Preparation of Various Tc-99m Dimercaptosuccinate Complexes and their Evaluation as Radiotracers

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The organ distributions of four different Tc-99m dimercaptosuccinate complexes ("Tc-DMS", Complexes 1 to 4) were determined using mice and were evaluated as renal imaging agents. The highest kidney uptake was observed with Complex 2: 21.7% of dose, 3 hr after injection. The biologic distributions and gel chromatographic analyses using carrier Tc-99 and Sn-113 indicate that there is little possibility of mixed metal complexes of the type Tc-Sn-DMS; rather they contain only Tc + DMS. The labeling procedure for Tc-99m DMS as a renal agent proceeds in two steps: a rapid formation of Complex 1, and a slower, rate-determining step from Complex 1 to Complex 2. A reproducible lyophilized kit has been prepared. The yield of Complex 2 greatly depends on the reconstitution volume of $^{99m}TcO_4^-$; yield averages 89% using 2 ml of $^{99m}TcO_4^-$ eluate.

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A rapid growth and use of Tc-99m-labeled radiopharmaceuticals in nuclear medicine requires high-quality compounds as well as rapid and reproducible preparations (*1–3*).

We have mentioned previously that the preliminary study of Tc-dimercaptosuccinate complexes (Tc-DMS) indicates the formation of at least four different types of Tc-DMS (Complex 1 to Complex 4) using carrier Tc-99, and that Complex 2 showed the highest kidney uptake. When the preparation is carried out in acidic solution, Complex 1 and Complex 2 are the main products. Complex 2 (purple complex) is formed by way of Complex 1 (yellow complex), and its formation depends greatly on Tc-99 concentrations and Sn(II)/Sn(IV) ratios. Further, we have proposed that in the usual radiopharmaceutical preparation for renal scanning, Tc-DMS will exist as Complex 2 (*4*). Krejcarek et al. also demonstrated the possibility of the formation of different types of ^{99m}Tc-DMS complexes (*5*).

Unexpected and possibly important problems were encountered during the development of a Tc-99m DMS Complex 2 kit. As a result of the experiments described here, a reproducible lyophilized kit has been developed for the preparation of Complex 2 and the latter evaluated as a renal scanning agent both in vivo and in vitro.

MATERIALS AND METHODS

Chemicals. Tin-113 tracer was obtained commercially and stored under nitrogen gas after electrodeposition on a small helical platinum wire by Brown's method. In this study we used freshly redissolved ^{113}Sn(II) in $\lesssim$0.5 ml of 6M HCl under nitrogen gas. $^{99m}TcO_4^-$ was eluted from ^{99}Mo-^{99m}Tc generator. DMS was also obtained commercially. All the other reagents used in this study were of analytical grade.

Preparation of various Tc-DMS complexes. *Complex 1.* This was prepared by two different methods. In one, a molar ratio of Sn(II)/Sn(IV) < 0.1 was used for the preparation, as reported previously (*4*). The other method was as follows. One milliliter of DMS solution (0.01 M, pH 2.5) was mixed with 0.1 ml of $^{99m}TcO_4^-$, and the mixture was heated in a water bath at 80–90°C for 2 hr. After cooling, the reaction mixture was passed through a 0.45-μm membrane filter to remove any insolubles. Complex 1 prepared by latter method was used for the organ-distribution study.

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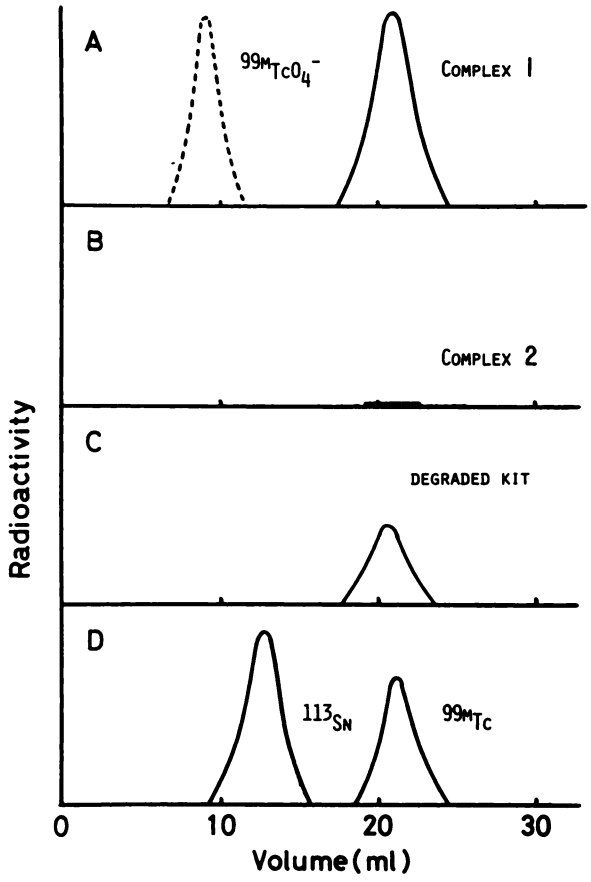

FIG. 1. Gel chromatograms of: (A) $^{99m}TcO_4^-$ and Complex 1; (B) a mixture of Complex 2 (95.1%) and Complex 1 (4.9%), showing that Complex 2 is adsorbed on gel while Complex 1 eluted in 19–22 ml; (C) degraded lyophilized kit containing 27.1% of Complex 1; and (D) $^{99}Tc(^{113}Sn)$-DMS using 1.36 mole of $^{99}TcO_4^-$ as shown in Table 1. Column was eluted with 1.5×10^{-3}M DMS, pH 2.5.

Complex 2. This was prepared by dissolving 7.5 mg of $SnCl_2 \cdot 2H_2O$ in 5 ml of 0.02 M DMS, adjusting the pH to 2.5, then diluting the total volume to 10 ml under nitrogen atmosphere. One-tenth milliliter of $^{99m}TcO_4^-$ eluate was added to 2 ml of the Sn(II)-DMS solution and allowed to stand for 10 min under nitrogen atmosphere.

Complex 3. This was prepared by adjusting the pH of Complex 1 to 9.0 with 1 N NaOH.

Complex 4. Prepared by adjusting the pH of Complex 2 to 9.0 with 1 N NaOH.

The yields of these complexes were determined by gel chromatography with Bio-Gel P-10 and were found to be: Complex 1 96.7%, Complex 2 95.1%, Complex 3 99.0%, and Complex 4 97.8%.

$^{99}Tc(^{113}Sn)$-DMS complexes. These were also derived from freshly prepared Sn(II)-DMS solution. Seven and one-half milligrams of $SnCl_2 \cdot 2H_2O$ were dissolved in 5 ml of 0.02 M DMS and 0.1 ml of ^{113}Sn(II) tracer was added. The pH was adjusted to 2.5 and the total volume was diluted to 10 ml under nitrogen atmosphere. The solution contains Sn(II) and DMS with a 1:3 molar ratio. One-half ml of various concentrations of $^{99}TcO_4^-$ and 0.1 ml of $^{99m}TcO_4^-$ were mixed, then 2 ml of the Sn(II) were added and the solution was allowed to stand for 10 min under nitrogen atmosphere.

Preparation of lyophilized kit. One-tenth gram of DMS was dissolved in 50 ml of 0.1 N NaOH and, to it 1 ml of $SnCl_2 \cdot 2H_2O$ solution containing 41.2 mg in 6M HCl was added under nitrogen atmosphere. The pH of the solution was adjusted to 2.5 and 1M HCl, and the total volume was diluted to 100 ml with distilled water. To each of a series of 10-ml vials, 1 ml of this solution was transferred and the contents freezed-dry. Each vial contains 1 mg of DMS and 0.412 mg of $SnCl_2 \cdot 2H_2O$. A "degraded kit" was prepared by lyophilization of Sn(II)-DMS solution in the above manner but without nitrogen treatment and incubating the kit at 60°C for 3 days.

Analysis. Reduction yields of pertechnetate were determined by thin-layer chromatography. Gel chromatography with Bio-Gel P-10 was used for the analysis of complexes as reported previously (*4*). In this study, rapid analysis using a Bio-Gel P-10 minicolumn (1 × 1 cm) was also used for the separation of Complex 1 and Complex 2. The column was eluted with 6 ml of 15 mM DMS, pH 2.5. In this system, Complex 1 and $^{99m}TcO_4^-$ passed through the gel, whereas Complex 2 was retained. The separation of Complex 1 from free $^{99m}TcO_4^-$ was performed by thin-layer chromatography (TLC) on silica-gel sheets with acetone as the developer. In this system, free $^{99m}TcO_4^-$ runs with an R_f value of 0.95–1.0, whereas Complex 1 remains at the origin.

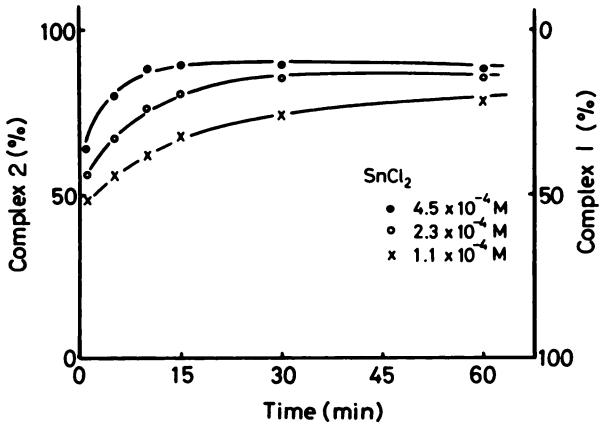

FIG. 2. Formation of Complex 2 as function of time after preparation, showing slow reaction from Complex 1 to Complex 2. Total reaction volume was 2 ml and reaction was performed under nitrogen atmosphere. Molar ratio of Sn(II) to DMS = 1:3.

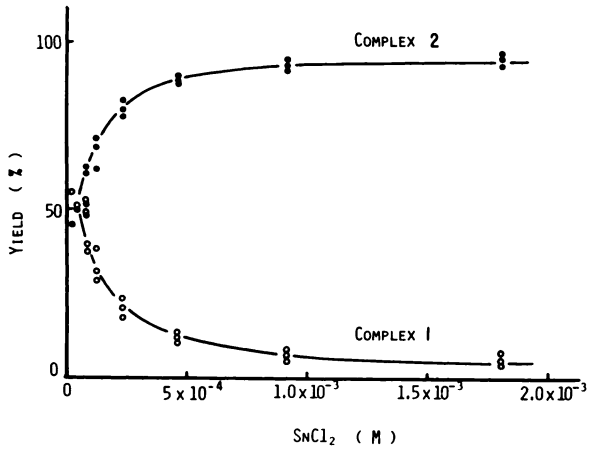

FIG. 3. Effect of $SnCl_2$ concentrations on Complex 2 formation. Free $^{99m}TcO_4^-$ was negligible in all cases. Total reaction volume was 2 ml, reacted for 15 min under nitrogen atmosphere. Molar ratio of Sn(II) to DMS = 1:3.

Animal experiment. One-twentieth milliliter of the complex of interest was injected into the tail vein of d-d mice (*6*) weighing 20–25 g. The animals were subsequently killed at various intervals after injection, and the activities in various tissues were determined in a well scintillation counter by comparison with a standard.

Male Donryu rats weighing 150–200 g were used for scintigraphic study. Five hundred μCi of the complex were injected. The rats were killed 3 hr after injection and whole-body images were obtained using a 7.5 cm (3 in.) NaI(Tl) scintiscanner.

Measurement of Tc-99m and Sn-113 radioactivity. The activity of these nuclides was determined by counting samples in a well scintillation counter 18 hr later to permit re-establishment of ^{113}Sn-^{113m}In parent-daughter equilibrium.

RESULTS AND DISCUSSION

Gel chromatography. Quality control for Tc-99m DMS complexes is an important consideration. Figure 1 shows the typical elution curves of various Tc-DMS complexes using Bio-Gel P-10. Complex 1 was found in one peak, whereas Complex 2 remained on the gel. This retention of Complex 2 (purple complex) was confirmed by spectrometric studies using ^{99}Tc. In Figure 1 the elution curve of $^{99m}TcO_4^-$ is also shown (dashed curve). When Tc-99m DMS complexes were analyzed by paper chromatography using 85% methanol (*7*), both Complex 1 and Complex 2 remained at the origin and only unreacted free $^{99m}TcO_4^-$ could be determined. The analysis by Sephadex G-25 gel chromatography was also unsuccessful because of its interaction with Complex 1 as well as Complex 2 (*8,9*). Thus Bio-Gel P-10 chromatographic analysis is considered more useful for the determination of two different Tc-DMS complexes; the analysis can be performed within 15 min by using TLC and a Bio-Gel P-10 minicolumn.

Effect of $SnCl_2$ concentration. Various concentrations of Sn(II)-DMS solution at pH 2.5 (molar ratio Sn(II):DMS = 1:3) were made under nitrogen atmosphere. One milliliter of each solution was added to 1 ml of $^{99m}TcO_4^-$ eluate (0.5–5 mCi) and allowed to stand under nitrogen atmosphere. Figure 2 shows the formation of Complex 2 as a function of time, under various concentrations of Sn(II)-

TABLE 1. BEHAVIOR OF Sn-113 IN THE COMPLEXES*, AT VARIOUS Tc-99 CONCENTRATIONS

$^{99}TcO_4^-$ (mole)	$^{99m}TcO_4^-$ (ml)	Reduction yield† (%)	Complex 2‡ (%)	Sn-113 adsorbed on gel‡ (%)	Sn/Tc	Kidney uptake§	
						Tc-99m (%)	Sn-113 (%)
136×10^{-8}	0.1	99.3	37.9	2.5	4.85	6.2 (5.1–7.2)	1.5 (1.4–1.6)
68×10^{-8}	0.1	99.9	56.8	11.1	9.71	8.8 (8.2–9.5)	1.5 (1.3–1.7)
34×10^{-8}	0.1	99.6	64.9	10.7	19.4	10.4 (9.4–11.1)	1.7 (1.5–2.2)
8.5×10^{-8}	0.1	99.8	80.6	5.1	77.7	15.3 (14.8–15.8)	1.5 (1.4–1.5)
4.3×10^{-8}	0.1	99.5	82.3	5.3	153	18.0 (16.8–18.9)	1.5 (1.2–1.7)
–	0.1	99.9	91.1	1.1	>>200	21.2 (20.8–21.9)	1.6 (1.3–1.9)

* Sn(II), 6.6×10^{-6} mole; DMS, 2×10^{-5} mole.
† Reduction yield by thin-layer chromatography.
‡ Determined by Bio-Gel P-10 gel chromatography.
§ Kidney uptake 3 hr after injection in mice. Figures in parentheses indicate range (n = 3).

TABLE 2. ORGAN DISTRIBUTION OF VARIOUS Tc-DMS COMPLEXES 3 HR AFTER I.V. INJECTION IN MICE (% DOSE/ORGAN)*

Organ	Complex 1	Complex 2	Complex 3	Complex 4
Blood	0.67	5.83	0.84	2.73
	(0.60–0.80)	(5.50–6.39)	(0.75–0.88)	(2.40–3.21)
Liver	1.01	5.93	0.97	2.33
	(0.77–1.16)	(5.71–6.28)	(0.91–1.05)	(2.18–2.51)
Lung	0.06	0.40	0.07	0.27
	(0.05–0.07)	(0.32–0.60)	(0.06–0.09)	(0.20–0.41)
Kidney	1.20	21.70	0.95	16.50
	(1.01–1.55)	(20.90–22.51)	(0.81–1.02)	(15.63–18.00)
Spleen	0.03	0.20	0.02	0.08
	0.02–0.04)	(–)	(–)	(0.06–0.10)
Stomach	0.33	0.40	0.12	0.40
	(0.10–0.55)	(0.30–0.50)	(0.10–0.13)	(0.22–0.71)
Small intestine	0.07	0.40	0.04	0.17
	(0.03–0.12)	(0.33–0.50)	(0.03–0.05)	(0.12–0.20)
Muscle	1.38	7.33	2.52	5.20
	(0.88–1.41)	(6.44–9.22)	(1.06–3.78)	(4.63–6.35)
Bone	20.5	5.03	17.1	8.87
	(17.2–23.8)	(4.79–5.51)	(10.2–20.8)	(6.42–10.2)

* Blood was assumed to be 7% of body weight, muscle 40%, and bone 10%. Figures in parenthesis indicate range ($n = 3$).

DMS. Unreacted $^{99m}TcO_4^-$ as determined by TLC was negligible; Complex 1 was formed instantly and was then gradually changed to Complex 2. The shift from Complex 1 to Complex 2 proceeds more rapidly as the Sn(II)-DMS concentration is increased, and maximum yield is obtained within 15 min with 4.5×10^{-4}M $SnCl_2$ and 1.35×10^{-3}M DMS. On the other hand, equilibrium was not observed even after 1 hr with 1.1×10^{-4}M $SnCl_2$ and 3.3×10^{-4}M DMS. To permit practical use as a radiopharmaceutical, the labeling time must be kept short. Figure 3 shows the yields of Complex 1 and Complex 2 for a reaction time of 15 min. Complex 2 is formed with high yields in $SnCl_2$ concentrations above 0.5 mM. Furthermore, the same findings could be obtained when various concentrations of $SnCl_2$ (3 to 12 mM) were used along with a constant amount of DMS. Therefore, the radiopharmaceutical kit should be prepared with a fixed volume of $^{99m}TcO_4^-$, so that the concentration of Sn(II)-DMS remains constant, as will be discussed later.

Behavior of Sn-113 in Tc-DMS complexes. In order to establish the behavior of Sn-113 in Tc-DMS complexes, gel chromatography and organ-distribution studies were performed. As shown in Fig. 1D, the radioactivity of Sn-113 was recovered in one peak, and the elution pattern was quite different from that of Tc-99m. It is impossible, however, to discuss the existence of a mixed metal complex using Sn-113 and Tc-99m since the amount of Sn(II) is considerably larger than Tc-99m in the usual radiopharmaceutical preparations. Comparable specific activities of Tc and Sn are required to evaluate the coexistence of the two cations in the complex. Accordingly, various amounts of $^{99}TcO_4^-$ were used with a constant amount of Sn(II)^{113}Sn(II) DMS, so that the molar ratios of Sn to Tc ranged from 4.85 to >>200. Complex 2 yields, and Sn-113 activities in the eluate as well as in the column not eluted, were determined by gel chromatography. The samples were also used for the organ-distribution studies in mice.

Table 1 shows that the formation of Complex 2 and the renal uptake of Tc-99m were both decreased as the concentrations of $^{99}TcO_4^-$ increased. On the other hand, most of the Sn-113 radioactivity passed through the gel and its kidney uptake was lower than that of Tc-99m. Further, if Tc and Sn had coexisted in Complex 1, about 20% of total Sn-113 activity should have appeared in the Complex 1 peak (Fig. 1D); if they coexisted in Complex 2, Sn-113 activity on the gel and its renal uptake should have varied as the Sn/Tc ratios varied. Our experimental results, however, showed no significant evidence for the coexistence of the two cations in either Complex 1 or Complex 2. The findings indicate that there are marked differences in behavior between Tc-DMS and Sn-DMS, both in vivo and in vitro.

Organ distribution of various complexes. Successive scintiphotos of the kidney following injection of Tc-99m DMS showed progressive accumulation of Tc-99m in the renal parenchyma at least up to 3 hr (*7,10*). This indicates that Complex 2 could be used clinically for renal scintigraphy with good-quality

visualization. Table 2 summarizes the organ distributions of various Tc-DMS complexes 3 hr after i.v. injection in mice. It is apparent that Complex 1 and Complex 3 have bone-seeking properties, whereas Complex 2 and Complex 4 have good affinity for the kidney.

Figure 4 shows the scintiscans of the complexes 3 hr after injection in rats. Complex 2 and Complex 4 gave prompt visualization of the kidneys with no interference from other focal accumulations. On the other hand, the scintigrams with Complex 1 and Complex 3 showed prompt visualization of skeleton,

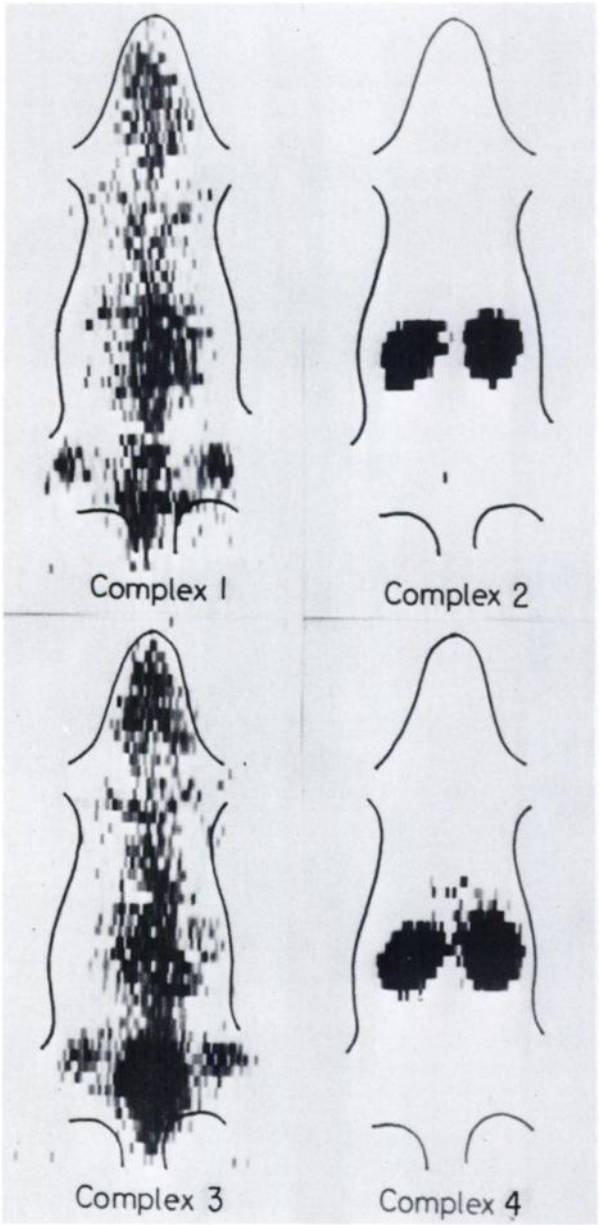

FIG. 4. Scintiscans of four different ^{99m}Tc-DMS complexes 3 hr after injection in rats.

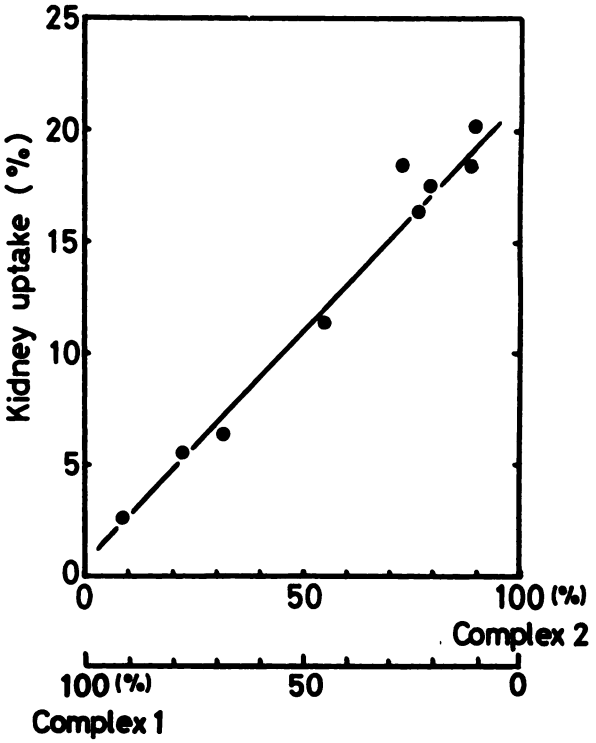

FIG. 5. Relationships between kidney uptake 3 hr after injection in mice, and indicated mixture of Complex 1 and Complex 2.

though the uptake was relatively lower than that of Tc-99m HEDP (*11*) and Tc-99m pyrophosphate (*12*).

In order to verify that Complex 2 has high affinity for the kidney, the renal uptake in mice was determined by injection of Complex 1 and Complex 2 mixed in various ratios. The percentage of Complex 2 in the complexes correlated well with kidney uptake, as is shown in Fig. 5. The plot suggests that a renal uptake of 20–22% in mice can be expected if pure Complex 2 is injected, and this agrees well with the 21.7% renal uptake shown in Table 2.

Krejcarek et al. have pointed out that Tc-99m DMS prepared under acidic conditions concentrates in the kidney, but that preparation at alkaline pH causes more rapid excretion in the urine (*5*). In the light of our data, these two preparations very likely contained mostly Complex 2 and Complex 3, respectively. The possibility of Complex 1 formation, however, must also be considered, since both Complex 1 and Complex 2 are formed under acidic conditions and give quite different organ distributions.

Evaluation of the lyophilized kit as a radiopharmaceutical. When the kit was reconstituted with 2 ml of $^{99m}TcO_4^-$ solution and reacted for 10 min, the yield of Complex 2 was 89.4 ± 1.0% (n = 5),

Complex 1 10.6 ± 1.0%, and negligible free $^{99m}TcO_4^-$. The kit, stored at 2–8°C, had a shelf life of at least 6 mo after preparation (after 6 mo Complex 2 ≃ 85%). The stability of the labeled product after reconstitution depends greatly on the temperature, with Complex 2 decreasing as the temperature rises (Fig. 6). Although a slight decrease of Complex 2 was observed in a few hours, it was stable for at least 24 hr when stored at a temperature below 25°C.

Organ distributions of Tc-99m DMS prepared by the lyophilized Complex 2 kit are shown in Table 3. The general trends of organ distribution in mice indicate that the renal uptake increases with time after injection at least up to 6 hr. After 3 hr 18.9% of the administered dose was found in the kidney, this being slightly lower than the expected uptake (21.7%) shown in Table 2. The result in this study is due mainly to the formation of Complex 1 (≃10.6%) during the preparation by the lyophilized Complex 2 kit. We believe, however, that the kit will be useful in renal scanning because of the high kidney uptake and favorable kidney/blood, kidney/liver, and kidney/muscle ratios. Our organ distributions, however, should not be used for calculations of radiation dose in man, since the renal distributions of various radiopharmaceuticals depend greatly on animal species—being especially different between rodents and non-rodents (*13*). Accordingly, we have based our estimates of human radiation doses entirely on human distribution data, using the methods of the MIRD Committee (*14*). For Tc-99m DMS prepared by the lyophilized Complex 2 kit, the doses in mrad/mCi are: 19 for total body, 674 for the kidneys, and 21 for the ovaries.

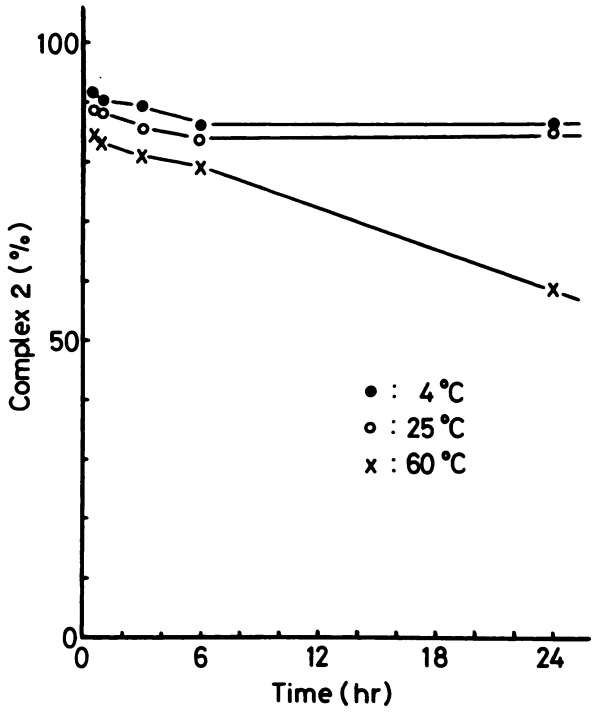

FIG. 6. Stability of Complex 2 stored at various temperatures after preparation. Kit was reconstituted with 2 ml of $^{99m}TcO_4^-$.

Figure 7 shows the formation of Complex 2 as a function of time after reconstitution of the kit. Unreacted free $^{99m}TcO_4^-$ disappeared within a minute. Complex 2 gradually increased with time, while Complex 1 correspondingly decreased. The formation of Complex 2 was greatly dependent on the reconstitution volume of $^{99m}TcO_4^-$. Yokoyama et al. have similarly pointed out the effect of $^{99m}TcO_4^-$ solution volume on the labeling of penicillamine (*3*). Furthermore, effects of Tc-99m concentration on the labeled product have been reported by Smith et al. (*2*). In fact, the yield of Complex 2 is greatly affected by Tc concentration. The calculated technetium concentrations in generator's eluate, however, are much less than 10^{-8} mole, and it follows from Table 1 that effects on Complex 2 formation will be minimal at such concentrations. We believe, therefore, that the results in Fig. 7 are caused neither by $^{99m}TcO_4^-$ concentrations, nor by any impurities in the eluate, but are due mainly to the Sn(II)-DMS concentrations. The unimportance of $^{99m}TcO_4^-$ concentrations in the eluate was further confirmed by finding that no significant difference in labeling yield was observed between the original eluate and its dilution to give $\frac{1}{10}$ the pertechnetate concentration.

When the kit was reconstituted with 2 ml of $^{99m}TcO_4^-$, the concentration of $SnCl_2$ became 9×10^{-4}M, and 88–90% of Complex 2 was obtained after a 15-min reaction (Fig. 7). This yield was slightly lower than the 90–95% expected from Fig. 3. We infer a slight degradation of Sn(II) during the lyophilization. On the other hand, when the kit was reconstituted with 10 ml of $^{99m}TcO_4^-$ (producing 1.8×10^{-4}M $SnCl_2$), the yield of Complex 2 was significantly lower than the expected yield. The experiments of Figs. 2 and 3 were performed with total volume of 2 ml under nitrogen atmosphere. Then, in order to clarify this situation, 9 ml of $^{99m}TcO_4^-$ eluate were added to 1 ml of Sn(II)-DMS solution ($SnCl_2 \simeq 1.8 \times 10^{-4}$M) and the mixture reacted with and without the usual nitrogen atmosphere. As seen in Fig. 7, the formation of Complex 2, exposed to the laboratory air, is similar to that of lyophilized kit reconstituted with 10 ml $^{99m}TcO_4^-$ eluate. On the other hand, the formation of Complex 2 under nitrogen protection, is higher than in the unprotected sample, and the yield agrees well

TABLE 3. ORGAN DISTRIBUTION OF Tc-99m DMS (AS PREPARED BY LYOPHILIZED DMS KIT) AFTER I.V.INJECTION IN MICE

Organ	Percent dose in organ*			
	0.5 hr	1 hr	3 hr	6 hr
Blood	8.9 ± 2.4	5.3 ± 0.6	3.2 ± 0.7	2.1 ± 0.4
Liver	4.6 ± 1.2	4.2 ± 0.3	3.5 ± 0.3	3.6 ± 0.4
Lung	0.8 ± 0.3	0.6 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
Kidney	12.4 ± 3.2	16.6 ± 2.5	18.9 ± 2.2	20.5 ± 2.1
Spleen	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Small intestine	0.4 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.2 ± 0.1
Muscle	8.3 ± 2.2	7.3 ± 1.8	4.7 ± 1.2	3.2 ± 0.6
Bone	12.4 ± 3.7	11.4 ± 6.8	9.7 ± 4.7	5.3 ± 1.0
Feces	<0.1	0.1 ± 0.1	0.3 ± 0.2	0.2 ± 0.1
Urine	20.9 ± 2.5	33.1 ± 4.9	39.6 ± 5.6	40.7 ± 4.1
	Ratios†			
Kidney/blood	7.3	12.6	32.8	61.4
Kidney/liver	11.7	14.8	22.2	24.8
Kidney/muscle	44.8	60.7	128	198

* Nine mice in each group; average ±1 s.d. Blood was assumed to be 7% of body weight, muscle 40%, and bone 10%.
† Ratios derived from percent dose/g body weight.

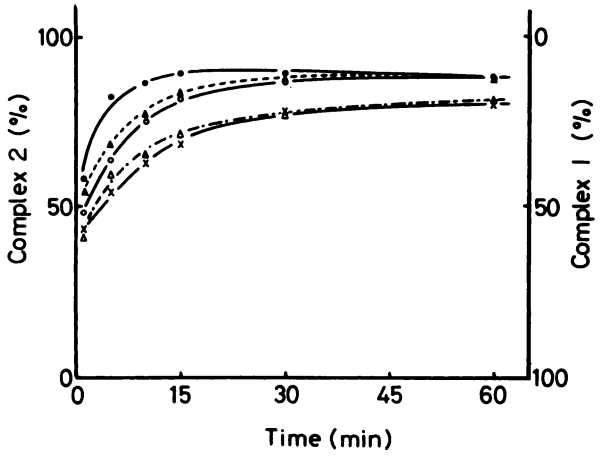

FIG. 7. Formation of Complex 2 as function of reaction time from the lyophilized kit, reconstituted with 2 ml (●), 5 ml (○), and 10 ml (×) of $^{99m}TcO_4^-$ eluate. The Complex 2 was formed from Sn(II)-DMS solution, with 10 ml of reaction volume ($SnCl_2$ concentration corresponds to lyophilized kit reconstituted with 10 ml), and reacted with nitrogen gas bubbling (▲) and without nitrogen treatment (△).

with the results shown in Figs. 2 and 3. These findings indicate that the formation of Complex 2 is greatly affected by oxygen in the $^{99m}TcO_4^-$ eluate and that the preparation of Tc-99m DMS for renal imaging (Complex 2) from the lyophilized kit should be reconstituted with a small portion of $^{99m}TcO_4^-$ and should not be used for at least 10 min after reconstitution. Although this problem can be partially resolved by using larger amounts of $SnCl_2$ and DMS, as shown in Fig. 3, we can not neglect the influence of Sn(II) on the patient (*15*).

Our findings demonstrate that the labeling reaction of Tc-99m DMS for renal imaging proceeds in two steps: rapid formation of Complex 1, followed by a slower, rate-determining step from Complex 1 to Complex 2, the latter being greatly affected by oxygen. Further, when the degraded kit was used, the contamination with Complex 1 was greatly increased, as shown in Fig. 1C. This indicates that the preparation of Tc-99m DMS for renal imaging (Complex 2)—even if done with a commercial kit —may involve contamination of the product with Complex 1 to some extent, depending on the labeling conditions. Therefore, for consistent high-quality renal images the Complex 2 yield should be carefully controlled and the yields of other complexes having the different biologic distributions should be determined in order to avoid misinterpretation of the results.

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