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Whole-body Autoradiographic Studies on the Distribution of ^{14}C-Labeled D- and L-5-Hydroxytryptophan, 5-Hydroxytryptamine and 5-Hydroxyindole-3-acetic Acid in Rats[1]

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The distribution of D- and L-isomers of ^{14}C-labeled 5-hydroxytryptophan (5-HTP) was comparatively investigated by means of whole-body autoradiography following intravenous and oral administration to rats. The distribution of ^{14}C-labeled 5-hydroxytryptamine (5-HT, serotonine) and 5-hydroxyindole-3-acetic acid (5-HIAA), the main end product of 5-HTP metabolism, was also studied for comparison purposes. The following differences were found in the distribution pattern of radioactivity between D- and L-isomers of 5-HTP: i) a rapid and appreciable uptake of radioactivity in the brain only by L-5-HTP, but none by D-5-HTP and 5-HT, ii) a high uptake and accumulation of radioactivity in the adrenal medulla by L-5-HTP and 5-HT, but none by D-5-HTP, iii) a high distribution of radioactivity in the skeletal muscle by L-5-HTP, but none by D-5-HTP and 5-HT, iv) a gradual accumulation of radioactivity in the spleen by L-5-HTP and 5-HT, but none by D-5-HTP, v) a high accumulation and retention in the pancreas by both D- and L-5-HTP, but none by 5-HT, and vi) a high absorbability of L-5-HTP from the intestine in contrast to a very limited absorbability of the D-isomer. The distribution pattern, particularly in regard to the brain uptake, of L-5-HTP-^{14}C did not change substantially upon increasing the oral dosage, in contrast to a significant change observed for L-DOPA. 5-HIAA, when injected intravenously, was found to be eliminated from the body extremely rapidly through the urinary route. These results were discussed with respect to the possible use of L-5-HTP orally as a brain serotonine precursor.

In our previous papers,[3,4] the distribution of radioactivity was compared between the D- and L-isomers of ^{14}C-labeled 3,4-dihydroxyphenylalanine (DOPA) following intravenous and oral administration to rats. The results revealed marked differences in the fate of the two isomers and indicated the presence of high specificities in the transport and metabolic systems of DOPA with respect to configuration of the isomer. The results thus provided an explanation for the therapeutic effect of only the L-isomer against Parkinsonism, when administered as a precursor of brain dopamine.[5]

It has been suggested[6,7] that mental depression is associated in some way with a deficiency in brain serotonine, 5-hydroxytryptamine (5-HT), and the oral use of L-5-hydroxytryptophan (L-5-HTP) as a precursor of brain 5-HT has been tested clinically.[8,9] In the present investigations, the distribution of D- and L-isomers of ^{14}C-labeled 5-HTP was comparatively studied by means of whole-body autoradiography following intravenous and oral administration to rats. The distribution of ^{14}C-labeled 5-HT and 5-hydroxyindole-3-acetic acid (5-HIAA),

1) A part of this work was presented at the 94th Annual Meeting of Pharmaceutical Society of Japan, Sendai, April, 1974.
2) Location: *Hiromachi 1-chome, Shinagawa-ku, Tokyo.*
3) H. Shindo, N. Miyakoshi, and I. Takahashi, *Chem. Pharm. Bull.* (Tokyo), **19**, 2490 (1971).
4) H. Shindo, N. Miyakoshi, and E. Nakajima, *Chem. Pharm. Bull.* (Tokyo), **20**, 966 (1972).
5) G.C. Cotzias, P.S. Papavasiliou, and R. Gellene, *New Engl. J. Med.*, **280**, 337 (1969).
6) I.P. Lapin and G.F. Oxenkrug, *Lancet*, **1**, 132 (1969).
7) A. Coppen, *J. Psychiat. Res.*, **9**, 163 (1972).
8) I. Sano, *Folia Psychiatrica et Neurologica*, **26**, 7 (1972); H.M. Van Praag, J. Korf, L.C.W. Dols and T. Schut, *Psychopharmacologia*, **25**, 14 (1972).
9) S. Takahashi, H. Kondo, and N. Kato, *J. Psychiat. Res.*, **11**, 1 (1975).

the main end product of 5-HTP metabolism was also studied. The distribution of 5-HTP-^{14}C has been studied in mice by the same technique by Ritzen, *et al.*,[10] however they used the DL-racemate.

Material and Method

Materials——D- and L-5-HTP-3-^{14}C were purchased from the New England Nuclear Corporation, Boston, U.S.A. The specific activity was 28.7 and 28.2 μCi/mg, respectively, and the radiochemical purity was ascertained to be over 98% by thin-layer chromatography. 5-HT-3-^{14}C with specific activity of 14.3 μCi/mg was purchased from the Radiochemical Center, Amersham, England and 5-HIAA-carboxyl-^{14}C with specific activity of 52.1 μCi/mg from the New England Nuclear Corporation. Non-radioactive D- and L-5-HTP were supplied from Kyowa Hakko Co., Ltd, and 5-HT and 5-HIAA were purchased from Sigma Chemicals Co.

Autoradiography——Male rats of Wistar-Imamichi strain weighing about 100 g were used without fasting. D- and L-5-HTP-^{14}C and 5-HT-^{14}C were diluted with the non-radioactive compound to 10 μCi/mg and dissolved in physiological saline at the concentration of 1 mg/0.2 ml. The solution was administered to rats intravenously from the tail vein or orally with a stomach tube at a constant dose of 10 mg/kg (10 μCi/100 g rat). 5-HIAA-^{14}C was administered intravenously at the same dose. One, 10, 30, and 60 min and 3, 6, 24, and 72 hr after intravenous administration and 30 and 60 min and 3, 6, 24, and 72 hr after oral administration, the rats were lightly anesthetized with ether and sacrificed by immersion in a dry ice-hexane mixture at about −70°. After a frozen animal was embedded on a microtome stage with aqueous carboxymethyl cellulose gel, sagittal 50 μ sections were cut with a heavy microtome (Yamato Type 1111) at −10° and freeze-dried overnight. The dried sections were brought to contact with Sakura Type N X-ray film and exposed for a constant period of 10 days.

Result

Distribution of D- and L-5-HTP-^{14}C after Intravenous Administration

Whole-body autoradiograms obtained from rats after intravenous administration of L- and D-5-HTP-3-^{14}C are presented in Fig. 1 and 2, respectively. One minute after injection of L-5-HTP-^{14}C, the highest uptake of radioactivity was shown by the kidney, gastric mucosa and adrenal, followed by the pancreas and intestinal mucosa. A high concentration was also shown in the salivary gland, lung and cardiac muscle as well as in the circulating blood. Only a slight but noticeable radioactivity was already detected in the brain. After injection of D-5-HTP-^{14}C, on the other hand, a high uptake of radioactivity was observed in the kidney, lung, pancreas and cardiac muscle as well as in the circulating blood, but the uptake by the adrenal and gastric and intestinal mucosa was much lower than the L-isomer. No radioactive uptake was detected in the brain parenchyma.

Ten to 30 min after injection of L-5-HTP-^{14}C, the highest concentrations were observed in the kidney, pancreas, urinary bladder, intestinal mucosa and adrenal medulla. The concentration in the gastric mucosa declined significantly. An appreciable uptake of radioactivity was shown by the central nervous system, which appeared to build up gradually up until 1 hr after administration. A slightly higher concentration was noted in the gray matter than in the white matter. No accumulation of radioactivity exceeding the blood level was shown in the liver, where L-DOPA-^{14}C accumulates in a high concentration.[3] An appreciable uptake of radioactivity was observed throughout the skeletal muscle. After injection of D-5-HTP-^{14}C, on the contrary, a high concentration of radioactivity was shown only in the kidney, urinary bladder and pancreas, while almost no radioactivity was observed in the gastric and intestinal mucosa, adrenal and skeletal muscle and no radioactive uptake was detected in the brain.

One to 3 hr after injection, an appreciable radioactivity remained in the circulating blood for both isomers, suggesting that the blood level of radioactivity continues for a longer period than after injection of DOPA-^{14}C.[3] After injection of L-5-HTP-^{14}C, the highest concentration of radioactivity was observed in the kidney, adrenal medulla, urinary bladder,

10) M. Ritzen, L. Hammarstrom, and S. Ullberg, *Biochem. Pharmacol.*, **14**, 313 (1965).

pancreas and intestinal mucosa. A moderate to weak radioactivity was distributed in the liver, skeletal muscle, lung, spleen, thymus, bone marrow and brain. Three hr after injection, some localization of radioactivity was noted in the brain, although the concentration in the whole brain decreased considerably. A higher concentration was observed in the thalamus, hypothalamus, amygdala and caudate nucleus. A high radioactivity retained in the adrenal medulla for more than 24 hr and the concentration in the spleen appeared to increase gradually with time. After injection of the D-isomer, a high concentration was maintained only in the kidney and pancreas and most of the radioactivity disappeared from the body, indicating

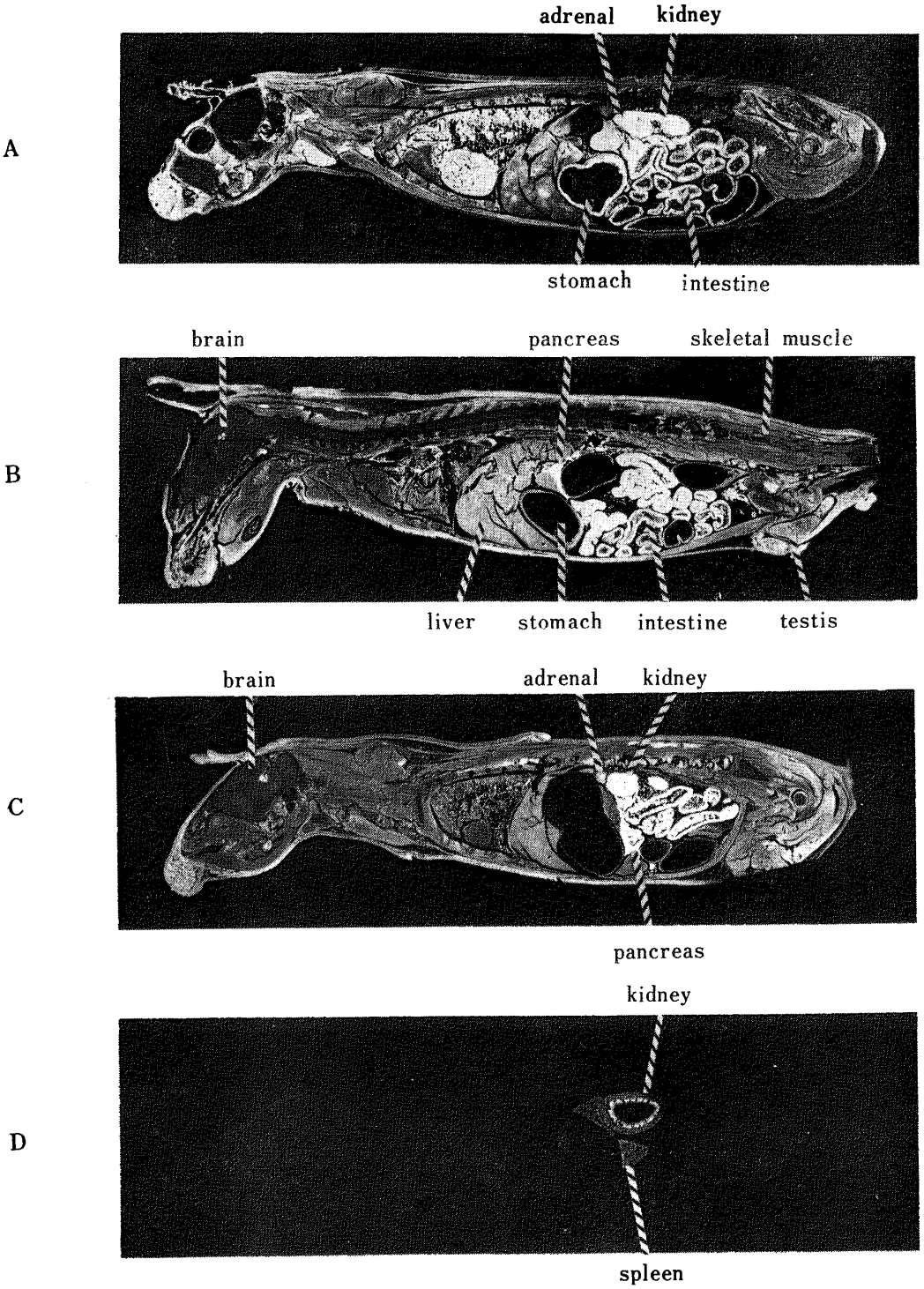

Fig. 1. Autoradiograms from Rats 1 min (A), 30 min (B), 1 hr (C) and 24 hr (D) after Intravenous Administration of L-5-HTP-^{14}C

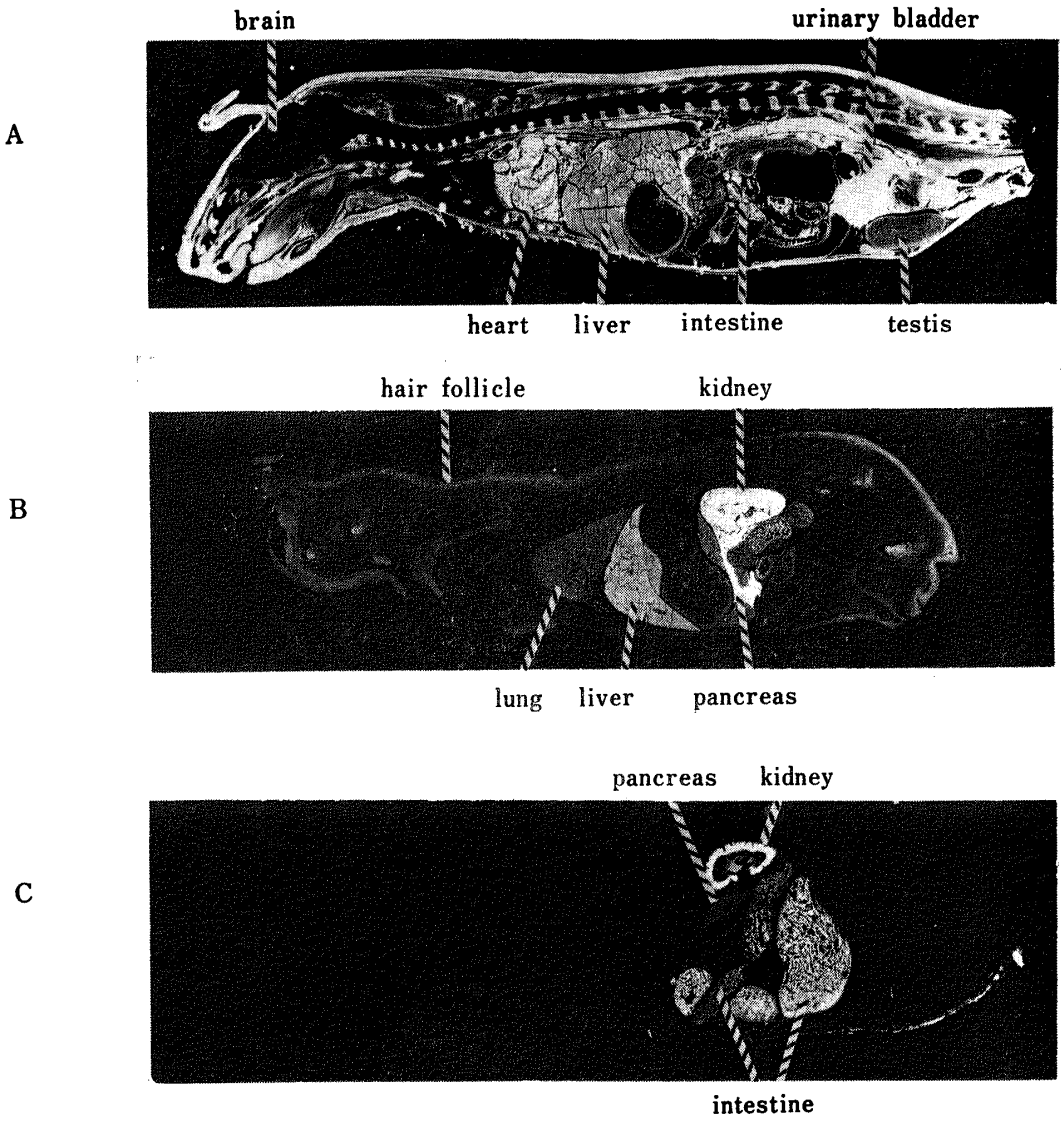

Fig. 2. Autoradiograms from Rats 10 min (A), 1 hr (B) and 6 hr (C) after Intravenous Administration of D-5-HTP-^{14}C

that the elimination of radioactive D-5-HTP from the body is considerably faster than that of the L-isomer.

Twenty four hr after injection of L-5-HTP-^{14}C, a high concentration of radioactivity remained only in the adrenal medulla and kidney medulla and an appreciable concentration in the spleen and kidney cortex. Radioactivity in the brain had disappeared by this time. After 72 hr, a high concentration of radioactivity was found to remain only in the kidney medulla for both isomers, while most of the radioactivity in other organs had disappeared.

Distribution of D- and L-5-HTP-^{14}C after Oral Administration

Following oral administration of 10 mg/kg L-5-HTP-^{14}C to rats, the concentration of radioactivity in the circulating blood and in most tissues reached a maximum within 30 min after administration (Fig. 3-A), indicating that the absorption of L-5-HTP from the intestine is quite rapid. The highest concentration was shown in the gastric and intestinal contents as well as their mucosa, the kidney, pancreas and adrenal followed by the blood, liver, spleen and skin. An appreciable radioactivity was already observed in the central nervous system with a slightly higher concentration in the gray matter than in the white matter.

The blood concentration declined considerably after 1 hr, as can be seen from Fig. 3-B, and the highest concentration was shown in the adrenal medulla, pancreas and kidney (Fig.

4-A), followed by the gastric mucosa, spleen and lung. In the brain, a slightly higher concentration was noted in the mesencephalon, diencephalon and medulla oblongata than in the cerebral cortex (Fig. 4-B). No accumulation of radioactivity exceeding the blood concentration was shown in the liver. In the spleen, a prominent radioactivity was observed only in the red pulp surrounding the white pulp, the latter being devoid of radioactivity.

After 3 to 6 hr, a high radioactivity was maintained only in the adrenal medulla, kidney, gastric mucosa and spleen, whereas the concentration in the pancreas declined considerably

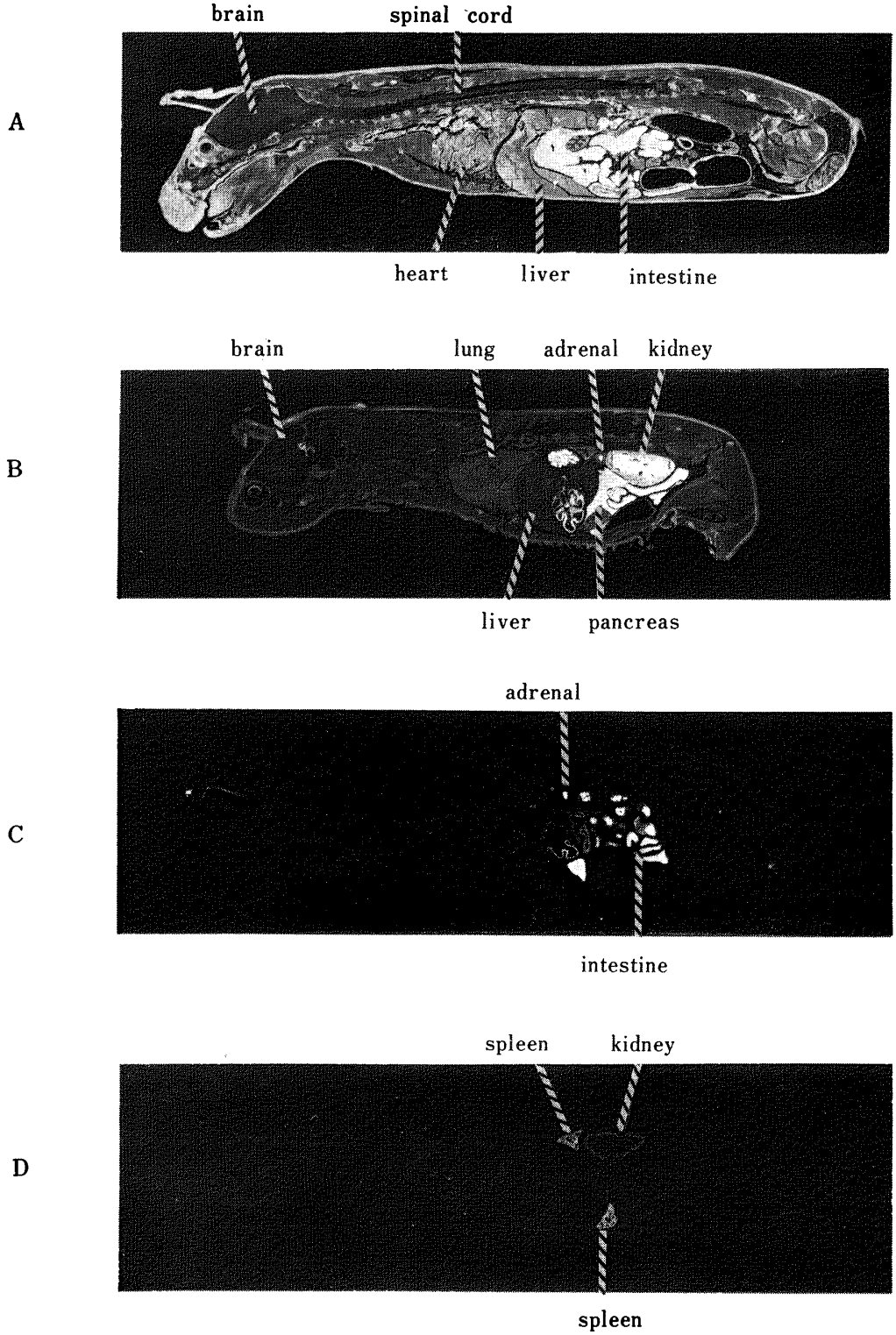

Fig. 3. Autoradiograms from Rats 30 min (A), 1 hr (B), 3 hr (C) and 24 hr (D) after Oral Administration of L-5-HTP-^{14}C (10 mg/kg)

(Fig. 3-C). The concentration in the spleen appeared to increase gradually up to the period of 3 to 6 hr. Only a very low radioactivity remained in the brain and this almost disappeared after 6 hr. After 24 hr, a high radioactivity remained only in the adrenal medulla and an appreciable radioactivity only in the spleen and kidney medulla; most of the radioactivity

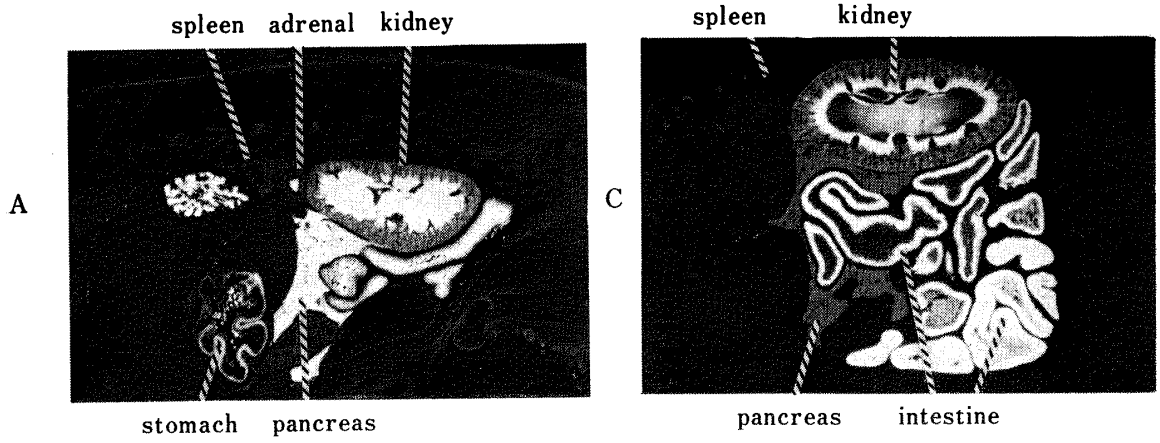

Fig. 4. Enlargement of Autoradiograms from Rats 1 hr after Oral Administration of L-5-HTP-^{14}C (A, B) and 3 hr after That of D-5-HTP-^{14}C (C)

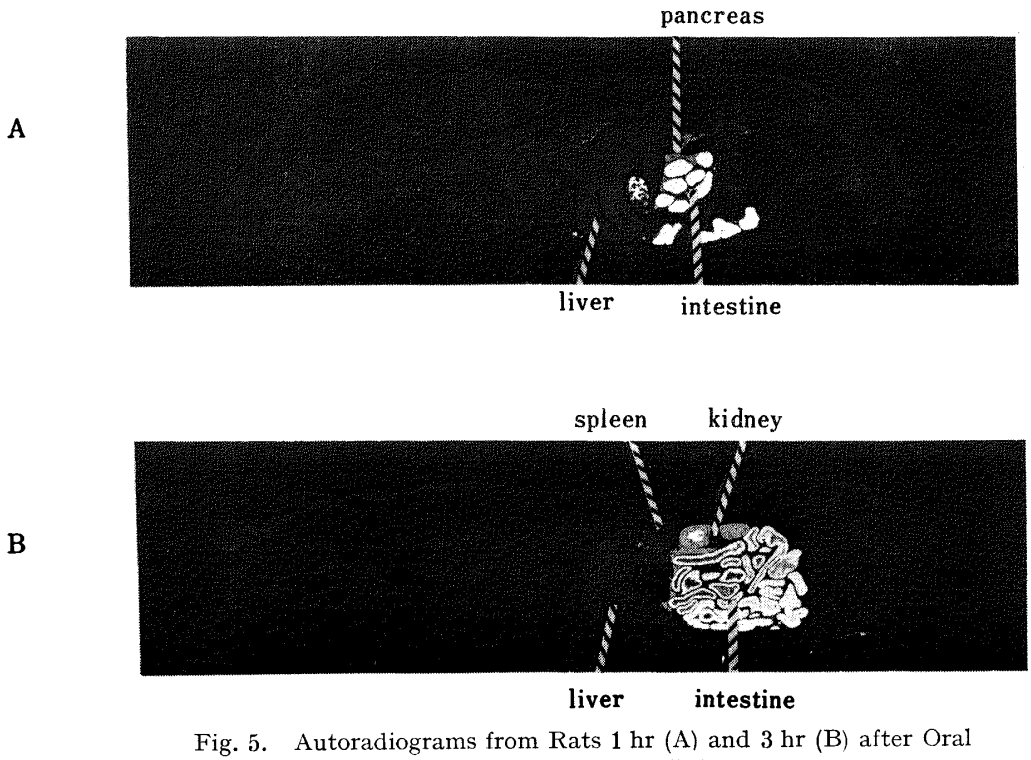

Fig. 5. Autoradiograms from Rats 1 hr (A) and 3 hr (B) after Oral Administration of D-5-HTP-^{14}C (10 mg/kg)

disappeared from the body (Fig. 3-D). Radioactivity in the adrenal medulla continued for more than 72 hr.

Contrary to the L-isomer and to the intravenous administration, as shown in Fig. 5, only a very low distribution of radioactivity was shown in organs and tissues after oral administration of D-5-HTP-^{14}C. Thirty to 60 min after administration, the highest concentration of radioactivity was shown in the gastric and intestinal contents and a high radioactivity only in the kidney and pancreas. Only an appreciable concentration of radioactivity was observed in the circulating blood, liver, lung and pancreas, indicating that the absorption of D-5-HTP from the intestine is extremely limited. Absolutely no radioactive uptake was detected in the central nervous system. After 3 to 6 hr, the distribution of radioactivity was mainly restricted to the gastro-intestinal tracts, as can be seen from Fig. 4-C and 5-B. Only an appreciable radioactivity was continued in the kidney and pancreas. Most of the radioactivity disappeared from the body after 24 and 72 hr.

In order to see if there is a dose dependency in the distribution pattern of L-5-HTP-^{14}C, particularly in the brain uptake, the amount of the oral dose was varied from 2 mg/kg to 10, 50 and 100 mg/kg, wherein the amount of radioactivity was kept constant. The autoradiography was then performed on animals sacrificed after 1 and 3 hr. The results revealed that the distribution pattern of radioactivity does not change to any appreciable extent with respect to the amount administered. The brain uptake of radioactivity and the concentration in the liver and pancreas appeared to be only slightly increased when the dosage was increased to over 50 mg/kg. It might be concluded, therefore, that L-5-HTP-^{14}C penetrates into the brain even at low doses of 2 and 10 mg/kg and that there is no significant dependency of the distribution pattern on the amount of oral dose as was observed in the case of L-DOPA-^{14}C, wherein the brain uptake of radioactivity was detected only when the dose amount was increased to over 50 mg/kg.[4]

Distribution of 5-HT-^{14}C and 5-HIAA-^{14}C after Intravenous Administration

Whole-body autoradiograms obtained from rats after intravenous administration of 5-HT-^{14}C are presented in Fig. 6. One to 10 min after injection of 5-HT-^{14}C, a high uptake of radioactivity was shown in the adrenal medulla, kidney, lung and spleen as well as in the circulating blood. In the liver, a spotted pattern of radioactivity was observed after 1 min corresponding to the hepatic venous vessels and the radioactivity was distributed over the liver parenchyma after 10 min. In the central nervous system, radioactivity was shown only in the choroid plexus and no radioactivity was detected in the brain parenchyma. No radioactive uptake was observed in the pancreas, where L-5-HTP-^{14}C accumulates in a high concentration. In the skeletal muscle, the radioactivity was distributed only in the extracellular spaces and/or the connective tissues. An appreciable uptake of radioactivity was observed in the bone marrow and thymus.

After 1 hr, the tissue radioactivity declined considerably and the highest radioactivity was distributed in the adrenal medulla, followed by the kidney medulla, lung, spleen and intestinal contents. An appreciable concentration continued in the bone marrow and liver. After 3 and 6 hr, a prominent radioactivity continued only in the adrenal medulla, spleen, lung, bone marrow and intestinal contents, revealing a very characteristic distribution pattern. In the spleen, a high radioactivity was distributed in the red pulp surrounding the white pulp, consistent with the distribution of L-5-HTP-^{14}C. After 24 hr, most of the radioactivity disappeared from the body, except that in the spleen and adrenal medulla (Fig. 6-D). In contrast to L-5-HTP-^{14}C (Fig. 1-D), no radioactivity was remained in the kidney medulla.

Whole-body autoradiograms of 5-HIAA-^{14}C, the main final product of L-5-HTP and 5-HT metabolism, after intravenous administration to rats are shown in Fig. 7. It was found that 5-HIAA is eliminated from the body extremely rapidly through the urinary route. One and 10 min after injection, a high radioactivity was distributed only in the kidney and urinary bladder as well as in the circulating blood and an appreciable uptake of radioactivity by tissue

was shown only by the liver. No radioactivity was detected in the central nervous system and an appreciable radioactivity was distributed only in the extracellular spaces of the skeletal muscle. After 30 min, a high radioactivity remained only in the kidney and urinary bladder and some radioactivity in the intestinal contents. After 1 hr, most of the radioactivity had already disappeared from the body and a high concentration was observed only in the urinary bladder and pelvis.

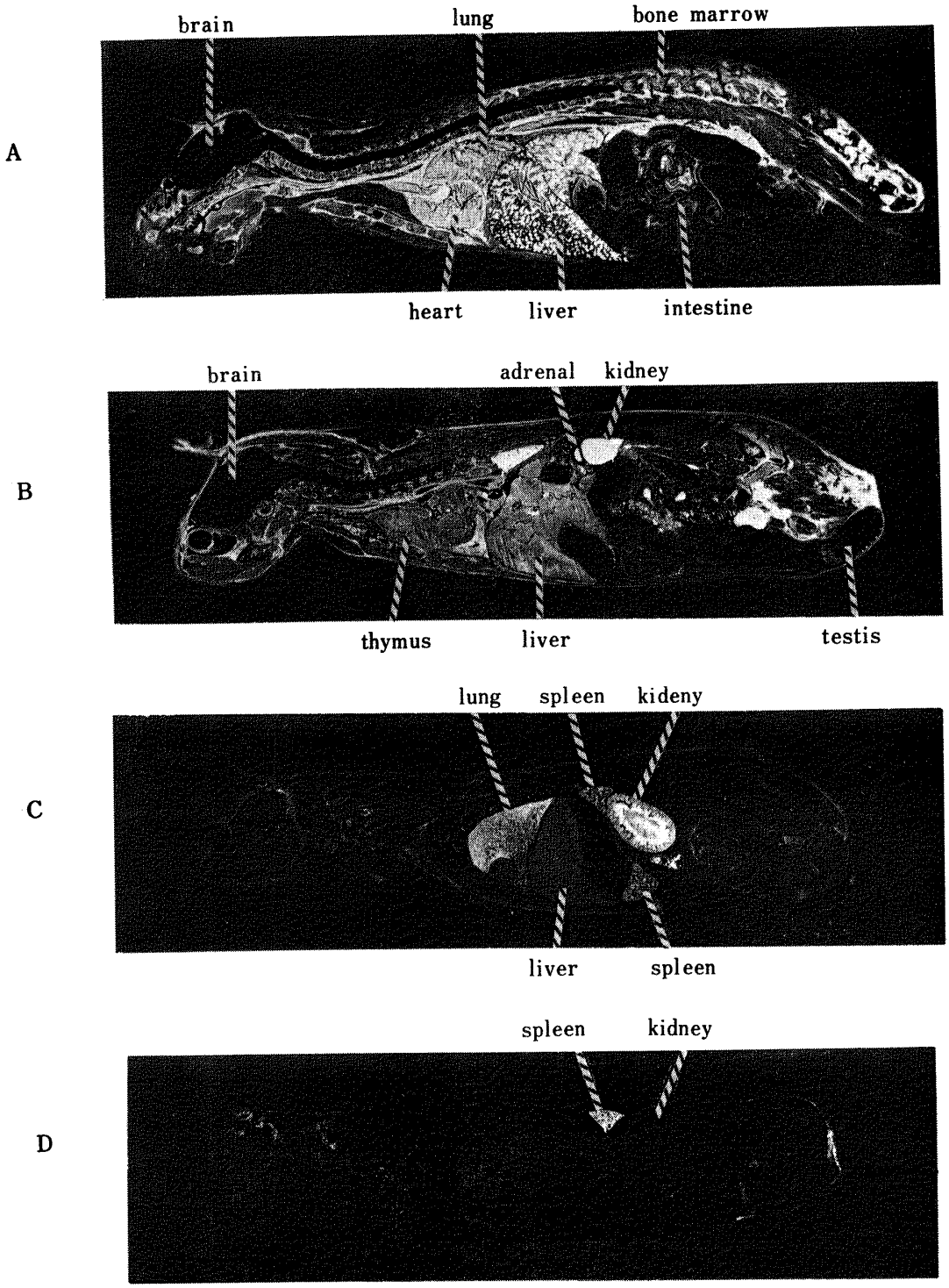

Fig. 6. Autoradiograms from Rats 1 min (A), 10 min (B), 1 hr (C) and 24 hr (D) after Intravenous Administration of 5-HT-^{14}C

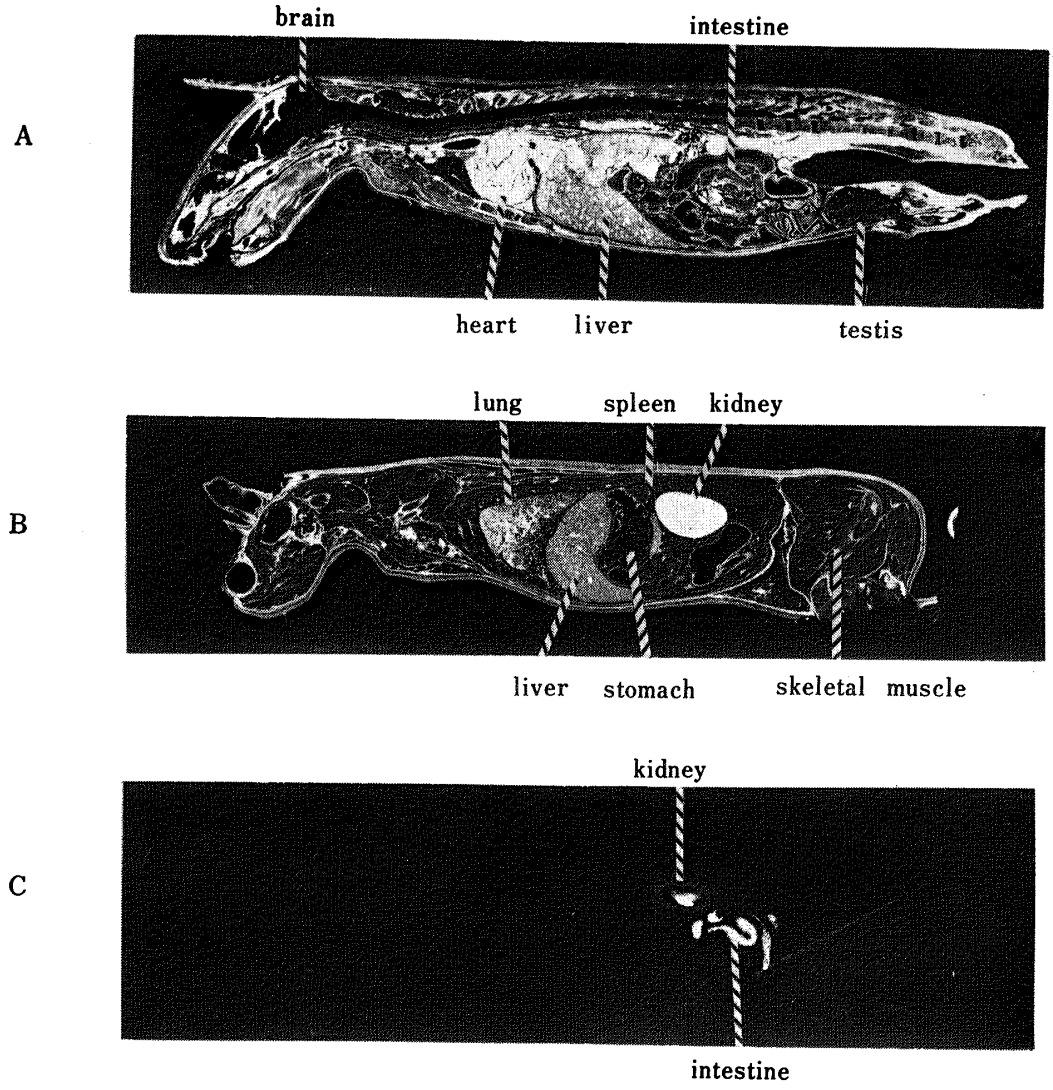

Fig. 7. Autoradiograms from Rats 1 min (A), 10 min (B), and 1 hr (C) after Intravenous Administration of 5-HIAA-^{14}C

Discussion

Distribution of ^{14}C-labeled 5-HTP and 5-HT in mice has been investigated by means of whole-body autoradiography by Ullberg, *et al.*[10] and Matsuoka, *et al.*,[11] however they used DL-5-HTP. Since significant differences were expected in the distribution pattern between the optical isomers, as had been found between D- and L-DOPA-^{14}C previously,[3,4] in the present work the D- and L-isomers of ^{14}C-labeled 5-HTP were investigated to compare their distribution after intravenous and oral administration to rats.

The following differences were found between the D- and L-isomers in the distribution characteristics. i) As early as 1 min after intravenous injection of the L-isomer, an appreciable uptake of radioactivity was observed in the brain; the maximum concentration was attained during 30 min to 1 hr after administration. The radioactivity was localized in the thalamus, hypothalamus, amygdala and caudate nucleus. After injection of the D-isomer, on the contrary, no appreciable uptake of radioactivity was detected in the brain parenchyma; radioactivity was noted only in the choroid plexus. ii) A very high uptake of radioactivity was observed in the adrenal immediately after injection of the L-isomer and a high accumulation

11) M. Kashima, and O. Matsuoka, *Nippon Acta Radiologica*, 27, 315 (1967).

continued in the medulla for more than 3 days. After injection of the D-isomer, only a slight uptake was shown in the adrenal during the first 30 min. iii) After injection of the L-isomer, a high radioactivity was distributed in the skeletal muscle and continued for more than 1 hr. After that of the D-isomer, no appreciable uptake was shown in the skeletal muscle, the radioactivity being distributed only in the extracellular spaces. iv) Only the L-isomer showed a gradual accumulation of radioactivity in the spleen, which continued for more than 24 hr. v) Only the L-isomer showed a high concentration of radioactivity in the intestinal mucosa, while no accumulation of radioactivity exceeding the blood level was shown in the liver by both isomers. vi) Both isomers showed a rapid and high accumulation and a long retention of radioactivity in the pancreas and kidney. vii) After oral administration, the L-isomer showed the same distribution pattern as that after intravenous administration, while the D-isomer showed only a very low tissue distribution of radioactivity because of its low absorbability.

These characteristics are mostly similar to those observed for the optical isomers of DOPA,[3,4] but some important differences are also noted: i) after administration of L-DOPA-^{14}C, a more prominent localization of radioactivity was shown in the caudate nucleus of the brain, ii) a much higher accumulation of radioactivity was observed in the liver after administration of L-DOPA-^{14}C, iii) in contrast to L-5-HTP-^{14}C, radioactive L-DOPA did not show any appreciable uptake by the spleen and bone marrow, iv) D-DOPA-^{14}C showed an appreciable uptake by the brain, in contrast to almost no uptake of D-5-HTP-^{14}C, and v) L-DOPA-^{14}C showed a more significant and substantial change of the distribution pattern depending upon the amount of oral dose.

The present results that no radioactivity was detected in the brain parenchymal tissues after intravenous administration of 5-HT-^{14}C, while a prominent uptake of radioactivity by the brain was demonstrated after administration of L-5-HTP-^{14}C, however none for the D-isomer, confirm that when exogeneously administered only L-5-HTP can elevate the brain 5-HT level. As will be described in the subsequent paper,[12] it has been clarified *in vitro* that only L-5-HTP, but not the D-isomer and 5-HT, penetrates into the brain tissue by an active transport mechanism. A more detailed localization of radioactivity in the brain has been studied after administration of L-5-HTP-^{14}C to cats and, as will be reported separately,[13] the radioactivity was found to accumulate in the hypothalamus, nuclear Raphe, substantia nigra and nuclear Oliviaris inferior as well as in the caudate nucleus.

A rapid and high accumulation of both D- and L-5-HTP in the pancreas may be attributed to their uptake by tissues with rapid protein synthesis, as in the case of D- and L-DOPA.[3] It is thus understood that 5-HT is not taken up by the pancreas. The uptake of 5-HT and L-5-HTP by the spleen and bone marrow, though more slowly in the latter, may be concerned in some way to the uptake of 5-HT, but not of dopamine, by the blood platelets. A present finding that a characteristic retention of radioactivity was observed in the kidney medulla for a long period after administration of both D- and L-5-HTP-^{14}C, but not by 5-HT-^{14}C and 5-HIAA-^{14}C suggests that the substance remaining in the kidney medulla is not that derived from 5-HT or 5-HIAA, but that from 5-HTP. It has been noted that[3,14] a similar retention in the kidney medulla occurrs after administration of 3-O-methyl-DOPA-^{14}C as well as DOPA-^{14}C, but not by dopamine-^{14}C and DOPAC-^{14}C.

A high accumulation of L-DOPA-^{14}C in the liver has been attributed[15] to its suffering from decarboxylation in the liver and L-DOPA-^{14}C was found to accumulate in the liver mostly as dopamine glucuronide. The present finding that L-5-HTP-^{14}C accumulates in the liver

12) H. Shindo, T. Komai, and K. Kawai, *Chem. Pharm. Bull.* (Tokyo), in press.
13) N. Miyakoshi, Y. Nishijima, and H. Shindo, *Jap. J. Pharmacol.*, to be published.
14) N. Miyakoshi and H. Shindo, *Chem. Pharm. Bull.* (Tokyo), to be published.
15) H. Shindo, T. Komai, K. Tanaka, E. Nakajima, and N. Miyakoshi, *Chem. Pharm. Bull.* (Tokyo), **21**, 826 (1973).

to a much lesser extent than L-DOPA-^{14}C might, therefore, suggest that L-5-HTP is decarboxylated in the liver to a much lesser extent than L-DOPA. In fact, as will be described in the subsequent paper,[12] L-DOPA was found to be decarboxylated in the rat liver homogenates approximately 7 times more rapidly than L-5-HTP. It was also found in the present study that L-5-HTP-^{14}C penetrates into the brain even at low doses of 2 and 10 mg/kg and there is no such dependency of the distribution pattern on the amount of oral dose as was observed in L-DOPA-^{14}C. In the latter case,[4] the brain uptake of radioactivity was detected appreciably only when the oral dose was increased to over 50 mg/kg and this was attributed to the extensive decarboxylation in the peripheral organs, in particular in the intestine and liver. Thus, the above finding must be again attributed to a much lower decarboxylation activity of the rat liver and intestine against L-5-HTP than L-DOPA. Therefore, it might be reasonable and promising that the clinical trials of L-5-HTP[8,9] have been performed using a relatively low oral dosage of 300 to 500 mg L-5-HTP in contrast to a much larger therapeutic oral dosage of 2 to 3 g L-DOPA.

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