# Human Pharmacokinetics and Radiopharmacological Studies of the Myocardial Perfusion Agent: Technetium (2-Carbomethoxy-2-Isocyano-Propane)$_6^+$

James F. Kronauge, Alba S. Leòn, E. Silvia Verdera, Henia S. Balter, Elsa T. Leòn, Fernando Mut, M. Cecilia Oliveira, Fernando A. Garcia, B. Leonard Holman, Alan Davison and Alun G. Jones

*Department of Radiology, Harvard Medical School, Boston, Massachusetts and Department of Radiology, Brigham & Women's Hospital, Boston, Massachusetts; Departamento de Radiofarmacia, Centro de Investigaciones Nucleares, Universidad de la Republica, Montevideo, Uruguay; Centro de Medicina Nuclear, Hospital de Clinicas, Universidad de la Republica, Montevideo, Uruguay, and Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts*

The myocardial perfusion agent technetium (2-carbomethoxy-2-isocyano-propane)$_6^+$ ($^{99m}$Tc-CPI) is unique from other cationic technetium isonitrile complexes in that it exhibits moderate washout from the heart and rapid hepatobiliary clearance in animal models and human volunteers. Dynamic imaging and HPLC analysis were performed in humans and guinea pigs to outline the pharmacological basis of its pharmacokinetics. Enzymatic hydrolysis of the terminal ester groups in blood was found to occur at a moderate rate producing new species that have been shown not to accumulate in heart tissue. However, after extraction by the heart, liver or kidneys, the $^{99m}$Tc-CPI complex undergoes metabolism at a much slower rate than observed in the blood. Differences in hydrolysis rate and products obtained indicate separate mechanisms of hydrolysis occurring in blood and other organs. It is proposed that the heart washout occurring after hydrolysis produces a neutral compound which is no longer retained by the negative cytosolic and mitochondrial membrane potentials in myocardial tissue.

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Technetium 2-carbomethoxy-2-isocyano-propane)$_6^+$ ($^{99m}$Tc-CPI) is a lipophilic cationic complex consisting of six identical ester isocyanide ligands coordinated to a central technetium(I) atom (*1*). The terminal ester groups of the coordinating isocyanide ligand were specifically designed to undergo in vivo hydrolysis, providing a pathway for metabolism and bioelimination of the agent. The radiopharmaceutical containing $^{99m}$Tc has demonstrated localization, proportional to blood flow, in perfused myocardial tissue of normal animals and infarct models (*2*), and provided images of myocardial perfusion in humans (*3*). As with the other lipophilic cations tested, i.e. technetium (t-butyl isocyanide)$_6^+$ ($^{99m}$Tc-TBI) and technetium (2-methoxyisobutyl-isocyanide)$_6^+$ ($^{99m}$Tc-MIBI), this agent freely diffuses into myocytes and accumulates in mitochondria in proportion to cytosolic and mitochondrial membrane potentials or tissue viability (*4–7*).

Although comparison in cultured heart cell models (*7*) with other isonitrile complexes indicated $^{99m}$Tc-CPI had an extraction efficiency lower than the first successful agent $^{99m}$Tc-TBI, its pharmacokinetic properties made it a more desirable myocardial imaging agent (*8*). Specifically, the rapid clearance of this compound from lung and liver tissue produced enhanced target-to-background ratios with better visualization of the apex of the heart (*9*). Also, the moderate washout rate of this agent from the heart muscle enabled same-day stress/rest imaging protocols (*10*). Subsequently, clinical emphasis of technetium isonitriles shifted to the ether containing analogue $^{99m}$Tc-MIBI because of its prolonged retention in myocardial tissue combined with rapid background clearance and increased renal excretion (*11*). The desirability of same-day stress/rest imaging protocols and the interest in developing a metabolically sensitive agent (*12*) has encouraged the continued investigation of the ester isocyanide complexes. Model studies in animals have confirmed the metabolism of $^{99m}$Tc-CPI (*13*), however, the direct identification of the metabolites produced in humans in vivo has not been shown. A comparison of biodistribution and radiochemical analysis of metabolites in bile, urine and blood from guinea pigs and humans, two species which show similar $^{99m}$Tc-CPI accumulation in heart tissue, was performed to outline the mechanistic basis of the agent's pharmacokinetic behavior.

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For reprints contact: James F. Kronauge, PhD, Department of Radiology, Brigham & Women's Hospital, 75 Francis St., Boston, MA 02115.

## MATERIALS AND METHODS

The metastable radionuclide $^{99m}$Tc, as sodium pertechnetate (30–150 mCi/ml, 1.1–5.5 GBq/ml), was obtained from a commercial ($^{99}$Mo/$^{99m}$Tc) generator in aqueous NaCl (0.15 *M*) (DuPont/Biomedical Products, N. Billerica, MA or CIS Bio International). Synthesis and characterization of the ligand 2-carbomethoxy-2-isocyano-propane (CPI), the $^{99}$Tc(CPI)$_6^+$ and Cu(CPI)$_4$BF$_4$ complexes have been published elsewhere (*1,14*).

### Chromatography

High-performance liquid chromatography (HPLC) was performed on a dual detection gradient system employing both UV (254 nm) and NaI-based radiometric detection as described previously (*14,15*). Analytical HPLC analysis of $^{99m}$Tc-CPI and its metabolites was carried out in a reversed-phase mode (RP-HPLC) on two separate systems. The first system RP-HPLC-A consisted of a C-8 bonded (5 $\mu M$) spherical silica particle stationary phase (Brownlee OS-MP cartridge, 100 mm × 4.1 mm, Rainin Instruments, Woburn, MA) with a gradient mobile phase of 100% aqueous buffer (ammonium sulfate, 0.05 *M*, pH 5.5) to 95% methanol in a 5-min linear gradient at a flow rate of 1 ml/min (*1*). On this system, the $^{99m}$Tc-CPI species was confirmed by comparison with a known sample of $^{99}$Tc(CPI)$_6^+$ and shown to have a retention time of 9.0 min (k′ = 4.20). The second system RP-HPLC-B consisted of a C-18 stationary phase (MICROPAK MCH-10 column, 300 mm × 4.1 mm, Varian Associates) with a linear ammonium sulfate/methanol gradient of (4:1, v:v) to (1:19, v:v) in 10 min at 2.0 ml/min (*14*). On this system $^{99m}$Tc-CPI eluted with a retention time of 14.0 min (k′ = 5.36). Reversed-phase thin-layer chromatography (RP-TLC), as described elsewhere (*13*), was routinely performed on $^{99m}$Tc preparations to quantitate the presence of any reduced hydrolyzed or polymeric technetium colloid that would not elute from the RP-HPLC column.

Preparation of biological samples for RP-HPLC analysis was performed using a pre-wet ($C_{18}$) Sep-Pak® Cartridge (Waters Associates, Milford MA), prepared by first injecting absolute ethanol (5 ml) through the cartridge, followed by distilled water (5 ml) and finally air (10 ml).

### Radiochemical Preparations

Technetium-99m-CPI was synthesized by addition of $^{99m}$TcO$_4^-$ (20–200 mCi, 0.74–7.4 GBq, 1–2 ml) as commercial generator eluate (NaCl, 0.15 *M*) to a vial containing sodium dithionite (5.0 mg, 0.029, ethanol (0.25 ml mmol, 95%) and the liquid CPI ligand (5 $\mu$l, 0.039 mmol followed by heating at 65°C for 30 min. Separation of the radiolabeled complex from reagents was performed by loading the cooled solution (1.0 ml) onto a pre-wet $C_{18}$ Sep-Pak® followed by saline (0.15 *M*, 10 ml) then an ethanol/water (30%, 5 ml) wash and eluted in ethanol/saline (95%, 5 ml). RP-HPLC-A retention time: 9.0 min (k′ = 4.20); RP-TLC $R_f$ = 0.7. Radiochemical purity was determined by both RP-HPLC and RP-ITLC to be >95%. The $^{99m}$Tc-CPI complex, in this kit formulation, is stable for >6 hr at pH 4–7.

Alternatively, human and some animal studies were performed using a lyophilized kit formulation consisting of Cu(CPI)$_4$BF$_4$ (1.0 mg), L-Cysteine (1.0 mg), $SnCl_2$ (0.075 mg), sodium citrate (2.6 mg), and mannitol (20.0 mg). With this formulation, quantitative radiochemical yield (>95%) was obtained by adding $^{99m}$TcO$_4^-$ (20–210 mCi, 0.7–7.8 GBq, 1–3 ml) and incubating for 10 min at 100°C or 20 min at 25°C (*14*). RP-HPLC-B retention time: 14.0 min (k′ = 5.36); RP-TLC $R_f$ = 0.7.

### Identification of $^{99m}$Tc-CPI Hydrolysis Products

Comparison of integrated RP-HPLC peak ratios over time for the base catalyzed hydrolysis products of the characterized $^{99}$Tc(CPI)$_6^+$ complex enabled the assignment of molecular structures to peaks with specific retention times for the carrier-free $^{99m}$Tc-CPI preparations. The characteristic RP-HPLC retention times were used to identify metabolites of the parent $^{99m}$Tc-CPI agent after exposure to biological samples. Synthesis and characterization of the base catalyzed hydrolysis products of $^{99}$Tc(CPI)$_6^+$ have been described elsewhere (*1*). Correlations with the products obtained from the short lived $^{99m}$Tc-CPI starting material were made by incubation of this cationic complex in aqueous base (NaOH, pH 10.0) at 25°C, which over time produced a series of nine new technetium-containing compounds. These are the results expected for a rigid octahedral complex undergoing six sequential alterations of the coordinated ligands. In addition to the structurally unique mono-, penta- and hexa-hydrolyzed products, concurrent pairs of *cis-trans*, *fac-mer* and *cis-trans* geometrical isomers for the di-, tri- and tetra-hydrolyzed products are produced (*1,13*).

### Animal Biodistribution and Pharmacokinetics

Male albino guinea pigs (250–300 g) were anesthetized by ether inhalation and injected intravenously, through an exposed femoral vein, with $^{99m}$Tc-CPI (100 $\mu$Ci, 3.7 MBq/100 $\mu$l) for biodistribution. Each time point represents the mean of six animals ± s.d. ($\sigma_{n-1}$). Dynamic imaging studies were performed after intravenous injection of $^{99m}$Tc-CPI (1.0 mCi, 37 MBq/0.2 ml) under nembutal anesthesia (i.p.) and positioning the animal for an anterior view directly on top of a LEAP collimated GE 400T gamma camera (GE Medical Systems, Milwaukee, WI). Images were collected at 60 sec intervals continuously for 1 hr in a 64 × 64 matrix. Time activity curves for heart, lungs, liver and kidneys were obtained without background subtraction and corrected for physical decay of the $^{99m}$Tc. The time post-maximum mean counts per pixel ($T_{MAX}$) for activity to clear to half its maximal value ($t_{½}$) was obtained directly from decay-corrected time-activity plots and is reported as the mean value for three guinea pigs.

### Analysis of In Vivo $^{99m}$Tc-CPI Hydrolysis in Guinea Pigs

For analysis of in-vivo metabolism of the technetium ester isonitrile complex, guinea pigs were fasted for 12 hr, anesthetized by ether inhalation and injected with $^{99m}$Tc-CPI (25 mCi, 0.9 GBq/0.3 ml) through an exposed femoral vein. Animals were killed at 30 min postinjection, the time of maximal gall bladder activity as determined by dynamic gamma camera studies. Bile and urine samples were removed immediately from the gall bladder and urinary bladder and treated with cold ethanol (1:2, v:v) followed by cooling to 4°C and centrifugation at 5000 × g for 10 min. Separation of supernatant from precipitated proteins and volumetric quantitation of activity indicated less than 3% of the $^{99m}$Tc-CPI was associated with proteins. The bile and urine supernatants were analyzed by RP-HPLC and RP-ITLC under conditions identical to those used to test for radiochemical purity.

### Human Pharmacokinetic Studies

Eleven male volunteers (ages 29–50, average 37.4) without previous history or clinical evidence of cardiopulmonary disease (four smokers/seven nonsmokers) were studied in a dynamic mode to measure pharmacokinetics of $^{99m}$Tc-CPI. All subjects had been fasting 3–4 hr prior to the rest intravenous injection of

$^{99m}$Tc-CPI (18.3–23.7 mCi, 0.68–0.88 GBq/1.5 ml). Image acquisition was performed with subjects in a supine position using a large field-of-view Anger scintillation camera (GE 400T) with a low-energy, high-resolution, parallel-hole collimator centered over the thoracic abdominal region in an anterior view. Dynamic studies were begun immediately following injection and continued for 60 min with images recorded in a 64 × 64 matrix at a rate of 15 sec/frame. Regions of interest (ROIs) were hand drawn for left ventricular myocardium, ventricular cavity, right lung, liver (free of biliary structures), spleen, left kidney and gall bladder. Time-activity curves were generated, normalized to cps/pixel, corrected for physical decay and used to calculate $T_{max}$ and $t_{½}$.

Periodic venous blood samples were drawn from the opposite arm of the administered dose in four normal volunteers. Beginning at 30 sec postinjection, a total of 17 samples (2–3 ml each) were obtained up to 1 hr. Blood clearance data were expressed as %ID in the whole blood (assuming total blood = 7.8% of body weight) (*16*) and %ID in blood cells. Immediately after collection, samples were centrifuged (3000 × g, 3 min) and cells were separated from plasma to determine cell associated activity.

### Analysis of In Vivo $^{99m}$Tc-CPI Hydrolysis in Humans

Plasma samples, obtained from blood drawn at 1 min and 2 min postintravenous injection of $^{99m}$Tc-CPI, were analyzed for radiochemical species present by RP-HPLC. To 1.0 ml of plasma was added 2.0 ml ice cold ethanol followed by chilling and centrifuging to separate precipitated proteins. The supernatant was analyzed by RP-HPLC with the eluate collected in 0.2 ml samples and counted in an auto gamma counter and plotted as elution time versus cpm/ml.

Bile samples were obtained from volunteer patients who had undergone cholecystectomy where an external t-tube to the common bile duct had been emplaced. In one patient injection of $^{99m}$Tc-CPI (10 mCi, 0.37 GBq) followed by gamma camera imaging was performed to evaluate patency of the bile duct. Bile samples were drawn via the t-tube from this patient at 5-min intervals at volumes of 1.3–2.5 ml up to 60 min postinjection. At maximum bile activity, 30 min postinjection, a 1.9-ml sample of bile was collected and mixed with ethanol (0.5 ml), chilled in ice for 10 min and centrifuged at 5000 × g for 10 min. The supernatant, which contained >95% of the activity, was analyzed by RP-HPLC. Control experiments to test for continued hydrolysis in bile after elimination by hepatocytes were performed by incubating $^{99m}$Tc-CPI (20 mCi, 0.74 GBq/400 μl) in freshly drawn human bile (20 ml) at 37°C and withdrawing 1.0-ml samples at 0, 30, 60 and 120 min to analyze by RP-HPLC as above. Tests for possible retention of protein bound species on the column demonstrated <5% of bile associated activity was retained.

Urine samples were obtained from blood clearance study volunteers at 1, 3, 6, 12 and 24 hr postinjection to determine %ID excreted through the kidneys. Samples obtained at 1 hr postinjection were subjected to RP-HPLC analysis after being processed the same as guinea pig samples above.

### In Vitro Hydrolysis of $^{99m}$Tc-CPI

Tests for in vitro hydrolysis of the radiolabeled compound were performed using plasma obtained by centrifuging freshly drawn heparinized blood. Human blood and urine was obtained from fasted (>8 hr) male and female volunteers. For interspecies comparison, heparinized blood was taken by cardiac puncture of anesthetized male albino guinea pigs. Blood samples were centrifuged for 5 min at 3000 × g, to separate cells, and the plasma kept cold (4°C) until each experiment. In each hydrolysis experiment, a fluid sample (0.20 ml) was pipetted into a borosilicate culture tube and equilibrated to 37°C in a water bath. The $^{99m}$Tc-CPI complex (10–50 mCi/ml, 0.37–1.85 GBq/ml, 20 μl) in an ethanol/saline solution (25%, 0.15 *M*) was added, the contents were shaken and incubation was continued for various lengths of time. Enzymatic hydrolysis was halted by addition of cold (4°C) absolute ethanol (1.0 ml) and cooling in an ice bath to precipitate the plasma proteins. The samples were centrifuged (5 min, 5000 × g, 4°C) and the supernatant was analyzed by radiometric quantitation of the RP-HPLC separated products. Repeated RP-HPLC analysis of the ethanolic supernate solution over time proved that further hydrolysis did not occur.

Urine, bile, heart, liver and kidney were also obtained from killed animals. Samples of urine and bile were processed undiluted in a manner identical to the human samples above. Tissue homogenate of guinea pig heart, liver or kidney was prepared by mincing the organ into 1–2 ml pieces, diluting with an equal weight of phosphate-buffered saline (pH: 7.4, 4°C) and homogenizing in a Potter-Elvehjem (teflon and glass) tissue grinder. To 1 ml of the tissue homogenate was added $^{99m}$Tc-CPI (10–50 mCi/ml, 0.37–1.85 GBq/ml, 40 μl) followed by mixing and incubation for varying lengths of time at 37°C. To halt the hydrolysis, the homogenate was mixed with an equal volume of ethanol (95%, 4°C), centrifuged at 5000 × g for 5 min at 4°C and the supernatant analyzed by RP-HPLC.

## RESULTS

### Biodistribution and Pharmacokinetics

Quantitative biodistribution measurements of $^{99m}$Tc-CPI in the guinea pig demonstrated a high initial accumulation of activity in myocardial tissue (Table 1). At increasing times postinjection the concentration of $^{99m}$Tc-CPI in the heart was observed to decrease with a half-life of 1.1 hr. Technetium-99m-CPI cleared from the blood very rapidly, with less than 1% of the injected activity remaining at 5 min and lower quantities observed at later times. The distribution at 5 min postinjection indicated that most of the activity was cleared through the liver and kidneys with a significant amount remaining in the peripheral muscles. Technetium-99m-CPI activity localized in peripheral muscle cleared at a moderate rate which was significantly faster than rates reported for $^{99m}$Tc-MIBI (*17*).

Dynamic gamma camera imaging performed on anesthetized guinea pigs enabled plotting of ROI time-activity curves for the heart, lung, liver and kidneys (Fig. 1) with measured biological half-lives of 68 ± 12 min, 6.6 ± 0.5 min, 12.5 ± 2.3 min and 36.6 ± 9.2 min respectively for each organ (n=3). The more rapid pharmacokinetic behavior in nontarget organs produces an increase in heart-to-lung and heart-to-liver ratios to maximums of 2.0 and 1.0 respectively at 25 min postinjection. The gradual myocardial clearance of $^{99m}$Tc-CPI is consistent with the biodistribution data and is faster than the reported whole heart clearance of Tc-MIBI (*17*) but not nearly as fast as washout of the neutral myocardial perfusion agent Cardiotec® (*18*).

**TABLE 1**
Biodistribution of $^{99m}$Tc-CPI in Guinea Pigs in %ID/g

| Organ | 5 min* | 30 min* | 60 min* | 4 hr* |
|---|---|---|---|---|
| Heart | 0.90 ± 0.20 | 0.86 ± 0.35 | 0.51 ± 0.21 | 0.097 ± 0.033 |
| Blood | 0.053 ± 0.008 | 0.035 ± 0.018 | 0.023 ± 0.014 | 0.007 ± 0.001 |
| Lung | 0.47 ± 0.06 | 0.221 ± 0.029 | 0.047 ± 0.013 | 0.020 ± 0.009 |
| Liver | 0.87 ± 0.29 | 0.15 ± 0.10 | 0.066 ± 0.028 | 0.016 ± 0.002 |
| Kidneys | 3.04 ± 0.78 | 2.10 ± 0.90 | 1.40 ± 0.50 | 0.10 ± 0.03 |
| Muscle | 0.12 ± 0.08 | 0.14 ± 0.05 | 0.07 ± 0.05 | 0.05 ± 0.02 |
| Fat | 0.34 ± 0.13 | 0.10 ± 0.07 | 0.10 ± 0.04 | 0.04 ± 0.02 |

* Mean of n = 6 animals ± s.d. ($\sigma_{n-1}$).

Gamma camera imaging in humans, following rest administration of $^{99m}$Tc-CPI, demonstrated good visualization of the myocardium in all volunteers. Myocardial activity was visually resolved early after injection, by 3 min in most subjects, with rapid clearance of blood and lung activity. Figure 2 shows three representative images obtained at 1 hr postinjection with 5 min acquisition times in the anterior, LAO and lateral projections. Imaging studies in humans qualitatively mimicked the initial distribution observed in guinea pigs in that $^{99m}$Tc-CPI extraction from the bloodstream by the heart, liver and kidneys was rapid.

Human blood clearance curves, from serial blood sampling, exhibited an exponential clearance to 4% of the injected activity by 1 hr. Figure 3 plots the decrease in total blood activity over time, for a single subject, as well as the relative percent of cell associated counts in the cells. The percent of activity in the blood that was cell associated remained approximately constant with 19% and 27% associated with blood cells at 1 min and 90 min postinjection respectively. Rapid extraction of activity from blood into the liver can be appreciated from the time-activity curves, as exemplified in Figure 4 for a single subject. The mean measured $T_{max}$ in the liver and kidney was 11.6 ± 3.1 and 3.8 ± 1.4 min respectively (n=11 subjects). A comparison of guinea pig and human organ ratios from these curves are favorable at early and late time points. For the guinea pig, heart-to-lung ratios went from 1.5 to 2.0 and heart-to-liver ratios went from 0.6 to 1.0 at 5 and 60 min postinjection respectively. The mean of human heart-to-lung ratios went from 1.51 ± 0.14 to 3.25 ± 0.44 and heart-to-liver ratios went from 0.48 ± 0.10 to 0.87 ± 0.25, respectively, at 5 and 60 min postinjection.

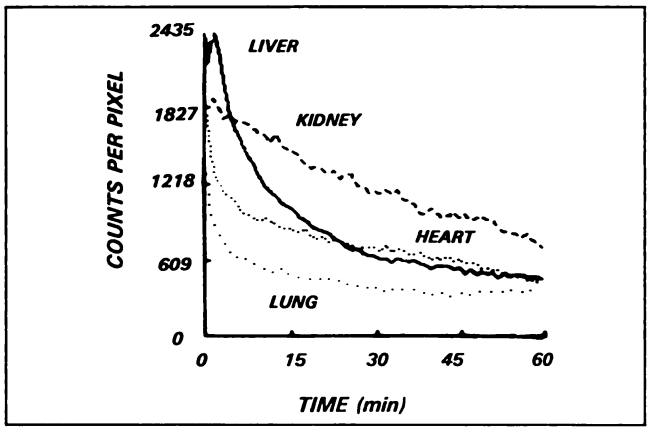


**FIGURE 1.** Representative time-activity curves for fasted anesthetized guinea pigs up to 60 min postinjection of $^{99m}$Tc-CPI. Regions of interest are normalized to cpm/pixel and corrected for physical decay.

Hepatobiliary and renal clearance as determined from dynamic imaging studies for 11 fasted male volunteers are summarized in Table 2. The clearance rates vary greatly for this series of volunteers, especially for liver clearance with a biological half-life range of 22.0–82.6 min. The residual activity levels as measured by the retention index at 30 min postinjection (cpm @ 30 min/$cpm_{MAX}$) also varied greatly in the liver with a range of 48%–94%. Renal clearance times were much shorter and fell within a more narrow range: 11.2–19.9 min. Based on a linear extrapolation of heart time-activity curves, a measured mean value of 4.9 ± 1.8 hr for the biological myocardial washout was also determined which was slower than rates observed in guinea pigs.

### Technetium-99m-CPI Hydrolysis in Guinea Pigs

Radiochemical analysis by RP-HPLC of bile samples taken from fasted guinea pigs 30 min after injection of $^{99m}$Tc-CPI revealed numerous species not present before injection as seen in Figure 5. The first new technetium-containing compound in the bile was the mono-hydrolyzed species. The observed quantity of this neutral species at 30 min varied greatly between animals with a mean value of 56.2% ± 13.3% (n=4). The next most prevalent compound in the guinea pig bile at 30 min was the di-hydrolyzed product 37.7% ± 7.6% with a geometric isomer ratio for *cis* to *trans* approaching 4:1, the theoretical ratio for random hydrolysis.

Analysis of urine samples, taken at 30 min postinjection from the same animals, also indicate the most prevalent species present were the mono-hydrolyzed product at 55.2% ± 19.8% (n=4) and the di-hydrolyzed product 34.3% ± 9.9%, as shown in Figure 5. In contrast to the bile samples however, the ratio of *cis* to *trans* for the di-hydrolyzed product approached 1:2, indicating sequential enzymatic hydrolysis that was not random.

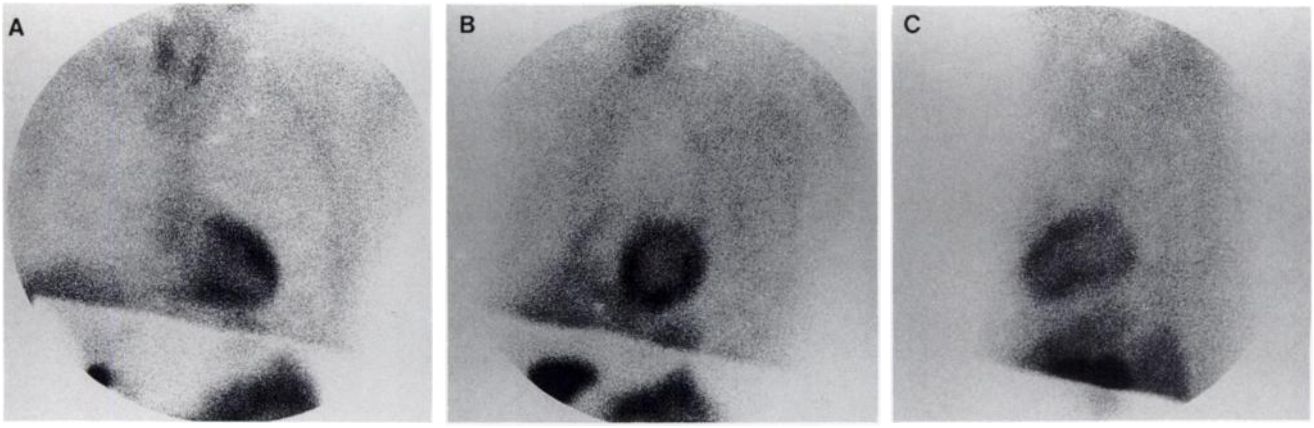


**FIGURE 2.** Representative images of a normal male volunteer at 60 min after rest injection of $^{99m}$Tc-CPI. Images are 5 min acquisitions in the (A) anterior, (B) LAO and (C) lateral projections.

Control studies to determine possible compartmentalization of this hydrolysis reaction consisted of in vitro incubation in plasma, urine, bile, buffer solution and heart, liver or kidney homogenate. It was noted that bile and urine samples could often exhibit rather high pH values and since alkaline hydrolysis of esters is known to occur, incubation in phosphate buffered bile and urine were also performed. Table 3 summarizes these experiments and shows the consistent decrease in percent of the initial cationic $^{99m}$Tc-CPI over time. Even in physiologically buffered Krebs salt solution almost 50% of the parent $^{99m}$Tc-CPI was hydrolyzed by 2 hr. Incubation with guinea pig bile in vitro produced more rapid hydrolysis of $^{99m}$Tc-CPI than observed in buffer solution with only 48.8% of the cation remaining at 30 min. However, the pH for this bile sample was extremely high (pH=9.0) and when the same bile was neutralize to 7.4, much slower decomposition rates were observed.

Table 3 shows that the rate of $^{99m}$Tc-CPI hydrolysis in guinea pig plasma was at least three times faster than in any other tissue homogenate or fluid. Incubation of $^{99m}$Tc-CPI in homogenate of guinea pig heart, liver and kidney showed significant hydrolysis of the parent cationic complex over time with only 27%, 19% and 25% of the original complex present respectively after 60 min at 37°C. None of the tissue homogenates, however, produced hydrolysis as rapid as was observed in plasma which resulted in only 3% of the initial $^{99m}$Tc-CPI being present after 30 min of incubation at pH 7.4. In addition to a greater rate of hydrolysis, in vitro incubation of $^{99m}$Tc-CPI in guinea pig plasma produced different isomer ratios from those observed in bile or organ homogenates. Repeated experiments demonstrated that, in guinea pig plasma, the ratio for *cis/trans* isomers of the di-hydrolyzed products was closer to 0.5 rather than the 4.0 predicted for a sequential random process.

### Technetium-99m-CPI Hydrolysis in Humans

A bile sample obtained from a patient 30 min after intravenous injection of $^{99m}$Tc-CPI and subject to RP-HPLC analysis revealed significant hydrolysis of $^{99m}$Tc-CPI occurring in vivo (Fig. 6). In this analysis, none of the initially injected cationic complex was observed, with the

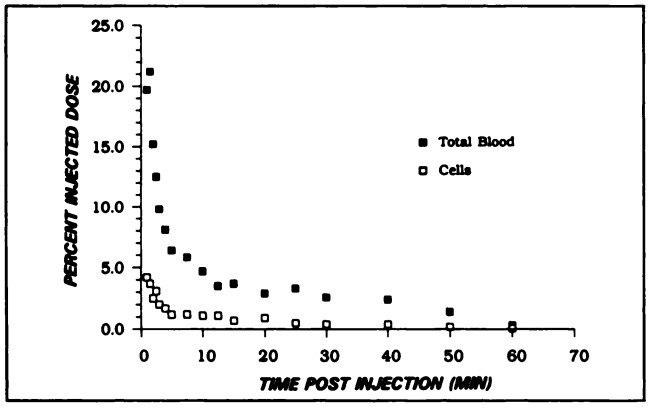


**FIGURE 3.** Blood clearance curves of $^{99m}$Tc-CPI in a fasted human volunteer, including percent of dose associated with whole cells.

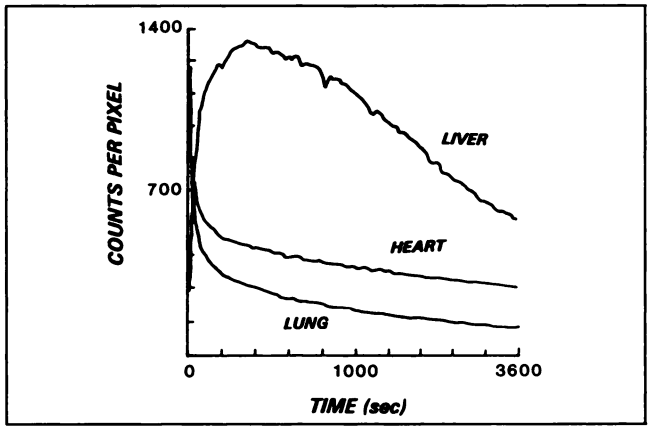


**FIGURE 4.** Representative time-activity curves for liver, heart and lung in a representative fasted human volunteer up to 60 min postinjection of $^{99m}$Tc-CPI. Regions are normalized to counts/15 sec/pixel and corrected for physical decay.

**TABLE 2**
Excretion of $^{99m}$Tc-CPI in Humans

| Patient | Hepatic clearance | | Renal clearance | |
|---|---|---|---|---|
| | $T_{½}$ (min) | RI (%) | $T_{½}$ (min) | RI (%) |
| 1 | 53.0 | 75.4 | — | — |
| 2 | 22.0 | 47.9 | 29.2 | 52 |
| 3 | 47.0 | 78.0 | 19.9 | 41 |
| 4 | 36.2 | 79.8 | 19.2 | 31 |
| 5 | 40.8 | 85.1 | 15.6 | 31 |
| 6 | 31.7 | 80.1 | 15.9 | 32 |
| 7 | 38.2 | 67.6 | 11.2 | 23 |
| 8 | 31.0 | 68.1 | 17.5 | 33 |
| 9 | 34.5 | 84.1 | 15.8 | 31 |
| 10 | 82.6 | 93.8 | 22.2 | 42 |
| 11 | 42.0 | 62.8 | — | — |
| mean | 41.7 | 74.8 | 18.5 | 36.2 |
| s.d. ($\sigma_{n-1}$) | 15.9 | 12.5 | 5.1 | 8.5 |

$T_{½}$ = time to half-value of maximal activity and RI = retention index (cpm @ 30 min/cpm max).

**TABLE 3**
In Vitro Hydrolysis of $^{99m}$Tc-CPI* in Guinea Pig Tissue at 37°C

| | 0 min | 2 min | 30 min | 60 min | 120 min | pH |
|---|---|---|---|---|---|---|
| Krebs | 97.4 | 95.7 | 87.6 | 72.1 | 54.6 | 7.4 |
| Bile | 95.3 | 89.7 | 48.8 | 15.0 | 2.0 | 9.0 |
| Bile† | 97.4 | 96.6 | 86.6 | 75.0 | 47.3 | 7.4 |
| Urine | 95.3 | 95.1 | 82.2 | 68.5 | — | 8.0 |
| Liver | 95.5 | 93.4 | 42.8 | 19.5 | 7.2 | 7.4 |
| Kidney | 95.5 | 92.5 | 47.3 | 25.0 | 11.9 | 7.4 |
| Heart | 98.4 | 96.0 | 52.2 | 27.3 | 9.8 | 7.4 |
| Plasma | 98.5 | 89.2 | 3.1 | 1.7 | ≤0.1 | 7.4 |

* Values represent percent of activity present as initial cationic $^{99m}$TC-CPI starting material.
† Repeat of same bile sample with pH adjusted to control buffer.

majority of products present as the mono- and di-hydrolyzed species. Also, the ratio of *cis/trans* isomers for the di-hydrolyzed octahedral complex was 4:1, which is what would be expected for a random sequential reaction.

Analysis of urine samples obtained at 60 min postinjection from several patients also exhibited hydrolysis of the injected lipophilic cation. The major components present in the urine were the mono- and di-hydrolyzed species with the noted similarity to bile products in that the ratio of *cis/trans* isomers was 4:1.

HPLC analysis of blood samples drawn at 1 and 2 min postinjection indicated that 85% ± 9% (n=4) of the radiolabel was present as the parent lipophilic cationic complex with the remainder present as the mono- and di-hydrolyzed species. In vitro incubation of the $^{99m}$Tc-CPI complex with human plasma from normal subjects for 2 min showed a similar rate of hydrolysis, with 85.2% ± 6.6% present as $^{99m}$Tc-CPI.

A summary of the analysis for in vitro hydrolysis of $^{99m}$Tc-CPI in plasma, bile and urine from human is shown in Table 4. Comparison of hydrolysis rates between different fluids parallels the results obtained in guinea pigs in that only plasma demonstrated a rate significantly greater than control Krebs buffer. In vitro incubation in human plasma for longer times produced an extended series of new technetium-containing species. Figure 7 shows the RP-HPLC separation of products obtained after incubation for 70 min at 37°C and pH 7.4. Nine separated species were observed under these conditions indicating continued sequential hydrolysis.

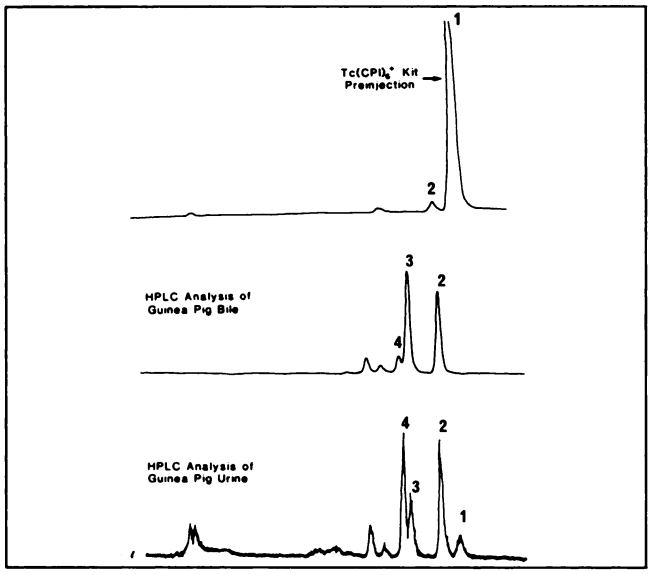


**FIGURE 5.** Radiographic detection for RP-HPLC analysis of guinea pig bile and urine 30 min postintravenous administration of $^{99m}$Tc-CPI and a comparison with the agent preinjection. Peak numbers correspond to species in Table 5.

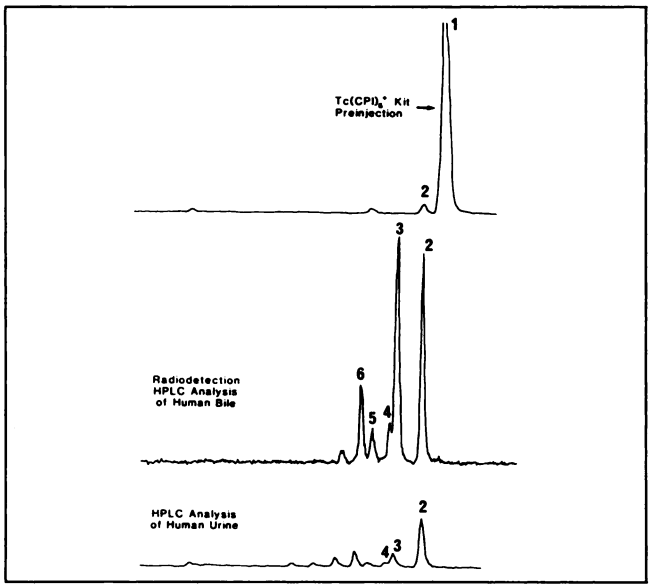


**FIGURE 6.** Radiographic detection for RP-HPLC analysis of human bile (30 min) and urine (60 min) postintravenous administration of $^{99m}$Tc-CPI and a comparison with the agent preinjection.

**TABLE 4**
In Vitro Hydrolysis of $^{99m}$Tc-CPI* in Human Fluids at 37°C

| | 0 min | 2 min | 30 min | 60 min | 120 min | pH |
|---|---|---|---|---|---|---|
| Krebs | 97.4 | 95.7 | 87.6 | 72.1 | 54.6 | 7.4 |
| Bile | 98.5 | 95.5 | 89.9 | 79.5 | 62.8 | 7.4 |
| Urine | 99.8 | 97.3 | 93.9 | 96.6 | 96.3 | 6.0 |
| Serum | 97.1 | 85.2 | 1.7 | 0.6 | ≤0.1 | 7.4 |

* Values represent percent of activity present as initial cationic $^{99m}$Tc-CPI starting material.

Table 5 summarizes the products obtained when $^{99m}$Tc-CPI is incubated in either human or guinea pig plasma for increasing lengths of time. Although the initial rate of hydrolysis is similar for the two species, the ratio of *cis/trans* isomers of the di-hydrolyzed products was consistently different with an observed mean of 3.6:1 (n=6) for human plasma and 0.47:1 (n=4) in guinea pig plasma. These results indicate that hydrolysis of $^{99m}$Tc-CPI occurs enzymatically in plasma of both human and guinea pig, however, the enzyme systems are most probably quite different.

## DISCUSSION

### Biodistribution

Guinea pigs were chosen for quantitative biodistribution studies because of their similarity to human cardiophysiology and the observation of slow in-vitro serum hydrolysis of $^{99m}$Tc-CPI (*13*). In contrast to the reported distribution in rat and mouse (*13*), guinea pigs showed efficient extraction and localization of $^{99m}$Tc-CPI in the heart with gradual washout over time. Quantitative biodistribution data at 15 min and greater postinjection demonstrated a rapid decrease in liver and kidney activity without increases in blood concentrations, indicating both hepatobiliary and renal excretion without reabsorption. This behavior qualitatively correlated with human dynamic imaging studies.

The measurement of clearance rates from biodistributions correlated well with dynamic gamma camera imaging, although ratios of heart-to-lung or heart-to-liver on a per gram basis did not. In the absence of real human biodistribution data, heart-to-lung and heart-to-liver ratios from dynamic camera images were used to compare $^{99m}$Tc-CPI between humans and guinea pigs. At early times postinjection, heart-to-lung and heart-to-liver ratios of 1.5 and 0.6 compared favorably with human values of 1.5 and 0.5, respectively. The rapid clearance of background activity from blood and lung, through extraction by liver and kidney, meets the requirements for a "nonredistributing" myocardial perfusion agent to evaluate ischemia (*19*).

At longer times postinjection, target-to-background ratios for the smaller guinea pigs improved, due to the more rapid pulmonary and hepatobiliary clearance, resulting in heart-to-lung and heart-to-liver ratios of 2.0 and 1.0, respectively, at 60 min postinjection. A similar trend was also observed in humans with ratios increasing to 3.2 and 0.9 for heart-to-lung and heart-to-liver, respectively. Moderate washout of activity from the heart was measured in both species but at a rate that was much slower than blood, lung, liver or kidney clearance. Consequently, high contrast images were obtainable even at greater than 1 hr postinjection.

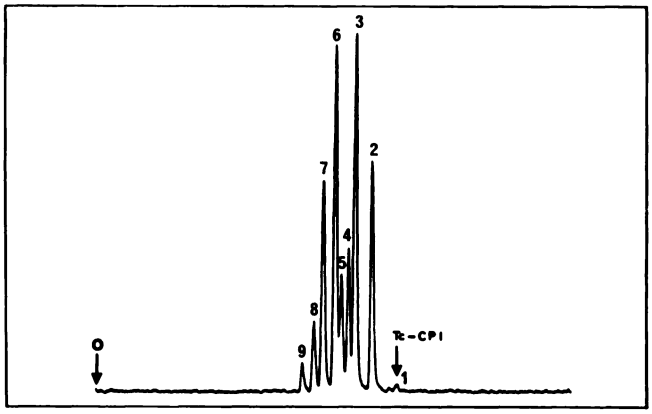


**FIGURE 7.** Radiographic detection for RP-HPLC analysis of $^{99m}$Tc-CPI following 70 min of in vitro incubation with human serum at 37°C. Peak numbers correspond to species in Table 5.

### Radiopharmacology

The kit formulation of $^{99m}$Tc-CPI is stable and found to contain the cationic radiolabeled complex in radiochemical yields ≥95%, for more than 24 hr (*14*). However, when diluted in physiological buffer at pH 7.4 significant hydrolysis occurs, although not as rapid as the hydrolysis observed in plasma. Incubation of $^{99m}$Tc-CPI in plasma from guinea pigs or humans produces hydrolysis of the terminal ester groups far too rapidly to be explained by chemical hydrolysis at pH 7.4. The initial rates of $^{99m}$Tc-CPI metabolism are similar in plasma from humans and guinea pigs but still more than 500 times slower than hydrolysis rates observed in rat or mouse plasma (*13*). This increased rate over buffer solutions along with the different ratios of *cis/trans* isomers of di-hydrolyzed products observed in guinea pig blood are evidence of an enzymatic hydrolysis process occurring in the blood.

The first product of hydrolysis, although still lipophilic with a net overall neutral charge, unlike other neutral complexes (*18*), does not accumulate significantly in the heart of guinea pig or rabbit (*13*) and is not taken up by isolated cultured chick heart cells (*7*). Also, the amount of cationic $^{99m}$Tc-CPI or $^{99m}$Tc-MIBI, a compound of similar lipophilicity, accumulation into myocytes due to nonspecific lipid partitioning is low, representing only about 5%–10% of the activity at equilibrium (*5,22,23*). This is explainable because the lipophilicity of $^{99m}$Tc-MIBI, $^{99m}$Tc-CPI and its mono-hydrolyzed product are significantly less than the neutral myocardial perfusion against Cardiotec® (*20,21*). These data infer that any hydrolyzed $^{99m}$Tc-CPI in the blood, at extended times postinjection, would not

**TABLE 5**
Metabolites of $^{99m}$Tc-CPI After In Vitro Incubation in Plasma at 37°C

| Peak no. | Identity | 5 min | | 30 min | | 60 min | |
|---|---|---|---|---|---|---|---|
| | | Guinea pig | Human | Guinea pig | Human | Guinea pig | Human |
| 10 | $[Tc(H\text{-}CPI)_6]^{-5}$ | — | — | — | — | 2.5 | — |
| 9 | $[Tc(CPI)(H\text{-}CPI)_5]^{-4}$ | — | — | 2.8 | 1.8 | 12.9 | 2.7 |
| 8 | *trans*-$[Tc(CPI)_2(H\text{-}CPI)_4]^{-3}$ | — | — | 11.2 | 2.1 | 7.6 | 5.1 |
| 7 | *cis*-$[Tc(CPI)_2(H\text{-}CPI)_4]^{-3}$ | — | — | 6.3 | 6.2 | 5.7 | 13.2 |
| 6 | *mer*-$[Tc(CPI)_3(H\text{-}CPI)_3]^{-2}$ | — | — | 33.1 | 14.9 | 29.2 | 22.6 |
| 5 | *fac*-$[Tc(CPI)_3(H\text{-}CPI)_3]^{-2}$ | — | — | 17.9 | 4.3 | 15.9 | 7.2 |
| 4 | *trans*-$[Tc(CPI)_4(H\text{-}CPI)_2]^{-1}$ | 6.9 | 2.6 | 11.4 | 8.4 | 12.5 | 8.0 |
| 3 | *cis*-$[Tc(CPI)_4(H\text{-}CPI)_2]^{-1}$ | 2.3 | 8.8 | 4.6 | 24.5 | 6.8 | 25.3 |
| 2 | $[Tc(CPI)_5(H\text{-}CPI)]^{0}$ | 11.7 | 14.1 | 9.6 | 36.1 | 5.6 | 15.3 |
| 1 | $[Tc(CPI)_6]^{+}$ | 79.1 | 74.5 | 3.1 | 1.7 | 1.3 | 0.6 |

Values represent percent of each compound present in HPLC chromatograph.
(CPI) = $CNC(CH_3)_2COOCH_3$ and (H-CPI) = $CNC(CH_3)_2COO^-$.

accumulate in myocardial tissue to produce a redistribution phenomenon in a stress image.

Even though enzymatic hydrolysis of $^{99m}$Tc-CPI occurs in human blood, the rate is slow enough that more than 85% of the activity is present as cation during the first pass through the coronary arteries and is available for myocardial extraction. The experiments in guinea pig heart homogenate indicate that hydrolysis of $^{99m}$Tc-CPI occurs, after extraction by this tissue, at a moderate rate that is slower than rates observed in plasma. This rate, as well as the products obtained, suggests that, in myocardial tissue, chemical and/or a slow enzymatic hydrolysis of $^{99m}$Tc-CPI may be occurring. If, as has been proposed, myocardial retention of the cationic isonitrile complexes is determined by the large negative cytosolic and mitochondrial membrane potentials (*5*), then a neutral compound would not be expected to be intracellularly retained. Thus, the moderate washout of $^{99m}$Tc-CPI from myocardial tissue, not observed for other isonitrile complexes (*8,11*), probably results from gradual hydrolysis of the cation to a neutral, more hydrophilic species after it has been extracted by the heart.

Analysis of activity excreted in the urine and bile, following in vivo injection of $^{99m}$Tc-CPI, primarily revealed only hydrolyzed technetium complexes present by 60 and 30 min postinjection, respectively. However, in vitro incubation in urine and bile at pH ≤7.4 did not produce hydrolysis significantly different from buffered salt solutions. Assuming the agent once injected does not experience a pH significantly greater than 7.4 and that >90% of the activity is cleared from the blood by 2 min, it is concluded that to some extent enzymatic hydrolysis occurs within the heart, liver and kidney.

In vitro incubation in guinea pig urine, bile and tissue homogenate for extended lengths of time produced products that were consistent with a random sequential hydrolysis process. However, guinea pig plasma and in vivo samples of urine contained different ratios of the di-hydrolyzed products. These results, combined with hydrolysis rates greater than those observed in buffered solutions, suggest at least two esterase enzyme systems in guinea pigs. The more rapid enzymatic reaction occurs in the blood with the resulting mono-hydrolyzed product being excreted through either the renal or hepatobiliary systems, but the anionic di-hydrolyzed species is cleared primarily through the kidney.

It is not known if hydrolysis is necessary for renal or hepatobiliary clearance since human bile and urine samples at earlier times postinjection could not be obtained. Considering that the related $^{99m}$Tc-MIBI compound is excreted primarily unchanged in the bile and urine of guinea pig (*24*) and that some cationic $^{99m}$Tc-CPI is present in the urine of guinea pigs, it is feasible that excretion of the original cationic $^{99m}$Tc-CPI also occurs.

## ACKNOWLEDGMENTS

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