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# An Evaluation of $^{99m}$Tc-Labeled Hepatobiliary Agents

Brian W. Wistow, Gopal Subramanian, Ronald L. Van Heertum, Robert W. Henderson, George M. Gagne, Robert C. Hall, and John G. McAfee

*State University of New York Upstate Medical Center, Syracuse, New York*

***Scintigraphic imaging of the hepatobiliary system has been significantly improved with the development of $^{99m}$Tc-labeled compounds. Two of the most promising agents, pyridoxylideneglutamate and HIDA, each formed the basis for the development of a group of structural analogs. Condensation of pyridoxal with leucine and arginine (in place of glutamate) produced pyridoxylideneleucine and pyridoxylidenearginine. Since increasing the molecular weight and the lipid solubility of compounds tends to enhance their biliary excretion, several new IDA derivatives were synthesized by altering the lipophilic substituents on the ring of HIDA. The substitutions included ethyl and ethoxy groups as well as iodine. All compounds were compared with $^{131}$I-rose bengal using a baboon model that allowed blood, bile, and urine collection.***

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Gamma-emitting radiopharmaceuticals excreted primarily through the biliary tract are potentially useful for assessment of hepatobiliary function and for the scintigraphic diagnosis of acute cholecystitis and dilatation or obstruction of the biliary tract. Scintigraphy avoids one of the disadvantages of intravenous cholangiography (i.e., the high incidence of reactions to the contrast material) and may allow improved visualization of the biliary system compared with intravenous cholangiography when the serum bilirubin is mildly elevated.

Iodine-131-rose bengal was the first agent used for hepatobiliary studies (*1,2*). Iodine-labeled bromsulphthalein (*3*), indocyanine green (*4*), and asialo-$\alpha_1$-glycoprotein (*5*) have also been proposed. The physical characteristics of the nuclide $^{131}$I, however, are not optimal for imaging. Rose bengal is quickly excreted through the biliary tract and small bowel, but the long residence time in the large-bowel contents and the beta emissions of $^{131}$I result in a relatively high radiation dose to the bowel mucosa. Consequently the administered dose of this agent is restricted to 100–200 $\mu$Ci, thereby limiting the resolution of the images of the biliary tract. Iodine-123-rose bengal overcomes this limitation, but is expensive and, because of its physical half-life of 13 hr, not practical for unscheduled emergency examinations (*6*).

A $^{99m}$Tc-labeled agent would be preferable, because of the excellent physical characteristics of $^{99m}$Tc for imaging and the availability of $^{99m}$Tc in almost all nuclear-medical facilities. Many complexes of $^{99m}$Tc have been proposed as hepatobiliary agents, including penacillamine (*7*), dihydrothioctic acid (DHT) (*8*), tetracycline (*9,10*), mercaptoisobutyric acid (MIBA) (*11*), pyridoxylideneglutamate (PyG) (*12,13*), and 2,6-dimethylacetanilideiminodiacetic acid (HIDA) (*14*). In preliminary experiments reported elsewhere (*15*), several $^{99m}$Tc-labeled amino-acid complexes of pyridoxal also showed excellent hepatobiliary excretion. Of these, three complexes of pyridoxal (glutamate, leucine, and arginine) were studied here for comparison purposes. HIDA formed the basis for the other group of agents. The biliary excretion of compounds may

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For reprints contact: Brian W. Wistow, Div. of Nuclear Medicine, State Univ. of New York Upstate Medical Center, 750 E. Adams St., Syracuse NY 13210.

be enhanced by increasing their molecular weight and their lipid solubility. Accordingly various substitutions were made on the benzene ring of HIDA, creating the following compounds: 2,6-diethyl-IDA, p-ethoxy-IDA, and p-iodo-IDA (Fig. 1). These compounds are referred to in Figs. 1–6 as "diethyl," "p-ethoxy," and "p-iodo," respectively.

Following preliminary evaluation in mice, rabbits, and dogs, the most promising $^{99m}Tc$ agents were compared in the baboon. This animal was selected because the biliary excretion of many stable drugs in man is known to be markedly different from that in lower animals (*16*). Iodine-131-rose bengal was administered simultaneously with the various $^{99m}Tc$ agents to serve as a reference standard and to normalize the variations in biliary excretion rates.

## METHODS

The preparation of pyridoxylideneglutamate has been described elsewhere (*12,13*). Similar procedures were used to prepare pyridoxylideneleucine (PyL) and pyridoxylidenearginine (PyA). The iminodiacetic acid (IDA) derivatives shown in Fig. 1 were prepared by reacting various alpha-chloroacetanilides with IDA in ethyl alcohol and water solution under conditions similar to those described by Loberg et al. (*17*). The 2,6-dimethyl-alpha-chloride derivative is commercially available* and the other three derivatives were prepared by methods outlined by Borovansky et al. (*18*). All the components were purified by recrystallization before use. Detailed methods of preparation and their initial evaluation will be reported elsewhere (*19*). These compounds were made into freeze-dried kits containing 20–40 mg of the IDA derivative and 200–400 $\mu$g of $SnCl_2 \cdot 2H_2O$. The pH was adjusted to 5.5–6.5 and the kits were stored under nitrogen or vacuum. Standard methods of formulation were used. Labeling was achieved by adding 2–5 ml of $^{99m}TcO_4^-$ containing 5–75 mCi of activity. Labeling yields and radiochemical purities were studied using paper chromatography in both saline and in acetonitrile–water (3:1) as in methods described elsewhere (*17*). Consistent labeling yields of greater than 98% were obtained with all four compounds. No colloids were found by chromatography.

All of the previously mentioned pyridoxal and IDA derivatives were tested in a baboon model (Fig. 2) (*20*). Six different baboons weighing approximately 10–12 kg were surgically prepared to allow bile, blood, and urine samples to be collected. A T-tube was placed in the common bile duct with its distal end (a rubber septum) in the subcutaneous tissue of the anterior abdominal wall. A 22-gage needle with tubing was inserted through the septum for bile collection. Inflatable cuff occluders on the distal common bile duct and cystic duct (if necessary) directed the bile through the T-tube. A routine intravenous line was maintained for injections and

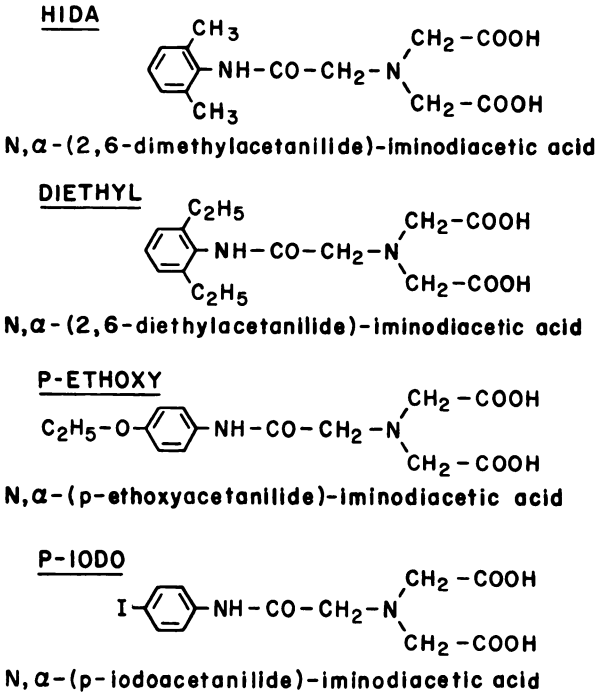


**FIG. 1.** HIDA-N,α-(2,6-dimethylacetanilide)-iminodiacetic acid and its derivatives evaluated in this study. Note various substituents on the benzene ring—2,6-diethyl, p-iodo, and p-ethoxy substitutions were made. Note: "diethyl," "p-ethoxy," and "p-iodo" refer to diethyl-IDA, p-ethoxy-IDA, and p-iodo-IDA, respectively, for Figs. 1–7.

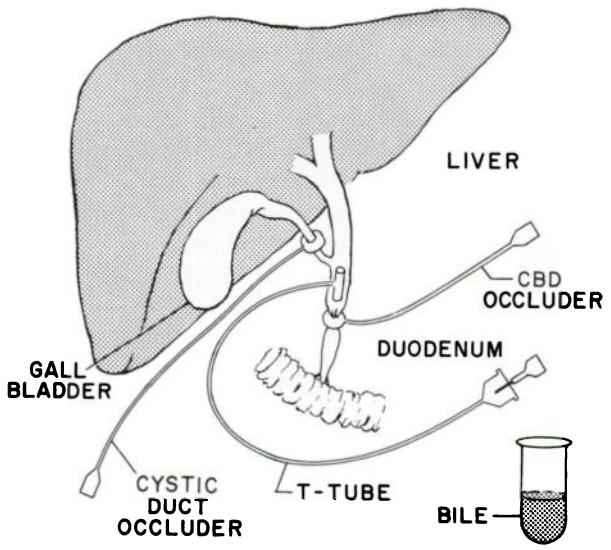


**FIG. 2.** Baboon model for collection of bile, blood and urine samples. Inflatable cuff occluders on common bile duct and cystic duct direct bile flow through T-tube in common bile duct.

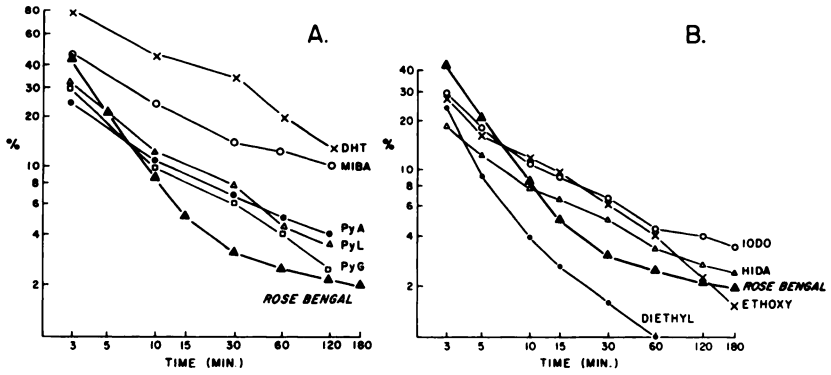


**FIG. 3.** Baboon blood clearances (double-log). Percent injected dose in total blood volume vs time (assuming blood volume to be 7% of body weight). Note only diethyl-IDA consistently remains below rose bengal throughout study, with less than 1% remaining in the blood at 1 hr.

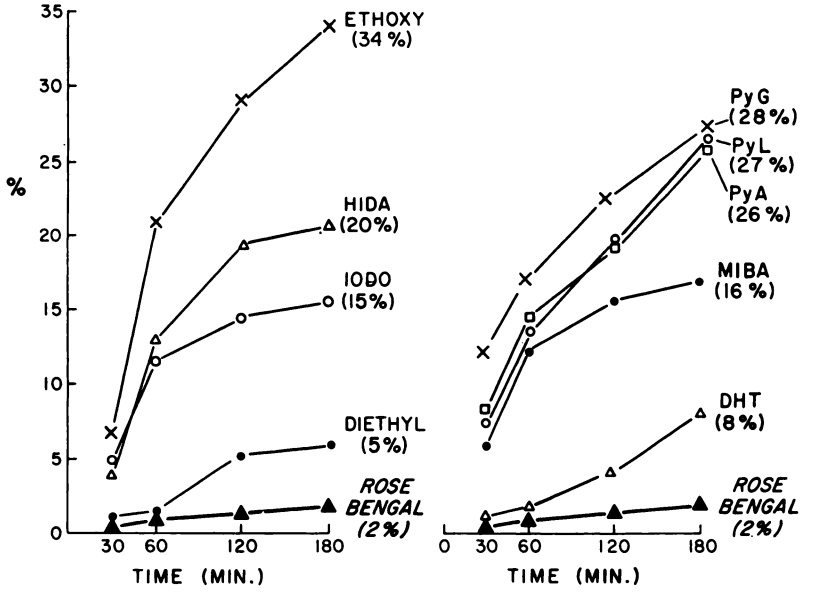


**FIG. 4.** Cumulative urinary excretion in baboons. Rose bengal has lowest value, 2% of the injected dose recovered from the urine in first 3 hr. Diethyl-IDA has the next lowest level (5%). Others range from DHT (8%) to ethoxy (34%).

hydration. Urine was drained from the bladder through a straight catheter.

One hundred microcuries of the $^{99m}$Tc-labeled hepatobiliary agent and 10 $\mu$Ci of $^{131}$I-rose bengal were injected simultaneously through the intravenous line in one of the extremities. Blood samples were collected at 3, 5, 10, 15, 30, 60, 120, and 180 min after injection. The first three samples were obtained from the opposite extremity. Urine samples were obtained for the 0–30, 30–60, 60–120, and 120–180-min intervals. Bile was collected continuously over 30-min periods for three hr. A minimum of three experiments was performed for each compound.

A single experiment in a human volunteer was performed following the intravenous injection of 15 mCi of diethyl-IDA. Serial images were taken during the first hour after injection utilizing a wide field of view scintillation camera with medium-resolution collimation. Images were also taken at 24 hr after injection.

## RESULTS

**Blood clearances.** Blood clearances in the baboons are recorded as percent administered dose in total blood volume and are plotted against time in Fig. 3. During the first 3–5 min, all the compounds of both the pyridoxal and the IDA-based groups have lower blood levels than rose bengal. Thereafter, however, only the diethyl-IDA is lower than rose bengal. The levels of the other compounds in these two groups are similar to each other and slightly higher than rose bengal. The two commercially available kits, MIBA and DHT, are considerably slower to clear from the blood.

**Urinary excretion.** Considerable differences in the

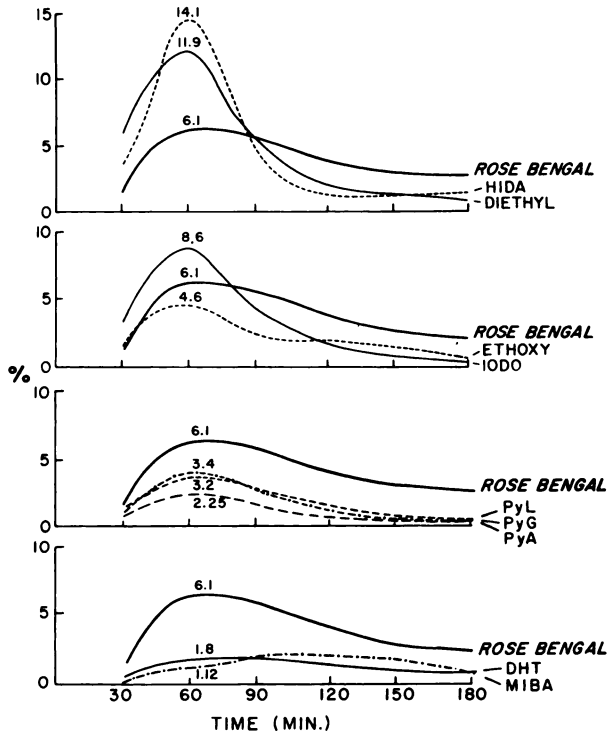


**FIG. 5.** Percent dose per cc bile. Note: at 30 min diethyl-IDA has highest value, 5.8%. This is a factor of 1.6 greater than HIDA and four times the rose bengal levels. Iodo-IDA is also substantially better than rose bengal during the first hour. Pyridoxylidene group and two commercially available kits have much lower levels.

cumulative urinary excretions of these compounds are apparent (Fig. 4). Rose bengal consistently has the lowest value of less than 2% of the injected dose recovered in the 3 hr. All the pyridoxal compounds are similar to each other and have 3-hr cumulative totals of 26–28%. The IDA derivatives show great variation. Ethoxy-IDA, HIDA, iodo-IDA, and diethyl-IDA range from 34% (ethoxy-IDA) to 5% (diethyl-IDA). MIBA measured 16%, and DHT (8%) was the only compound other than diethyl-IDA and rose bengal to be under 10%.

**Biliary excretion.** Figure 5 illustrates the biliary concentration of these agents as the percent dose/cc compared with time. During the first 30 min, diethyl-IDA has the highest level (5.8%). This is four times the rose-bengal concentration and 1.6 times that of HIDA. Somewhat lower levels are observed with iodo-IDA, PyG, and PyL. Concentrations of less than 1% are found with PyA, DHT, and MIBA. In the 30–60-min interval, HIDA has the highest value of 14.1% compared to 11.9% for diethyl-IDA and 6.1% for rose bengal. The only other compound to surpass rose bengal during this interval is iodo-IDA (8.6%). All compounds except MIBA peak during that 30–60-min period. MIBA peaks during the next 30 min with a maximum of only 1.9%. After 1 hr, the diethyl-IDA and HIDA levels drop below those of rose bengal.

The cumulative percent dose in the bile is plotted vs time in Fig. 6, normalized to an average bile flow of 1 cc/10 min (the average bile flow from the 132 half-hour periods recorded). Using this assumption, 67% of the injected rose bengal is recovered in the bile during the first 3 hr. This correlates well with previously reported experience (*2*). Only diethyl-IDA (80%) and HIDA (75%) have higher cumulative totals than rose bengal throughout the first 3 hr. Other agents in order of cumulative percent dose at 3 hr are iodo-IDA (62%), ethoxy-IDA

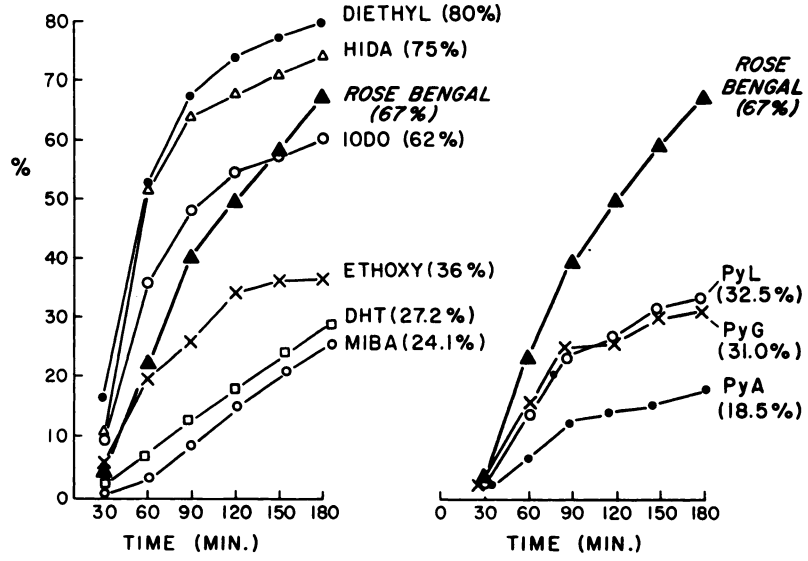


**FIG. 6.** Cumulative percent dose in bile (baboons). The 3-hr cumulative percent dose of rose bengal is 67%. Only diethyl-IDA (80%) and HIDA (75%) exceed this number. However, iodo-IDA is higher than rose bengal throughout the first 2.5 hr of the study. Note considerably lower cumulative totals of pyridoxylidene group and commercially available preparations.

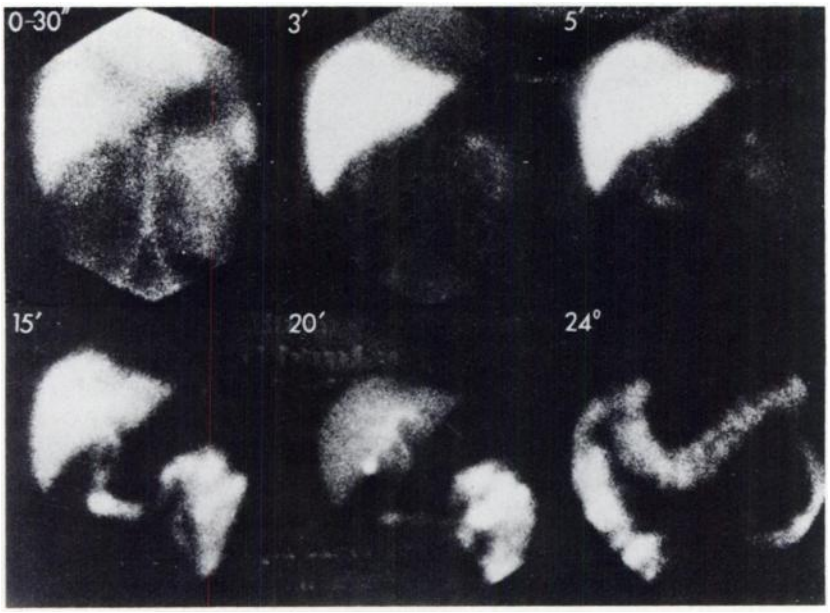


**FIG. 7.** Human distribution images, diethyl-IDA. 15 mCi of diethyl-IDA injected I.V. The 0–30-sec image shows cumulative blood flow and blood pool in abdominal organs. Note portal vein just to right of aorta. At 3 min, liver activity predominates, with rapid clearing of cardiac pool and faint renal visualization. By the 5-min image, activity is already seen in the duodenum. At both 15 and 20 min common bile duct, cystic duct, and gallbladder are all visualized. Twenty-four hour abdominal image shows activity to be confined to colon, suggesting there is little if any reabsorption. Colon is critical organ for radiation dosimetry calculations.

(36%), PyL (32%), PyG (31%), DHT (27%), MIBA (24%), and PyA (18%). Note that although the iodo-IDA has a lower 3 hr total, it is only during the last hour that it drops below that of rose bengal.

**Human images.** Following the intravenous injection of 15 mCi of diethyl-IDA, the 0–30-sec image shows the combined blood flow and blood pool of the various abdominal organs and vessels (Fig. 7). The cardiac blood pool noted at this time can be compared with that on later images to show its rapid clearance. Liver, spleen, and small-bowel blood pools are clearly demonstrated. Note the linear structure to the right of the abdominal aorta (the portal vein).

By 3 min there has been rapid clearance of the cardiac blood pool and almost all the activity is located in the liver. Faint visualization of the kidneys is noted. By 5 min, activity is already in the common bile duct and duodenum. By 15 min there is excellent identification of the common bile duct and cystic duct. The small-bowel position is also easily identified. At 20 min, the gallbladder is filling. The 24-hr image demonstrates the entire large bowel, complete with haustral markings. There is no evidence of reabsorption from the gastrointestinal tract. No activity is seen in other organs except the gallbladder.

## DISCUSSION

Hepatobiliary images superior to $^{131}$I-rose bengal images can be achieved in two different ways: by using $^{99m}$Tc as the labeling nuclide or by employing a compound with physiologic advantages over rose bengal. An ideal hepatobiliary agent for scintigraphy should have the following characteristics: (A) rapid extraction from the plasma by the polygonal cells of the liver; (B) rapid transit through these cells; (C) high biliary concentration; (D) little or no absorption from the intestine, (E) minimal concentration in the urinary tract; (F) a high labeling yield with $^{99m}$Tc; (G) and availability as a sterile, nontoxic instant kit.

The biliary excretion of compounds depends on the following factors: molecular size and weight, polarity (lipid solubility), molecular structure (relation of polar and nonpolar groups) and, possibly, protein binding (*16,21,22*). Substances with a molecular weight between 300 and 1000 are preferentially excreted in the bile. Increasing the molecular weight of a compound within this range may favorably affect its biliary excretion. A strong polar group is necessary: anion, cation, or nonionized molecule with polar and lipophilic groups. The intramolecular relationship between the hydrophilic and lipophilic portions of the molecule can greatly influence biliary excretion, for if these two groups are too close together, biliary excretion may be severely decreased. Cyclic structures arranged in different planes are preferred. Such changes in molecular configuration can influence biliary excretion in addition to differences in molecular weight or polarity. A degree of protein binding is probably necessary to prevent these biliary agents from being promptly filtered by the glomeruli of the kidney. Protein binding may play a role in the transport of the agent to the sinusoids of the liver and into the hepatocyte. Rapid active transport across the hepatocyte into the biliary canaliculi then occurs. It is likely that none of these agents is conjugated in its passage through the hepatocyte (*15,17*).

The pyridoxylideneglutamate represents the acidic amino-acid Schiff's base with pyridoxal. Leucine and arginine represent the neutral and basic amino acids, respectively. The condensed pyridoxal-amino-acid Schiff's bases do form stable complexes with $^{99m}$Tc, but autoclaving is an essential and time-consuming step in their preparation. Moreover, their biliary concentrations remain consistently below those of rose bengal.

The biliary clearances of PyG and PyL are approximately equal. PyA, DHT, and MIBA are much less effective because of their low concentrations in the bile. The advantages of this group of compounds for the imaging of the hepatobiliary system therefore seem to result only from the technetium label.

On the other hand, the IDA derivatives have better characteristics for biliary-tract imaging. They are readily labeled with $^{99m}$Tc in instant kits, without any heating step. They are extracted from the blood and excreted through the hepatocyte, resulting in an extremely fast blood clearance, a short hepatocyte transit time, and prompt appearance in the bile. Consequently, during the first hour their biliary concentrations are considerably higher than those of rose bengal. Chemically they have many of the features listed earlier as necessary for significant biliary excretion. HIDA has a molecular weight of 367, with hydrophilic and lipophilic ends separated by substantial distance. Chelation with $^{99m}$Tc probably creates a dimer, thus decreasing the hydrophilic nature of the carboxyl groups and doubling the effective molecular weight of the compound. Replacing the methyl groups with ethyl groups (as in diethyl-IDA) has at least two advantages: slightly increasing the molecular weight and substantially increasing the lipid solubility. The p-iodo substitution of p-iodo-IDA increases the molecular weight and changes the polarity, resulting in biliary excretion greater than that of rose bengal but less than that of dimethyl-IDA (HIDA), or diethyl-IDA. A p-ethoxy substitution reduces lipid solubility, though slightly increasing the molecular weight. This change substantially reduces the biliary excretion of this agent to the lowest level among the IDA derivatives.

As predicted from the preceding observations, the change from methyl groups to ethyl groups at the 2,6 position of the benzene ring of HIDA does increase blood clearances and biliary concentrations (in the early stages of the experiments) and reduces the renal excretion from 20% to 5% over the first 3 hr. This should reduce the visual interference from the right kidney and upper ureter, especially during the initial appearance of activity in the common bile duct and duodenum. The blood clearance of the diethyl-IDA is rapid despite its relatively low renal excretion. This suggests that the extraction of the diethyl-IDA by the hepatocytes is considerably higher than that of HIDA, which has a slightly slower blood clearance even with its higher urinary excretion. As a result of the characteristics mentioned before, camera images of the hepatobiliary system with diethyl-IDA are excellent.

In conclusion, comparative assays of blood, urine, and bile in the baboon indicate that the IDA derivatives are superior to the other $^{99m}$Tc-labeled complexes evaluated for hepatobiliary imaging. Of this group, the diethyl-IDA has the advantage of having the lowest renal excretion. This compound combines the markedly increased biliary concentrations compared with rose bengal, and the physical advantages of $^{99m}$Tc over $^{131}$I, thus providing vastly improved visualization of the hepatobiliary system and offering an alternative to the intravenous cholangiogram, especially for those patients presenting with mildly elevated bilirubin or known sensitivity to radiographic contrast media.

## FOOTNOTE

* Aldrich Chemicals, Milwaukee, Wisc.

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