Chemical and Biological Characterization of Different Tc Complexes of Cysteine and Cysteine Derivatives

Bernd Johannsen, Rosemarie Syhre, Hartmut Spies, and Rudolf Münze

Central Institute of Nuclear Research, Rossendorf, German Democratic Republic

The labeling of cysteine and its derivatives (penicillamine, N-acetylcysteine, cysteine ethyl ester) with $^{99}Tc(^{99m}Tc)$ was studied as a model for the radiopharmaceutical preparation of Tc-99m mercaptide complexes. After the use of TcO_4^-, $TcOCl_5^{2-}$, and $TcBr_6^{2-}$ as labeling agents for the Tc oxidation states VII, V, and IV, respectively, complexes of Tc(V) and Tc(IV) were prepared and characterized. The biologic behavior of these complexes was studied in rats. The substitutions in cysteine are responsible for substantial changes of net charge and lipophilicity in the Tc complexes, and the consequences on the excretion patterns in Wistar rats are discussed.

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Several investigators (*1–9*) have labeled sulfhydryl-containing amino acids, carbonic acids, and heterocyclic compounds with Tc-99m as a new approach to radiopharmaceutical design. Some of these new Tc mercaptide complexes proved to be suitable scintigraphic agents. The published labeling procedures, however, differ in many details and seem to influence strongly the in vivo behavior of the radiopharmaceuticals. This is most obvious in the case of Tc-99m DMSA (*10*) and Tc-99m penicillamine preparations. As recently reported by Yokoyama et al. (*11,12*), several different labeling products of penicillamine can be observed to occur with slight changes in the labeling conditions.

As a consequence, more insight into the labeling reactions of Tc mercaptide complexes, and a chemical characterization of them, should be provided. For a study of the labeling process and the relationship of chemical structure to biologic behavior, the choice of cysteine as ligand molecule seems to be advantageous because this amino acid is a physiologic compound and lends itself to substitution at different positions in the molecule. We have explored the chemical and biologic behavior of $^{99}Tc(^{99m}Tc)$ complexes of cysteine, and of ligands that are derived from cysteine, by introducing a two-carbon residue at three different positions within the parent molecule, thus obtaining N-acetylcysteine, penicillamine, and cysteine ethyl ester.

MATERIALS AND METHODS

Cysteine hydrochloride, penicillamine, and N-acetylcysteine were commercial products, whereas cysteine ethyl ester has been synthetized (*13*).

Pertechnetate. $^{99m}TcO_4^-$ in saline solution (5–10 mCi/ml) was obtained by elution of commercial generators. Dried or high-activity pertechnetate was prepared by methylethylketone (MEK) extraction of the saline eluate and evaporation of the MEK. $^{99}TcO_4^-$ was obtained commercially as a solution of NH_4TcO_4. Before starting carrier experiments, the two pertechnetate analogs were mixed.

$TcOCl_5^{2-}$. In a chilled vial, 35 μmole dried $^{99m}TcO_4^-/^{99}TcO_4^-$ are added to 0.5–2 ml cold concentrated HCl. The solution is kept at 4°C under nitrogen. The purity is checked by uv spectroscopy (λ_{max} at 294 and 228 nm) (*14*).

$TcBr_6^{2-}$. Two milliliters of concentrated HBr are added twice to 35 μmole of mixed pertechnetate,

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For reprints contact: B. Johannsen, Zentralinstitut für Kernforschung, Akademie der Wissenschaften der DDR, Rossendorf, GDR.

with evaporation to dryness after each addition. HBr (0.5 ml) is then added again and the solution is kept under nitrogen. The quality control is performed as in the case of $TcOCl_5^{2-}$ [λ_{max} at 443, 385, 325, 287 nm (*15*)].

Labeling experiments. Studies were carried out in order to define appropriate labeling conditions and to assess the kinetics of the labeling reactions. At various pH values, temperatures, and concentrations of ligands and Tc as specified below, TcO_4^- was reduced and chelated under nitrogen, both in the presence and absence of $SnCl_2$.

Preparation of Tc(V) complexes. The preparation of Tc(V) complexes of cysteine and cysteine derivatives is achieved: (a) by reduction of TcO_4^- with the aid of the thiol compound itself if the kinetics of the labeling process permits the use of suitable conditions; or (b) more directly by reaction of a reactive Tc(V) species [$TcOCl_5^{2-}$ or Tc(V)-Sn citrate] with the ligand.

The Tc(V) cysteine is prepared as follows:

1. A mixture of 35–40 μmole $^{99m}TcO_4^-/^{99}TcO_4^-$ in 0.2 ml saline, and 100 μmole cysteine hydrochloride in 0.1 ml water is kept at 25°C for 40 min under nitrogen (pH ≈ 1). The yellow Tc complex is separated from free pertechnetate (60% TcO_4^- after 40 min) on Bio-Gel P-2, exactly as described below for analytical gel chromatography. The complex is eluted with 20–25 ml saline.

2. To the solution of 35 μmole $TcOCl_5^{2-}$ is added a chilled, freshly prepared solution of 70 μmole cysteine hydrochloride in 1 ml 2 N NaOH. After 1 min the complex is purified on Bio-Gel P-2, as described above.

For spectroscopic studies, Tc(V)-Sn citrate (*16*) is used instead of $TcOCl_5^{2-}$. To 10 μmole TcO_4^- in 10 ml 0.5 M sodium citrate (pH 6.5) are added 10 μmole $SnCl_2 \cdot 2H_2O$ in 0.1 N HCl. After verification of the absence of TcO_4^- by TLC (cellulose, methanol-water 85:15, R_f Tc(V)-citrate = 0.05 — 0.1, R_f TcO_4^- = 0.7 — 0.8) sequential amounts of 10 m*M* cysteine solution are added to aliquots of this pale blue-green solution of Tc(V) citrate.

A yellow Tc-penicillamine complex was prepared from $TcOCl_5^{2-}$ and penicillamine by the same procedure, as described for Tc(V) cysteine.

A complex of Tc(V) with cysteine ethyl ester could not be prepared in a similar way because the product is decomposed under the conditions used. A suitable way to prepare a yellow Tc complex is by the direct reduction of pertechnetate with cysteine ethyl ester in ethanolic solution. Thus, a solution of 125 μmole cysteine ethyl ester in 0.5 ml absolute ethanol is added to a solution of 37 μmole TcO_4^- in 0.5 ml ethanol. After 30 min at 25°C the reaction is stopped by adding 2 ml of cold 0.9% NaCl solution and the product is purified on CM-Sephadex C-25 using stepwise elution with aqueous NaCl solution. The free ligand is removed by 20 ml 0.9% NaCl, and the yellow complex is then eluted with 20–35 ml 1.8% NaCl.

Preparation of Tc(IV) complexes. Green or red Tc-99 complexes of cysteine and cysteine derivatives are prepared both by reduction of pertechnetate with an excess of ligand in neutral or alkaline solution, and by reaction of $TcBr_6^{2-}$ with the ligand.

To the solution of 35 μmole $TcBr_6^{2-}$ is added a solution of 570 μmole cysteine hydrochloride, 580 μmole N-acetylcysteine, or 2 mmole penicillamine, in 1 ml N NaOH. After 1 min the complexes are purified on Bio-Gel P-2.

The green Tc complex of cysteine is also prepared by reaction of pertechnetate with an excess of cysteine at pH 7.

A red Tc complex of cysteine is obtained when the solution of 35 μmole $TcBr_6^{2-}$ is mixed with a solution of only 70 μmole cysteine hydrochloride in 1 ml 2 N NaOH. After 1 min the complex is purified on Bio-Gel P-2.

Polymer. A mixture of 30–50 μmole $^{99m}TcO_4^-/^{99}TcO_4^-$ in 0.2 ml saline and a solution of 100 μmole cysteine in 0.1 ml water is kept at 30–40°C for 2 hr at pH ≈ 1. The reaction mixture at this point consists of polymer, the yellow complex, pertechnetate, and reduced hydrolyzed Tc. Bio-Gel P-2 is used to fractionate the mixture, exactly as described below for analytical gel chromatography. The polymer fraction is eluted with 10–13 ml saline solution ($V_e \approx V_o$: V_e = elution vol; V_o = void vol), the yellow complex with 20–25 ml, and pertechnetate with 35–45 ml. The radiochemical purity is determined by TL chromatography on silica gel (solvent water: R_f for polymer = 0.8; solvent MEK: polymer remains at the origin).

Analytical methods. Gel chromatography was performed on Bio-Gel P-2 (column dimensions: 1 × 26 cm), both in a conventional manner and with the GCS technique introduced by Persson et al. (*17*). Saline was used as eluant. Experimental details have been published previously (*18*). Labeling yields and radiochemical purities were studied using thin-layer chromatography on SILUFOL strips (Czechoslovakia), silica gel, and cellulose strips, with (a) MEK, (b) water, or (c) methanol-water (90:10 or 85:15), as solvents. Ultraviolet and visible spectra were registered with a Beckman DK 2 spectrophotometer (England). The electrophoretic behavior of the Tc compounds, in comparison with pertechnetate and cysteine, was studied using acetate, phosphate or

TABLE 1. SCHEMATIC REPRESENTATION OF THE STEPS INVOLVED IN THE LABELING PROCESS OF CYSTEINE WITH TECHNETIUM

Tc oxidation state	Labeling agents		Products		By-products	
VII	TcO_4^-					
			a			
V	$TcOCl_6^{2-}$	d	Complex I			
			b			
		e		c		
IV	$TcBr_6^{2-}$	→	Complex II	→ Complex III	Polymer	TcO_2
		f				

a) in acid, neutrol or alkaline solution when TcO_4^- is reduced whether by cysteine or by stannous ions without excess of Sn
b), c) especially in neutral or alkaline solution when reaction a) proceeds
d) in strong HCl solution, Tc:cys ratio = 2
e) in strong HBr solution, Tc:cys ratio = 2
f) in strong HBr solution, Tc:cys ratio > 2

glycine buffers, ionic strength 0.1, 1000 V, FN 4 paper (GDR), 1 hr.

The water-octanol partition coefficients for the radioactive compounds, whose purity was verified, were determined. Aliquot portions of less than 1 ml of the material being tested were diluted with distilled water to 5 ml. The solution in each vial was treated separately with NaOH or HCl to adjust to the desired pH within a range of 3–9. Mixture of 5 ml of the pH-adjusted aqueous solutions and 5 ml of 1-octanol were shaken under nitrogen at room temperature for 20 min. After centrifugation, aliquots of each phase were withdrawn and counted.

Sulfhydryl-determination in the labeling mixture was performed polarographically at −90 mV vs. SCE in saline solution, and at −450 mV in 0.1 N NaOH.

In vivo distribution study. The in vivo distribution was determined in male Wistar rats (150–200 g) by injecting into the tail vein 0.1–0.5 ml of the $^{99m}Tc(^{99}Tc)$ compound at a concentration range of 0.1–5 mg/kg body weight. At least four rats were used for each data point. The rats were killed at times ranging from 5 min to 3 hr postinjection. The kidneys, bladder, liver, intestines, heart, spleen, stomach, and blood (obtained by heart puncture) were wet-weighted and counted. For the blood clearance curve, blood was continuously withdrawn from the tail vein.

RESULTS

The reaction of cysteine with TcO_4^-. The labeling of cysteine with $^{99}TcO_4^-$, without reducing agents like stannous ions, proceeds via a yellow complex (Complex I) and a red intermediate complex (Complex II) to a green complex (Complex

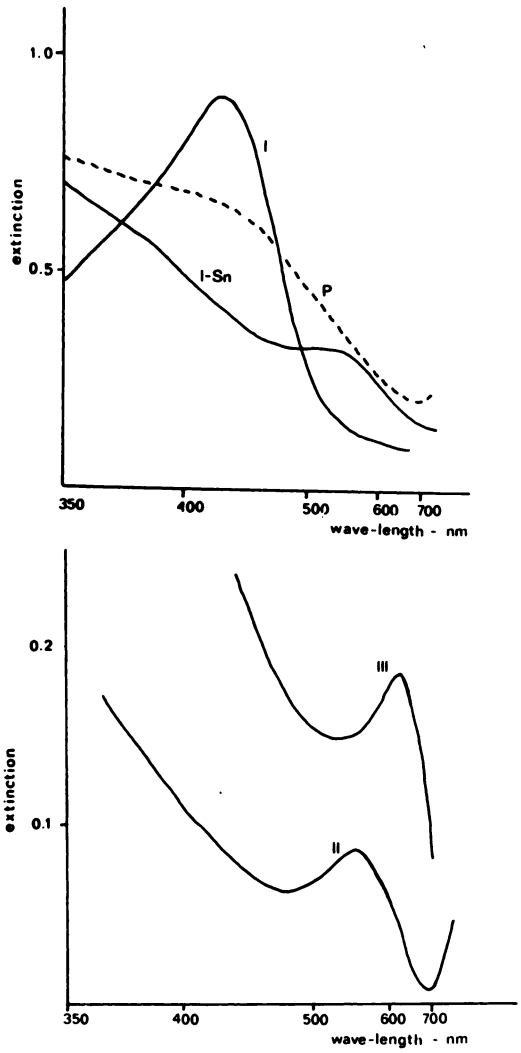

FIG. 1. Absorption spectra of purified labeling products: I = aqueous solution of Complex I, I-Sn = Sn-reduced Complex I, II = Complex II, III = Complex III and P = polymer.

III). The corresponding Sn-free reaction is considerably slower than the rapid labeling of cysteine in the presence of stannous ions. For example, the pertechnetate concentration in 0.33 *M* cysteine (pH $\approx$ 1, 25°C, $c_{Tc} = 0.12$ *M*) decreased in 40 min only to 60%, whereas the reduction by Sn(II) ions is complete within a few minutes. At low concentrations of pertechnetate ($c_L >> c_{Tc} = 10^{-9} - 10^{-3}$ m*M*), the decrease of TcO_4^- can be expressed as a first-order reaction with $k = 4.3 \cdot 10^{-4}$ sec^{-1} ($c_{cysteine} = 0.33$ *M*, T = 25°C, pH = 1.3). The decomposition constant of the yellow Tc-cysteine complex is approximately $5 \cdot 10^{-5}$ sec^{-1}. In acid solution, Complex I exists as the favored product, but in neutral or alkaline media the intermediate steps were quickly passed, so that the green complex (Complex III) appeared as the predominant labeled product.

Reduced hydrolyzed Tc and a polymer are formed as by-products. The former compound moves neither on Bio-Gel nor on silica-gel layers in water, whereas the polymer migrates on Bio-Gel P-2 almost with the void volume ($V_e \approx V_o$), and on silica-gel layers in water with an R_f of 0.8. The polymer remains at the origin when MEK is used as solvent. The polymer can therefore easily be distinguished from pertechnetate, the Tc complexes, or reduced hydrolyzed Tc.

The chromatographic analysis of the labeling mixture in procedure 1, described above (pH $\approx$ 1, $c_L =$ 0.33 *M*, $c_{Tc} = 0.12$ *M*), indicated the absence of by-products within the first 40–45 min of reaction. A decrease of 2.8 mole-SH per mole TcO_4^- was found. During the further reaction an increasing amount of by-products were formed.

Preparation, purification, and chemical characterization of Tc-cysteine complexes. Complexes I, II, and III were obtained as schematically indicated in Table 1. The complexes and the polymer were purified on Bio-Gel P-2 columns. The radiochemical purity was consistently greater than 98%, as judged by TL chromatography in the solvent systems described and by GCS analysis. The free ligand was not separated from the labeling product.

The absorption spectra of the purified complexes and the by-products are shown in Fig. 1. For Complexes I, II, and III, characteristic absorption maxima in the visible range of the spectrum were registered at 420 nm ($\epsilon = 2100$), 540 nm ($\epsilon = 260$), and 620 nm ($\epsilon = 480$), respectively. The data in Fig. 1 indicated that Complex I was destroyed by stannous chloride added to the purified complex. In contrast, the other products were stable in the presence of Sn(II) ions.

Titration of pertechnetate with stannous chloride solution at pH 1 in the presence of an excess of cysteine at low concentration yielded increasing amounts of yellow complex (increasing extinction at 420 nm) up to a molar Sn/Tc ratio of 1:1 (Fig. 2). Excess stannous chloride up to a molar ratio of about 1:1.5 decreased the extinction. Chromatographic data and water-octanol partition coefficients are listed in Table 2. The electrophoretic migration of Complex I corresponded to that of a carbonic acid—i.e. an anionic behavior down to a pH of approximately 2. In contrast, Complex III proved to be an amphoteric compound with the amino and carboxyl groups of the ligand molecule free. Above the isoelectric point of about 2.5, additional negative charge due to the chelation of Tc contributes to the negative net charge.

Chemical characterization of Tc complexes of cysteine derivatives. Two groups of Tc complexes of cysteine derivatives were obtained, one group analogous to Tc cysteine Complex I and the other to Tc-cysteine Complex III.

Yellow Tc-99 complexes of penicillamine and cysteine ethyl ester belong to the group whose parent complex is the Tc-cysteine Complex I. At the start, with pertechnetate in aqueous solution, the labeling process of cysteine ethyl ester passes the first step quickly without sufficient steady-state concentration of the yellow complex. The different kinetics in water and ethanol permit a preparation of the complex in ethanolic solution.

Technetium-99m-labeled products of penicillamine, cysteine ethyl ester, and N-acetylcysteine could be obtained analogous to the Tc-cysteine Complex III. The preparation of a green Tc-N-acetyl-cysteine complex did sometimes fail, resulting in the formation of polymer.

Some analytical data for the complexes purified

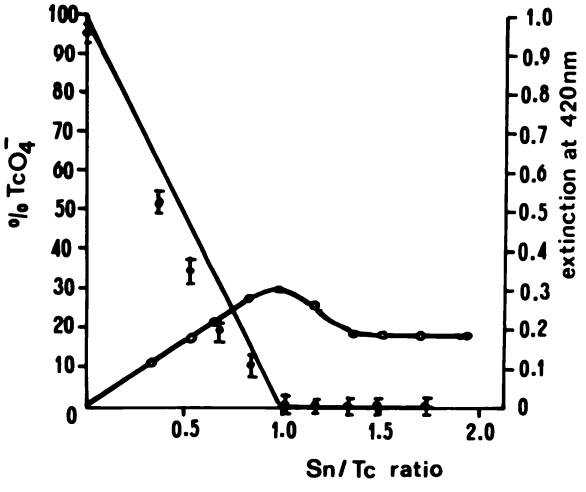

FIG. 2. Titration of TcO_4^- in cysteine solution with $SnCl_2$ ($c_{TcO_4^-} = 0.23 \cdot 10^{-3}$ *M*, $c_L = 0.063$ *M*).

TABLE 2. ANALYTICAL DATA OF PURIFIED LABELING PRODUCTS OF CYSTEINE

			Tc cysteine				
			Complex I	Complex II	Complex III	Polymer	TcO_4^-
V_e (ml)*			22–27	28–33	25–30	11–16	35–45
R_f†	(A)		0.9	0.9	0.9	0.9	0.9
	(B)		0.8	0	0	0	0.8–0.9
$\ln \frac{a_w}{a_o}$‡	at pH	1	3.2	6.5	7.5	5.4	—
		3	3.5	6.9	7.1	5.9	5.4
		7	6.8	7.8	8.6	7.2	6.5

* V_e is the elution volume on Bio-Gel P-2 columns (1 × 26 cm).
† R_f values were obtained on Silufol-TL-plates with (A) water and (B) methanol-water 9:1 as eluant.
‡ Natural logarithm of the water-octanol partition coefficients.

by gel chromatography are shown in Table 3. The electrophoretic migration as a function of pH is shown in Fig. 3. The yellow complex of cysteine ethyl ester did not migrate, and the yellow complexes of cysteine and penicillamine behave identically.

In vivo distribution. The labeling products of cysteine purified by gel chromatography did not exhibit important differences in their in vivo distribution in rats. There was no specific accumulation in a special organ. Blood elimination and cumulative urinary excretion are compared in Fig. 4. Figure 5 shows the percentages of renal and hepatobiliary excretion (concentration in kidney and urine in comparison with concentration in liver, gut, and feces) of the Tc complexes of cysteine derivatives together with those of the two parent cysteine complexes (Complexes I and III). In Tables 4 and 5 the concentrations of the complexes in kidney, liver, blood, urine, and gut are listed.

The color of the urine corresponded with that of the injected Tc compound. When the urine of rats given $^{99}Tc(^{99m}Tc)$-cysteine Complex I was reinjected into the animals, similar distribution and excretion patterns were observed. Urinary samples also exhibited R_f values similar to those of the injected compounds when tested by TLC on silica gel in methanol-water (85:15).

DISCUSSION

The chemical properties of the transition element technetium suggest the ability of Tc to be coordinated at different oxidation states, whereby complexes with different compositions and configurations may be formed at defined oxidation levels. As shown in Table 1, different Tc complexes occur during the labeling of cysteine with Tc-99 in addition to a "polymer" ($V_e \approx V_o$ on Bio-Gel P-2) and reduced hydrolyzed Tc. Comparative studies on the labeling of systematically varied mercapto ligands verify the occurrence of at least two groups of Tc mercaptide complexes, which may be considered as derived from the corresponding parent compounds Tc cysteine Complex I and Tc cysteine Complex III.

In order to ensure valid conditions, Tc-99m was simulated by carrier Tc-99, though we must realize that reactions at the carrier-free level may differ

TABLE 3. ANALYTICAL DATA FOR Tc-99 COMPLEXES OF CYSTEINE DERIVATIVES

			Penicillamine		Cysteine ethyl ester		N-acetylcysteine
			Tc(V)	Tc(IV)	Tc(V)	Tc(IV)	Tc(IV)
Ve (ml)*			21–25	19–23	—	18–26	21–27
R_f†	(A)		0	0		0	0
	(B)		0.7	0.7	0.5		0.7
	(C)		1.0	0.7			1.0
	(D)		0.7	0.7			0.7
$\ln \frac{a_w}{a_o}$‡	at pH	3	0.3	0.9	1.3	3.1	7.2
		5	3.0	1.0	0.9	1.9	4.7
		8	4.8	4.3	2.1	0.1	9.8

* V_e is the elution volume on Bio-Gel P-2 columns (1 × 26 cm).
† R_f values were obtained on Silufol-TL-plates with (A) methyl ethyl ketone, (B) ethanol, (C) water, or (D) methanol-water 9:1, as eluants.
‡ Natural logarithm of the water-octanol partition coefficients.

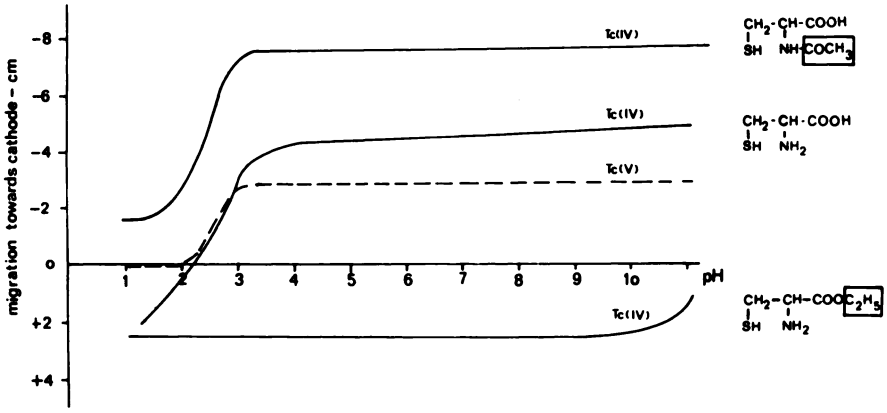

FIG. 3. Electrophoretic migration distances of Tc complexes of cysteine, penicillamine, N-acetylcysteine, and cysteine ethyl ester (acetate, phosphate and glycine buffers, ionic strength 0.1; 1000 V, 1 hr).

from those of Tc-99 in some respects. This implies that the results obtained can be compared with results on Tc-99m preparations only with some reservations.

Cysteine can fulfill the functions of a reducing agent and that of the ligand of Tc. Obviously, the rate of the complexing reaction seems to be faster than the rate of reduction, since no ligand-free Tc(V) compounds are formed. The reduction rate can be diminished by decreasing the concentration of cysteine, which offers the possibility of determining the formal oxidation state of Tc in the complex by Sn(II) titration. Richards et al. (*19,20*) have succeeded in establishing the oxidation states V, VI,

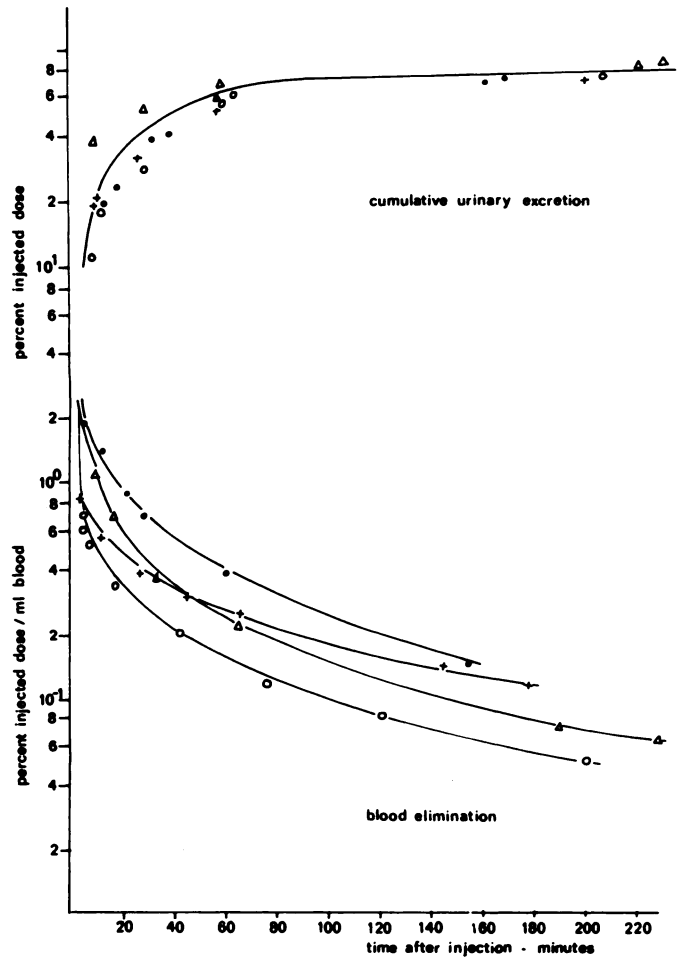

FIG. 4. Blood clearances and cumulative urinary excretion of purified labeling products in rats [Complex I (-··-), II (-+-), III (-o-), and Tc polymer (-△-)].

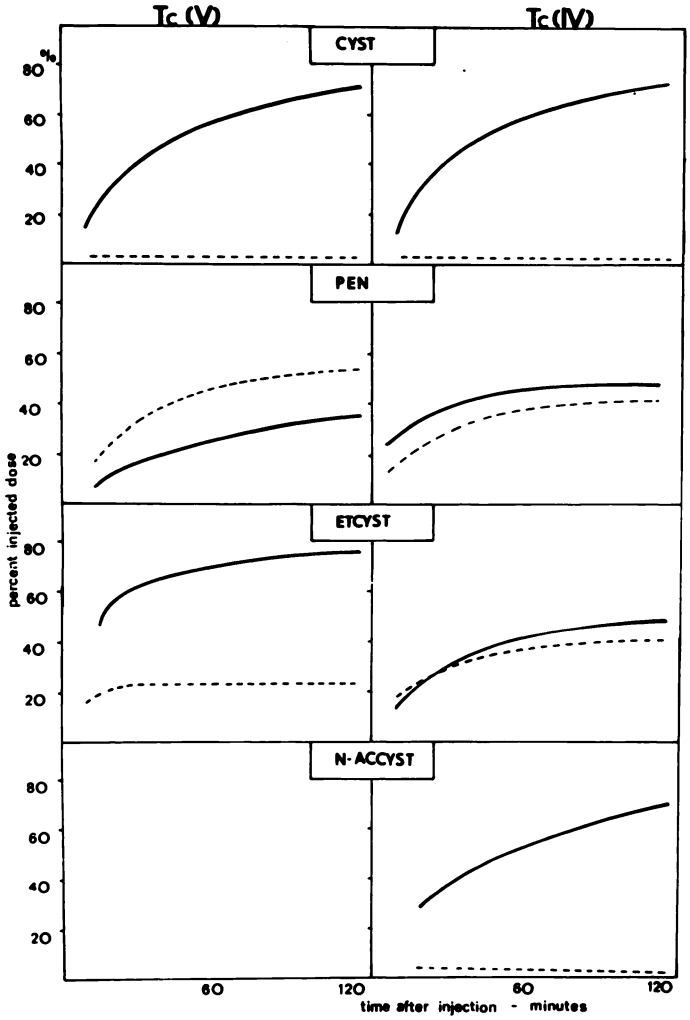

FIG. 5. Excretion of Tc-99 complexes of cysteine, penicillamine, cysteine ethyl ester, and N-acetylcysteine, in rats (solid lines = renal excretion; dashed lines = hepatobiliary excretion).

and III of some Tc-99 complexes prepared under defined conditions. The usual oxidation state in most radiopharmaceuticals seems to be IV. According to the analytical data we obtained in the Sn(II)-titration experiment, cysteine chelates Tc in the yellow complex (Complex I) at the formal oxidation state V. The alternative preparation of the yellow complex from a Tc(V) species [$TcOCl_5^{2-}$ or Tc(V) citrate] supports this conclusion. The ^{99}Tc(V)-mercaptide complexes are stable for weeks, even in the presence of atmospheric oxygen or in solution with low excess concentrations of ligand. The decomposition rate of the yellow Tc-cysteine complex in cysteine solution at pH $\approx$ 1 is approximately one-tenth that of its formation rate. Thus, Complex I appears to be the predominant labeling product in acid solution under appropriate conditions. The pertechnetate disappearance rate proved to be independent of the pertech-

TABLE 4. DISTRIBUTION OF LABELING PRODUCTS OF CYSTEINE IN RATS AT 1 HR POSTINJECTION

	^{99}Tc-cysteine			
Organ	Complex I (n = 5)	Complex II (n = 4)	Complex III (n = 5)	Polymer (n = 6)
Kidney*	3.4 ± 0.4	0.7 ± 0.2	0.5 ± 0.1	2.3 ± 0.5
Liver*	0.2 ± 0.05	0.1	0.1	0.1
Blood*	0.5 ± 0.1	0.1	0.3	0.2
Urine†	53.2 ± 2.5	55.8 ± 2.0	56.2 ± 2.5	65.1 ± 2.5
Gut†	1.7 ± 0.2	1.0 ± 0.1	0.6 ± 0.1	1.2 ± 0.2

* In % dose per gram organ or ml blood.
† In % dose per organ.

TABLE 5. DISTRIBUTION OF Tc-99 COMPLEXES OF CYSTEINE, N-ACETYLCYSTEINE, PENICILLAMINE, AND ETHYL ESTER IN RATS AT 2 HR POSTINJECTION

Organ	Tc oxidation state	Ligand: Cysteine [Tc(V): n = 6] [Tc(IV): n = 5]	Ligand: N-acetylcysteine [Tc(IV): n = 6]	Ligand: Penicillamine [Tc(V): n = 5] [Tc(IV): n = 5]	Ligand: Cysteine ethyl ester [Tc(V): n = 6] [Tc(IV): n = 4]
Kidney*	V	2.0 ± 0.3	—	0.8 ± 0.2	20.4 ± 3.1
	IV	0.5 ± 0.1	1.0 ± 0.1	1.7 ± 0.2	0.9 ± 0.1
Liver*	V	0.5 ± 0.1	—	2.5 ± 0.2	0.8 ± 0.3
	IV	0.1	0.1 ± 0.05	0.9 ± 0.1	0.2 ± 0.1
Blood	V	0.2	—	0.1	0.7 ± 0.2
	IV	0.2	0.1	0.1	0.2
Urine†	V	70.0 ± 2.8	—	34.8 ± 1.2	46.6 ± 2.6
	IV	69.7 ± 3.0	70.4 ± 3.0	35.6 ± 1.4	39.4 ± 2.1
Gut†	V	0.5 ± 0.2	—	25.9 ± 1.1	16.6 ± 0.8
	IV	0.6 ± 0.3	0.6 ± 0.2	34.5 ± 1.6	34.8 ± 1.4

* In % dose per gram organ or ml blood.
† In % dose per organ.

netate concentration. This implies that the results obtained at the carrier level are applicable to the kinetic behavior of carrier-free Tc-99m systems.

Whereas in acid solution Complex I exists as the favored product, in neutral or alkaline solutions of cysteine and TcO_4^- the reaction proceeds via a red species (Complex II) to the green Complex III. Like excess ligand, stannous chloride reduces the Tc(V) complex to products of Tc(IV). This can be seen in Fig. 2. The extinction at 420 nm changes only up to a molar Sn/Tc ratio of about 1:1.5, which corresponds to the formal oxidation state IV. The postulation of the oxidation state IV is also supported by the alternative preparation route of the corresponding complexes and polymer using $TcBr_6^{2-}$.

The labeling of cysteine and cysteine derivatives with Tc results in products whose physicochemical properties differ substantially from those of the free ligands. There is an unavoidable increase in the molecular weight and a change of the net charge. The free parent ligand, cysteine, has a negligible net charge in the pH range of 3–7. Chelation of Tc with cysteine results in products with anionic behavior at physiological pH (Fig. 3). The net charge of the labeling products can be regulated by substitutions in the ligand: in the case of N-acetylcysteine the modification causes an increased anionic migration. Ethylation of cysteine produces a negligible electrophoretic migration of the Tc(V) complex, and cationic behavior of the Tc(IV) complex. Therefore, the use of suitable metabolic derivatives, instead of the corresponding unsubstituted metabolites, enables one to adjust the charge produced by chelation and to prepare a certain Tc complex with the desired net charge.

Lipophilicity is another important factor determining the biologic fate of the complexes. For example, Yokoyama et al. (*21*) assumed the lipophilic character of the uncharged Tc-mercaptid complex, ^{99m}Tc(Sn)-kethoxal-bis(thiosemicarbazone), to be responsible for its rapid biliary excretion. The lipid solubility of the Tc-cysteine complexes, as reflected in the water-octanol partition coefficients, is very low. Whereas N-acetylation of the ligand does not increase it, the introduction of two methyl groups in the ligand molecule (penicillamine) contributes much to the lipid solubility. Ethylation of the carboxyl group causes both elimination of the ionic group in the Tc(V) complex and introduction of a hydrophobic moiety (Table 3).

Such a directed alteration of the net charge and lipophilicity—by substitution in the ligand molecule without loss of the Tc binding affinity—is suggested as another area of derivative approach to overcome the problems associated with the labeling of metabolites or drugs. Promising attempts at alternative derivatization recently has been started; Eckelman and Levenson (*22*) discuss the use of chelates containing derivatives in order to produce a high Tc-binding affinity or to avoid undesired alterations of the in vivo behavior.

Firnau (*23*) has recently published an interesting attempt to adapt known pharmacologic rules to the specific case of hepatobiliary Tc-99m compounds. However, unless the chemical properties of the Tc compounds are confirmed, conclusions are difficult to draw. The systematically altered properties of the Tc complexes of mercapto compounds are reflected in their excretion patterns (Fig. 5, Table 5), thus providing valid information on the interrelationship of physicochemical properties and excretion routes of Tc-99m radiopharmaceuticals.

The two parent cysteine complexes are quickly excreted by the kidneys without any important extra-

renal help. Owing to the blockade of functional groups or the introduction of alkyl groups in the cysteine molecule, the hepatobiliary excretion of the complexes increases substantially if the lipophilic character of the complexes is raised by modification of the ligand. This is obvious in the case of penicillamine and cysteine ethyl ester, whereas the acetyl group in N-acetylcysteine neither increases the lipid solubility nor the hepatobiliary excretion.

FOOTNOTE

* Radiochemical Centre, Amersham.

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