## RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

# Tc-99m(Sn)-*N*-Pyridoxylaminates: A New Series of Hepatobiliary Imaging Agents

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**Three derivatives of *N*-pyridoxylamino acid were synthesized: Tc-99m(Sn)-*N*-pyridoxylphenylalanine, -*N*-pyridoxyltryptophan, and -*N*-pyridoxyl-5-methyltryptophan. The chemical and biologic properties of each complex was found to be quite analogous to those of the corresponding Tc-99m(Sn)pyridoxylideneaminate. In rats, Tc-99m(Sn)-*N*-pyridoxyl-5-methyltryptophan [Tc-99m(Sn)PHMT] showed rapid blood clearance, fast hepatobiliary transit, low urinary excretion, and no intestinal reabsorption. Over 90% of the dose arrived in the intestine through the liver at 30 min after i.v. administration, whereas only 2% of the dose escaped through the kidneys. In rabbits the gallbladder was clearly visualized within 5 min of injection and no renal or bladder images were noted on any scintiphotos. The BSP (sulfobromophthalein) intervention study revealed that the biliary excretion of Tc-99m(Sn)PHMT is much more resistant than that of Tc-99m(Sn) diethyl-IDA to the intervention of BSP and hence to serum bilirubin. Toxicity studies on PHMT and Sn-PHMT indicated a wide margin of safety for the proposed human dose.**

**J Nucl Med 23: 517–524, 1982**

For the past several years, our efforts have been focusing on the stannous preparation of Tc-99m(Sn)pyridoxylideneaminates, aiming toward superior hepatobiliary imaging agents (*1–14*). In the course of this work, we observed that an increase in lipophilicity and rigidity of the Tc-99m complex enhances its hepatic uptake and also accelerates its hepatobiliary transit (*6,10,11*). Low solubility of the lipophilic amino acid component, however, arose as the major problem when an amino acid with a bulky lipophilic side group (as in tryptophan derivatives) was adopted as the constituent (*12,13*). In this event, relatively high concentrations (at least 20 mmole/l) of the amino acid and pyridoxal are needed for the efficient stannous preparation of a Tc-99m(Sn)-pyridoxylideneaminate, in which the pyridoxylideneaminate (a Schiff's base) is in equilibrium with the amino acid and pyridoxal through hydrolysis/dehydration reactions (*3*).

One approach to the above problem is to "fix" the ligands so as to make them stable against hydrolytic cleavage even under dilute conditions. Our current investigation is based on: (a) synthesis of stable, isolatable *N*-pyridoxylamino acids through catalytic hydrogenation of the imine moiety of the corresponding pyridoxylideneaminate, (b) preparation of Tc-99m(Sn)-*N*-pyridoxylaminates by the stannous reducing method, and (c) chemical and biological evaluation of these Tc-99m complexes as candidates for a new series of hepatobiliary imaging agents.

## MATERIALS AND METHODS

**Synthesis of *N*-pyridoxylamino acids.** Three derivatives of *N*-pyridoxylamino acid were synthesized and purified according to the method reported by Heyl et al.

Received Dec. 1, 1981; revision accepted Jan. 22, 1982.

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(*15*). They were *N*-pyridoxyl-L-phenylalanine, -L-tryptophan, and -DL-5-methyltryptophan (Fig. 1). Structure and purity of each compound (colorless crystals) were confirmed by elemental analysis, melting point, high-performance liquid chromatography, infrared absorption spectroscopy, ultraviolet absorption spectroscopy, and nuclear magnetic resonance spectroscopy.

**Preparation of a Sn-*N*-pyridoxyl-L-tryptophan (Sn-PHT) kit reagent.** L-(+)-ascorbic acid (the stabilizer, 704 mg, 4.0 millimol) and anhydrous stannous chloride (94.8 mg, 0.5 millimol) were dissolved successively in 1,000 ml of sterile, pyrogen-free water, made oxygen free by nitrogen purging. *N*-pyridoxyl-L-tryptophan (2.132 g, 6.0 millimol) was suspended in the solution, then 2*N* NaOH was added dropwise with vigorous magnetic stirring until the *N*-pyridoxyl-L-tryptophan dissolved completely and the pH of the solution reached 10.0. Finally, 2.0 ml of the resultant clear colorless solution was dispensed through a 0.2-$\mu$m Millipore-Dualex (Teflon) filter into sterile nitrogen-purged 3-ml vials. All the above processes were carried out under nitrogen atmosphere. The Sn-PHT kit reagent thus prepared was stored at −30 °C until used (*5*).

**Preparation of other Sn-*N*-pyridoxylaminate kit reagents.** Sn-*N*-pyridoxyl-L-phenylalanine (Sn-PHP) and Sn-*N*-pyridoxyl-DL-5-methyltryptophan (Sn-PHMT) were prepared by methods analogous to that described above, with the replacement of *N*-pyridoxyl-L-tryptophan by *N*-pyridoxyl-L-phenylalanine and *N*-pyridoxyl-DL-5-methyltryptophan (6.0 millimol each, pH 9.7–10.2).

**Preparation of Tc-99m(Sn)-*N*-pyridoxyl-L-tryptophan [Tc-99m(Sn)PHT] and other Tc-99m(Sn)-*N*-pyridoxylaminates.** These Tc-99m complexes were prepared in 3.5-ml vials by mixing 1.5 ml of the freshly thawed kit reagent with 1.0 ml of $^{99m}TcO_4^-$ solution (5–10 mCi in physiological saline) and heated for 7 min in a boiling-water bath to convert a trace of Tc-99m ascorbate into the desired Tc-99m *N*-pyridoxylaminate.

**Preparation of Tc-99m(Sn)pyridoxylideneisoleucine [Tc-99m(Sn)PI] and Tc-99m(Sn)-*N*-(2,6-diethylphenylcarbamoylmethyl)-iminodiacetate [Tc-99m(Sn)diethyl-IDA].** The preparation of Tc-99m(Sn)PI has been described previously (*2*,*3*,*5*). Tc-99m(Sn)diethyl-IDA was prepared according to the instructions from the kit manufacturer.*

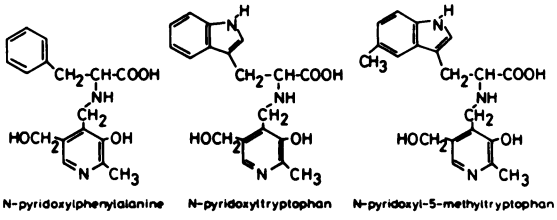


**FIG. 1.** Structures of three *N*-pyridoxylamino acids.

**Chromatographic studies.** Radiochemical purity of Tc-99m(Sn)PHP, Tc-99m(Sn)PHT, and Tc-99m(Sn)PHMT was evaluated by thin-layer chromatography using silica-gel plates (0.25 mm thick) developed with three different solvent systems: (a) MEK: methanol:2*M* aqueous KCl (10:9:1 v/v); (b) pyridine: *n*-hexane:water (40:4:1 v/v); and (c) ethyl acetate: MEK:DMSO:water (200:100:40:7 v/v). A drop (2–3 $\mu$l) of each complex solution was charged on the plate and was developed (10–12 cm) with the solvent before the charged spot dried. A radiochromatogram scanner was used for the analysis.

**In vivo studies in normal rats.** Sprague-Dawley female rats, 220–260 g, were injected intravenously with 0.1 ml of the Tc-99m-labeled complex solution. At various time intervals the animals were killed by blood drainage (6–9 ml) through the aorta with a heparinized syringe. Isolated organs were collected in plastic cups and counted on a scintillation counter.

**Scintigraphic studies using rabbits.** The in vivo behavior of Tc-99m(Sn)PHMT was compared with that of Tc-99m(Sn)diethyl-IDA using male rabbits (2.8–3.2 kg) anesthetized by the procedure of Jansholt et al. (*16*). Each animal was placed under the detector of a scintillation camera (high-resolution collimator) and injected by ear vein with 1–2 mCi of the Tc-99m complex solution. Serial scintigrams were then made on x-ray film.

**Sulfobromophthalein (BSP) intervention studies in rats.** The biliary excretion of Tc-99m(Sn)PHMT, with and without BSP intervention, was compared with that of Tc-99m(Sn)diethyl-IDA and Tc-99m(Sn)PI in rats by the method of Fritzberg et al. (*17*). A female Sprague-Dawley rat was continuously infused through the femoral vein with BSP solution (11.26 m*M*, pH 8.5) at a rate of 2.5 $\mu$mol/min of BSP per kg body weight over a period of 60 min, the infusion starting 15 min before the injection of a Tc-99m complex. After the bolus i.v. administration of a Tc-99m complex solution (0.1 ml), each 3-min output of bile from the cannulated common bile duct was collected on a chip of filter paper. The successive radioactivity measurements of these bile fractions provided differential and cumulative biliary excretion curves for each of the Tc-99m agents. In the control study, physiological saline was infused intravenously instead of the BSP solution.

**Toxicity studies on *N*-pyridoxyl-DL-5-methyltryptophan (PHMT).** Our preliminary toxicity study on PHMT revealed that the $LD_{50}$ for i.v. administration cannot be obtained because of its low toxicity and low solubility in water. In order to evaluate the toxicity on i.p. and oral administrations, mice (ICR) and rats (Sprague-Dawley) were administered, intraperitoneally or orally, a DMSO suspension of 2.0 g of PHMT/kg body wt.

**Toxicity studies on Sn-PHMT.** Mice were injected i.v. with 50 ml of Sn-PHMT solution per kg of body weight;

**TABLE 1. CHROMATOGRAPHIC BEHAVIOR OF Tc-99m(Sn)-*N*-PYRIDOXYLAMINATES (TYPICAL $R_f$ VALUES)**

| TLC system* | Tc-99m species | | | | |
|---|---|---|---|---|---|
| | Tc-99m(Sn)PHP | Tc-99m(Sn)PHT | Tc-99m(Sn)PHMT | $^{99m}TcO_4^-$ | $^{99m}TcO_2$, colloid |
| A | 0.76 | 0.72 | 0.72 | 0.98 | origin |
| B | 0.78 | 0.76 | 0.77 | 0.68 | origin |
| C | 0.20 | 0.18 | 0.18 | 0.97 | origin |

* Silica-gel plate (0.25 mm thick) developed with (A) MEK:methanol:2*M* aqueous KCl (10:9:1 v/v), (B) pyridine:*n*-hexane:water (40:4:1 v/v), and (C) ethyl acetate:MEK:DMSO:water (200:100:40:7 v/v).

rats received 10 ml/kg. For mice the dose corresponds to 2,000 times the proposed human dose; for rats it is 400 times the dose. At the same time, control animals were injected with equivalent volumes of physiological saline. Each group consisted of 15 animals, and both males and females were tested. The visual inspection and the measurement of body weight were continued for 10 days, after which all the animals were killed and dissected for histologic study.

## RESULTS

**Chromatographic behavior.** Table 1 lists the $R_f$ values for Tc-99m(Sn)PHP, Tc-99m(Sn)PHT, and Tc-99m(Sn)PHMT with each of the three TLC systems. Clearly, each TLC system discriminated the Tc-99m complex from $^{99m}TcO_4^-$ or insoluble Tc-99m species such as $TcO_2$ and Tc-99m-(Sn)-colloid. Each of the three Tc-99m preparations showed a sharp single peak on the chromatogram, and neither free pertechnetate nor Tc-99m-Sn-colloid ($^{99m}TcO_2$) was detected in any preparation. Furthermore, the chromatographic behavior of the Tc-99m complexes remained unchanged for more than 24 hr after the preparation. Partial radiolytic decomposition (about 10% of the total radioactivity) was observed at 24 hr after the preparation when $^{99m}TcO_4^-$ of high concentration (130 mCi/ml at preparation) was used for the labeling, but an increase in ascorbate concentration successfully eliminated the decomposition even when pertechnetate of 200 mCi/ml was used.

**In vivo distribution in rats.** The distributions of the three Tc-99m(Sn)-*N*-pyridoxylaminates at 1 hr after i.v. administration in rats were almost identical to those of the corresponding Tc-99m(Sn)pyridoxylideneaminates (Table 2). Among the three Tc-99m(Sn)-*N*-pyridoxylaminates (Table 2, column A), the 5-methyltryptophan derivative [Tc-99m(Sn)PHMT] showed the highest biliary excretion, the lowest blood and renal retention, and the smallest urinary output. The distributions as a function of time (Figs. 2–5), also indicate the superiority of Tc-99m(Sn)PHMT as a hepatobiliary imaging agent. The blood clearance (due to hepatic uptake) of Tc-99m(Sn)PHMT was significantly faster than that of Tc-99m(Sn)PHP (Fig. 2), and almost identical with those of Tc-99m(Sn)pyridoxylidenetryptophan and Tc-99m(Sn)PI (*13*). The biliary excretion of Tc-99m(Sn)PHMT was also faster and more concentrated than those of Tc-99m(Sn)PHP, Tc-99m(Sn)PI, and Tc-99m(Sn)diethyl-IDA (Figs. 3, 4, 8, 9). The urinary excretion of Tc-99m(Sn)PHMT, on the other hand, was smaller than those of the other Tc-99m complexes (Figs.

**TABLE 2. COMPARISON OF IN VIVO DISTRIBUTION; Tc-99m(Sn)-*N*-PYRIDOXYLAMINATE VS. Tc-99m(Sn)PYRIDOXYLIDENEAMINATE***

| Organ | Amino acid moiety | | | | | |
|---|---|---|---|---|---|---|
| | Phenylalanine | | Tryptophan | | 5-Methyltryptophan | |
| | A† | B† | A | B | A | B |
| Liver | 6.5 ± 0.4 | 7.4 ± 0.5 | 1.0 ± 0.2 | 1.0 ± 0.1 | 1.5 ± 0.1 | 1.3 ± 0.4 |
| Small intestine | 79.6 ± 2.6 | 76.8 ± 3.1 | 92.2 ± 1.3 | 92.0 ± 1.4 | 93.0 ± 0.9 | 91.3 ± 0.6 |
| Kidneys | 0.8 ± 0.3 | 0.7 ± 0.2 | 0.6 ± 0.1 | 0.5 ± 0.1 | 0.3 ± 0.1 | 0.3 ± 0.2 |
| Blood (1 ml) | 0.05 ± 0.01 | 0.05 ± 0.01 | 0.03 ± 0.00 | 0.04 ± 0.00 | 0.03 ± 0.00 | 0.03 ± 0.00 |
| Urine | 9.7 ± 2.1 | 9.2 ± 1.8 | 2.9 ± 0.3 | 2.5 ± 0.3 | 2.1 ± 0.1 | 1.9 ± 0.2 |

* For the preparation of Tc-99m(Sn)pyridoxylideneaminates, see Refs. *3, 10, 12, 13.*

† A = N-pyridoxyl derivative, B = pyridoxylidene derivative. Data are % of administered dose, mean ± s.d. for five rats, at 1 hr after injection. Blood levels were normalized to a body weight of 250 g.

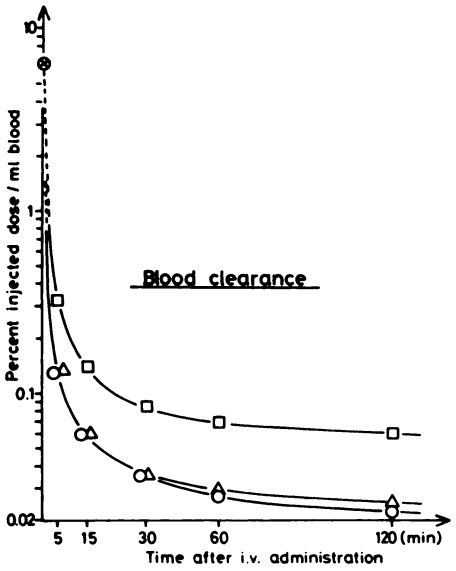


**FIG. 2.** Blood clearance of radioactivity after i.v. administration. Each point represents mean result for five female rats normalized to body weight of 250 g. O = Tc-99m(Sn)PHMT, Δ = Tc-99m(Sn)PHT, and □ = Tc-99m(Sn)PHP. (See text for abbreviations.)

5, 10); its 2%/two-hour excretion rate is comparable with those of I-131- or I-123-labeled rose bengal and Tc-99m(Sn)-*p*-butyl-IDA (*18*).

The results of a long-term (5 min–36 hr) distribution study on Tc-99m(Sn)PHMT in rats are summarized in Table 3. The radioactivity excreted into the small intestine through the liver was quantitatively transferred to the large intestine and then discharged in the feces, where it approached 97% of the injected dose at 36 hr after administration. On the other hand, the hepatic radioactivity, as well as the blood level, declined continuously. These findings indicate the absence of any intestinal reabsorption of radioactivity.

**In vivo distribution in rabbits.** Several series of experiments were performed, and Fig. 6 shows typical scintiphotos of the distribution of Tc-99m(Sn)PHMT and Tc-99m(Sn)diethyl-IDA in rabbits. The in vivo behavior of Tc-99m, injected into a rabbit as Tc-99m(Sn)PHMT, showed a time dependence similar to that observed in rats (Table 3).

The biliary excretion of Tc-99m(Sn)PHMT was significantly faster than that of Tc-99m(Sn)diethyl-IDA: almost all of the radioactivity has left the liver at 10 min and the gallbladder was clearly visualized. The 5-min image of the liver, gallbladder, and intestine with Tc-99m(Sn)PHMT is nearly identical to the 20-min image with Tc-99m(Sn)diethyl-IDA. An intense image of the urinary bladder was observed with Tc-99m(Sn)diethyl-IDA. The urinary excretion of Tc-99m(Sn)-PHMT, on the other hand, was so small that no renal or bladder images were seen on any scintiphoto.

**BSP intervention studies.** The infused dose of 2.5 μmol/min of BSP per kg wt was approximately twice the

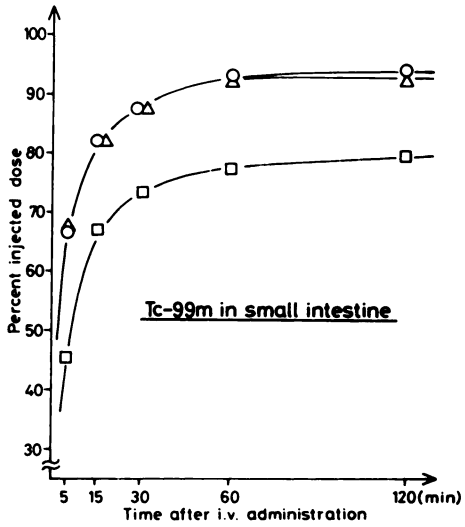


**FIG. 4.** Radioactivity in small intestine after i.v. administration in rats. Symbols as in Fig. 2.

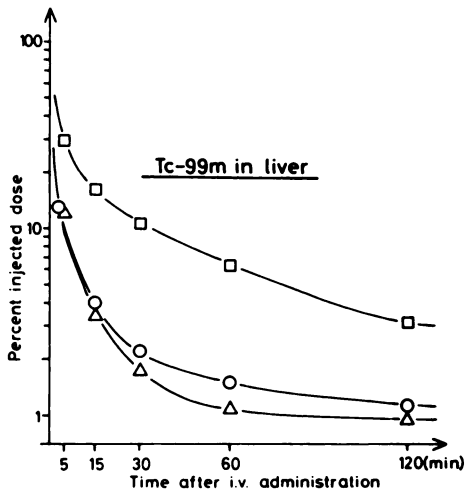


**FIG. 3.** Radioactivity in liver after i.v. administration in rats. Symbols as in Fig. 2.

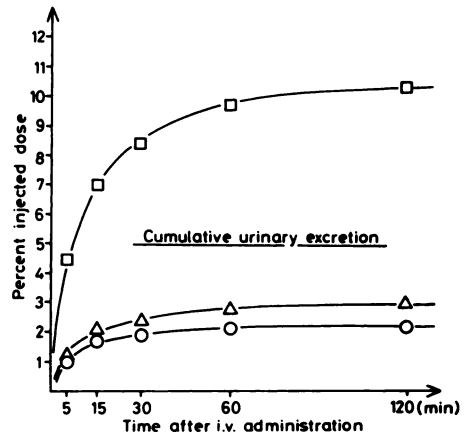


**FIG. 5.** Cumulative urinary excretion of radioactivity after i.v. administration in rats. Symbols as in Fig. 2.

**TABLE 3. ORGAN DISTRIBUTION OF Tc-99m(Sn)PHMT IN RATS AT VARIOUS TIMES AFTER I.v. ADMINISTRATION**

| Organ | 5 min | 15 min | 30 min | 1 hr | 2 hr | 3 hr | 6 hr | 12 hr | 24 hr | 36 hr |
|---|---|---|---|---|---|---|---|---|---|---|
| Liver | 13.10 | 2.50 | 1.86 | 1.48 | 1.21 | 0.76 | 0.75 | 0.64 | 0.32 | 0.33 |
| | (3.25) | (0.54) | (0.24) | (0.10) | (0.11) | (0.05) | (0.04) | (0.03) | (0.07) | (0.04) |
| Small intestine | 72.20 | 89.58 | 92.98 | 93.44 | 95.19 | 96.21 | 0.94 | 0.22 | 0.04 | 0.02 |
| | (3.79) | (1.22) | (0.96) | (0.98) | (0.54) | (0.37) | (0.10) | (0.08) | (0.01) | (0.00) |
| Large intestine | 0.42 | 0.18 | 0.09 | 0.07 | 0.05 | 0.21 | 96.37 | 79.74 | 6.05 | 0.48 |
| | (0.05) | (0.03) | (0.01) | (0.01) | (0.02) | (0.21) | (0.24) | (4.72) | (0.86) | (0.14) |
| Stomach | 0.13 | 0.08 | 0.06 | 0.04 | 0.05 | 0.04 | 0.02 | 0.01 | 0.004 | 0.005 |
| | (0.02) | (0.02) | (0.01) | (0.00) | (0.02) | (0.00) | (0.00) | (0.00) | (0.001) | (0.002) |
| Spleen | 0.07 | 0.03 | 0.02 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 | 0.006 | 0.005 |
| | (0.01) | (0.01) | (0.00) | (0.00) | (0.00) | (0.00) | (0.01) | (0.01) | (0.002) | (0.002) |
| Heart | 0.07 | 0.02 | 0.02 | 0.02 | 0.006 | 0.003 | 0.003 | 0.003 | 0.001 | 0.001 |
| | (0.01) | (0.00) | (0.00) | (0.00) | (0.002) | (0.001) | (0.001) | (0.001) | (0.000) | (0.001) |
| Lung | 0.38 | 0.18 | 0.10 | 0.08 | 0.05 | 0.02 | 0.01 | 0.01 | 0.004 | 0.003 |
| | (0.05) | (0.06) | (0.01) | (0.02) | (0.01) | (0.00) | (0.00) | (0.00) | (0.001) | (0.000) |
| Kidneys | 0.46 | 0.23 | 0.23 | 0.24 | 0.19 | 0.13 | 0.14 | 0.15 | 0.13 | 0.087 |
| | (0.07) | (0.07) | (0.04) | (0.02) | (0.01) | (0.02) | (0.01) | (0.01) | (0.03) | (0.010) |
| Ovaries | 0.03 | 0.01 | 0.005 | 0.003 | 0.003 | 0.001 | 0.001 | 0.001 | 0.000 | 0.000 |
| | (0.00) | (0.00) | (0.001) | (0.001) | (0.001) | (0.000) | (0.000) | (0.000) | (0.000) | (0.000) |
| Blood (1 ml) | 0.11 | 0.029 | 0.031 | 0.023 | 0.013 | 0.005 | 0.004 | 0.003 | 0.001 | 0.001 |
| | (0.01) | (0.007) | (0.002) | (0.001) | (0.002) | (0.001) | (0.000) | (0.000) | (0.000) | (0.000) |
| Carcass | 11.01 | 5.22 | 2.73 | 2.19 | 1.23 | 0.50 | 0.38 | 0.28 | 0.12 | 0.12 |
| | (0.95) | (0.37) | (0.44) | (0.61) | (0.23) | (0.04) | (0.02) | (0.05) | (0.01) | (0.03) |
| Urine | 1.07 | 1.78 | 1.82 | 2.21 | 2.08 | 2.13 | 1.96 | 1.94 | 1.98 | 1.97 |
| | (0.14) | (0.27) | (0.05) | (0.24) | (0.17) | (0.10) | (0.21) | (0.10) | (0.17) | (0.08) |
| Feces | — | — | — | — | — | — | 0.003 | 17.03 | 91.51 | 96.97 |
| | | | | | | | (0.003) | (4.68) | (0.82) | (0.14) |

Data express mean results and s.d. (in parentheses) for five female rats as % of administered dose. Blood levels were normalized to body weight of 250 g, and physical decay of Tc-99m was corrected.

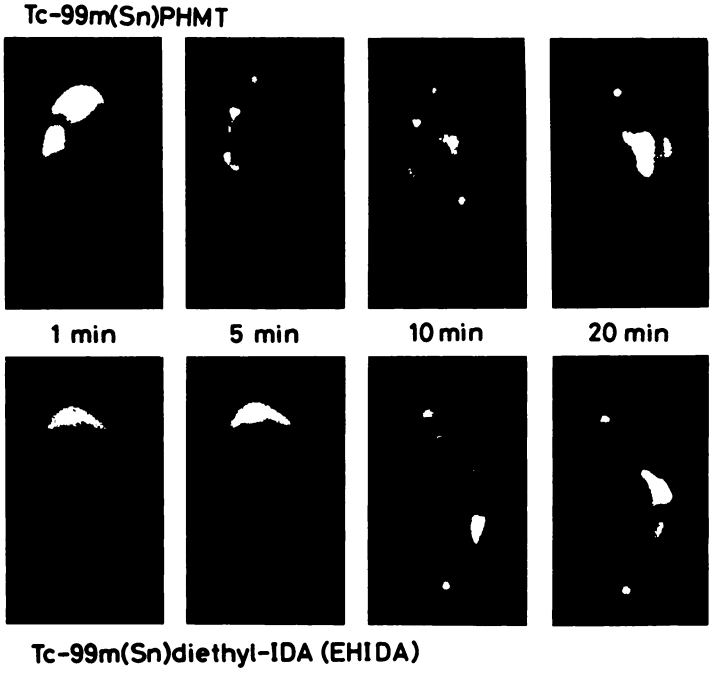


**FIG. 6.** Scintigrams showing distribution of Tc-99m(Sn)PHMT (upper row) and Tc-99m(Sn)diethyl-IDA (lower row) in rabbits. See text for details.

transport maximum (Tm) of BSP in rats (*17,19*). Without the intervention of BSP, Tc-99m(Sn)PHMT was rapidly excreted into the bile, almost reaching completion at 30 min after the i.v. administration (Fig. 7). The biliary excretion of each of the three Tc-99m species was suppressed by the Tm state of BSP, but the effect was minimal on Tc-99m(Sn)PHMT (Figs. 8, 9). The cumulative value for Tc-99m(Sn)PHMT at 90 min under Tm of BSP was equivalent to the control value (without BSP intervention) for Tc-99m(Sn)diethyl-IDA at the same time. The biliary excretion of Tc-99m(Sn)PI, on the other hand, was seriously compromised by the intervention of BSP. Urinary excretion of these Tc-99m complexes with or without BSP intervention showed parallel trends (Fig. 10). Even under the Tm of BSP, the urinary excretion of Tc-99m(Sn)PHMT was lower than that of Tc-99m(Sn)diethyl-IDA without BSP.

**Toxicity of PHMT.** No death was caused by the i.p. or oral administration of a DMSO suspension of 2.0 g PHMT/kg body weight, whether in mice or rats. This was not pursued further since Tc-99m(Sn)PHMT will be administered intravenously, and the proposed human dose is some 0.06 mg/kg body weight.

**Toxicity of intravenous Sn-PHMT.** No adverse effects were noted, whether in mice given 2,000 times the proposed human dose, or in rats given 400 times the human dose. No significant differences in body weight were observed between the tested animals and the controls during the 10 days after administration. No significant histologic differences were found between the organs of test animals necropsied at 10 days and those of the controls.

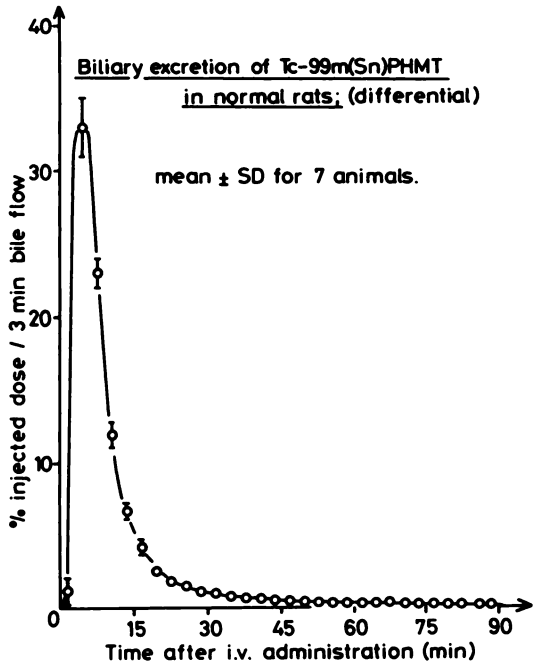


**FIG. 7.** Differential biliary excretion of Tc-99m(Sn)PHMT in normal female rats. Each point represents mean result for seven animals ± s.d., as % of injected dose in each 3-min bile flow.

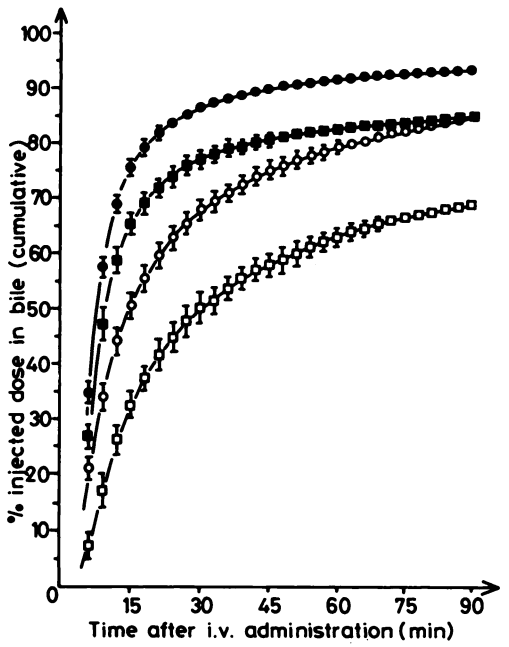


**FIG. 8.** Cumulative biliary excretion with and without BSP intervention. Each point represents mean result ± s.d. for seven female rats. Tc-99m(Sn)PHMT: control (●), under Tm of BSP (○). Tc-99m(Sn)diethyl-IDA: control (■), under Tm of BSP (□).

## DISCUSSION

The phenylalanine, tryptophan, and 5-methyltryptophan derivatives of Tc-99m(Sn)-*N*-pyridoxylaminate were studied at this time because each of the corre-

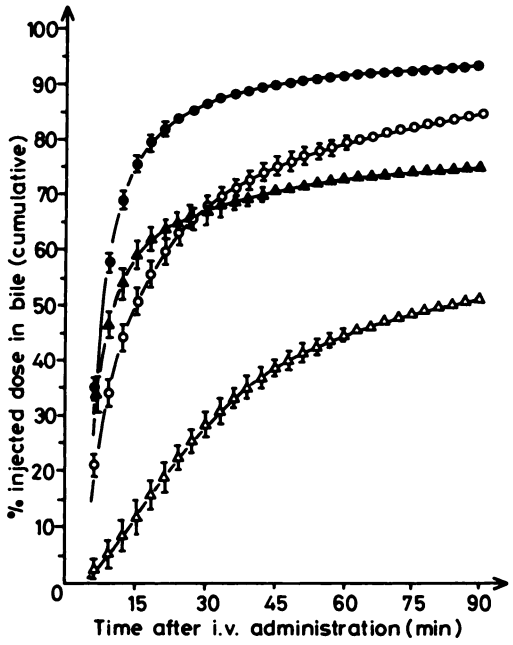


**FIG. 9.** Cumulative biliary excretion with and without BSP intervention. Each point represents mean result ± s.d. for seven female rats. Tc-99m(Sn)PHMT: control (●), under Tm of BSP (○). Tc-99m(Sn)PI: control (▲), under Tm of BSP (△).

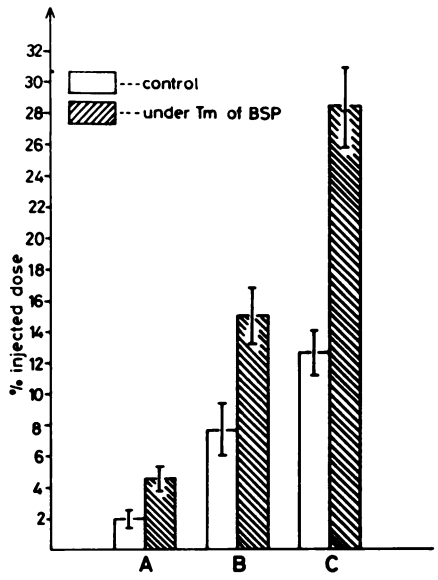


**FIG. 10.** Cumulative urinary excretion during 90 min after administration period with and without BSP intervention. Each column represents mean result ± s.d. for five female rats. A = Tc-99m(Sn)PHMT, B = Tc-99m(Sn)diethyl-IDA, and C = Tc-99m(Sn)PI.

sponding Tc-99m(Sn)pyridoxylideneaminates has been intensively studied in our laboratory (*6,10–13*). Our earlier investigation revealed that rapid hepatobiliary transit is compatible with low urinary excretion when the Tc-99m(Sn)pyridoxylideneaminate is designed to have a lipophilic and rigid structure (*6,10,11*), and Tc-99m(Sn)pyridoxylidenetryptophan and its 5-methyl derivative were introduced through the extrapolation of our structure/distribution relationship (SDR) approach (*12,13*). Although the in vivo dynamics of Tc-99m(Sn)pyridoxylidene-5-methyltryptophan as a hepatobiliary imaging agent are nearly ideal, the low solubility of the amino acid component generated a problem, and the viscosity of the preparation increased (*12,13*). We will overcome this disadvantage if *N*-pyridoxyl-5-methyltryptophan (PHMT) forms a Tc-99m complex similar in structure to Tc-99m(Sn)pyridoxylidene-5-methyltryptophan, because we expect to use stable *N*-pyridoxylamino acids in relatively dilute solution (1–10 m*M*) for the stannous preparation, thus preventing precipitation and elevation of viscosity.

Our preliminary investigation revealed that Tc-99m complexes with common chemical and biologic properties were formed with the D, L, or DL forms of *N*-pyridoxyltryptophan. Accordingly, only the DL form of *N*-pyridoxyl-5-methyltryptophan was evaluated in this study, since 5-methyltryptophan is commercially available in its DL form. As expected, these Tc-99m(Sn)-*N*-pyridoxylaminates showed biologic properties essentially identical to those of the corresponding Tc-99m(Sn)pyridoxylideneaminates (Table 2). This finding, as well as the chromatographic data, suggest the structural resemblance between Tc-99m(Sn)-*N*-pyridoxylaminates and Tc-99m(Sn)pyridoxylideneaminates (*14*).

Rapid blood clearance, fast hepatobiliary transit, and low urinary excretion are all compatible with each other for Tc-99m(Sn)PHMT (Table 3, Figs. 2–5). The urinary excretion of Tc-99m(Sn)-*N*-(*p*-butylphenylcarbamoylmethyl)iminodiacetate [Tc-99m(Sn)*p*-butyl-IDA] is reported to be as low as 2.0–2.5% of the injected dose under normal hepatobiliary function (*13,18*). Slow blood clearance and delayed hepatobiliary transit of Tc-99m(Sn)*p*-butyl-IDA, however, discourage us from adopting this agent for routine clinical use (*18*). Tc-99m(Sn)diethyl-IDA, on the other hand, shows significantly faster blood clearance and biliary excretion compared with Tc-99m(Sn)*p*-butyl-IDA (*13,18*), while its 8–12% urinary excretion under normal hepatobiliary function is 4–6 times greater than those of Tc-99m(Sn)*p*-butyl-IDA and Tc-99m(Sn)PHMT (*13,18*, Fig. 10). In these Tc-99m-labeled *N*-(phenylcarbamoylmethyl)iminodiacetic acid derivatives, therefore, the rapid biliary excretion (around 90% at 30 min in normal rats) is incompatible with the low urinary excretion (around 2% at 2 hr). Recently, Nunn and his co-workers (*20*) reported that Tc-99m(Sn)-*N*-(3-bromo-2,4,6-trimethylphenylcarbamoylmethyl)iminodiacetate (SQ-26, 962) shows faster blood clearance and biliary excretion along with low urinary excretion, but precise in vivo distribution data for this new agent are not yet available.

Estimation of the inhibitory effect of serum bilirubin on the biliary excretion of Tc-99m complexes is another important factor in the evaluation of new Tc-99m-labeled hepatobiliary imaging agents (*17,18,21–24*). The condition of high serum bilirubin (as in jaundice) can be simulated in animals by continuous i.v. infusion of BSP (*17,22,23*), since BSP shares same biliary excretion pathway with bilirubin (*25*). Tc-99m(Sn)diethyl-IDA and Tc-99m(Sn)PI were chosen as the reference agents in our present BSP intervention study of Tc-99m(Sn)-PHMT, and this tracer was found much more resistant to the BSP intervention (Figs. 8–10). Pauwels and coworkers (*21*) reported that "a bilirubin level close to 20 mg/dl sets the upper limit of reliability of Tc-diethyl-IDA in the detection of obstructive jaundice." The results of our present investigation, therefore, suggest the possibility of successful detection of obstructive jaundice with Tc-99m(Sn)PHMT even when serum bilirubin exceeds 20 mg/dl.

*N*-pyridoxyl-DL-5-methyltryptophan (PHMT) was found essentially nontoxic in animals, and the Sn-PHMT kit reagent was also found nontoxic in two animal species—even at 400–2,000 times the proposed human dose. These results indicate a wide margin of safety in Tc-99m(Sn)PHMT for human use.

We are currently supplying Tc-99m(Sn)PHMT to some hospitals in its Tc-99m-labeled form (i.e., ready to

inject), and its clinical trials are now in progress. Pilot scale production of the freeze-dried kit reagents is also under way.

In summary, *N*-pyridoxylamino acids form Tc-99m complexes with biologic properties analogous to that of the corresponding Tc-99m pyridoxylideneaminate. Tc-99m(Sn)-*N*-pyridoxyl-5-methyltryptophan [Tc-99m(Sn)PHMT] was found to be a promising hepatobiliary imaging agent with: (a) low toxicity, (b) rapid blood clearance (hepatic uptake), (c) fast hepatobiliary transit, (d) no intestinal reabsorption, (e) low urinary excretion, and (f) stout resistance to serum bilirubin.

## FOOTNOTE

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## ACKNOWLEDGMENTS

The author expresses his appreciation to Drs. S. Hamada, N. Yasuda, and K. Tanaka, members of the Nara Cancer Center, for their assistance in the scintigraphic studies using rabbits, and to Mr. H. Ueda of our laboratory for his help in designing the TLC system. The author also thanks Dr. M. Hazue and all the colleagues of the Research and Development Section, Nihon Medi-Physics Co., Ltd., for useful discussions and valuable assistance in the animal studies. Grateful thanks also go to Miss Kikuko Itoh for typing the manuscript.

This paper was presented in part at the 21st Annual Meeting of the Japanese Society of Nuclear Medicine, held in Sapporo, October 1981.

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