nm/PRELIMINARY NOTE

^{99m}Tc-PENICILLAMINE, A NEW CHOLESCINTIGRAPHIC AGENT

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This communication reports the development and use of a new radiopharmaceutical containing ^{99m}Tc that may be useful as a cholescintigraphic agent. The new agent would circumvent the difficulties and inconveniences associated with the existing oral nonradioactive cholecystographic agents and would obviate nephrotoxicity and problems associated with iodine sensitivity (*1,2*).

In our laboratory we have successfully combined ^{99m}Tc with D-penicillamine and this complex has been used as an intermediate to prepare other radiopharmaceuticals (*3–5*).

Technetium-99m-penicillamine (Tc-Pen) appears to be specifically useful as a cholescintigraphic agent. The radionuclide has a short half-life and ideal characteristics for dynamic and static imaging. The new agent has little or no toxicity permitting the use of millicurie doses with the possibility of early repeat studies and would require no thyroid blocking. In addition, it can be rapidly and simply prepared.

METHODS

Tc-Pen is prepared by reducing $^{99m}TcO_4^-$ with D-penicillamine (D-Pen) in HCl solutions of approximately 2.4 ± 0.2 normal. Under these conditions, the "reduced" ^{99m}Tc binds to the penicillamine by way of the SH and NH_2 groups forming a stable chelate which resists reversion to a spectrum of valence states. The excess penicillamine constitutes a reducing medium for the ^{99m}Tc.

Pertechnetate can be reduced from the Tc (VII) valence state by D-Pen at other pH levels and these are being investigated. The stability of Tc-Pen is demonstrable by paper chromatography in several solvents. Also it is excreted intact in the bile and urine.

The preparation is as follows: The pertechnetate solution, in a small volume containing the desired amount of radioactivity, is mixed with 60 mg D-Pen. Concentrated HCl is then added in the ratio of 0.3 to 1 ml TcO_4^- solution in a serum vial which is then capped, sealed, and heated to 100°C for 10 min. The contents are cooled, approximately 0.3 ml 11 *N* NaOH is added to nearly neutralize the acid, and the pH is finally adjusted to 7.0 with 1 *N* or 0.1 *N* NaOH. The solution is then filtered through a 0.22-micron membrane filter and calibrated. All preparations have been sterile and apyrogenic. Preparation time is approximately 20 min. The preparation is quite stable over several hours.

The complex has been characterized by descending paper chromatography on Whatman No. 1 using a solvent of *N* butanol:acetic acid:water, 4:1:1 by volume.

The average rf values in this system are as follows: TcO_4^- 0.25, Tc-Pen 0.7, and D-Pen 0.6. The D-Pen is identified by spraying with ninhydrin. The quantities of pertechnetate remaining or formed are always minimal.

RESULTS AND DISCUSSION

Organ distribution studies following intravenous injection were performed in mice. The results are plotted in Fig. 1. It can be seen that the greatest percent of dose per milligram of the Tc-Pen appears in the gallbladder bile very rapidly, is present at all time periods, and at 45 min, the ratio of gallbladder radioactivity to liver is 200:1. Other organs do not acquire nor retain significant radioactivity.

Chromatographic analysis of the bile has shown that 60–70% of the radioactivity is in the form of

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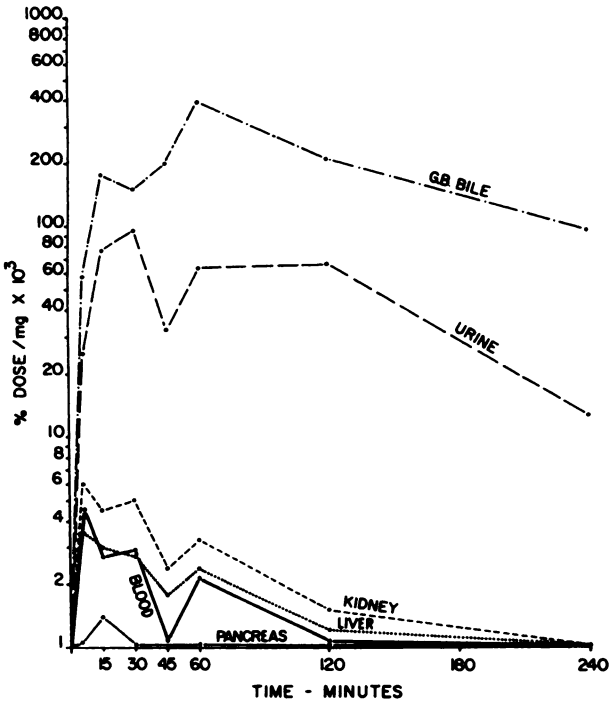

FIG. 1. Distribution of Tc-Pen in mice tissues and body fluids at various times after intravenous injection. Note early, rapid rise and high concentration in gallbladder bile.

Tc-Pen; the remainder is in the form of some other complex. Urinary radioactivity was shown to be associated with similar compounds. No significant amount of TcO_4^- was found in the plasma or urine of dogs.

Toxicity studies were performed in several species as follows: Twenty albino mice weighing 21–34 gm were injected i.v. (tail vein) at the rate of 15 mg Tc-Pen/20 gm body weight. This level is equivalent to 3,500 times the postulated dose for a 70-kilo man. There were no reactions, no evidence of toxicity and no fatalities. Mice injected intraperitoneally with 8,500 times the postulated human level dose showed no reactions or death. Rats injected intravenously with 1,000 times the level of the human dose, based on comparable body weight, also showed no reactions or death. Rabbits and dogs injected at 1,000 times the equivalent human dose, based on comparable body weights, showed no reactions or death. Thus in four animal species, no toxic reactions have been manifest after relatively massive doses, demonstrating a very high factor of safety.

Reported drug reactions to D-Pen in humans (*6,7*) have been due to repeated use of multimilligram or gram quantities.

Gallbladder visualization has been demonstrated in dogs. In four dogs, following intravenous injection of 2–3 mCi, the liver was well visualized within 30–40 min, with radioactivity appearing to be uniformly distributed throughout the organ. With time, the radioactivity gradually cleared from the liver and accumulated in the gallbladder. Forty-five to 60 min postinjection, the gallbladder was clearly demonstrated with the scintillation camera. Maximum gallbladder radioactivity is visible 2–3 hr after injection. Fat in the form of evaporated whole milk given orally, induced the gallbladder to empty the radioactivity into the intestine in 20–30 min. As the gallbladder radioactivity decreased, intestinal radioactivity rose concomitantly. Gallbladder and liver studies have been performed with the rectilinear scanner and scintillation camera.

Preliminary studies in human patients confirm the observations in other species. A scintillation camera photograph of one of the studies is shown in Fig. 2.

We have noticed that in our human patients there was a transient accumulation of radioactivity in the kidneys after 5–10 min, followed by a complete disappearance later.

CONCLUSIONS

The new radiopharmaceutical, Tc-Pen, may fill the need for a ^{99m}Tc-labeled agent for cholescintigraphy and may also aid in the differential diagnosis of space-occupying lesions in the liver due to either primary or secondary tumor. This agent may prove helpful in the differential diagnosis of obstructive jaundice and the diagnosis of congenital biliary atresia in the newborn. Tc-Pen is simply and readily prepared and has been shown to be of very low toxicity and to utilize the favorable properties of

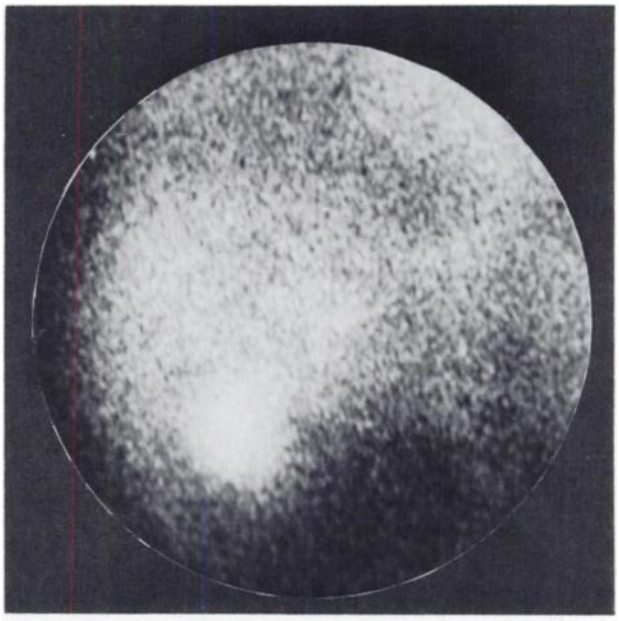

FIG. 2. Scintiphoto of human patient showing high concentration of radioactivity in gallbladder against low liver background.

^{99m}Tc. The potentialities of this new radiopharmaceutical for cholescintigraphy are very promising.

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