RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

Complex Formed from 3-Hydroxy-4-formylpyridine, Glutamic Acid, and Technetium—A Possible Cholescintigraphic Agent

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In order to obtain a technetium-99m-labeled cholescintigraphic agent suitable for kit preparation, reactions of a variety of aromatic aldehydes, amino acids, and [^{99m}Tc] pertechnetate were examined under mild conditions. Aqueous solutions of the three reactants were heated in a boiling-water bath and the products analyzed by means of thin-layer chromatography for the formation of organic technetium complexes. 3-Hydroxy-4-formylpyridine (HFP) and 1-methyl-3-hydroxy-4-formylpyridinium chloride (N-Me-HFP) formed the complex with excellent yields, whereas 3-hydroxy-2-formylpyridine, 4-nitrosalicylaldehyde, salicylaldehyde, and isonicotinaldehyde did not. The complex is concluded to be the Tc-99m chelate of the Schiff base formed from the aldehyde and amino acid. Scintigrams, biliary excretion, and the effect of pancreozymin were examined in rabbits administered with the complex of HFP, glutamic acid, and Tc-99m. The results indicate that it is promising as a cholescintigraphic agent.

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Recently, Tc-99m-labeled compounds for assessment of the hepatobiliary system have attracted much interest. The complex formed from pyridoxal, glutamic acid (Glu), and Tc-99m was claimed to be an excellent cholescintigraphic agent (*1,2*). This finding has been confirmed by several workers (*3–6*). The autoclave treatment was employed for the preparation. A stannous compound is not required for reduction of pertechnetate. This Sn-free labeling method seems favorable for clinical use, but is unsuitable for kit preparation.

In search of Tc-99m compounds suitable for this purpose, the reactions of a number of aldehydes and amino acids with pertechnetate were investigated under mild conditions. Herein are the results as well as the biologic data.

MATERIALS AND METHODS

The following compounds were synthesized according to the methods cited: 3-hydroxy-4-formylpyridine (HFP); 3-hydroxy-2-formylpyridine; 3-hydroxyisonicotinic acid; 3-hydroxy-4-aminomethylpyridine (*7*); 1-methyl-3-hydroxy-4-formylpyridinium chloride (N-Me-HFP); 1-methyl-3-hydroxy-2-formylpyridinium iodide (*8*); 1-methyl-4-formylpyridinium iodide (*9*); 1-methylpyridoxal chloride (*10*); and 4-nitrosalicylaldehyde (*11*). Other aldehydes and amino acids used in this study were

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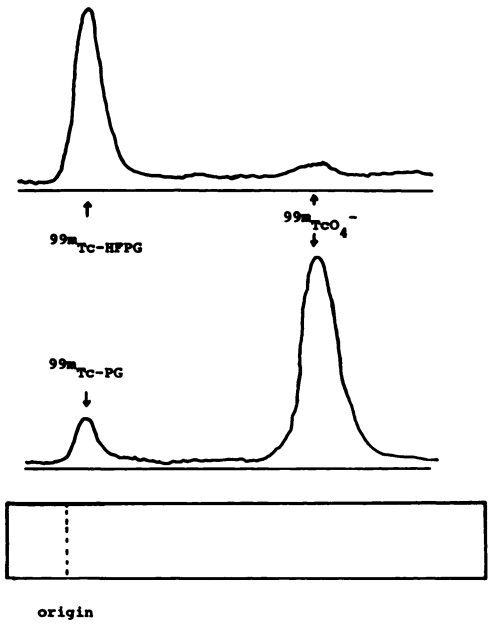

FIG. 1. Typical thin-layer chromatograms of the reaction mixture of pyridoxal (or HFP), glutamic acid, and $^{99m}TcO_4^-$. Solvent system = 1-butanol:acetic acid:water (4:1:1) Plate = Silicagel.

obtained from commercial sources, as were Tc-99m and Tc-99 as pertechnetates. The specific activity of $NH_4{}^{99}TcO_4$ was 17 mCi/g and the concentration was 0.043 g/ml.

To an aqueous solution of equivalent amounts (0.1 millimol) of an aldehyde and an amino acid, adjusted to pH 7.0–7.5 with 0.1 *N* NaOH, a saline solution of pertechnetate was added, the final volume being 2 ml. The mixture was heated in a boiling-water bath for 15 min. After cooling, the solution was analyzed by silicagel thin-layer chromatography (TLC) using two solvent systems, methanol : 10% ammonium acetate (1:1), and 1-butanol:acetic acid:water (4:1:1).

Male white rabbits weighing 3.5 kg were used for the biologic studies. Sequential scintigrams were obtained at 5-min intervals for 1 hr with a scintillation camera collimated with 30,000 parallel holes. Time-radioactivity curves were obtained simultaneously. With the Tc-99m compounds, a pancreozymin test was performed 30 min after the injection. Blood clearance of radioactivity was measured at 5-min intervals. Bile samples were collected from the common bile duct, cannulated with a polyethylene tube, and the recovery of the injected radioactivity in the bile measured.

RESULTS

Figure 1 shows typical thin-layer chromatograms of the reaction mixtures. Two radioactive spots can be seen. One was identified as inorganic pertechnetate and the other as organic material. This indicates that part of the Tc-99m was converted to the organic complex. There was no sign of the complex formation in unheated solutions; neither was it formed in the absence of one of the three reactants. The yield of the complex was estimated from the relative radioactivity. Table I lists the yields in the reactions of glutamic acid and pertechnetate with various aromatic aldehydes and related compounds. Excellent yields were attained with HFP and N-Me-HFP under these mild conditions. With pyridoxal and 1-methylpyridoxal chloride, prolonged heating enhanced the yield. However, the reproducibility of the data was poor, probably because of side reactions.

The reactions of HFP with various amino acids

TABLE 1. ALDEHYDES AND THEIR ANALOGS USED FOR Tc-99m COMPLEX FORMATION WITH GLUTAMIC ACID

Compounds	Yield (%)
3-Hydroxy-4-formylpyridine (HFP)	100
3-Hydroxy-2-formylpyridine	0
1-Methyl-3-hydroxy-2-formyl-pyridinium iodide	50
1-Methyl-3-hydroxy-4-formylpyridinium chloride (N-Me-HFP)	100
Isonicotinic aldehyde	0
3-Hydroxyisonicotinic acid	0
Salicylaldehyde	0
4-Nitrosalicylaldehyde	0
1-Methyl-4-formylpyridinium iodide	0
3-Methoxy-4-formylpyridine	0
3-Hydroxy-4-aminomethylpyridine	0
3-Hydroxypyridine-4-aldoxime	0
Pyridoxal	10
1-Methylpyridoxal chloride	10
Pyridoxal phosphate	3

TABLE 2. AMINO ACIDS AND THEIR ANALOGS USED FOR THE Tc-99m COMPLEX FORMATION WITH 3-HYDROXY-4-FORMYLPYRIDINE (HFP)

Compounds	Yields (%)
Glutamic acid	100
Aspartic acid	100
Alanine	100
β-Alanine	100
α-Methylalanine	100
Sarcosine	0
Dimethylglutamate	20
Alanine ethyl ester	20
Alanine amide	0
Serine	35
Cysteine	0
α-Ketobutyric acid	0
Glycylglycine	30

TABLE 3. THE R_f VALUES FROM THIN-LAYER CHROMATOGRAPHY OF Tc-99m HFP-GLU COMPLEX AND RELEVANT SUBSTANCES ON SILICAGEL PLATES

Substances	R_f Values, Solvent System: Methanol:10% ammonium acetate (1:1)	R_f Values, Solvent System: 1-Buthanol:Acetic acid:Water (4:1:1)
3-Hydroxy-4-formylpyridine (HFP)	0.74	0.65
N-(3-Hydroxy-4-pyridylmethylene)-glutamic acid* (HFP-Glu)	0.55	0.13
Pyridoxal (Pal)	0.72	0.43
Pyridoxylideneglutamate* (PG)	0.73	—
$^{99m}TcO_4^-$†	0.96	0.66
Tc-99m HFP-Glu†	0.53	0.086
Tc-99m PG†	—	0.09

* These are prepared by mixing HFP (or Pal) with an equivalent amount of glutamic acid in methanol (*17*).
† R_f values of these substances were determined by radioactivity.

TABLE 4. EFFECT OF PERTECHNETATE CONCENTRATION ON THE Tc-99-HFP-GLU COMPLEX FORMATION

$NH_4{}^{99}TcO_4$ concentration (M)	Yield (%)
0.5	0
0.3	0
0.175	3
0.008	90

HFP 0.5 *M*; Glutamic acid 0.5 *M*; pH-7

were then examined, with the results summarized in Table 2. Most of α-amino acids and β-alanine gave the complex in good yield. The same complex was obtained from 3-hydroxy-4-aminomethyl-pyridine, α-ketobutyric acid, and [^{99m}Tc] pertechnetate in about 15% yield.

The effect of temperature on the yield was examined in the HFP-Glu-$^{99m}TcO_4^-$ system. All the pertechnetate was converted to the organic complex by heating in an 80°C bath for 30 min, but only 30% in a 50°C bath for the same time. No radioactivity was incorporated into organic compound at room temperature. The yield of the complex decreased in acidic conditions, no complex being formed at pH 4.0. The absorption and PMR spectra of the reaction mixtures of HFP and Glu with and without [^{99m}Tc] pertechnetate were identical, and showed that the substances present in significant amounts are the Schiff base (aldimine) and its components (*12–14*). The R_f values for thin-layer chromatography of the complex and relevant substances are listed in Table 3.

In an attempt to isolate and characterize the complex, [^{99}Tc] pertechnetate was used for the reaction. Only a small part of the radioactivity was incorporated into the complex (Table 4) and a pure substance was not isolated.

Biologic studies were made on rabbits administered intravenously with one of the following Tc-99m compounds: the complex of HFP, Glu, and Tc-99m (Tc-99m HFPG; 310 μCi) prepared as described above, and 1 mCi of Tc-99m pyridoxylideneglutamate (Tc-99m PG) prepared according to Baker et al. (*1*).

Scintigrams of the animal receiving Tc-99m HFPG (Fig. 2) show that the radioactivity was rapidly cleared from the blood through the liver—in 5 min the radioactivity was on its way from the liver to the small intestine. The gallbladder became visible in 10 min and was very clearly visualized at 25 min after the injection. The image of the liver faded out in 40 min.

Technetium-99m PG was also cleared from the blood, though the clearance was slower than for Tc-99m HFPG. The kidneys were imaged initially, then faded into the background within 30 min. The

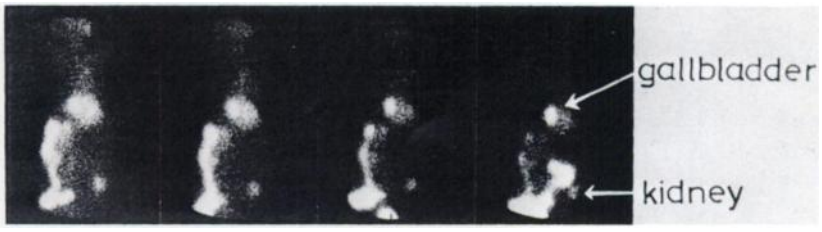

FIG. 2. Distribution of radioactivity after 15, 20, 25, and 30 min in rabbit pictured with scintillation camera using Tc-99m-3-hydroxy-4-formyl-pyridine-glutamate.

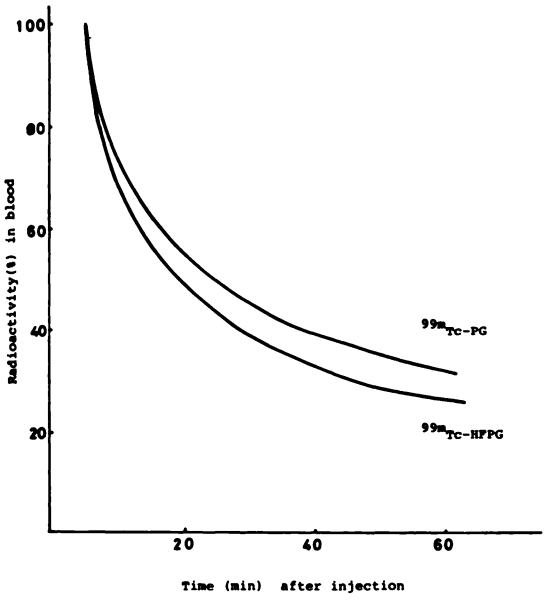

FIG. 3. Clearance of radioactivity from blood after injection of Tc-99m PG and Tc-99m HFPG.

thyroid was faintly imaged. At 40 min, the gallbladder, the small intestine, and the urinary bladder were visualized.

Figure 3 shows the blood radioactivity curves from the rabbits injected with Tc-99m HFPG and Tc-99m PG. The radioactivity at 5 min after injection was taken as 100%. The clearance curves are quite similar for the two compounds.

In a rabbit injected with Tc-99m HFPG, the radioactivity showed an exponential decrease in the liver, whereas the falloff was slower in the gallbladder. Gallbladder radioactivity was the same as that in the liver at 10 min after the injection, and became 2.35 times as much as the latter at 40 min. The injection of pancreozymin accelerated the movement of radioactivity from gallbladder to gut (Fig. 4).

Figure 5 shows the cumulative radioactivity curves from the bile samples collected at 5-min intervals. The recovery of the radioactivity at 60 min was 33.8% of the injected dose for Tc-99m HFPG, and 42.1% for Tc-99m PG.

DISCUSSION

In spite of the failure to isolate and characterize the Tc-99m HFPG complex, for the following reasons we conclude it to be the Tc-chelate of the Schiff base (aldimine) formed from the aldehyde and amino acid (Fig. 6).

1. As shown in Table 2, only the ortho-hydroxypyridinealdehydes can form the complex. It is well known that the Schiff base derived from the aldehydes can form stable metal chelates (*15*).

2. Only the amino acids that can form the Schiff base with the aldehydes can produce the complex. Consequently, sarcosine, a secondary amine, did not give rise to the complex. Low yields of cysteine and serine can be explained in terms of thiazolidine and oxazolidine derivative formation, respectively (*16,17*). Low yields in esters and amides of amino acids may be caused by the low complexing ability of their Schiff bases.

3. It might be possible that the Schiff bases undergo further chemical changes by metal chelation and that the complex is the product of the reaction, such as a transaminated Schiff base (ketimine) and compounds where the α-position of the amino acid is substituted (*18,19*). This possibility however, can be ruled out because 2-methylalanine formed the complex with a good yield. This amino acid lacks an α-proton, the loss of which is the essential step for the subsequent reactions (*18,19*).

4. α-Ketobutyric acid did not form the complex with HFP but did form with 3-hydroxy-4-aminomethylpyridine. 3-Hydroxy-4-aminomethylpyridine, α-keto acid, and a metal ion can yield the

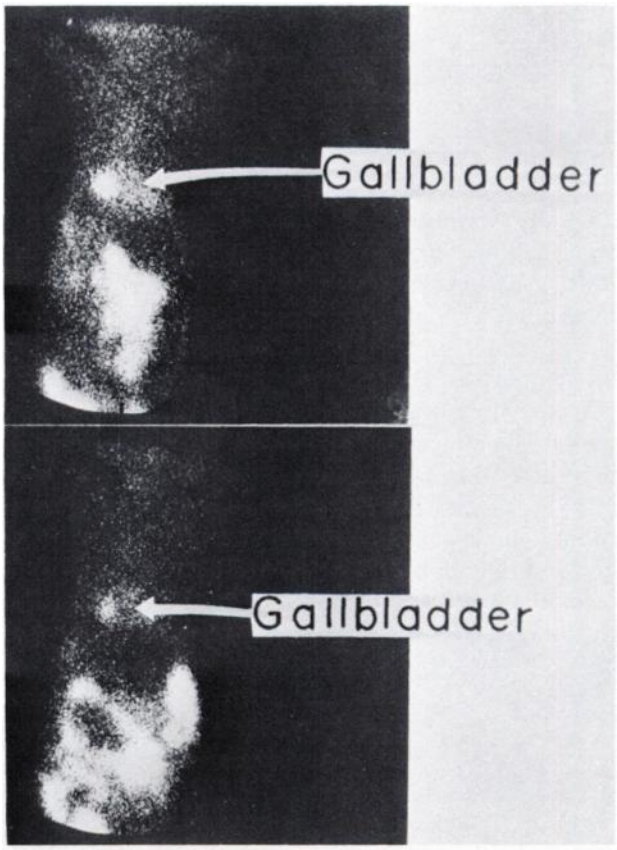

FIG. 4. Scintigrams before (top) and 10 min after (bottom) pancreozymin injection in rabbit shown in Fig. 2.

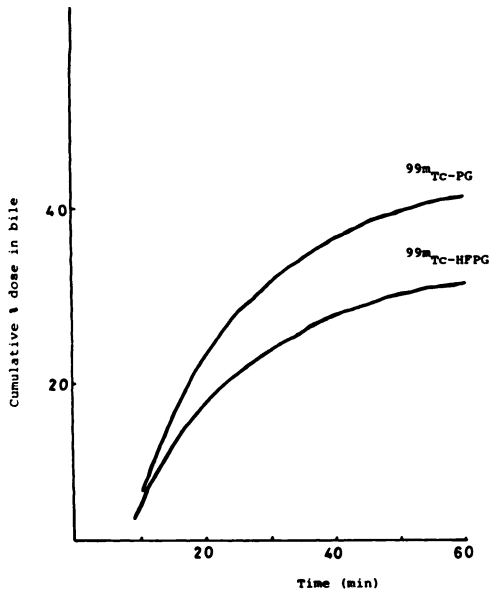

FIG. 5. Cumulative biliary radioactivity curves from 5-min samples after i.v. injection of Tc-99m PG or Tc-99m HFPG.

metal chelate of the aldimine by nonenzymatic transamination (*20*).

The aromatic aldehydes in the present system function as reducing agents and also as Schiff-base-forming substances. Under these conditions pyridoxal may be present predominantly as the intramolecular hemiacetal species, whereas HFP is present as the free-aldehyde species (*21,22*). These reasons are suggested for the difference in reactivity between the two pyridinealdehydes.

The results with Tc-99 indicate that a large excess of the reducing aldehyde is required for the complete incorporation of the radioactivity into the complex.

Scintigraphic study of Tc-99m HFPG suggests its potential utility for assessment of the hepatobiliary system. It was cleared from the blood more rapidly than Tc-99m PG. However, the less prominent and slower biliary excretion of Tc-99m HFPG would result in urinary excretion. Since the two pathways of excretion are competetive, the increased urinary excretion works against a good cholescintigram.

In conclusion, the complex formed from HFP, amino acid and Tc-99m can be prepared with an excellent yield by a 15-min heating. The labeling requires no nitrogen supply and no stannous compound. A toxicity test is in progress in our laboratory, and no adverse reactions have been detected so far.

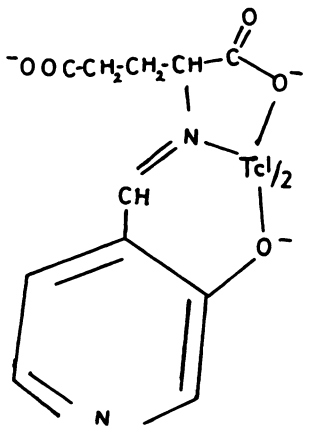

FIG. 6. A reasonable structure for Tc-HFPG.

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