Tc-99m(Sn) Pyridoxylideneaminates: Preparation and Biologic Evaluation

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A new method of labeling pyridoxylideneaminates with Tc-99m has been developed using divalent tin as the reductant. Nearly 100% efficiency is obtained by the simple mixing of a kit reagent with pertechnetate at room temperature. We have prepared several Tc-99m(Sn) pyridoxylideneaminates and have compared their chromatographic and in vivo properties with those of the corresponding Tc-99m pyridoxylideneaminates prepared by the autoclaving method. Biliary excretion of the new compounds correlates well with molecular hydrophobicity; the valine and isoleucine analogs [Tc99m(Sn) P.Val and Tc-99m(Sn) P.isoL] show marked and rapid biliary excretion in rats, with only 10–15% of the injected dose escaping through the kidneys during the first hour, whereas over 90% of the retained body burden has arrived in the gut through the liver. Scintigraphic studies in rabbits gave parallel results; the gallbladder appeared within 5 min of injection. Toxicity studies in four animal species indicated a wide margin of safety for the doses of Tc-99m(Sn) P.Val and Tc-99m(Sn) P.isoL proposed for the diagnosis of hepatobiliary disorders in human patients. In a normal volunteer the hepatic duct, cystic duct, gallbladder, and common bile duct were clearly visualized scntigraphically within 15 min of i.v. injection of Tc-99m(Sn) P.isoL. The preparation, structure, and chemistry of the new complexes are intensively discussed.

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In recent years two groups of technetium-labeled radiopharmaceuticals have been attracting the interest of many investigators because of their utility in the diagnosis of hepatobiliary disorders. One group comprises Tc-99m pyridoxylideneglutamate (Tc-99m P.Glu) (*1–5*), and some of its analogues (*5*); the other consists of technetium-tagged, N-substituted iminodiacetic acid derivatives (*5,6*). An autoclaving method has been developed by Baker et al. to prepare Tc-99m P.Glu (*1*), but the chemistry of the preparation and of the Tc-99m-labeled species still remains obscure.

We have established a new method for the technetium labeling of pyridoxylidene aminates in an alkaline medium using divalent tin as the reductant. Our early findings on Tc-99m(Sn) pyridoxylidenevaline and Tc-99m(Sn) pyridoxylideneisoleucine have been reported previously (*7*). In this paper we present the details of our investigation.

MATERIALS AND METHODS

Preparation of a Sn-pyridoxylidenevaline (Sn P.Val) kit reagent. Pyridoxal hydrochloride (3.67 g, 18 millimol), L-(+)-ascorbic acid (the stabilizer, 70 mg, 0.4 millimol), and anhydrous stannous chloride (37.92 mg, 0.2 millimol) were dissolved successively in 100 ml of sterile, pyrogen-free and oxygen-free (by nitrogen bubbling) water (solution A). In another vessel, sodium hydroxide (1.44 g, 36 millimol) and L-valine (2.1 g, 18 millimol) were dissolved in 100 ml of sterile, pyrogen-free and oxygen-free water (solution B). Solution B was then poured, with stirring, into solution A. Finally, 2.2

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TABLE 1. CHROMATOGRAPHIC RESULTS FOR Tc-99m PYRIDOXYLIDENEAMINATES PREPARED BY THE AUTOCLAVING AND STANNOUS REDUCING METHODS*

Chromatographic system†	Autoclaving method								Tc-Sn colloid
	Tc P.Val	Tc P.isoL	Tc P.Leu	Tc P.phAl	Tc P.Al	Tc P.Gly	Tc P.Glu	TcO_4^-	
A	0.01–0.25	0.01–0.25	0.02–0.28	0.02–0.22	0.02–0.28	0.00–0.05	0.01–0.09	0.55–0.60	origin
B	0.62–0.74	0.61–0.73	0.35–0.68‡	0.62–0.72	0.34–0.68‡	0.62–0.72	0.26–0.68‡	0.95–0.97	origin
C	0.67–0.78	0.58–0.76	0.60–0.74	0.52–0.70	0.51–0.66	0.72–0.86	0.63–0.73	0.67–0.70	origin

Chromatographic system†	Stannous reducing method						
	Tc(Sn) P.Val	Tc(Sn) P.isoL	Tc(Sn) P.Leu	Tc(Sn) P.phAl	Tc(Sn) P.Al	Tc(Sn) P.Gly	Tc(Sn) P.Glu
A	0.29–0.38	0.29–0.38	0.09–0.20	0.10–0.20	0.01–0.07	0.01–0.03	origin
B	0.80–0.85	0.80–0.85	0.78–0.82	0.78–0.84	0.80–0.84	0.68–0.70	0.70–0.74
C	0.85–0.94	0.85–0.94	0.78–0.90	0.66–0.80	0.75–0.80	0.90–0.98	0.90–0.98

* Data express Rf range of distribution of radioactivity with solvent front as the reference.

† A-TLC; silica gel-chloroform; methanol (75:25 v/v). B-TLC; silica gel-methanol:water:MEK (45:5:50 v/v). C-PC; Toyo No. 51-physiological saline.

‡ Three broad activity peaks appeared in this Rf range (see Fig. 6).

TABLE 2. ORGAN DISTRIBUTION OF Tc-99m PYRIDOXYLIDENEAMINATES, PREPARED BY THE AUTOCLAVING METHOD, IN RATS AT 1 HR AFTER I.V. ADMINISTRATION

Organ	Percent dose per organ*						
	Tc P.Val	Tc P.isoL	Tc P.Leu	Tc P.phAl	Tc P.Al	Tc P.Gly	Tc P.Glu
Liver	6.15 ± 0.72	5.36 ± 0.56	8.70 ± 1.25	2.97 ± 1.24	14.12 ± 3.29	3.30 ± 1.25	7.87 ± 2.96
	(4.32 ± 0.51)	(3.85 ± 0.40)	(5.58 ± 0.80)	(2.03 ± 0.85)	(8.60 ± 2.00)	(1.64 ± 0.62)	(4.11 ± 1.54)
Small intestine	77.16 ± 2.21	78.16 ± 1.90	84.17 ± 4.28	88.65 ± 3.86	73.08 ± 3.58	75.33 ± 4.90	67.82 ± 4.73
	(54.26 ± 1.55)	(56.11 ± 1.36)	(53.95 ± 2.74)	(60.67 ± 2.64)	(44.53 ± 2.18)	(37.53 ± 2.44)	(35.40 ± 2.47)
Large intestine	0.55 ± 0.18	0.43 ± 0.20	0.27 ± 0.10	0.23 ± 0.09	0.29 ± 0.09	0.57 ± 0.23	0.79 ± 0.50
	(0.39 ± 0.13)	(0.31 ± 0.14)	(0.17 ± 0.06)	(0.16 ± 0.06)	(0.18 ± 0.05)	(0.28 ± 0.11)	(0.41 ± 0.26)
Stomach	0.81 ± 0.21	0.70 ± 0.29	0.11 ± 0.08	0.20 ± 0.08	0.13 ± 0.03	2.40 ± 1.28	0.28 ± 0.10
	(0.57 ± 0.15)	(0.50 ± 0.21)	(0.07 ± 0.05)	(0.14 ± 0.05)	(0.08 ± 0.02)	(1.20 ± 0.64)	(0.15 ± 0.05)
Spleen	0.27 ± 0.09	0.27 ± 0.10	0.27 ± 0.00	0.09 ± 0.03	0.44 ± 0.10	0.09 ± 0.03	0.21 ± 0.03
	(0.19 ± 0.06)	(0.19 ± 0.07)	(0.17 ± 0.00)	(0.06 ± 0.02)	(0.23 ± 0.06)	(0.04 ± 0.01)	(0.11 ± 0.02)
Lung	0.67 ± 0.19	0.65 ± 0.21	0.22 ± 0.10	0.22 ± 0.09	0.58 ± 0.13	0.38 ± 0.10	0.93 ± 0.02
	(0.47 ± 0.13)	(0.47 ± 0.15)	(0.14 ± 0.06)	(0.15 ± 0.06)	(0.35 ± 0.08)	(0.19 ± 0.05)	(0.49 ± 0.01)
Heart	0.09 ± 0.01	0.06 ± 0.02	0.00 ± 0.01	0.08 ± 0.00	0.07 ± 0.04	0.20 ± 0.03	0.13 ± 0.00
	(0.06 ± 0.01)	(0.04 ± 0.01)	(0.00 ± 0.01)	(0.05 ± 0.00)	(0.04 ± 0.02)	(0.10 ± 0.01)	(0.07 ± 0.00)
Kidneys	2.16 ± 0.45	1.98 ± 0.34	0.93 ± 0.10	0.83 ± 0.26	2.11 ± 0.39	2.28 ± 0.43	2.17 ± 0.37
	(1.52 ± 0.32)	(1.42 ± 0.24)	(0.60 ± 0.06)	(0.57 ± 0.18)	(1.29 ± 0.24)	(1.14 ± 0.21)	(1.13 ± 0.19)
1 ml blood	0.17 ± 0.03	0.16 ± 0.02	0.57 ± 0.08	0.11 ± 0.03	0.22 ± 0.02	0.27 ± 0.06	0.43 ± 0.06
	(0.12 ± 0.02)	(0.11 ± 0.01)	(0.36 ± 0.05)	(0.08 ± 0.02)	(0.13 ± 0.01)	(0.13 ± 0.03)	(0.22 ± 0.03)
Carcass	11.28 ± 1.12	10.34 ± 1.24	4.77 ± 1.93	6.16 ± 1.86	7.81 ± 2.35	14.00 ± 2.63	18.12 ± 3.87
	(7.93 ± 0.79)	(7.42 ± 0.89)	(3.06 ± 1.23)	(4.22 ± 1.27)	(4.76 ± 1.43)	(6.97 ± 1.31)	(9.46 ± 2.02)
Urine	(29.68 ± 2.32)	(28.21 ± 3.62)	(35.90 ± 3.33)	(31.56 ± 2.29)	(39.06 ± 3.38)	(50.18 ± 4.01)	(47.81 ± 3.29)

* Mean results for five rats ± 1 s.d. Data are expressed as % of activity remaining in body except for urine (without parenthesis), and % of total administered activity (in parentheses).

ml of the resultant bright-yellow solution was dispensed through a 0.22-μm Millipore filter into sterile 3-ml ampules and each was flame-sealed. All the above processes were carried out under nitrogen atmosphere. The Sn-P.Val kit reagent (pH 8.52) was stored at 4°C until used.

Preparation of other Sn-pyridoxylideneaminate kit reagents. A method analogous to that described above was used, with the replacement of L-valine by L-isoleucine, L-leucine, L-phenylalanine, L-alanine, L-glycine, and L-monosodium glutamate monohydrate (18 millimol each, pH 8.3–8.7).

Preparation of Tc-99m(Sn) pyridoxylidenevaline [Tc-99m(Sn) P.Val] and other Tc-99m(Sn) pyridoxylideneaminates. These Tc-labeled complexes were prepared in 3.5-ml vials by mixing 1.5 ml of each kit reagent with 1.5 ml of $^{99m}TcO_4^-$ solution (5–10 mCi in physiological saline, obtained by MEK

TABLE 3. ORGAN DISTRIBUTION OF Tc-99m PYRIDOXYLIDENEAMINATES, PREPARED BY STANNOUS REDUCING METHOD, IN RATS AT 1 HR AFTER I.V. ADMINISTRATION

Organ	Percent dose per organ*						
	Tc(Sn) P.Val	Tc(Sn) P.isoL	Tc(Sn) P.Leu	Tc(Sn) P.phAl	Tc(Sn) P.Al	Tc(Sn) P.Gly	Tc(Sn) P.Glu
Liver	1.46 ± 0.30	0.78 ± 0.21	1.99 ± 0.37	17.92 ± 2.26	3.48 ± 0.97	3.20 ± 0.87	3.73 ± 0.49
	(1.24 ± 0.26)	(0.68 ± 0.18)	(1.72 ± 0.32)	(16.27 ± 2.05)	(2.20 ± 0.61)	(0.88 ± 0.24)	(1.37 ± 0.18)
Small intestine	93.68 ± 2.78	96.43 ± 1.90	91.04 ± 2.00	77.70 ± 2.77	77.32 ± 3.26	23.64 ± 2.73	14.61 ± 3.21
	(79.89 ± 2.37)	(84.88 ± 1.67)	(78.59 ± 1.73)	(70.55 ± 2.52)	(48.80 ± 2.06)	(6.51 ± 0.75)	(5.38 ± 1.18)
Large intestine	0.13 ± 0.02	0.05 ± 0.01	0.13 ± 0.03	0.15 ± 0.04	0.22 ± 0.10	1.42 ± 0.13	1.97 ± 0.31
	(0.11 ± 0.02)	(0.04 ± 0.01)	(0.11 ± 0.03)	(0.14 ± 0.04)	(0.14 ± 0.06)	(0.39 ± 0.04)	(0.73 ± 0.11)
Stomach	0.03 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.14 ± 0.03	0.46 ± 0.12	0.64 ± 0.26
	(0.03 ± 0.01)	(0.00 ± 0.00)	(0.00 ± 0.00)	(0.00 ± 0.00)	(0.09 ± 0.02)	(0.13 ± 0.03)	(0.23 ± 0.10)
Spleen	0.02 ± 0.00	0.01 ± 0.00	0.03 ± 0.00	0.00 ± 0.00	0.06 ± 0.04	0.46 ± 0.09	0.36 ± 0.05
	(0.02 ± 0.00)	(0.01 ± 0.00)	(0.03 ± 0.00)	(0.00 ± 0.00)	(0.04 ± 0.03)	(0.13 ± 0.02)	(0.13 ± 0.02)
Lung	0.18 ± 0.03	0.08 ± 0.02	0.14 ± 0.04	0.23 ± 0.04	0.44 ± 0.12	1.09 ± 0.53	1.91 ± 0.33
	(0.15 ± 0.03)	(0.07 ± 0.02)	(0.12 ± 0.03)	(0.20 ± 0.04)	(0.26 ± 0.08)	(0.30 ± 0.15)	(0.70 ± 0.12)
Heart	0.04 ± 0.01	0.05 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	0.16 ± 0.06	0.03 ± 0.01	0.40 ± 0.10
	(0.03 ± 0.01)	(0.04 ± 0.00)	(0.03 ± 0.00)	(0.01 ± 0.00)	(0.16 ± 0.03)	(0.01 ± 0.00)	(0.15 ± 0.04)
Kidneys	0.99 ± 0.13	0.78 ± 0.15	1.71 ± 0.21	0.80 ± 0.20	4.94 ± 1.23	12.50 ± 2.03	9.83 ± 2.27
	(0.84 ± 0.11)	(0.69 ± 0.13)	(1.48 ± 0.18)	(0.73 ± 0.18)	(3.11 ± 0.78)	(3.44 ± 0.56)	(3.62 ± 0.84)
1 ml blood	0.09 ± 0.02	0.03 ± 0.01	0.14 ± 0.04	0.07 ± 0.01	0.27 ± 0.03	0.83 ± 0.10	1.67 ± 0.27
	(0.08 ± 0.02)	(0.03 ± 0.01)	(0.12 ± 0.03)	(0.06 ± 0.01)	(0.17 ± 0.02)	(0.23 ± 0.03)	(0.61 ± 0.10)
Carcass	2.71 ± 0.34	1.98 ± 0.48	4.15 ± 1.37	2.66 ± 0.57	11.36 ± 2.26	52.54 ± 5.34	57.22 ± 3.86
	(2.31 ± 0.29)	(1.74 ± 0.42)	(3.58 ± 1.18)	(2.42 ± 0.52)	(7.17 ± 1.43)	(14.46 ± 1.47)	(21.07 ± 1.42)
Urine	(14.72 ± 2.28)	(11.98 ± 2.15)	(13.68 ± 1.93)	(9.20 ± 2.01)	(36.89 ± 4.60)	(72.48 ± 4.73)	(63.18 ± 4.47)

* Mean results for five rats ± 1 s.d. Data are expressed as % of activity remaining in body except for urine (without parentheses), and % of total administered activity (in parentheses).

extraction in our laboratory), and incubated for 1 hr at room temperature.

Preparation of Tc-99m pyridoxylideneaminates by the autoclaving method. This method of preparing Tc-99m pyridoxylideneglutamate has been described by Baker et al. (*1*). We used similar procedures to prepare other Tc-99m pyridoxylideneaminates, with L-valine, L-isoleucine, L-leucine, L-phenylalanine, L-alanine, and L-glycine as the constituent amino acid.

Chromatographic studies. Three chromatographic systems were used for the analysis: (a) silica gel plate (0.25 mm thick) with chloroform-methanol (75:25 v/v); (b) silica gel plate with methanol-water-MEK (45:5:50 v/v); and (c) paper with physiological saline. A drop of each complex solution was charged on the plate or paper 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 rats. Sprague-Dawley female rats, 160–180 g, were injected intravenously with 0.2 ml of the Tc-99m-labeled complex solution. At various time intervals, the animals were killed and dissected. The blood (6–9 ml) was collected by aortic puncture with a heparinized syringe; isolated organs were collected in plastic cups and counted on a scintillation counter.

Scintigraphic studies using rabbits. Tc-99m(Sn) P.Val and Tc-99m(Sn) P.isoL were tested for the biliary excretion using male rabbits (2.8–3.2 kg) anesthetized by intraperitoneal sodium pentobarbital (30 mg/kg). Each animal was placed under the detector of a scintillation camera (high-resolution collimator, 25,800 parallel holes) and injected by ear vein with 4–5 mCi of the Tc-99m(Sn) complex of interest. Serial Polaroid pictures were then made.

Toxicity studies. Sn-P.Val and Sn-P.isoL were tested for acute toxicity using mice (ICR), rats (Sprague-Dawley), guinea pigs, and rabbits. The reagents were used in a concentration five times that of the standard preparation. The animals were injected over a period of 1–2 min with a 1:1 mixture of the concentrated reagent and physiological saline. Mice and rats were injected i.v. with 1 ml/100 g body weight, and guinea pigs and rabbits with 0.5 ml/100 g body weight. For mice and rats this dose corresponds to 1,000 times the proposed human dose; for guinea pigs and rabbits it is 500 times the dose. At the same time control animals were injected with equivalent volumes of physiological saline. Each group consisted of ten animals, and both males and females were tested.

The visual inspection and the measurement of the body weight were carried out for 10 days, after which all the animals were killed and dissected for histo-

TABLE 4. ORGAN DISTRIBUTION OF Tc99m(Sn) P.Val AND Tc-99m(Sn) P.isol IN RATS AT VARIOUS TIME INTERVALS AFTER I.V. ADMINISTRATION

	Percent dose per organ*							
	5 min		20 min		60 min		120 min	
Organ	PV	PI	PV	PI	PV	PI	PV	PI
Liver	27.33 ± 3.49	21.32 ± 3.39	4.63 ± 1.28	4.16 ± 0.92	1.46 ± 0.30	0.78 ± 0.21	1.41 ± 0.26	0.70 ± 0.20
	(25.33 ± 3.23)	(19.60 ± 3.12)	(3.98 ± 1.10)	(3.70 ± 0.82)	(1.24 ± 0.26)	(0.68 ± 0.18)	(1.20 ± 0.22)	(0.61 ± 0.17)
Small intestine	48.46 ± 6.28	59.34 ± 5.26	86.52 ± 3.29	87.39 ± 2.23	93.68 ± 2.28	96.43 ± 1.90	94.88 ± 1.68	96.62 ± 1.53
	(44.92 ± 5.82)	(54.55 ± 4.84)	(74.49 ± 2.83)	(77.69 ± 2.20)	(79.89 ± 2.37)	(84.88 ± 1.67)	(80.64 ± 1.43)	(84.47 ± 1.34)
Large intestine	0.94 ± 0.20	0.92 ± 0.25	0.22 ± 0.07	0.32 ± 0.04	0.13 ± 0.02	0.05 ± 0.01	0.10 ± 0.03	0.05 ± 0.01
	(0.87 ± 0.19)	(0.85 ± 0.23)	(0.19 ± 0.06)	(0.29 ± 0.04)	(0.11 ± 0.02)	(0.04 ± 0.01)	(0.08 ± 0.03)	(0.04 ± 0.01)
Stomach	0.52 ± 0.18	0.32 ± 0.07	0.08 ± 0.03	0.01 ± 0.00	0.03 ± 0.01	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
	(0.48 ± 0.17)	(0.29 ± 0.06)	(0.07 ± 0.03)	(0.01 ± 0.00)	(0.03 ± 0.01)	(0.00 ± 0.00)	(0.01 ± 0.00)	(0.00 ± 0.00)
Spleen	0.13 ± 0.32	0.07 ± 0.02	0.03 ± 0.00	0.00 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.00 ± 0.00
	(0.12 ± 0.30)	(0.06 ± 0.02)	(0.03 ± 0.00)	(0.00 ± 0.00)	(0.02 ± 0.00)	(0.01 ± 0.00)	(0.02 ± 0.02)	(0.00 ± 0.00)
Lung	0.60 ± 0.14	0.56 ± 0.20	0.42 ± 0.12	0.22 ± 0.04	0.18 ± 0.03	0.08 ± 0.02	0.14 ± 0.03	0.08 ± 0.02
	(0.56 ± 0.13)	(0.51 ± 0.18)	(0.36 ± 0.10)	(0.20 ± 0.04)	(0.15 ± 0.03)	(0.07 ± 0.02)	(0.12 ± 0.03)	(0.07 ± 0.02)
Heart	0.17 ± 0.06	0.16 ± 0.05	0.09 ± 0.02	0.06 ± 0.02	0.04 ± 0.01	0.05 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
	(0.16 ± 0.06)	(0.15 ± 0.05)	(0.08 ± 0.02)	(0.05 ± 0.02)	(0.03 ± 0.01)	(0.04 ± 0.00)	(0.03 ± 0.00)	(0.03 ± 0.00)
Kidneys	2.91 ± 0.63	1.92 ± 0.37	1.04 ± 0.28	0.99 ± 0.11	0.99 ± 0.13	0.78 ± 0.15	0.93 ± 0.10	0.76 ± 0.13
	(2.70 ± 0.58)	(1.77 ± 0.34)	(0.90 ± 0.24)	(0.88 ± 0.10)	(0.84 ± 0.11)	(0.69 ± 0.13)	(0.79 ± 0.08)	(0.66 ± 0.11)
1 ml blood	0.62 ± 0.10	0.43 ± 0.09	0.12 ± 0.03	0.08 ± 0.01	0.09 ± 0.02	0.03 ± 0.01	0.07 ± 0.02	0.03 ± 0.01
	(0.57 ± 0.09)	(0.40 ± 0.08)	(0.10 ± 0.03)	(0.07 ± 0.01)	(0.08 ± 0.02)	(0.03 ± 0.01)	(0.06 ± 0.02)	(0.03 ± 0.01)
Carcass	14.73 ± 1.23	12.54 ± 2.01	6.31 ± 1.13	5.86 ± 1.09	2.71 ± 0.34	1.98 ± 0.48	1.72 ± 0.21	1.90 ± 0.17
	(13.65 ± 1.14)	(11.53 ± 1.85)	(5.43 ± 0.97)	(5.21 ± 0.97)	(2.31 ± 0.29)	(1.74 ± 0.42)	(1.46 ± 0.18)	(1.66 ± 0.13)
Urine	(7.30 ± 1.54)	(8.07 ± 1.05)	(13.90 ± 2.27)	(11.10 ± 1.98)	(14.72 ± 2.28)	(11.98 ± 2.15)	(15.01 ± 1.73)	(12.58 ± 1.96)

* Mean results for five rats ± 1 s.d. Data are expressed as % of activity remaining in body except for urine (without parentheses), and % of total administered activity (in parentheses).

logic study. Sterility and pyrogenecity tests were also performed.

Scintigraphic study in normal human subject. Technetium-99m(Sn) P.isoL was tested for biliary excretion in a normal 23-year-old male volunteer without history of hepatobiliary disease. The volunteer, fasting for 4 hr and lying supine under a gamma camera (high-resolution collimator, 15,000 holes), received an i.v. injection of 5 mCi of Tc-99m(Sn) P.isoL while the camera monitored the liver and upper abdomen. A series of 30-sec exposures (anterior view) were made, using a Polaroid camera (Fig. 4).

RESULTS

Chromatographic behavior. The chromatographic analysis of Tc-99m pyridoxylideneaminates, prepared by the autoclaving and by the stannous reducing methods, yielded the results shown in Table 1. The distribution of the radioactivity was shown as the R_f range using the solvent front as the reference. To emphasize the distribution of radioactivity, the R_f range is shown instead of R_f value.

The chromatographic system B is the most suitable for the evaluation of labeling efficiency because $^{99m}TcO_4^-$ migrates close to the solvent front, whereas Tc-99m Sn colloid remains at the origin and the Tc-99m(Sn) pyridoxylideneaminates show sharp single peaks on the scanning chromatograms. Neither unreacted pertechnetate nor Tc-Sn colloid was detected in any preparation. The use of systems A and C for the analysis of Tc-99m P.Glu prepared by the autoclaving method has been reported by Kubota et al. (*3*). We adopted system A for the comparison of the hydrophobic properties of various Tc-99m pyridoxylideneaminates, since in this system the more hydrophobic the complex becomes, the greater is its R_f value. In this sense, the data shown in Table 1 indicate that Tc-99m(Sn) P.Val and Tc-99m (Sn) P.isoL have relatively hydrophobic characters compared with the other Tc-99m complexes studied here. The technetium complexes prepared by the autoclaving method, on the other hand, showed relatively broad distributions of radioactivity on chromatography compared with those prepared by the stannous reducing method. When Tc P.Leu, Tc P.Al and Tc P.Glu (by autoclaving) were analyzed with system B, three broad activity peaks appeared on a wide distribution of activity that covered the R_f ranges listed in Table 1 (see also Fig. 6), whereas each Tc-99m(Sn) pyridoxylideneaminate showed a sharp, single peak with system B.

In vivo distribution in rats. Tables 2 and 3 show the distributions in rats at 1 hr after administration of Tc-99m pyridoxylideneaminates prepared by the autoclaving and the stannous reducing methods. With

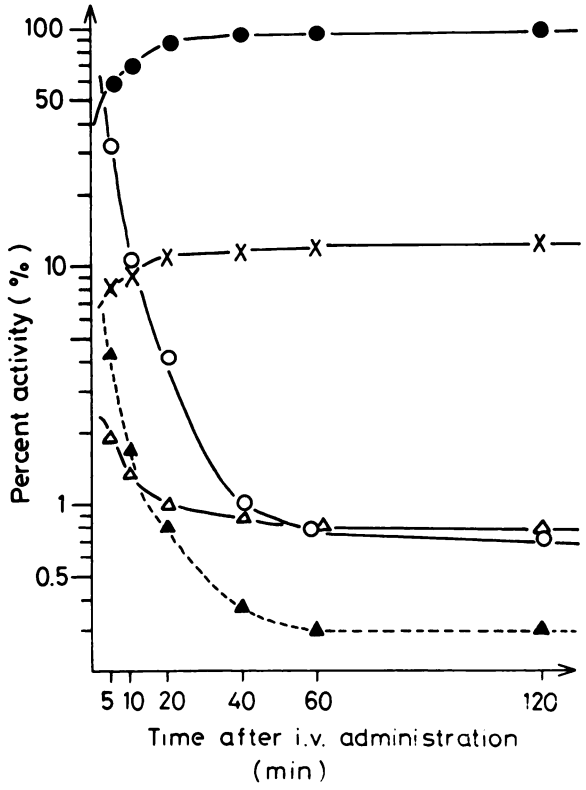

FIG. 1. Organ distribution of Tc-99m(Sn) P.isoL in rats at various time intervals after i.v. injection. Each point is a mean for five rats. Data are plotted as % of activity remaining in body—except for urine, in which it is plotted as % of total injected activity. (●) small intestine, (○) liver, (△) kidneys, (×) urine (cumulative), (▲) 1 ml blood × 10.

the autoclaving method, Tc-P.Phe showed the highest uptake ratio for small intestine to liver, the smallest distribution in kidneys and blood, and relatively small cumulative urinary excretion (Table 2).

On the other hand, the results of Table 3 indicate that Tc-99m(Sn) P.Val, Tc-99m(Sn) P.isoL and Tc-99m(Sn) P.Leu are promising agents for hepatobiliary tract imaging. Note also that the in vivo distribution of Tc-99m P.Glu prepared by Baker's autoclaving method was quite different from that of Tc-99m(Sn) P.Glu. The results shown in Table 4 and Fig. 1 also suggest that Tc-99m(Sn) P.Val and Tc-99m(Sn) P.isoL could serve as hepatobiliary tract imaging agents. These two Tc-99m complexes, injected intravenously, were rapidly cleared from the blood by the liver and were then excreted into the small intestine. The initial half-time of the blood concentration curve is less than 15 sec, and that for the liver activity is about 3.4 min. The amount of urinary excretion is relatively small compared with that of other Tc-99m complexes studied here, and reaches a plateau within a short time after the administration.

This chromatographic and in vivo behavior of the Tc-99m(Sn) pyridoxylideneaminates showed no change for 60 days after the preparation of the kit reagent and for 48 hr after the labeling with technetium.

In vivo distribution in rabbits. Figures 2 and 3 show scintiphotos of the distribution of Tc-99m(Sn) P.Val and Tc-99m(Sn) P.isoL, respectively, in rabbits. The dynamic behavior of radioactivity injected as Tc-99m(Sn) P.Val or Tc-99m(Sn) P.isoL in rabbits showed a time dependency similar to that observed in rats (Table 4, Fig. 1). Intense uptake of the radioactivity by the liver was observed clearly in the early phase (3 min) but the image faded rapidly, becoming faint at about 15 min. The gallbladder began to concentrate activity by about 5 min and excellent images of this organ were obtainable by 10 min. Faint renal uptake of the radioactivity was

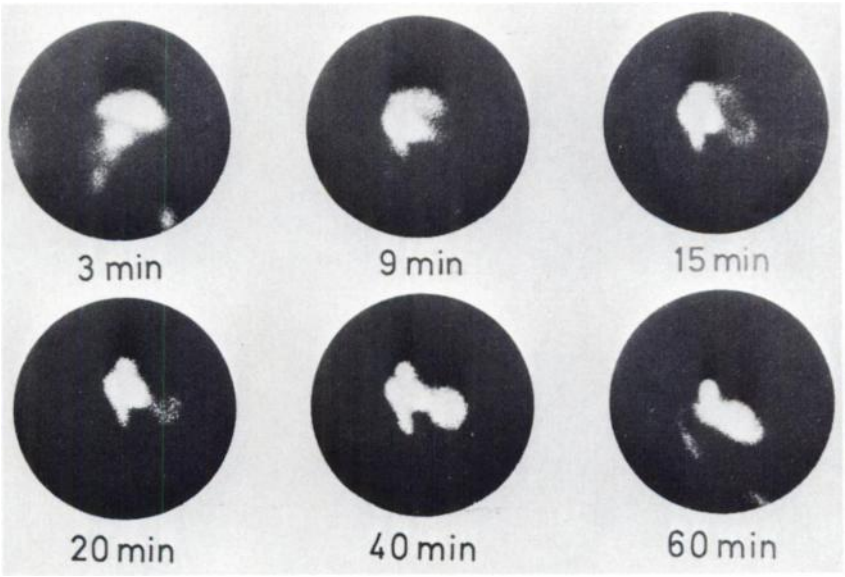

FIG. 2. Gamma-camera images showing distribution of Tc-99m(Sn) P.Val in rabbit (high-resolution, parallel-hole collimator).

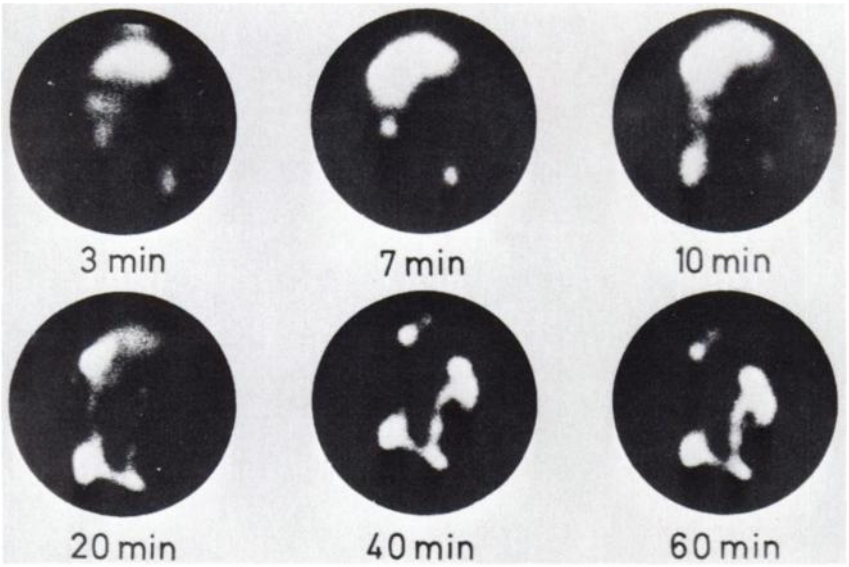

FIG. 3. Gamma-camera images showing distribution of Tc-99m(Sn) P.isoL in rabbit (high-resolution, parallel-hole collimator).

seen in the pictures taken at 10 min, but this was not observed after 15 min.

Toxicity studies. No adverse effects were noted, whether in groups of mice and rats given 1,000 times the proposed human dose, or in groups of guinea pigs and rabbits given 500 times the proposed human dose. No significant differences in body weight were observed between the tested animals and the controls during a period of 10 days after administration. The organs of all tested animals excised at 10 days after the administration revealed no significant histologic differences from those of controls.

The preparation was found to be sterile and pyrogen-free.

Normal human images. Following the i.v. injection of 5 mCi of Tc-99m(Sn) P.isoL, the 5-min image showed that essentially all the activity was already in the liver (Fig. 4). The kidneys were not observed in any pictures taken later than 5 min postinjection. By 10 min the hepatic duct began to contain activity, and by 15 min there was excellent identification of the hepatic duct, the cystic duct, the gallbladder, and the common bile duct. At 30 min the gallbladder was filled and activity was also visible in the small intestine.

By 60 min the image of the liver had disappeared almost completely and no activity was seen in organs other than the gallbladder and small intestine.

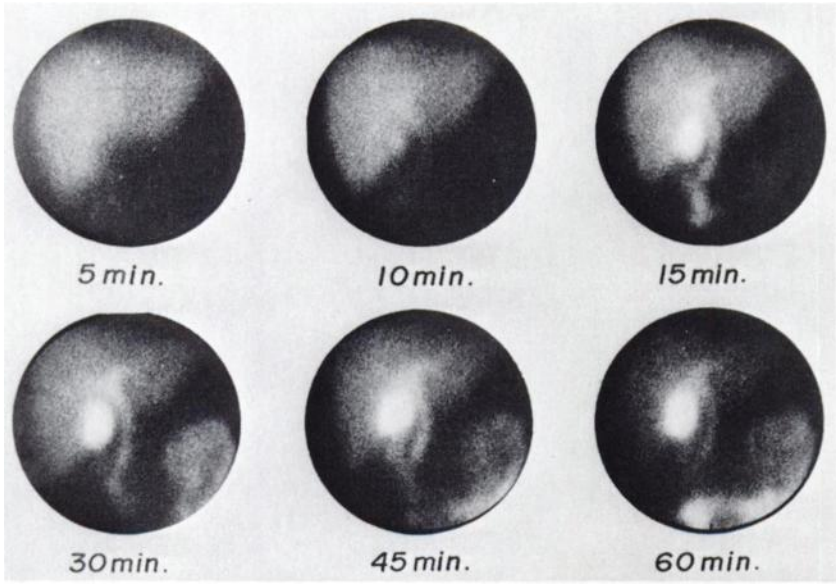

FIG. 4. Normal human distribution for Tc-99m(Sn) P.isoL at various times after 5-mCi i.v. dose (gamma camera as before). At 15 min, hepatic duct, cystic duct, gallbladder, and common bile duct were all visualized.

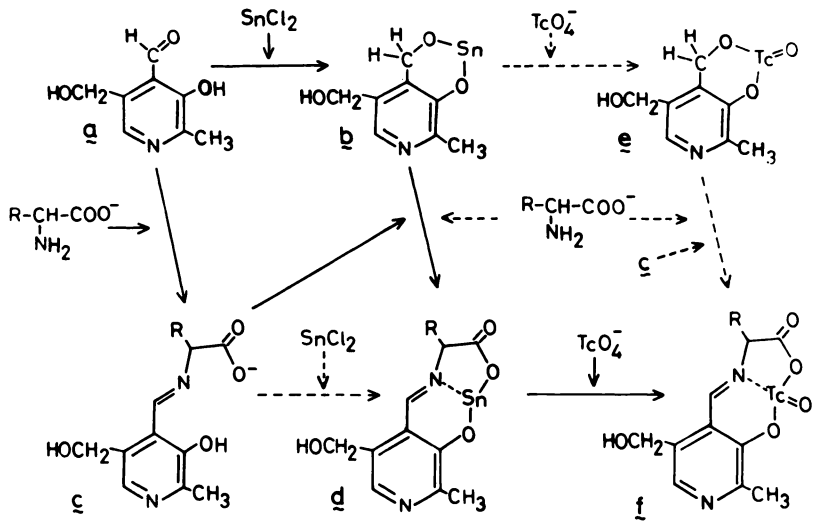

FIG. 5. Reaction path in preparation of Tc-99m pyridoxylideneaminate using divalent tin as reductant. Solid arrows indicate possible major path; dotted for possible minor path. Complexes are represented in their presumed structure.

DISCUSSION

In this report we have presented a new method for specific technetium labeling of organic molecules in an alkaline medium (pH 8–9) using divalent tin as the reductant. Many investigators have indicated that "if new methods of technetium labeling could be developed in alkaline media, the number of valuable technetium radiopharmaceuticals would rapidly expand" (*8*).

It is well known that ionic divalent tin—e.g. in the stannous halides—undergoes hydrolysis to form Sn colloid when the pH of the solution is raised to the alkaline region. If, however, a large excess of a strong chelating reagent, such as EDTA, is present together with divalent tin, chelate-complex formation occurs before hydrolysis even at a pH of 9–10 (*9*).

Pyridoxylideneaminate Schiff bases form stable metal complexes, especially with divalent metal ions —for example Cu^{2+}, Ni^{2+}, Mn^{2+}, and Co^{2+} (*10–12*)—and the equilibria in mixtures of pyridoxal with valine and a divalent metal ion have been intensively studied (*12*). We sought to prepare Sn^{2+} pyridoxylideneaminate complexes in alkaline media and to use these Sn^{2+} complexes as the reductant for heptavalent technetium.

Figure 5 shows the reaction path in the preparation of Tc-99m pyridoxylideneaminates using divalent tin. To date we have observed some evidence that divalent tin is first chelated by pyridoxal (a) (Fig. 5) in solution A (pH 2–3) and the resulting Sn^{2+} pyridoxal complex (b) is then converted into a Sn^{2+} pyridoxylideneaminate complex (d) in the mixture of solutions A and B. (See section on materials and methods for solutions A and B.)

The Tc pyridoxal complex (e) can be prepared specifically when pertechnetate is added to solution A, but the formation of (e) is not observed in the standard preparation of (f) with the kit reagents. The species (e) can be partially converted into the Tc pyridoxylideneaminate complex (f) by mixing it with the amino acid solution (solution B), though the conversion rate of (e) to (f) is quite slow at room temperature (2–3%/60 min).

The formation of a Sn^{2+} pyridoxylideneaminate complex (d) in the kit reagent seems to proceed by way of an exchange reaction of metal ion or Sn^{2+} from a pyridoxal ligand to a pyridoxylideneaminate ligand (c) since the latter affords a much stronger ligand field to Sn^{2+} than that of the former. Species (d) can be prepared by the direct addition of stannous chloride or other divalent tin into the solution of (c) (pH 8–9) but in the process a considerable amount of the added tin undergoes hydrolysis to form Sn colloid.

When the concentration of pyridoxal and aminates was decreased to 5 m*M* in the preparation of the kit reagents, Tc-99m pyridoxylideneaminate could not be formed, and only the formation of Tc-99m Sn colloid was observed after the mixing of the kit reagent and pertechnetate. This result also indicates the formation of a Sn^{2+} pyridoxylideneaminate complex (d) in the standard preparation of the kit reagent.

To summarize the kit preparation, the solid arrows in Fig. 5 indicate the possible major path to Tc-99m pyridoxylideneaminate (f). These mechanisms in the preparation of the kit reagents and technetium labeling enable one to prevent the formation of Tc-99m Sn colloid and to succeed in the specific labeling of pyridoxylideneaminates with technetium in an alkaline medium.

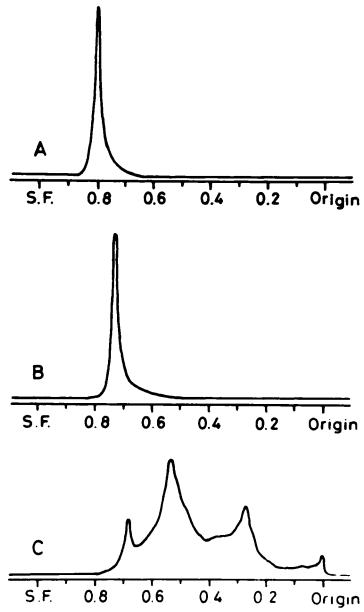

FIG. 6. Distribution of radioactivity on silica gel plate after development with methanol-water-MEK (45:5:50 v/v). (A) Tc-99m (Sn) P.isoL, (B) Tc-99m(Sn) P.Glu prepared by stannous reducing method, and (C) Tc-99m P.Glu prepared by Baker's autoclaving method.

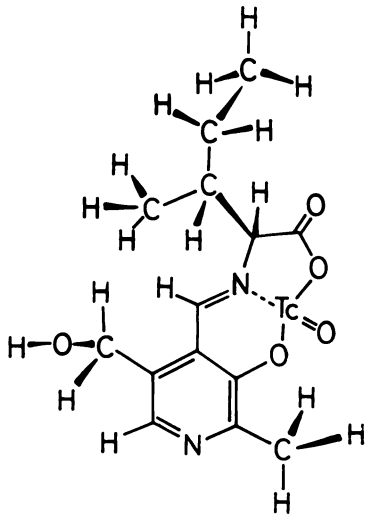

FIG. 7. Presumed three-dimensional structure of Tc-99m pyridoxylideneisoleucine prepared by stannous reducing method.

The chemistry of the autoclaving method, on the other hand, is rather complicated. Snell (*13*) reported that transamination proceeds up to 85% under the conditions (120°C, 30 min) adopted by Baker et al. (*1*). The resulting solution therefore contains 2-ketoglutarate and pyridoxamine as well as glutamate, pyridoxal and P.Glu when Tc-99m P.Glu is prepared by the autoclaving method. Glutamate, which is the least heat-stable species in the preparation, cyclizes to some extent to form α-pyrrolidonecarboxylate during the autoclaving process (*14,15*). At the same time the tautomerization of the Schiff base proceeds (*16*). These subreactions make the chemistry of the final Tc-99m P.Glu solution quite complicated, and it remains a question as to which species in the preparation reduces the heptavalent technetium to a low-valency state.

The results of chromatographic analysis suggests that the radioactive species obtained by Baker's method consists of several Tc-99m complexes. Figure 6 shows the radiochromatogram of Tc-99m(Sn) P.isoL (A), Tc-99m(Sn) P.Glu (B), both prepared by the stannous reducing method, and Tc-99m P.Glu prepared by Baker's autoclaving method. Analysis of Baker's Tc-99m P.Glu with this chromatographic system shows wide distribution of radioactivity (R_f 0.26–0.68), including three broad activity peaks at R_f 0.27, 0.53, and 0.67. In contrast with this result, Tc-99m(Sn) P.isoL, Tc-99m(Sn) P.Glu, and other Tc-99m(Sn) pyridoxylideneaminates show a sharp single peak on the chromatograms (see also Table 1).

The organ distributions of Tc-99m(Sn) P.Glu and Tc-99m P.Glu also suggested a chemical difference between the two preparations. Technetium-99m(Sn) P.Glu distributes mainly in the carcass, blood, and kidneys, and only about 14% of the activity remaining in the body is excreted into the small intestine during a period of 1 hr after administration in rats. The amount of cumulative urinary excretion is more than 60% of the total administered activity (Table 3). It can be concluded, therefore, that Tc-99m(Sn) P.Glu is *not* a good hepatobiliary agent.

The results of chromatographic analysis and in vivo distribution studies demonstrate the close relationship between the molecular hydrophobicity of Tc-99m(Sn) pyridoxylideneaminates and their organ distributions (Tables 1, 3). Wistow et al. reported that the increase in hydrophobicity of N-substituted iminodiacetic acid derivatives enhances the hepatobiliary-seeking properties of their Tc-99m complexes (*5*). The same effect was expected in our Tc-99m (Sn) pyridoxylideneaminate system, so we tested a series of amino acids that were suitable for the evaluation of this effect.

The expected result from Tc-99m(Sn) P.Val and Tc-99m(Sn) P.isoL show relatively high hydrophobicity among the tested complexes, and both of these proved to be promising hepatobiliary agents. Compared with other hepatobiliary radiopharmaceuticals thus far reported, Tc-99m(Sn) P.Val and Tc-99m (Sn) P.isoL appeared in the gallbladder and small intestine more rapidly. We note, moreover, that the dynamic distribution of these two complexes in rab-

bits shows a time dependency approximately identical with that observed in rats.

Let us consider the possible structure of Tc-99m (Sn) P.Val and Tc-99m(Sn) P.isoL. The presumed three-dimensional structure of Tc-99m(Sn) P.isoL is shown in Fig. 7. Assuming that the reduced state of technetium is tetravalent, pyridoxylideneisoleucine would provide a tridentate ligand to the TcO^{2+} ion. When methyl and ethyl groups (CH_3- and $CH_3 \cdot CH_2$-) attach themselves to the β-carbon of the isoleucine moiety, the more bulky ethyl group would orient itself in such a way as to minimize the repulsion of the ring framework. The methyl group must then occupy the space near the hydroxymethylene group fused on the pyridine ring of the pyridoxal moiety. Metzler (*17*) reported that branching in the β position of the amine (valine, isoleucine, isobutylamine) is particularly effective in increasing the amine stability. He indicated that van der Waals bonding of the branched alkyl side chains to the methylene bridge and to the hydroxymethyl side chain of the pyridoxal moiety would be involved. In this manner the methyl group would provide a shielding effect on the hydrophilic hydroxymethylene group and hence the global hydrophilicity of the molecule would be diminished. The same phenomenon would take place in Tc-99m(Sn) P.Val molecule.

When leucine is adopted as the amino acid moiety, however, the i-propyl group is the only one that fuses on the β carbon and there is no hydrophobic group to provide the shielding effect on the hydroxymethylene of the pyridoxal moiety. Therefore Tc-99m(Sn) P.Leu is expected to show slightly stronger hydrophilic properties than Tc-99m(Sn) P.Val and Tc-99m(Sn) P.isoL. This consideration is supported by the result of the chromatographic analysis of these three complexes using silica gel plate and chloroform-methanol (75:25 v/v) (Table 1).

Sn-P.Val and Sn-P.isoL kit reagents were found to be nontoxic in four experimental animal species —even at 500–1,000 times the proposed human dose. This result, along with the results of sterility and pyrogenecity studies, indicate that Tc-99m(Sn) P.Val and Tc-99m(Sn) P.isoL are suitable for use in humans with a wide margin of safety.

The distribution images in a normal human subject using Tc-99m(Sn) P.isoL showed a rapid accumulation of radioactivity in the biliary system, including hepatic duct, cystic duct, gallbladder, and common bile duct. The scintiphotos (Fig. 4) obtained with this new radiopharmaceutical appear superior to those reported with Tc-99m diethyl IDA (*5*), but further imaging studies in normal human subjects should be done, with special attention to the effect of food (*18*).

Clinical trials of Tc-99m(Sn) P.isoL in subjects with and without hepatobiliary disease are now being performed at about 80 hospitals in Japan.

In summary, Tc-99m(Sn) P.Val and Tc-99m(Sn) P.isoL are promising agents of low toxicity for clinical application in the diagnosis of hepatobiliary disorders.

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