*Int. J. Appl. Radiat. Isot.* Vol. 36, No. 12, pp. 965–969, 1985
Printed in Great Britain

0020-708X/85 $3.00 + 0.00
Pergamon Press Ltd

# $^{11}$C-Labeling of Indolealkylamine Alkaloids and the Comparative Study of their Tissue Distributions

TOSHIHIRO TAKAHASHI, KAZUHIRO TAKAHASHI*, TATSUO IDO, KAZUHIKO YANAI, REN IWATA, KIICHI ISHIWATA and SHIGEO NOZOE[1]

Cyclotron and Radioisotope Center, Tohoku University and [1]Pharmaceutical Institute, Tohoku University, Sendai 980, Japan

(*Received* 22 *March* 1985; *in revised form* 21 *May* 1985)

Five indolealkylamines (N,N-dimethyltryptamine, N-methyltryptamine, bufotenine, O-methylbufotenine, N,N,N-trimethyltryptamine iodide) were labeled with $^{11}$C by use of $^{11}CH_3I$. The labeled compounds were synthesized with a radiochemical yield of 2–50% (based on trapped $^{11}CH_3I$) in 20–35 min with radiochemical purities of more than 92%. The tissue distributions of these labeled compounds were investigated in rats. In all cases, the accumulations in the liver, lung and small intestine were high. [$^{11}$C]DMT and [$^{11}$C]OMB also accumulated to a large extent in the brain, where their accumulation was retained. Brain uptake of three other radiopharmaceuticals was low. [$^{11}$C]DMT is the radiopharmaceutical of choice for the study of the serotonin action mechanism in the brain, because it has the highest radiochemical yield and the highest brain uptake of these $^{11}$C-labeled compounds.

## 1. Introduction

In the indolealkylamine alkaloids, there are a series of hallucinogenic compounds such as N,N-dimethyltryptamine (DMT), N-methyltryptamine (NMT), bufotenine, O-methylbufotenine (OMB), psilocin, psilocybin[1,2] etc. The structure of these hallucinogenic indolealkylamines is similar to that of serotonin, which plays an important role in the central nervous transfer system. These compounds are also known to have an affinity for the serotonin$_1$ receptors.[3,4] Indolealkylamines labeled with positron emitters are thus interesting compounds for the study of the serotonin action mechanism and the *in vivo* receptor assay in the brain by PET, and for the preliminary study of an hallucinogenic action mechanism. We tried the $^{11}$C-labeling of DMT, NMT, bufotenine, OMB[5] and N,N,N-trimethyltryptamine iodide (TMT) by the reaction of N-monomethyl indolealkylamines with $^{11}CH_3I$. In this paper, we also report the comparative study of their tissue distribution in rats.

* Present address: Division of Radiology, Research Institute for Brain and Blood Vessels, Akita, Japan.

Address correspondence and reprint requests to: Dr T. Takahashi Division of Radioisotope Research, Cyclotron and Radioisotope Center, Tohoku University, Aoba, Aramaki, Sendai 980, Japan.

## 2. Materials and Methods

*Preparation of the starting materials*

The scheme for the preparation of starting materials is shown in Fig. 1. Tryptamine (**1a**) and 5-methoxytryptamine (**1c**) were purchased from Tokyo Kasei Kogyo Co., Ltd. N$\omega$-Methyltryptamine (N-methyltryptamine:NMT) (**5a**), N,N-dimethyl-5-methoxytryptamine (O-methylbufotenine:OMB) (**3c**) and 5-hydroxy-N$\omega$-methyltryptamine oxalate (5-hydroxy-N-methyltryptamine oxalate:5-HNMT oxalate) were purchased from Aldrich Chemical Co., Inc.

*Synthesis of N,N,N-trimethyltryptamine iodide (TMT)* (***2a***). To a solution of **1a** (1.0 g, 6.3 mmol) dissolved in acetone, $CH_3I$ (3.0 g, 21.1 mmol) was added. The mixture was stirred at room temperature for 1 day. The precipitate was filtered and washed with acetone. It was recrystallized from MeOH–EtOAc. An amount of 500 mg of **2a** was obtained. m.p. 233°C (decomp.). Yield: 24%. MS (m/e):188 ($M^+$-$CH_3I$).

*Synthesis of N,N-dimethyltryptamine (DMT)* (***3a***). To a solution of **2a** (400 mg, 1.2 mmol) dissolved in THF (5 mL), 1M $LiEt_3BH$-THF solution (1.5 mL, 1.5 mmol) was added.[6] The suspension was refluxed for 9 h under $N_2$ atmosphere. After cooling, the mixture was acidified with 3N HCl. After removal of the THF, the residue was made basic with 2N NaOH. The alkaline solution was extracted with ether. After

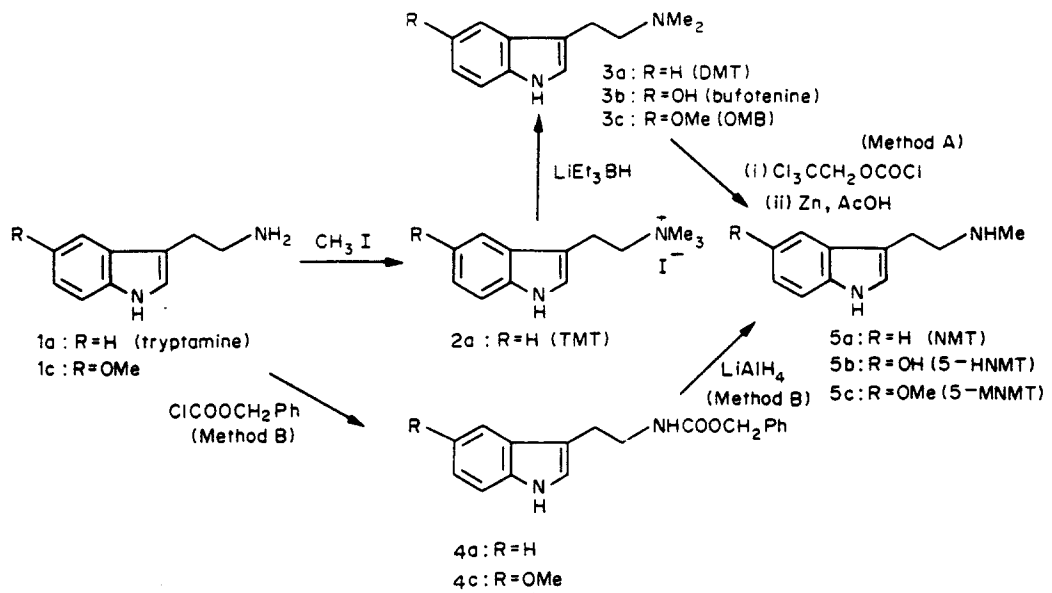


Fig. 1. Synthesis of N-methyl- or N,N-dimethylindolealkylamines.

evaporation of the solvent, the residue crystallized on standing. Thus, 120 mg of **3a** was obtained. m.p. 57–59°C. Yield: 53%. MS (m/e):188 ($M^+$).

*Preparation of 5-hydroxy-N-methyltryptamine (5-HNMT)* (**5b**). A solution of 5-HNMT oxalate (100 mg, 0.4 mmol) in $H_2O$ was passed through a column of weak base type ion exchange resin (AC-3) treated with 1N NaOH. From the effluent, the solvent was evaporated to dryness, and 6 mg of **5b** was obtained.

*Synthesis of 5-methoxy-N-methyltryptamine (5-MNMT)* (**5c**). (Method A)[7]: To a solution of **3c** (100 mg, 0.5 mmol) dissolved in benzene (5 mL), 2,2,2-trichloroethyl chloroformate (500 mg, 2.4 mmol) was added. The mixture was refluxed for 2 days. After cooling, ether (5 mL) was added and the organic solvent layer was rinsed with 3N HCl (20 mL × 2) and water (20 mL). The organic solvent was evaporated to dryness, and 120 mg of the adduct (N-(2,2,2-trichloroethoxycarbonyl)-N-methyl-5-methoxytryptamine) was obtained (Yield: 60%). To a solution of the adduct (120 mg, 0.3 mmol) dissolved in acetic acid (2 mL), zinc powder (150 mg, 2.3 mmol) was added. The mixture was stirred at room temperature for 4 h. After the zinc powder was filtered off, the filtrate was made basic with 3N NaOH. The alkaline solution was extracted with ether (20 mL × 3). The solvent was evaporated to dryness, and 30 mg of crude **5c** was obtained. The crude **5c** was purified by preparative TLC (developing solvent: n-BuOH/AcOH/$H_2O$ = 12/3/5). An amount of 13 mg of pure **5c** was obtained. m.p. 90–93°C. Yield: 43%. MS (m/e):204 ($M^+$).

(Method B): To a solution of **1c** (86 mg, 0.5 mmol) dissolved in 2N NaOH (0.5 mL) and dioxane (1 mL), benzyl chloroformate (0.2 mL) and 4N NaOH (0.25 mL) were added at the same time with vigorous stirring in an ice-water bath. The mixture was stirred at room temperature for an additional 10 min. After the mixture was acidified with c.HCl in the ice-water bath, $H_2O$ (10 mL) was added with vigorous stirring. The acid solution was extracted with ether (20 mL × 3). After evaporation of the solvent, the crude N-benzyloxycarbonyl-5-methoxytryptamine (5-methoxytryptamine-BOC) (**4c**) obtained was purified by silica-gel column chromatography (eluent:n-hexane/$CH_2Cl_2$ = 1/9). An amount of 114 mg of **4c** was obtained (Yield: 78%). To a suspension of $LiAlH_4$ (178 mg, 4.7 mmol) in abs THF (10 mL), the solution of **4c** (114 mg, 0.4 mmol) in abs THF (10 mL) was added slowly with stirring in an ice-water bath. The mixture was refluxed for 4 h. After cooling, the reaction mixture was acidified with 1N HCl, followed by removal of the THF. The water layer was rinsed with ether (50 mL) and made basic with $NaHCO_3$ powder. The alkaline solution was extracted with ether (50 mL × 3). After evaporation of the solvent, the residue was purified by preparative TLC (developing solvent:n-BuOH/AcOH/$H_2O$ = 12/3/5), and 16 mg of pure **5c** was obtained. m.p. 88–91°C. Yield: 17% (from **1c**).

*Synthesis of $^{11}CH_3I$.* $^{11}CO_2$ was produced from the proton bombardment of nitrogen gas by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction (cyclotron:CGR-MeV model 680, Tohoku University). $^{11}CH_3I$ was synthesized from $^{11}CO_2$ according to the following reaction scheme by an automated synthesis system:[8]

$$^{11}CO_2 \xrightarrow[-20°C]{LiAlH_4/THF} \xrightarrow[100°C]{THF\uparrow} \xrightarrow[-20°C]{H_2O} {}^{11}CH_3OH \xrightarrow[100°C]{HI} {}^{11}CH_3I$$

The total time required for the synthesis of $^{11}CH_3I$ from $^{11}CO_2$ was approximately 25 min.

*$^{11}$C-Labeling*

*$^{11}$C-N,N-Dimethyltryptamine ([$^{11}$C]DMT).* $^{11}CH_3I$ was trapped in an acetone solution (1 mL) of **5a** (6 mg, 34 $\mu$mol) at −78°C (dry ice–acetone). The reaction mixture was stirred at room temperature for 5 min. After removal of the solvent, the residue was dissolved in a small amount of $CHCl_3$ for chromatographic separation on a short silica-gel column (SEP-PAK (silica), solvent:$CHCL_3$/EtOH/50% $Me_2NH$ aq. sol. = 9 mL/1 mL/30 $\mu$L). [$^{11}$C]DMT was eluted with 10–20 mL of the solvent. From the combined fractions, the solvent was removed. To the residue, saline was added, followed by a pH adjustment with 0.1 N HCl. The neutral solution was filtered through a 0.22 $\mu$m pore size membrane filter for injection, and 10–20 mCi of [$^{11}$C]DMT was obtained from 50–100 mCi of $^{11}CH_3I$.

*$^{11}$C-N-Methyltryptamine ([$^{11}$C]NMT).* **1a** (6 mg, 38 $\mu$mol) was reacted with 45 mCi of $^{11}CH_3I$ and treated in a manner similar to [$^{11}$C]DMT, giving 500 $\mu$Ci of [$^{11}$C]NMT.

*$^{11}$C-5-Hydroxy-N,N-dimethyltryptamine ($^{11}$C-Bufotenine).* **5b** (6 mg, 32 $\mu$mol) was reacted with 44 mCi of $^{11}CH_3I$ in a MeOH solution (1 mL) at 40°C for 10 min and treated in a manner similar to [$^{11}$C]DMT, giving 2 mCi of $^{11}$C-bufotenine.

*$^{11}$C-5-Methoxy-N,N-dimethyltryptamine ($^{11}$C-O-Methylbufotenine ([$^{11}$C]OMB)).*[3] **5c** (6 mg, 29 $\mu$mol) was reacted with 28 mCi of $^{11}CH_3I$ at 40°C for 10 min, followed by chromatographic separation on a short reversed phase column [SEP-PAK (C-18), solvent:water (10 mL) and then EtOH (10 mL)] and treated in a manner similar to [$^{11}$C]DMT, giving 1.5 mCi of [$^{11}$C]OMB.

*$^{11}$C-N,N,N-Trimethyltryptamine iodide ([$^{11}$C]-TMT).* **3a** (6 mg, 32 $\mu$mol) was reacted with 76 mCi of $^{11}CH_3I$, followed by chromatographic separation on a short reversed phase column [SEP-PAK (C-18), solvent:water (10 mL)] and treated in a manner similar to [$^{11}$C]DMT, giving 12 mCi of [$^{11}$C]TMT.

*Radiochemical yield and radiochemical purity.* The radiochemical yields are summarized in Table 1. The radiochemical purities were determined by HPLC and TLC. The analytical conditions and the radiochemical purities are summarized in Table 2.

*Animal experiments*

A saline solution of each $^{11}$C-labeled compound was injected into Wistar Rats (150–180 g) through a lateral vein in the tail. At 5, 10, 30 and 60 min intervals after injection, the rats were killed by neck dislocation. The organs and tissues were excised, rinsed, blotted to remove adhering blood and weighed. They were then counted in an automated NaI counter. The uptake is expressed as the differential absorption ratio (DAR); DAR = [(the observed tissue activity) × (the injected activity)]/[(the body weight) × (the tissue weight)].

Table 1. $^{11}$C-Labeling conditions of indolealkylamines and their radiochemical yields

| Compound | Solvent | Time (stirring) (min) | Reaction temperature (°C) | Purification method | Radiochemical yield (%) | Time required[a] for the synthesis (min) |
|---|---|---|---|---|---|---|
| [$^{11}$C]DMT | Acetone | 5 | RT[b] | silica-gel column[c] | 50 | 35 |
| [$^{11}$C]NMT | Acetone | 5 | RT | silica-gel column | 2 | 20 |
| $^{11}$C-bufotenine | MeOH | 10 | 40 | silica-gel column | 9 | 20 |
| [$^{11}$C]OMB | Acetone | 10 | 40 | reversed phase column[d] | 18 | 30 |
| [$^{11}$C]TMT | Acetone | 5 | RT | reversed phase column | 31 | 25 |

[a] From the end of $^{11}CH_3I$ trapping.
[b] RT:room temperature.
[c] Sep-Pak, silica (Waters Ltd).
[d] Sep-Pak, C-18 (Waters Ltd).

Table 2. Analytical conditions of $^{11}$C-indolealkylamines and their radiochemical purities

| | HPLC[a] | | | TLC[b] | | |
|---|---|---|---|---|---|---|
| Compound | Column[c] | Eluent[d] | Retention time (min) | Developing[e] solvent | $R_f$ value | Radiochemical purity (%) |
| [$^{11}$C]DMT | P | I | 3.9 | A | 0.44 | 99 |
| | | | | B | 0.34 | |
| [$^{11}$C]NMT | P | I | 15.4 | A | 0.58 | 95 |
| | | | | B | 0.28 | |
| $^{11}$C-bufotenine | B | II | 2.8 | A | 0.41 | 98 |
| | | | | B | 0.46 | |
| [$^{11}$C]OMB | B | II | 5.5 | A | 0.41 | 92 |
| | | | | B | 0.55 | |
| [$^{11}$C]TMT | B | II | 2.0 | A | 0.43 | 99 |
| | | | | B | 0 | |

[a] Flow rate: 2 mL/min.
[b] plate:silica-gel (DC-Alufolien Kieselgel 60 $F_{254}$ (Merck)).
[c] P: $\mu$-Poracil, B: $\mu$-Bondapak C-18.
[d] I:$CHCl_3$/EtOH/50% $Me_2NH$ aq. sol. = 9/1/0.03; II:MeOH/$H_2O$ = 25/75, pH = 3.19 AcOH-AcONa buffer.
[e] A:n-BuOH/AcOH/$H_2O$ = 12/3/5; B:iso-PrOH/10% $NH_4OH$/$H_2O$ = 20/1/2.

## 3. Results and Discussion

The starting materials (N-monomethylamines) are prepared by either method A (N-demethylation)[7] or method B (N-monomethylation) because both primary amine and N,N-dimethylamine compounds are readily available. The N-monomethylation of tryptamine with HCHO and $NaBH_3CN$ is disadvantageous because of the formation of a cyclic by-product (1,2,3,4-tetrahydro-$\beta$-carboline).

In general, the ease of N-methylation of amines with $CH_3I$ increases in the following order: $-NH_2 \rightarrow -NHMe < -NHMe \rightarrow -NMe_2 < -NMe_2 \rightarrow -\overset{+}{N}Me_3I^-$. [$^{11}$C]DMT was synthesized easily and with a relatively high radiochemical yield. But the radiochemical yield of $^{11}$C-bufotenine was lower than that of the [$^{11}$C]DMT or [$^{11}$C]OMB (Table 1). [$^{11}$C]OMB has been synthesized with H$^{11}$CHO and $NaBH_3CN$,[5] but the $^{11}$C-labeling method with $^{11}CH_3I$ is simpler and more convenient.

The purification of these $^{11}$C-labeled compounds using a short silica-gel or reversed phase column was effective in removing most starting materials but was not sufficient to remove them fully (chemical purity: 90–98%). For the receptor mapping study, further purification is needed because of the hallucinogenic activity of the starting materials themselves. These $^{11}$C-labeled compounds were further purified by HPLC separation. For example, [$^{11}$C]DMT is well separated using the following conditions; column: $\mu$-Porasil (Waters Ltd), eluent:$CHCl_3$/MeOH/50% $Me_2NH$ aq. sol. = 900 mL/98 mL/2 mL, flow rate: 2 mL/min, detection:u.v. 254 nm.

The tissue distributions of [$^{11}$C]DMT, [$^{11}$C]NMT, $^{11}$C-bufotenine, [$^{11}$C]OMB and [$^{11}$C]TMT are shown in Figs 2–6.

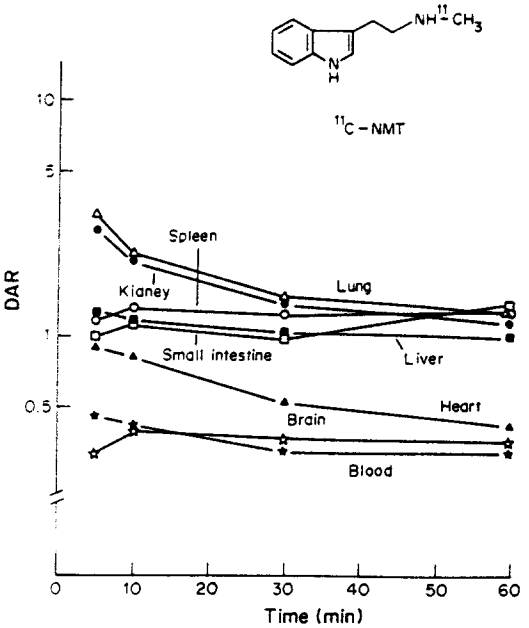


Fig. 3. Tissue distribution of [$^{11}$C]NMT in normal Wistar rats ($n$ = 3).

The four $^{11}$C-labeled compounds, excepting [$^{11}$C]TMT, were highly accumulated in the liver, kidney, lung and small intestine. [$^{11}$C]DMT, [$^{11}$C]NMT and [$^{11}$C]OMB were cleared quickly from the blood but $^{11}$C-bufotenine was cleared relatively slowly.

The time-course of brain-to-blood ratios of these $^{11}$C-labeled compounds are summarized in Table 3. [$^{11}$C]DMT and [$^{11}$C]OMB were relatively highly accu-

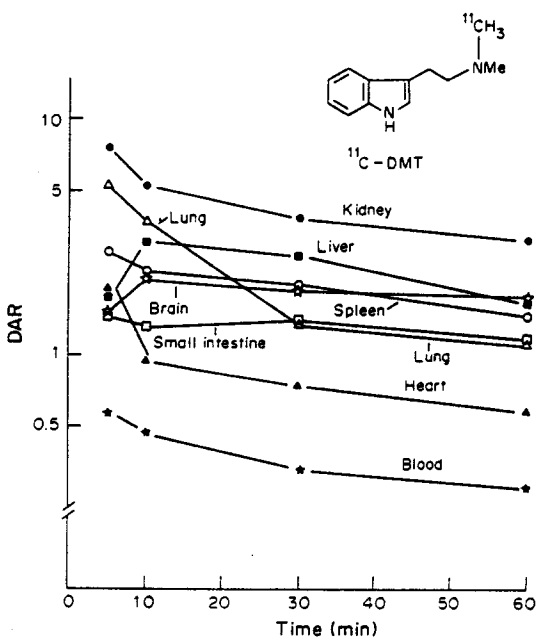


Fig. 2. Tissue distribution of [$^{11}$C]DMT in normal Wistar rats ($n$ = 3).

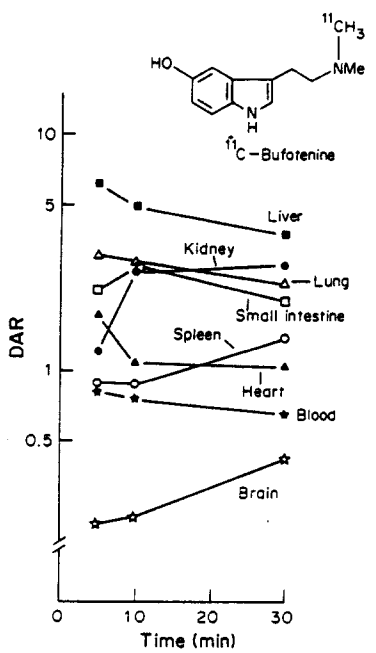


Fig. 4. Tissue distribution of $^{11}$C-bufotenine in normal Wistar rats ($n$ = 3).

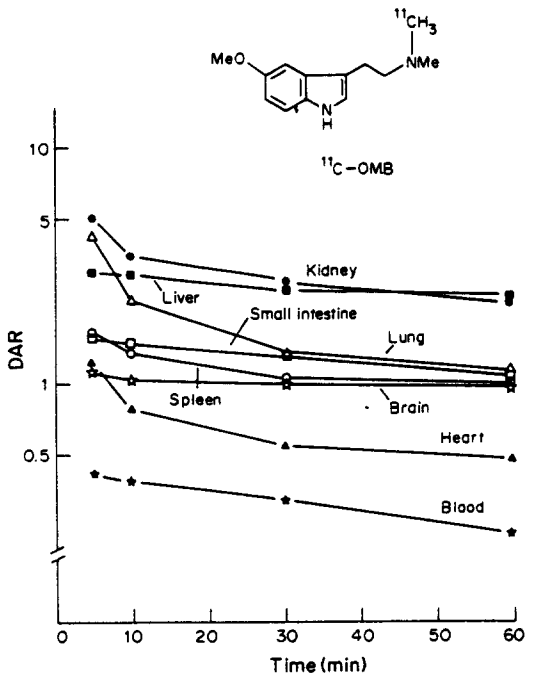


Fig. 5. Tissue distribution of [$^{11}$C]OMB in normal Wistar rats ($n = 3$).

Table 3. Time-course of brain-to-blood ratios

| Compound | Brain-to-blood | | | |
|---|---|---|---|---|
| | 5 min | 10 min | 30 min | 60 min |
| [$^{11}$C]DMT | 2.7 | 4.6 | 6.0 | 6.5 |
| [$^{11}$C]NMT | 0.7 | 0.9 | 1.1 | 1.1 |
| $^{11}$C-bufotenine | 0.3 | 0.3 | 0.6 | — |
| [$^{11}$C]OMB | 2.7 | 2.7 | 3.2 | 4.2 |
| [$^{11}$C]TMT | 0.07 | 0.07 | 0.07 | 0.07 |

mulated in the brain and their accumulations were retained. The brain uptake of [$^{11}$C]NMT and $^{11}$C-bufotenine was low, but that of $^{11}$C-bufotenine increased with time.

For [$^{11}$C]TMT, not a hallucinogenic compound, the tissue distribution pattern was different from those of the other four compounds as shown in Fig. 6. The differences were observed in the lower kidney uptake and the poor brain uptake. It may be due to the quaternary ammonium salt structure of [$^{11}$C]TMT. The gradually increasing blood uptake seems to be due to metabolic products.

In view of the radiochemical yield and the brain uptake, [$^{11}$C]DMT and [$^{11}$C]OMB are expected to be useful for serotonin$_1$ receptor studies as positron-emitting radiopharmaceuticals.

*Acknowledgements*—The technical assistance of Mr M. Monma in target handling and the assistance of Mr K. Kawashima in animal experiments are acknowledged and greatly appreciated. The operation of the cyclotron by the members of the Cyclotron and Radioisotope Center are also acknowledged and appreciated. The present investigation was supported by a Grant-in-Aid for Developmental Scientific Research No. 58870064, Ministry of Education, Science and Culture, Japan.

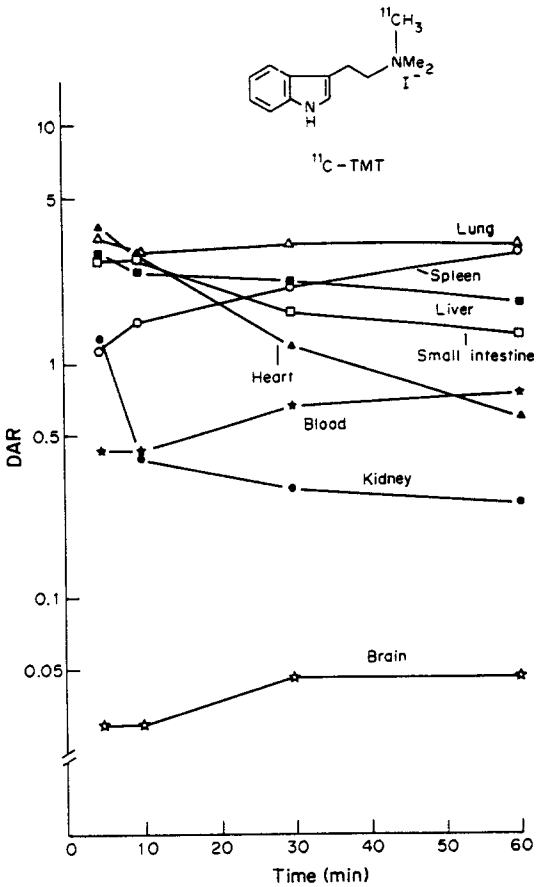


Fig. 6. Tissue distribution of [$^{11}$C]TMT in normal Wistar rats ($n = 3$).

## References

1. Dowing D. F. *Quart. Rev.* **16,** 133 (1962).
2. Koike Y., Wada K., Kusano G. and Nozoe S. *J. Natural Prod.* **44,** 326 (1981).
3. Glennon R. A. and Gessner P. K. *J. Med. Chem.* **22,** 428 (1979).
4. Fuxe K., Holmstedt B. and Johnson G. *Eur. J. Pharmac.* **19,** 25 (1972).
5. Berger G., Maziere M., Marazono C. and Comar D. *Eur. J. Nucl. Med.* **3,** 101 (1978).
6. Cooke M. P. Jr. and Parlman R. M. *J. Org. Chem.* **40,** 531 (1975).
7. Reinecke M. G. and Daubert R. G. *J. Org. Chem.* **38,** 3281 (1973).
8. Iwata R., Ido T., Saji H., Suzuki K., Yoshioka K., Tamate K. and Kashida Y. *Int. J. Appl. Radiat. Isot.* **30,** 194 (1979).