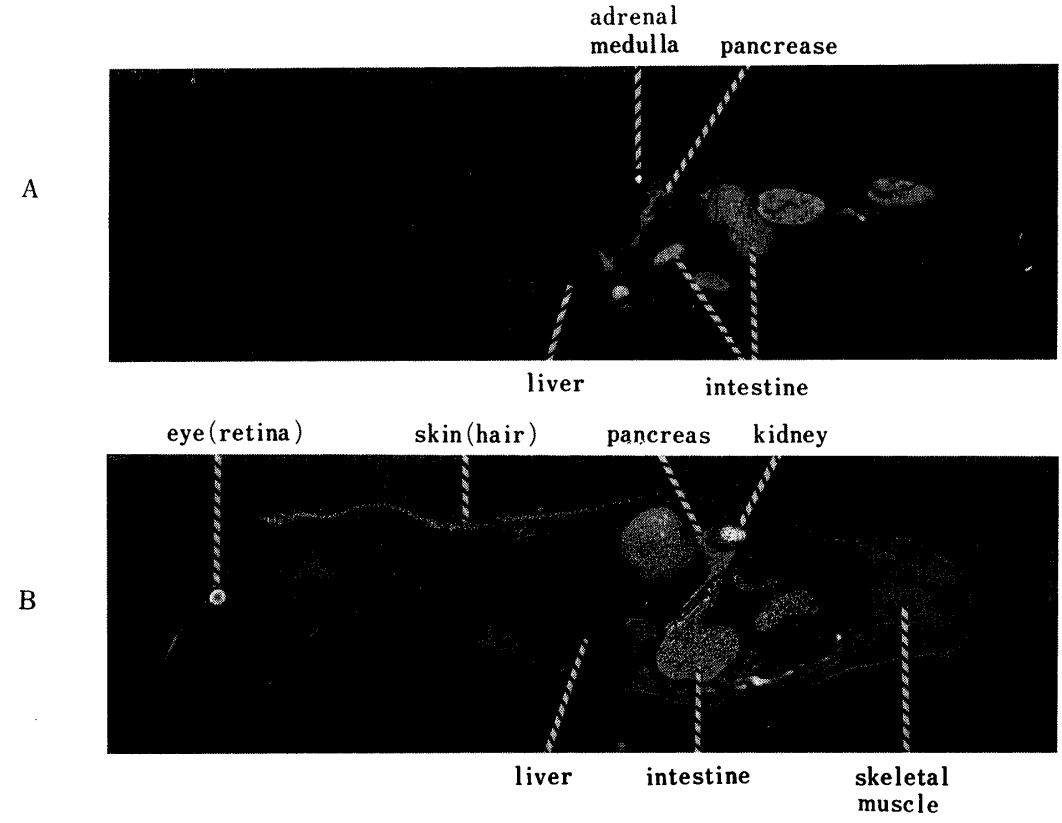

Fig. 6. Autoradiograms from Rats 6 hr after Intravenous Injection of L- (A) and D-^{14}C-DOPA (B)

appears to be present in the hair follicles. No radioactive accumulation was observed in the liver, skeletal muscle and brain, in contrast to the L-isomer. In the skeletal muscle, however, a uniform distribution of a low radioactivity was observed after this time of the survival period, indicating a slight and slow penetration of D-DOPA or its metabolites into the muscle fibers. In the adrenal, a slightly higher radioactivity was shown in the medulla, but the localization was not so specific as the L-isomer. Some accumulation which exceeded the blood level was observed in the spleen and thymus, which appear to be appreciably higher than those of the L-isomer (Fig. 4).

One hour after injection of L-^{14}C-DOPA (Fig. 5), the high concentration of radioactivity was continued to be observed in the adrenal medulla, pancreas, kidney, liver, retina and gastric and intestinal mucosa. The concentrations in the skeletal muscle and brain were appreciably decreased, but a high concentration was still evident in the caudate nucleus of the brain. In the stomach, the highest concentration was still localized in the submucosa and an weaker activity was distributed through the mucosa and the contents, suggesting an occurrence of some gastric secretion of radioactivity.

After injection of D-^{14}C-DOPA, as compared in Fig. 5, a high concentration of radioactivity was observed only in the pancreas, kidney and skin. The concentration in the liver and skeletal muscle was declined to a very low level. In the skin, a high radioactivity appears to be localized in the hair follicles in a concentration which is appreciably higher than the L-isomer. Some radioactivity was observed in the adrenal medulla and a very low radioactivity over the whole brain.

After 3 hr, the concentration of radioactivity in the body was generally still higher for the L-isomer than the D-isomer. The highest radioactivity was shown in the adrenal medulla followed by the kidney and pancreas after injection of the L-isomer, while in the pancreas followed by the kidney after that of the D-isomer, the radioactivity being almost disappeared from the adrenal.

After 6 hr, the radioactive concentration in the body was declined to a very low level and there was found almost no significant difference betweeen the two isomers, with an apparent exception of a long retention of a high radioactivity in the adrenal medulla for the L-isomer (Fig. 3-D). Retentions of some radioactivity were observed in the pancreas, hair follicles, renal medulla, retina and skeletal muscle for the both isomers. It was noted at this time, however, that these concentrations appear to be slightly higher for the D-isomer than the L-isomer (Fig. 6), suggesting an even longer retention of radioactivity of the D-isomer than that of the L-isomer.

After 24 and 72 hr, a very high radioactivity was found to be retained in the adrenal medulla for the L-isomer and a retention of a prominent radioactivity was observed in the renal medulla for both isomers (Fig. 3-E). After 72 hr, the radioactivity except in these two organs disappeared almost completely from the body.

Distribution of ^{14}C-Dopamine

One minute after intravenous injection of ^{14}C-dopamine, the highest uptake of radioactivity was shown in the kidney, adrenal medulla, cardiac muscle and intestinal mucosa. In the brain, a high radioactivity was noted in the choroid plexus and pituitary, but no radioactivity was detected in the brain parenchyma and spinal cord. In the skeletal muscle, the distribution pattern was similar to that observed after injection of D-DOPA which suggested the distribution of radioactivity only in the extracellular spaces.

After 10 min, the blood level was declined and the localization of radioactivity became more evident (Fig. 7-A). The highest concentration of radioactivity was shown in the kidney, adrenal medulla, cardiac muscle, salivary gland, liver and intestinal mucosa. The cardiac muscle showed a considerably higher uptake of radioactivity than that of radioactive DOPA. No appreciable uptake of radioactivity was shown in the pancreas, with some radioactivity only in the blood vessels (Fig. 7). A very high radioactivity was localized in the mucosal

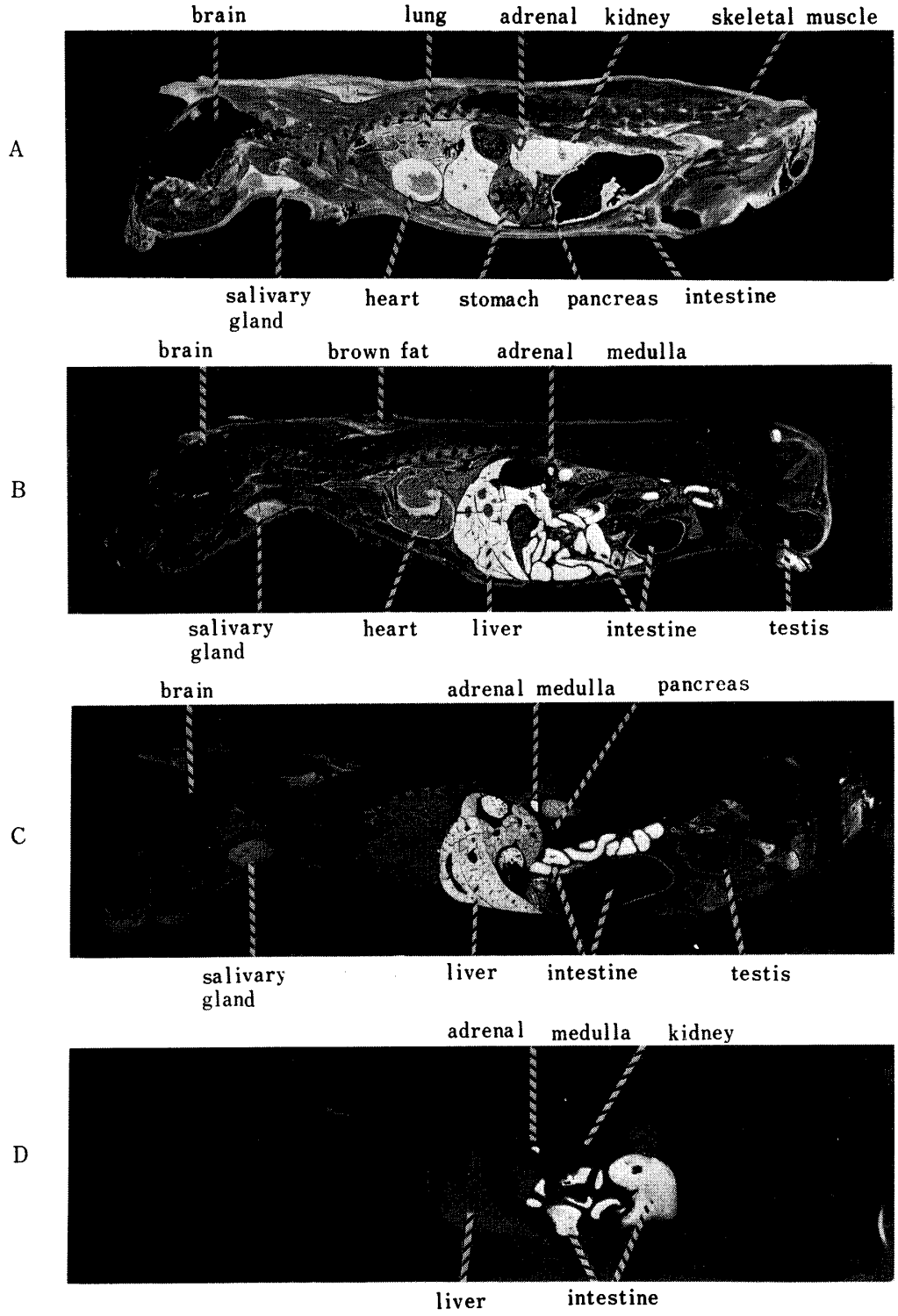

Fig. 7. Autoradiograms from Rats 10 (A) and 30 (B) min, 1 (C) and 3 (D) hr after Intravenous Injection ot ^{14}C-Dopamine

layer of the intestine, while only a slight radioactivity was observed in the gastric mucosa in contrast to radioactive L-DOPA. In the brain, a high concentration was observed in the pituitary and a low concentration in the choroid plexus, but no radioactivity in the parenchyma.

After 30 min, the high concentration of radioactivity was remained in the adrenal medulla, kidney, intestine and liver, followed by the salivary gland and cardiac muscle (Fig. 7-B). The lung showed a concentration comparable to the blood level. In the skeletal muscle, the concentration was decreased to a very low level, suggesting no appreciable penetration of dopamine into the fibers of skeletal muscle. No appreciable accumulation of radioactivity was observed in the pancreas.

After 1 hr, the high concentration was continued in the adrenal medulla and kidney, followed by the liver and intestinal mucosa (Fig. 7-C). The concentration in other tissues including the blood was declined to an extremely low level, indicating that the rate of elimination of radioactivity from the body appears to be faster than radioactive D- and L-DOPA. After 3 to 6 hr, a high radioactivity was retained only in the adrenal medulla and intestinal contents and after 24 hr no radioactivity was detected in all the organs and tissues other than the adrenal medulla, where a very high radioactivity was found to be still remained.

Discussion

DOPA has been established to be an effective agent against Parkinsonism clinically since Cotzias's finding[5] that the administration of the L-isomer rather than DL-DOPA reduces the side effects significantly with an increased therapeutic effect. It has been well known[9] that DOPA-decarboxylase in animal organisms is specific for the L-isomer. It has been also found from the *in vitro* studies[10] that an active transport mechanism is involved in the transfer of DOPA into rat brain only for the L-isomer. It might be expected, therefore, that there are significant differences in the distribution and the fate of DOPA between the L- and D-isomers. The present results revealed, in fact, significant differences between the distribution of radioactivity after intravenous injection of L- and D-^{14}C-DOPA, which are summarized as follows.

i) A higher uptake and localization of L-DOPA in the brain — As early as 1 min after injection of L-^{14}C-DOPA, a prominent radioactive uptake was shown by the brain, particularly by the caudate nucleus, cerebellar cortex and thalamus, and a localization of high radioactivity in the caudate nucleus was prominent for 6 hr after injection. After injection of D-^{14}C-DOPA, on the other hand, the transfer of radioactivity into the brain was much slower and a uniform distribution of a very low concentration was observed for the period from 30 min to 6 hr.

ii) A high accumulation and a long retention of L-DOPA in the adrenal medulla—After injection of L-^{14}C-DOPA, an extremely high radioactivity was concentrated in the adrenal medulla and remained for more than 72 hr. Though the D-isomer was also concentrated in the organ, the concentration was much lower than the L-isomer and disappeared after 24 hr.

iii) A high accumulation of L-DOPA in the liver and skeletal muscle and a faster elimination of D-DOPA from the body—The L-isomer showed a rapid and high penetration into the skeletal muscle, while the D-isomer only a low concentration mostly in the extracellular spaces. The concentration in the liver was considerably higher for the L-isomer than the D-isomer throughout the whole period after injection. The elimination from the body appears to be proceeded mainly through the urinary route and the rate to be considerably faster for the D-isomer.

iv) Accumulation of both isomers in the pancreas, skin, kidney and gastric and intestinal mucosa, with different concentrations and retentions—A rapid and high accumulation and a

9) W. Lovenberg, H. Weissbach and S. Udenfriend, *J. Biol. Chem.*, **237**, 89 (1962).
10) H. Yoshida, K. Kaniike and J. Namba, *Nature*, **198**, 191 (1963).

long retention in the pancreas was shown by both isomers. In the gastric and intestinal mucosa, a higher uptake of the L-isomer was observed, while in the skin, mostly in the hair follicles, the D-isomer showed an appreciably higher concentration. It was also noted that an appreciably higher concentration was continued in the thymus for the D-isomer.

It has been found[11] that the brain dopamine level is significantly depressed in Parkinson's disease, particularly those in the caudate nucleus, putamen and substantia nigra which belong to the so-called extrapyramidal motor system. Thus, the effectiveness of L-DOPA against Parkinsonism has been considered[4] to be attributable to that L-DOPA can pass through the blood-brain barrier, leading to an increase in dopamine concentration in the brain. In the present investigations, in fact, no radioactivity was detected in the brain parenchymal tissues after intravenous injection of ^{14}C-dopamine, confirming that dopamine itself cannot pass through the blood-brain barrier. On the other hand, a prominent uptake of radioactivity by the brain and its localization in the caudate nucleus was clearly demonstrated after intravenous injection of L-^{14}C-DOPA, while not after that of the D-isomer.

It has been indicated from the *in vitro* experiments[10] that only L-DOPA is penetrated into the brain tissue by active transport mechanism and it has been generally considered[7] that D-DOPA cannot pass through the blood-brain barrier. The present results indicated, however, a slow but an appreciable uptake of radioactivity by the brain after injection of D-^{14}C-DOPA and its duration for rather a long period. It might be possible, therefore, that the brain uptake of unbeneficial D-DOPA has some bearing on the clinical finding[5] of decreased side effects and an increased therapeutic effect when L-DOPA was used rather than DL-racemate and that D-DOPA has some toxicity which is different from or higher than the L-isomer.

Accumulation of DL-DOPA in the pancreas which is characterized by a rapid protein synthesis has been pointed out by Rosell, *et al.*[5] and interpreted as that the cell uptake of amino acids by the pancreas is not very selective and the more accurate discrimination might be involved in the next step of the incorporation into proteins. The present finding that both L- and D-isomers of DOPA was accumulated in the pancreas might indicate that the uptake mechanism of the pancreatic cells is not specific sterically for the L-isomer. It is probable that the compound accumulated in the pancreas is unchanged DOPA, at least at the earliest period, because of its extremely rapid uptake after injection and of the finding that dopamine injected intravenously was not accumulated to any appreciable extent in the pancreas. If an active transport mechanism is assumed to be involved in the cell uptake of amino acids by the pancreas, it is interesting to note that the mechanism is not specific sterically with respect to the optical isomers, since all the active transport systems for amino acids so far found appear to be more or less specific for the L-isomer.[12]

It was another interesting results that both L- and D-DOPA showed a high accumulation in the hair follicles and it might be possible, in a similar way to that in the pancreatic cells, that DOPA is transported actively into cells which are directly related to the hair growth. As an alternative explanation, it might be also possible that DOPA is accumulated in the melanocyte zone of the hair follicles, although melanin synthetic pathway is inactive in Albino rats. A rapid and high uptake of radioactivity by the intestinal mucosa which is another site of a rapid protein synthesis might also be interpreted in the same way as that in the pancreas, but a marked difference was that in the intestinal mucosa D-DOPA showed a much lower uptake and more rapid disappearance than the L-isomer.

It was another marked difference between the isomers that only L-DOPA was accumulated in a high concentration in the liver. This might bear a connection to the difference in their metabolism in the liver, since DOPA-decarboxylase which concerns the first step of DOPA metabolism has been known to be highly specific for the L-isomer and to be rich in the liver.[9]

11) O. Hornykiewicz, *Pharmacol. Revs.*, **18**, 925 (1966).
12) J.H. Quastel, *Proc. Royal Soc. Ser. B*, **163**. 169 (1965).

In the adrenal medulla, the radioactivity was accumulated as early as 1 min after injection of both L- and D-^{14}C-DOPA, but the concentration was much lower and disappeared much more rapidly for the D-isomer. A high accumulation in the medulla was also shown after injection of ^{14}C-dopamine, as was expected, and the accumulation of radioactivity after injection of L-^{14}C-DOPA might be derived from the uptake of both L-DOPA and dopamine in the circulating blood. Labeled dopamine and noradrenaline have been identified in the mouse adrenal medulla 30 min after injection of DL-^{14}C-DOPA.[6]

The elimination of injected radioactivity from the body appears to be mainly through the urinary route for both L- and D-DOPA and dopamine and a high accumulation of radioactivity was observed in the kidney from right after injection of all of these compounds. A long retention of radioactivity was, however, observed in the renal medulla 72 hr after injection of both D- and L-^{14}C-DOPA, which appears to be different from the excretion pattern. Because no such a retention was observed in the renal medulla after injection of ^{14}C-dopamine, it is probable that some metabolite was retained in the organ which was formed by any pathway other than that through dopamine.

Although the initial rate of elimination of radioactivity from the body was considerably faster for D-DOPA than the L-isomer, even an appreciably longer retention of radioactivity in the tissues such as the skeletal muscle, hair and brain was noted for the D-isomer. This might be considered to be due to a much slower rate of metabolism of D-DOPA in contrast to a rapid metabolism of L-DOPA and probably to a long retention of unchanged DOPA in the tissues. It has been suggested[13] from detection of dopamine and its metabolites in the urine that D-DOPA is partly metabolized to form dopamine possibly through a transformation to L-DOPA by deamination by D-amino acid oxidase followed by the action of transaminase. From a comparative study on the urinary metabolites, it was found in this laboratory[14] that after administration of D-DOPA, dopamine was excreted not as the glucuronide conjugate but mainly as the free form, in contrast to the L-isomer, suggesting that dopamine formed from D-DOPA is excreted into the urine without passing through the liver. It was thus further suggested that the metabolism of D-DOPA is proceeded mainly in the kidney, where D-amino acid oxidase has been known to be localized,[15] the metabolites being excreted directly into the urine. The fact that radioactivity derived from the D-isomer was disappeared from the adrenal medulla in the same rate as that from other tissues might also indicate that the amount of dopamine and/or L-DOPA in the circulating blood is insignificant. The metabolic changes of DOPA in various organs following the injection of the two isomers in rats are now under investigation

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13) T.L. Sourkes, M.H. Wiseman-Distler, J.F. Moran, G.F. Murphy and S.S. Cyr, *Biochem. J.*, 93, 469 (1964).
14) H. Shindo, T. Komai and K. Tanaka, *Chem. Pharm. Bull.* (Tokyo), to be published.
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