Technetium-99m-Kethoxal-bis(thiosemicarbazone), an Uncharged Complex with a Tetravalent ^{99m}Tc State, and Its Excretion into the Bile

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Technetium-99m-kethoxal-bis(thiosemicarbazone) complex (^{99m}Tc-KTS), a potentially useful radiopharmaceutical for cholescintigraphy, is prepared by the reaction of KTS with $^{99m}TcO_4^-$ in the presence of stannous chloride. Under the described conditions, a complex extractable with organic solvent was detected. Thin-layer chromatography, electrophoresis, and spectrometric analysis indicate the formation of an uncharged ^{99m}Tc(IV) complex. Organ distribution and biliary excretion studies are described. The complex showed a marked highly reproducible accumulation in the gallbladder.

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Several reports describe thiol compounds labeled with ^{99m}Tc as potentially useful cholescintigraphic agents. Among the thiols studied are ^{99m}Tc-penicillamine (*1*), ^{99m}Tc-β-mercaptoisobutyric acid (*2*), ^{99m}Tc-thioctoic acid (*3*), and ^{99m}Tc-mercaptopurine (*4*). Thus, the excretion of technetium into the bile has been uniquely linked to ^{99m}Tc-thiol compounds, and the Tc–S coordinating bond appears to relate closely to the behavior of technetium in vivo. Unfortunately, preliminary experiments have failed to show the expected accumulation of ^{99m}Tc in the gallbladder, even in animal studies. This curious failure might be caused by some chemical characteristic of the various ^{99m}Tc-labeled compounds, which are produced through a complicated mechanism involving complex formation, hydrolysis, and oxidation of the reduced ^{99m}Tc species. Although this controversial reaction has been discussed by several authors (*5,6*), a clear explanation for it has not been offered.

In our laboratory, studies on various methods of ^{99m}Tc labeling have suggested that a monomeric ^{99m}Tc(IV) ion, TcO^{+2}, is produced as a first step in the labeling reaction, after which a chemically stable complex can generally be obtained, provided that the TcO^{+2} ion reacts without being hydrolyzed or oxidized. A thiol compound whose chemical structure permits a stable complex with TcO^{+2} would be of interest as a means of studying the chemical character of ^{99m}Tc-labeled compounds and the possible correlation of the label's chemical state with its biologic behavior.

Kethoxal-bis(thiosemicarbazone) (KTS) has been introduced as an antivirus and anticancer drug with low toxicity (*7*). As shown in Fig. 1, the thiol group of KTS reacts with such bivalent metal ions (Me^{+2}) as Cu^{+2} and Zn^{+2} to form a stable uncharged complex with a conjugated chelate ring system (*8*). If ^{99m}Tc were coordinated with KTS through a bivalent ^{99m}Tc ion, $^{99m}TcO^{+2}$, a chemically stable uncharged ^{99m}Tc complex would be expected.

In this study, the preparation of the uncharged ^{99m}Tc-KTS complex is described and its biologic behavior examined.

MATERIALS AND METHODS

The KTS was synthesized according to the method described by Tiffany (*9*). The ^{99m}Tc generator was obtained from Mallinckrodt Inc. (St. Louis, Mo.), and the long-lived ^{99}Tc came from New England Nuclear Corp. (North Billerica, Mass.). All chemicals and solvents used were of reagent grade.

The analytic systems employed included thin-

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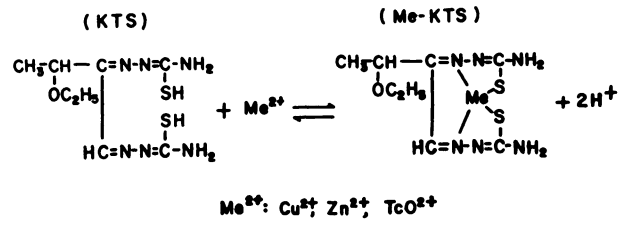

FIG. 1. Reaction of KTS with bivalent metal ion (Me^{+2}) to form uncharged KTS complex (Me–KTS).

layer chromatography (TLC) and electrophoresis. The TLC was performed with Merck silica gel with methanol–10% ammonium acetate (1:1) as the solvent. The electrophoresis was carried out with Toyo No. 50 filter paper on a Toyo C electrophoretic apparatus at constant voltage (500 V, 1 hr) with 0.2 *M* phosphate buffer (pH 7.0). A Fujitsu paper chromatoscanner was used for the analysis. Spectrometric measurements were carried out with a Shimadzu Spectrophotometer Double 40-R.

Preparation of the ^{99m}Tc-KTS uncharged complex. The KTS (2.76 mg) was first dissolved in 1 ml of 1 *N* NaOH; then 9 ml of 0.2 *M* acetate buffer (pH 5.6) was added to prepare the 10^{-3} *M* KTS solution. After 1 ml of KTS solution was deaerated by bubbling nitrogen gas through it for 5–10 min, 1 ml of freshly prepared 10^{-4} *M* $SnCl_2$ solution in 0.1 *N* HCl (also deaerated by nitrogen gas) was added and the mixture was stirred for 5 min. Finally, 1 ml of $^{99m}TcO_4^-$ (500–1,000 μCi) was added. The labeling solution was then purged with nitrogen for 15 min and filtered through a 0.22-μm Millipore filter.

In vivo distribution study. Mice (ddy), weighing 25–30 gm, were injected with 0.3 ml of the ^{99m}Tc-KTS uncharged complex solution. At specific time intervals, the mice were killed and autopsied. Excised organs were collected in tared plastic counting tubes and weighed immediately after removal. Blood was collected by cardiac puncture, and the gallbladder was carefully excised. All organs were counted against a standard of one-hundredth of the injected dose in a well scintillation counter.

Biliary excretion study. Male Wistar rats, weighing 250–300 gm, were anesthetized by intraperitoneal injection of sodium pentobarbital. The common bile duct was cannulated with a polyethylene tube. Body temperature was maintained at 37 ± 1°C, monitored by rectal probe during the experiment. Immediately after the cannulation, 0.5 ml of ^{99m}Tc-KTS uncharged complex solution was administered through the femoral vein. Bile samples were collected at 5–10-min intervals for 1 hr into preweighed vials, and the ^{99m}Tc activity in each sample was counted against a standard solution in a well scintillation counter.

RESULTS AND DISCUSSION

Chemical character of the ^{99m}Tc-KTS complex. In attempting to prepare the ^{99m}Tc-KTS uncharged complex, we postulated that the labeling should be performed under such conditions that the reduced ^{99m}Tc species (produced, we assumed, at the initial step in the reaction) would then react with KTS instead of undergoing hydrolysis, which it can do very easily. As the concentration of the anionic form of KTS increases, the complex-forming reaction will tend to prevail over the hydrolytic reaction. Thus, the KTS concentration and the pH become the essential considerations. Accordingly, a highly concentrated KTS solution, in which KTS is present as its thioenol form, was prepared as above. An optimal pH range of 5–6 was estimated from the acid dissociation constant of KTS and the hydrolysis constant of TcO^{+2} (*10*).

Furthermore, the hydrolysis of the reduced ^{99m}Tc species was found to occur faster with increasing concentrations of $SnCl_2$ reducing agent (*10*). Knowing this, we reasoned that using only a minute amount of Sn^{+2} would be best to reduce the $^{99m}TcO_4^-$, prevent the hydrolysis, and form the tetravalent monomeric ^{99m}Tc complex. Under an inert atmosphere, the KTS reacts with the minute amount of $SnCl_2$ to form a Sn-KTS complex, following which the reaction with $^{99m}TcO_4^-$ will proceed smoothly in the desired direction. This prevents the hydrolysis as well as the oxidation of the Sn^{+2} state.

The labeling solution was analyzed by TLC and electrophoresis. As shown in Fig. 2A, on TLC more than 95% of the total ^{99m}Tc activity is detected over a KTS spot (made visible by a chemical reaction) at an R_f value of 0.7, whereas only minute activity remains at the origin. The electrophoretic result (Fig. 2C) shows that most of the ^{99m}Tc activity remains at the origin and only a small portion migrates to the positive side. This minute activity at the positive side may correspond to that detected at the origin by TLC. These results strengthened our assumption that the main labeling species produced under the described conditions was an uncharged tetravalent complex.

In order to confirm this assumption, an organic extraction analysis was carried out with ethyl acetate. If the uncharged complex is the main product, then a large part of the ^{99m}Tc activity could possibly be extracted with this solvent. In fact, 90–95% of the ^{99m}Tc activity was transferred into the organic phase. Moreover, this extract gave the same results

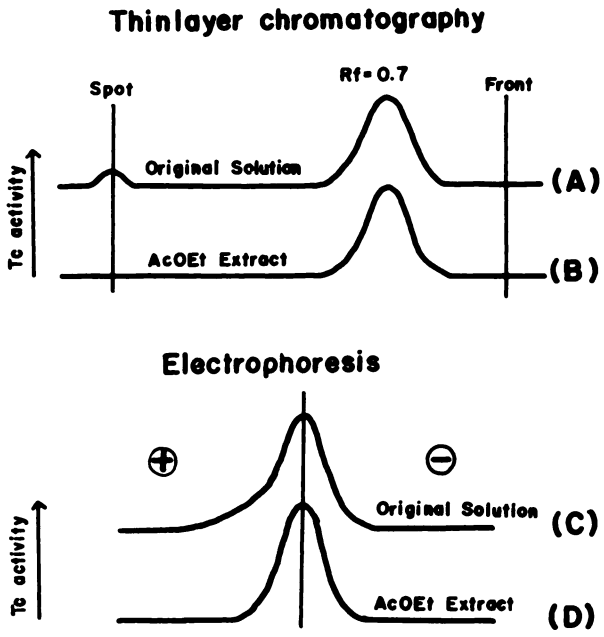

FIG. 2. Thin-layer chromatography and electrophoresis of ^{99m}Tc-KTS labeling solution before and after extraction with ethyl acetate. (A,C) Original labeling solution; (B,D) extracted solution.

as the original solution when tested by TLC and electrophoresis (Figs. 2B and 2D), confirming that the uncharged complex had moved into the organic phase without any chemical change.

The role of tetravalent technetium in forming this complex was investigated by spectrometric analysis using the long-lived ^{99}Tc. The organic-solvent-extractable complex, which was detected at R_f 0.7 on TLC, showed an absorption maximum at 450 nm. The same absorption maximum was observed in the spectrum obtained after the reaction of KTS solution with another tetravalent technetium species, $TcCl_6^{-2}$, prepared by the method described by Dalziel et al. (*11*). According to the absorption data for tetravalent technetium (*12,13*), this pronounced absorption band belongs to a charge-transfer band of a Tc(IV) complex. Recently, Hambright et al. reported that the tetravalent species is fairly general in the ^{99m}Tc complexes used in nuclear medicine; the ^{99m}Tc(IV) is coordinated with a type of oxygen-donor hard-base ligand (*6*). Our results with the ^{99m}Tc-KTS complex, in which ^{99m}Tc is coordinated through nitrogen and sulfur donor groups, indicate that the tetravalent state seems to be general for ^{99m}Tc complexes, irrespective of hard- or soft-base ligands.

The chemical character of the complex detected in small amounts at the origin of the TLC strip and on the positive side of the electrophoresis has not yet been clarified. Nevertheless, since this ^{99m}Tc species was not extracted into the organic phase and did migrate to the positive side in the electrophoresis, we assume that a small part of the labeling reaction proceeded toward $^{99m}TcO^{+2}$ hydrolysis, resulting in a negatively charged complex. Complexes with a hydrolyzed technetium state are generally seen to a greater or lesser extent in labeling reactions with reduced ^{99m}Tc. Investigation of the chemical character of the present hydrolyzed product, along with its biologic behavior, is now in progress.

In vivo distribution. The organ distribution of the ^{99m}Tc-KTS uncharged complex, following intravenous injection, was studied in mice. The greatest concentration of ^{99m}Tc was found in the gallbladder (Fig. 3), much as in the data reported for ^{99m}Tc-penicillamine (*1*) and ^{99m}Tc-β-mercaptoisobutyric acid (*2*). This ^{99m}Tc accumulation in the gallbladder was rapid and could be reproduced in every trial.

Excretion. In order to study the biliary excretion of the ^{99m}Tc-KTS uncharged complex in greater detail, the common bile ducts of male rats were can-

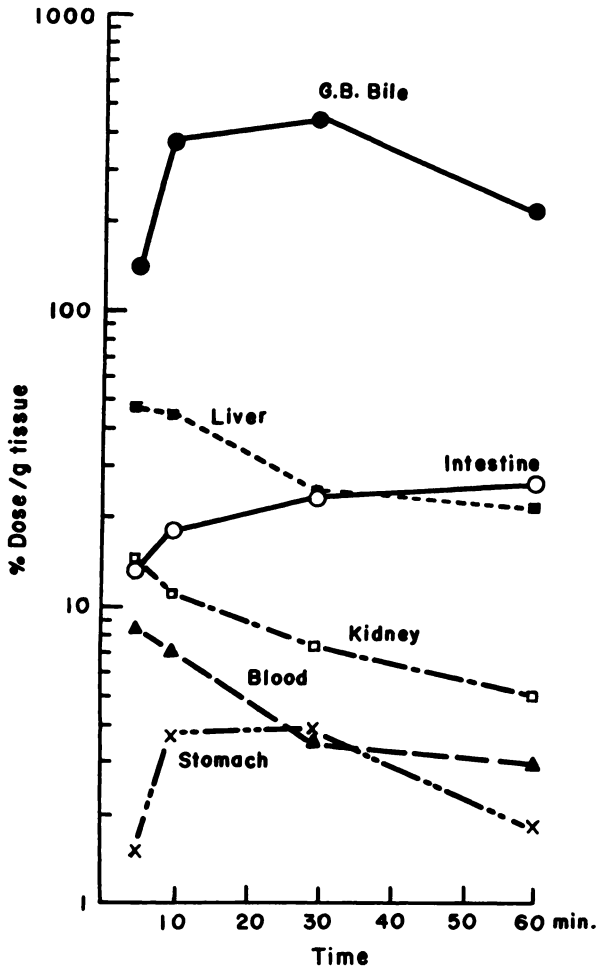

FIG. 3. Distribution of ^{99m}Tc-KTS uncharged complex in mouse tissues at various times after intravenous injection. Each point is mean for 4–6 animals.

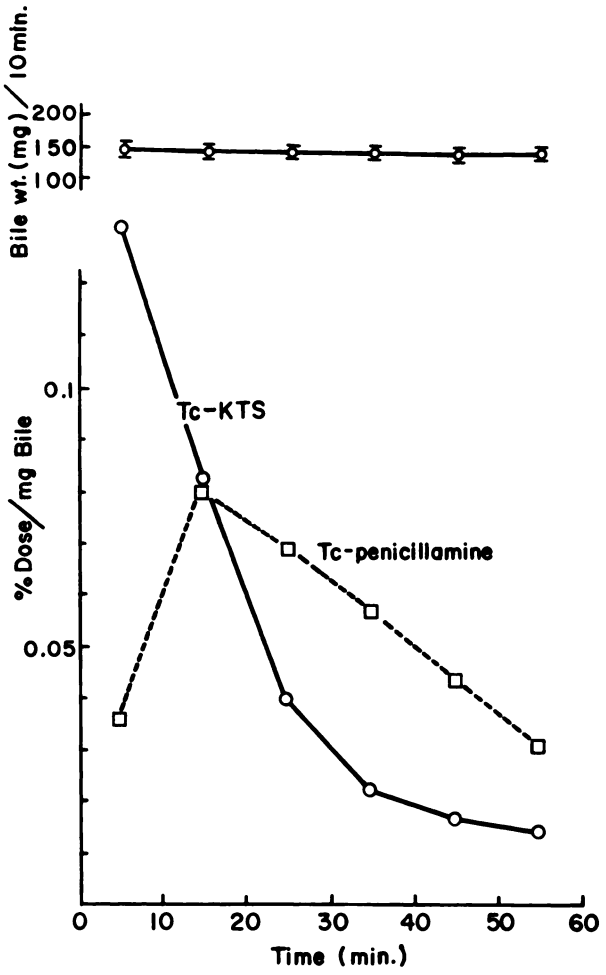

FIG. 4. Comparison of biliary excretion studies (by cannula) in rats for ^{99m}Tc-KTS uncharged complex and ^{99m}Tc-penicillamine. Tracer and total bile excretion rates are shown at various times after intravenous injection of ^{99m}Tc-labeled complexes. Each point is mean for 4–6 animals.

nulated and bile samples were collected as described above. As shown in Fig. 4, ^{99m}Tc is rapidly excreted: within 1 hr, 45–50% of the total activity injected is recovered from the bile. This excretion behavior is observed with high reproducibility. A similar result was obtained with a monomeric ^{99m}Tc-penicillamine complex, shown for comparison in Fig. 4. The ^{99m}Tc in that complex is considered to be in the same tetravalent monomeric state as in the ^{99m}Tc-KTS uncharged complex (*10*). The excretion of ^{99m}Tc-KTS is more rapid than that of ^{99m}Tc-penicillamine.

In summary, an enterohepatic pattern of ^{99m}Tc excretion can be observed when tetravalent monomeric ^{99m}Tc complexes are administered, and the chemical character of the molecule as a whole is reflected in this excretion behavior. The lipophilic character of the ^{99m}Tc-KTS uncharged complex may be responsible for its rapid excretion, a desirable property in an imaging agent.

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