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Localization of Low Molecular Weight ^{99m}Tc-Labeled Dimercaptodicarboxylic Acids in Kidney Tissue

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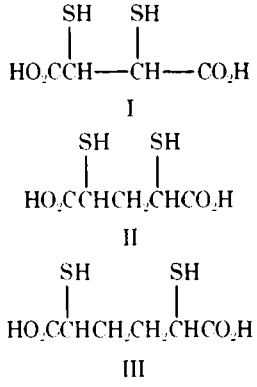
Abstract □ Kidney localization of low molecular weight ^{99m}Tc-dimercaptodicarboxylic acid complexes was examined in mice. The complexes ^{99m}Tc-dimercaptosuccinic acid, ^{99m}Tc-dimercaptoglutaric acid, and ^{99m}Tc-dimercaptoadipic acid were formed by reducing sodium ^{99m}Tc-pertechnetate with stannous chloride in the presence of 2–10-fold excess ligand at pH 2.5 or 7.5. Kidney specificity decreased as chain length between the mercapto groups increased. Optimum kidney retention occurred with complexes formed at pH 2.5. Complexes prepared at pH 7.5 were rapidly excreted through the urine and feces. Kidney localization of complexes prepared at one pH was not altered if the pH was later changed.

Keyphrases □ Technetium mercaptan complexes—localization in kidney tissue, synthesis, low molecular weight, structure–activity relationships □ Radionuclide imaging, renal—technetium mercaptan complexes, low molecular weight, localization in kidney tissue, synthesis, structure–activity relationships □ Kidney—radionuclide imaging, technetium mercaptan complexes, structure–activity relationships □ Structure–activity relationships—technetium mercaptan complexes, localization in kidney tissue

Radionuclide diagnosis of renal disease saves time in routine renal function tests and allows external kidney monitoring for clearance and morphological studies. The ^{203}Hg- and ^{197}Hg-labeled agents previously used have been replaced by ^{99m}Tc-labeled agents to decrease the high absorbed radiation dose *in vivo* (1).

Initial work on technetium mercaptan complexes as organ imaging agents led to the introduction of ^{99m}Tc-dihydrothioctic acid as a replacement for rose bengal in liver, gallbladder, and biliary tract studies (2). Other unpublished studies indicated that technetium complexes with short chain mono- and dimercaptodicarboxylic acids accumulated in kidney tissue. ^{99m}Tc-Dimercaptodicarboxylic acids were found to have better localization characteristics than their monomercapto analogs, especially ^{99m}Tc-dimercaptosuccinic acid, which has become well known as a renal imaging agent (1).

This *in vivo* study concerned organ localization of low molecular weight ^{99m}Tc-dimercaptodicarboxylic acids as affected by the number of carbon atoms between the mercapto groups and by the pH of complex formation.



I

II

III

EXPERIMENTAL

Dimercaptosuccinic acid[1] (I) was used without further purification.

Dimercaptoglutaric acid (II) was synthesized according to a literature procedure (3). Glutaric acid was brominated and esterified to form dimethyl 2,4-dibromoglutarate. The dibromide was reacted with potassium thioacetate to form the acetyl dithio ester. This derivative was subjected to alkaline hydrolysis in the presence of iodine and recrystallized from ethyl acetate to yield 1,2-dithiolane-3,5-dicarboxylic acid (the disulfide of II), mp 190–192° [lit. (3) mp 193–194°]. The structure for the disulfide was confirmed by elemental analysis, NMR, and mass spectrometry. Dimercaptoglutaric acid was generated from its disulfide, just prior to technetium complex formation, by reduction with aqueous sodium borohydride at pH 8, a technique that has been demonstrated in related compounds (4).

Dimercaptoadipic acid (III) was synthesized by incubating 2,2′-dibromoadipic acid[2] with aqueous potassium xanthogenate, followed by alkaline hydrolysis of the xanthate derivative in methanol and acidification. The solid was further purified by recrystallization from boiling water to yield pure crystalline III, mp 186–189° [lit. (5) mp 187–189°]. The structure for III was confirmed by elemental analysis and NMR.

^{99m}Tc-Dimercaptodicarboxylic Acid Complexes—These complexes were prepared in a nitrogen atmosphere by adding 0.1–2 ml of sodium pertechnetate ($Na^{99m}TcO_4$) (IV) eluate from ^{99}Mo-^{99m}Tc-generator[3] to 1–3 ml of stock tin–dimercaptodicarboxylic acid solution (pH 2.5 or 7.5), followed by 5–15 min of incubation at room temperature. The

[1] J. T. Baker, Phillipsburg, N.J.
[2] ICN Laboratories, Cleveland, Ohio.
[3] Mallinckrodt, St. Louis, Mo.

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Table I—Distribution of ^{99m}Tc-Dimercaptodicarboxylic Acid Complexes in Mice [a]

Organ	^{99m}Tc-Dimercaptosuccinic Acid		^{99m}Tc-Dimercaptoglutaric Acid		^{99m}Tc-Dimercaptoadipic Acid	
	pH 2.5[b]	pH 7.5	pH 2.5	pH 7.5	pH 2.5	pH 7.5
Kidneys	31.5 ± 2.9[c]	3.2 ± 0.8	15.3 ± 3.2	2.6 ± 0.9	8.7 ± 1.4	6.6 ± 0.6
Liver	6.1 ± 1.1	1.1 ± 0.3	2.6 ± 1.0	1.4 ± 0.2	10.3 ± 1.7	6.7 ± 1.1
Intestine	3.4 ± 0.8	1.3 ± 0.1	3.2 ± 0.2	3.8 ± 0.4	3.4 ± 0.5	6.2 ± 0.9
Blood	28.2 ± 3.2	13.9 ± 2.1	15.8 ± 4.6	5.1 ± 0.6	33.1 ± 5.1	24.8 ± 3.2
Urine and feces	28.4 ± 4.3	83.4 ± 9.6	65.4 ± 15.1	89.8 ± 10.8	30.5 ± 4.2	53.1 ± 9.3

[a] Four hours after injection of complexes (percent total). Complexes were prepared *via* reduction of sodium pertechnetate with stannous chloride. [b] The pH at which complexes were formed. [c] Percent total ± *SD*. Average results from four replications.

Table II—Distribution of ^{99m}Tc-Dimercaptosuccinic Acid in Mice [a]

Organ	Hours[b]					
	0.02	0.5	1.0	2.0	4.0	24.0
Kidneys	9.4 ± 1.3[c]	29.3 ± 3.8	39.5 ± 4.1	45.6 ± 6.7	54.8 ± 6.7	75.5 ± 12.2
Liver	28.6 ± 2.4	12.8 ± 1.7	9.4 ± 2.1	6.8 ± 1.8	3.8 ± 0.8	2.7 ± 0.7
Intestine	11.9 ± 2.1	7.2 ± 2.1	8.2 ± 1.9	4.8 ± 1.3	3.4 ± 1.0	2.1 ± 0.9
Blood	40.9 ± 5.8	30.4 ± 4.1	26.9 ± 4.0	20.4 ± 3.1	21.3 ± 4.5	8.4 ± 1.2
Urine and feces	9.2 ± 1.6	20.3 ± 3.8	16.0 ± 3.8	22.4 ± 2.6	16.7 ± 3.4	11.7 ± 3.1

[a] The complex was prepared *via* sodium hexabromotechnetate (action of hydrogen bromide on sodium pertechnetate). [b] Following injection. [c] Percent total ± *SD*. Average results from three replications.

stock solutions were made by mixing stannous chloride dissolved in oxygen-free 1 *M* HCl with 3 m*M* I, II, or III such that the ligand to tin ratio was 3:1. The stock solution pH was adjusted with 1 *M* $NaHCO_3$.

Following incubation, the complexes were analyzed for unreacted IV by TLC on silica gel 6060 paper[4] with acetone as the developing solvent; IV moved with the solvent front while reduced technetium remained at the origin. Unreacted IV was always less than 2%. If a mixture of acetone (100 parts) and 12 *M* HCl (0.5 part) was used as a developing solvent, the complexes migrated (R_f ~0.8) while IV moved with the solvent front.

To avoid accidental contamination of the complex with undesirable metal ions, all preparation steps were carried out in glass vessels and with highly pure reagents.

***In Vivo* Distribution Study**—The 84 female Swiss Webster mice[5] were from the same shipment and weighed 20–24 g. Prior to experimentation, the animals were housed in groups of six for 3 days. They were allowed free access to food and tap water.

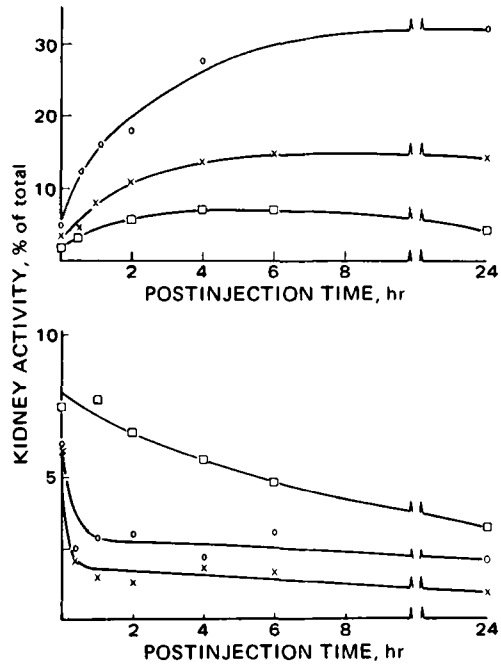

Figure 1—*Kidney localization of ^{99m}Tc-dimercaptosuccinic acid (○) compared with that of homologs ^{99m}Tc-dimercaptoglutaric acid (×) and ^{99m}Tc-dimercaptoadipic acid (□). Complexes were formed at pH 2.5 (top) and pH 7.5 (bottom).*

[4] Eastman.
[5] Bio-Lab, Centerville, Minn.

The *in vivo* distribution of ^{99m}Tc-dimercaptodicarboxylic acids in mice *versus* time was studied by intravenous injection of 1.5 mg/kg into the tail vein. The animals were injected at random and were killed at 0.02, 0.5, 1, 2, 4, 6, and 24 hr after injection by exposure to a carbon dioxide atmosphere. Blood samples were obtained by cardiac puncture immediately after death. The organs were assayed for radioactivity, and the results were reported as percent of total injected radioactivity. The experiment was replicated four times.

RESULTS AND DISCUSSION

Preparation of ^{99m}Tc-Dimercaptodicarboxylic Acid Complexes—This paper describes the reduction of radioactive pertechnetate IV and its reaction with excess dimercaptodicarboxylic acid complexes. Dimercaptodicarboxylic acids are by themselves reducing agents. Therefore, the technetium complexes could be formed simply by heating a solution of the acid with IV at 100° for 15–30 min. The addition of trace quantities (1 μg) of cupric sulfate or ferric chloride reduced the reaction time to 5 min.

A drawback of this method was that oxidation of mercaptan groups sometimes occurred, producing incomplete labeling and formation of labeled dimercaptodicarboxylic acid fragments. This problem was eliminated by room temperature labeling in the presence of an oxidizable, lower valence metal salt such as ferrous chloride or stannous chloride under a nitrogen atmosphere and use of double-distilled water diluent. The ligand to tin ratio could vary from 2:1 to 10:1, and the labeling could be done at any pH between 2 and 9. ^{99m}Tc-Dimercaptosuccinic acid (V), ^{99m}Tc-dimercaptoglutaric acid (VI), and ^{99m}Tc-dimercaptoadipic acid (VII) prepared under these conditions were stable for at least 6 hr at room temperature.

The stannous chloride reduction was adequate for the formation of the low molecular weight ^{99m}Tc-dimercaptodicarboxylic acid complexes described in this study; however, a modified method was required for several other mercaptan derivatives that precipitated in the presence of tin. ^{99m}Tc-Mercaptan complexes were made by first reacting the sodium pertechnetate eluate with 9 *M* HBr at 100° under nitrogen to form sodium hexabromotechnetate, $Na_2{}^{99m}TcBr_6$ (VIII) (6, 7). Then the mixture was evaporated to dryness over a nitrogen stream, and a slightly red solid (VIII) residue was obtained (8). This residue was dissolved in ethanol and mixed with an equal volume of an ethanolic solution of mercaptan ligand. Afterward, the organic solvent was evaporated and the resulting ^{99m}Tc-mercaptan complex was dissolved in saline.

In all studies, the extent of reduction and the complex stability were followed by TLC and radiation monitoring since IV had a distinctly different R_f value than its complexed forms.

Kidney Localization of ^{99m}Tc-Dimercaptodicarboxylic Acids—^{99m}Tc-Dimercaptodicarboxylic acids, prepared at pH 2.4 and 7.5 and having a 3:1 ligand–tin ratio, were injected. The complexes circulated in the blood and either accumulated in kidneys and, to a lesser extent, in the liver (<10%) or were excreted.

Table I shows the distribution and excretion of the complexes formed in acidic or basic solutions in mice killed 4 hr after injection. The average

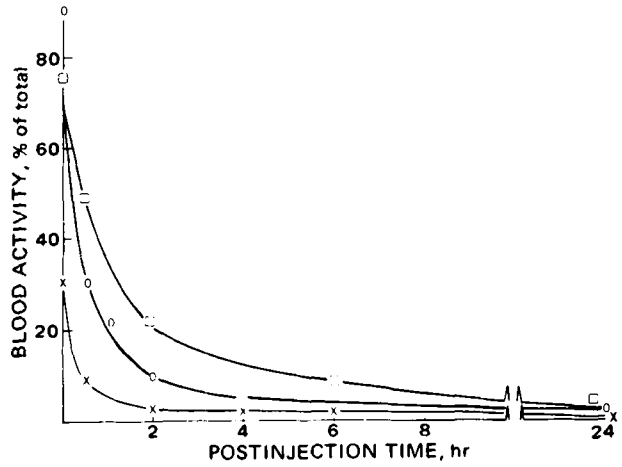

Figure 2—*Comparison of blood clearance rates of* ^{99m}*Tc-dimercaptosuccinic acid* (○), ^{99m}*Tc-dimercaptoglutaric acid* (×), *and* ^{99m}*Tc-dimercaptoadipic acid* (□) *formed at pH 2.5.*

kidney accumulation of V–VII *versus* postinjection time is shown in Fig. 1. Within a few hours after injection of the acidic complexes, V concentrated most highly in kidney tissue, plateauing at slightly above 30% of the total injected radioactivity. Five-carbon VI reached a maximum of 15%, and six-carbon VII concentrated least of all, plateauing at 8%. Paired *t* test analysis showed the curves to be significantly different ($p < 0.05$) for periods greater than 2 hr.

In contrast, the basic complexes showed an initial low kidney accumulation that radically dropped a short time after administration. Least sensitive of all to pH changes was VII, which showed very low kidney uptake of both acidic and basic preparations. In all cases, as the radioactivity in kidney tissue decreased, a marked increase in activity was found in the animal's excreta.

Figure 2 shows the average blood clearance of the three complexes formulated at pH 2.5. Slowest to clear was VII. The relative blood clearance rates were the same for complexes prepared at pH 7.5, but the individual clearance rates were faster. These complexes were excreted within 0.5 hr of injection.

Further studies indicated that low pH preparations were necessary for high kidney localization of the complexes. Complexes prepared at pH 2.5 and then raised to pH 7.5 by sodium bicarbonate addition prior to *in vivo* injection localized strongly in the kidney. However, complexes prepared at pH 7.5 and then lowered to pH 2.5 by hydrochloric acid addition still showed low kidney localization and were excreted rapidly. These observations are in agreement with those previously reported (9).

The ligand to tin ratio during complex formation and the injection time after complex preparation did not significantly affect the degree of complex localization in kidney tissue. During these studies, the ligand to tin ratio varied from 2:1 to 10:1, and the complexes were injected from 30 min to 6 hr after their preparation. If the complexes were not prepared using pure ligand and reagents and in an inert atmosphere, they rapidly decomposed into fragments that concentrated in the liver.

The nature of the technetium complexes formed is uncertain. The presence of two mercapto groups in the dicarboxylic acid molecules was necessary for good kidney uptake of the complexes. Comparative studies in this laboratory[6] using various monomercapto homologs such as ^{99m}Tc-mercaptoacetic acid and ^{99m}Tc-thiolactic acid showed that these complexes were more rapidly excreted and that their renal uptake was lower.

Reduction of pertechnetate with stannous chloride was used in this study because it was easy, rapid, and convenient. The complexes prepared by this method accumulated sufficiently in the kidney. However, reduction with hydrogen bromide, which can be used for a variety of mercaptan compounds, gave a higher deposition in the kidney with a considerable reduction in the excretion rate. Table II shows the distribution of V prepared by this method. The reason for the higher uptake is unknown. It may be due to the formation of a purer complex than that formed by tin reduction or to formation of an entirely different complex with higher specificity for kidney tissue.

Further studies are in progress to investigate the nature of complex formation as well as the chemical structure–kidney localization relationship.

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[6] Unpublished data.

Hydrocortisone Stability in Human Feces

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Received July 31, 1978, from the *Clinical Pharmacokinetics Laboratory, Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo,* and *Millard Fillmore Hospital, Buffalo, NY 14209.* Accepted for publication November 1, 1978.

Abstract □ Hydrocortisone stability in human feces was studied under various conditions to determine whether stability accounts for the variable effects of hydrocortisone enemas. Recovery from feces and assay specificity were assured using dual isotopes, TLC separation, and liquid scintillation counting. Hydrocortisone degraded slightly from 7 to 26% in 24 hr when incubated in fresh human feces at 37°. Less than 7% degradation occurred in feces stored at 10°, and negligible degradation occurred with hydrocortisone in water at 37°. Fecal bacteria may account for the observed degradation. Hydrocortisone stability in feces may contribute to local persistence and may account partly for its efficacy in ulcerative colitis treatment.

Keyphrases □ Hydrocortisone—degradation, human feces, various rectal dosage forms □ Dosage forms—hydrocortisone, various rectal dosage forms, degradation in human feces □ Stability—hydrocortisone in human feces, various rectal dosage forms

Rectally administered hydrocortisone (cortisol) is an established mode of long-term ulcerative colitis therapy. It has the proposed advantage of direct drug delivery without excessive systemic absorption and the attendant

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