RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

A Ga-68-Labeled Tetrabromophthalein (Ga-68 BP-IDA) for Positron Imaging of Hepatobiliary Function: Concise Communication

Jochen Schuhmacher, Ronald Matys, Harald Hauser, John H. Clorius, and Wolfgang Maier-Borst

Institute of Nuclear Medicine, German Cancer Research Center, Heidelberg, FRG

We describe the chemical synthesis of an iminodiacetic-acid-substituted tetrabromo-*o*-cresolphthalein (BP-IDA), which complexes Ga-68 tightly. The liver uptake, bile excretion, and urinary excretion of the complex were examined in rats. Maximum liver uptake reached 60%, and 1-hr cumulative bile excretion was 75% of injected dose. Urinary excretion in rats with ligated common bile duct remained below 1%. Competitive action of exogenous bilirubin on hepatobiliary excretion of the Ga complex was less pronounced than that of bromosulfophthalein. The absolute activity determination of the positron emitter Ga-68, the high accumulation in the liver, the low urinary excretion, and the weak competition from exogenous bilirubin are promising features of this radiopharmaceutical for the quantitative study of hepatobiliary function.

J Nucl Med 24: 593–602, 1983

For hepatobiliary scintigraphy, Tc-99m HIDA radiopharmaceuticals have proven unique in the rapid diagnosis of acute cholecystitis and acute obstruction of the common bile duct. There is a lack in accuracy, however, when small changes in the hepatic excretion rate must be evaluated in sequential studies during therapy or when dysfunctions of different patients are compared (*1*). The inaccuracy results primarily from scattering of the essentially single photon of Tc-99m. Positron imaging may overcome this disadvantage because it makes possible an activity determination in the patient, corrected for photon scattering. An accuracy better than 10% seems possible in the near future (*2*).

The synthesis of a new positron-labeled radiopharmaceutical and its pharmacological behavior with regard to hepatobiliary imaging is described. Gallium-68 ($T_{1/2}$ = 68.3 min, β^+ max. 1.9 MeV) has been chosen as the positron label because it is readily available for routine use as a generator product from its parent nuclide Ge-68 ($T_{1/2}$ = 287d), and because it undergoes quantitative and rapid complex formation with chelating agents containing hydroxy-, keto-, and carboxylic acid groups. The ligand we investigated is an iminodiacetic acid (IDA)-substituted tetrabromophthalein. This ligand was chosen since HIDA forms only a weak unstable complex with Ga-68, whereas the highly stable complex of Ga-68 with commercially available phenolphthalexon had a high renal excretion and an unsatisfactory hepatobiliary clearance. BP-IDA, however, combines the metal-chelating properties of phenolphthalexon with the affinity of tetrabrominated phenolphthaleins for hepatobiliary excretion.

Received Feb. 24, 1982; revision accepted Feb. 1, 1983.

For reprints contact: Dr. Jochen Schuhmacher, Institute of Nuclear Medicine, German Cancer Research Center, Im Neuenheimer Feld 280, D-6900 Heidelberg.

METHODS

Chemical synthesis of BP-IDA. The chemical structure of BP-IDA (4,5,6,7-tetrabromo-*o*-cresolphthalein-3′-methyliminodiacetic acid) is shown in Fig. 1. In a first step 4,5,6,7-tetrabromo-*o*-cresolphthalein was prepared from tetrabromophthalic anhydride and a 1.2-fold molar excess of *o*-cresol. $SnCl_4$ was added as a condensing agent according to a method of Baeyer for preparation of *o*-cresolphthalein (*3*). Purification from several by-products, mainly hydroxyanthraquinones and fluorans (*4*), was carried out by the method of Pratt et

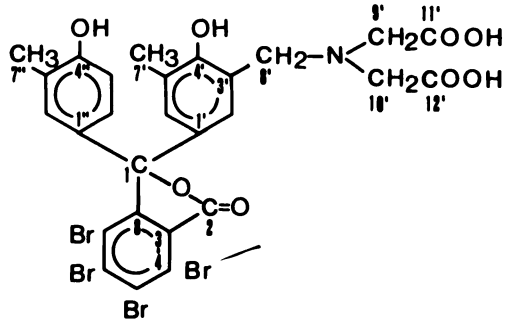

FIG. 1. Chemical structure of BP-IDA.

al. (5). A white crystalline product resulted. Data from microanalysis, C = 39.4% and H = 2.28%, correspond well with the theoretical values C = 39.9% and H = 2.13%. The substance is nearly insoluble in H_2O but is readily dissolved in a dilute sodium hydroxide solution. Solution is accompanied by a color change to violet. The chemical purity of the tetrabromo-*o*-cresolphthalein was checked by thin-layer chromatography (TLC) using silica gel plates*, developed with two different solvent systems: ethyl acetate/methanol/12.5% NH_3 (6:3:1), retention factor (R_f) = 0.86; and 1-propanol/H_2O/acetic acid (10:5:1), (R_f) = 0.84. The reaction yield was poor, never exceeding 5% of the theoretical value.

Subsequently a Mannich condensation of tetrabromo-*o*-cresol-phthalein with formaldehyde and iminodiacetic acid was carried out, according to the method of Anderegg et al., for preparation of phenolphthalexon (6), but with twice the amount of phthalein to favor monosubstitution. Purification of the phthalein-methyliminodiacetic acid was achieved by acidifying the reaction mixture with HCl to a pH of 1.8–1.9. This was followed by column chromatography of the resulting precipitate with silica gel mesh <100† using ethylacetate/methanol/25% NH_3 (6:3:1) as solvent. The monosubstituted compound migrates somewhat more slowly on the column than unchanged tetrabromo-*o*-cresolphthalein, whereas the disubstituted by-product showed a much higher retention. TLC of the 4,5,6,7-tetrabromo-*o*-cresol-phthalein-3′-methyliminodiacetic acid (BP-IDA) was carried out as described above for tetrabromo-*o*-cresolphthalein. R_f values were 0.05 for the ammoniacal solvent and 0.49 for the acid.

Microanalysis of the elemental composition of BP-IDA resulted in C = 39.1%, H = 2.38%, N = 1.60%, Br = 40.5%, which agree with theoretical values of C = 40.2%, H = 2.62%, N = 1.72%, Br = 39.7%. For the evaluation of the chemical structure of BP-IDA, C-13 NMR spectra were measured at 22.63 MHz with a WH 90 nuclear magnetic resonance spectrometer. Perdeuterated dimethylsulfoxide (DMSO-d_6) was used as a solvent, tetramethylsilan (TMS) as the internal standard. IR spectroscopy indicated only the presence of carboxylic acid groups. A typical yield for the preparation of BP-IDA from tetrabromo-*o*-cresolphthalein was 15% of the theoretical value.

Labeling of BP-IDA with Ga-68. Ionic Ga-68 was obtained from a Ge-68 → Ga-68 generator in a 0.5 *N* HCl solution containing 3 mCi per ml (7). After addition of 3 mg BP-IDA, dissolved in 2 ml of 0.01 *N* NaOH, 1.0 *N* NaOH was added to a pH of 6.0–6.5, and the product was ready for use. Radiochemical purity of the Ga-68 BP-IDA complex was checked by ascending paper chromatography: Whatman No. 1 with ethylacetate/methanol/H_2O (6:3:3). Lipid solubility of Ga-68 BP-IDA was tested by measuring the partition of Ga-68 activity between equal volumes of n-octanol and a 0.01 *M* phosphate buffer of pH 7.4, made isotonic with saline.

Electrophoresis. Gallium-68 BP-IDA was analyzed for its net charge by agarose gel electrophoresis in a buffer of 0.1 *M* glycine/Tris at pH 7.2. Bromophenol blue was added as a standard. Electrophoresis was allowed to proceed for 1 hr at 7mA and 600V. Subsequently the agarose strips were cut into eight pieces and the Ga-68 activity was determined in a Ge/Li well detector.

Animal experiments. Male Sprague Dawley rats (weight 250–420 g) were used for all experiments. Animals had free access to water and laboratory diet. Gallium-68 BP-IDA was administered intravenously in 0.5 ml solution (pH 6.5) containing, if not otherwise stated, 300 μg BP-IDA and about 100 μCi Ga-68.

To determine the organ distribution of Ga-68, the animals were anesthetized with ether and killed by heart puncture. Organs were removed and weighed. The 511-keV annihilation radiation was measured with a Ge/Li well detector. The radioactivity of the duodenum was always determined, including its content.

To investigate a possible change in the renal uptake and clearance pattern during hepatic obstruction, the distribution of Ga-68 BP-IDA was determined in rats with ligated common bile duct. Forty-eight hours after this surgical intervention, the hepatic uptake of the radiopharmaceutical was found drastically retarded. One hour after injection of activity, organ distributions were determined as described. Bladder urine and excreted urine were measured together.

The hepatobiliary excretion of Ga-68 was determined in animals narcotized by i.v. injection of 0.35 ml sodium pentobarbital (60 mg/ml). Subsequently the common bile duct was cannulated with a small polyethylene tube (PE 10). Bile was collected in 15-min intervals for 2 hr following i.v. injection of Ga-68 BP-IDA.

The competitive action of bilirubin and bromosulfophthalein (BSP) with Ga-68 BP-IDA was investigated using the same animal model. Bile was collected for 15 min to obtain a control value for the normal excretion of bilirubin. Following the control period the animals received an i.v. injection of either bilirubin (2 mg/100 g

body weight, p.A. crystalline 98%) or BSP (2 mg/100 g body weight). This priming dose was immediately followed by either an i.v. infusion of bilirubin in an isotonic Na_2CO_3/NaCl solution (pH 8.5, 2 mg/ml) or an infusion of BSP (6 mg/ml, pH 8.0, isotonic in saline). In one experiment animals were infused with bilirubin for 75 min at a rate of 0.12 μmole/min per 100 g body weight. In a second experiment animals received bilirubin at a rate of 0.09 μmole/min per 100 g body weight for 195 min. The BSP infusion continued for 195 min at a rate of 0.155 μmole/min per 100 g body weight. In all experiments Ga-68 BP-IDA was injected 15 min after the priming dose and start of infusion. Bile was collected in 15-min intervals. Bilirubin in serum and bile was determined photometrically as an azo dye in alkaline solution according to a method of Jendrassik (*8*). BSP in serum and bile was determined photometrically in alkaline solution (*9*).

Extraction of Ga-68 from liver homogenates was carried out to evaluate a change in the chemical binding of Ga-68 during passage through the liver. Two groups of four animals each were injected with 0.5 ml solution of Ga-68 BP-IDA containing 400 μg BP-IDA and 500 μCi Ga-68, and killed at 5 min and 60 min after injection. Livers were removed and homogenized (1 g liver + 4 vol of 3% sodium trichloracetate, pH 6.2). Subsequently 1 ml of homogenate was extracted with 5 ml of a 1:2 mixture of ethanol/isoamyl alcohol. After centrifugation at 3000 rpm for 15 min, activities in supernatant and precipitate were measured. For determination of blank values, an inactive liver homogenate was incubated with either Ga-68 BP-IDA or ionic Ga-68 and extracted as described above.

Investigations on the metabolism of stable Ga BP-IDA as well as BP-IDA were carried out by means of paper chromatography: Whatman No. 1 with ethylacetate/methanol/water (6:3:3). Rats of 250 g body weight were injected with 1.7 mg Ga BP-IDA (metal-to-ligand ratio 1:1) or 1.55 mg BP-IDA in isotonic saline, and bile was collected for 30 min. Undiluted bile was incubated for 17 hr at 37°C and subsequently analyzed, spots being visualized by NH_3 vapor. Similar runs were made with bile diluted 1:1 either with (a) 0.05*M* phosphate buffer (pH 7.2), or with (b) 0.1*M* acetate buffer pH 5.1; and bile buffered at pH 7.2 or pH 5.1 and mixed with β-glucuronidase (200 μl bile, 200 μl buffer, 4.6 mg [0.04 U] β-glucuronidase from bovine liver in 100 μl H_2O).

To evaluate the influence of an enhanced ligandin (gluthathione S-transferase) content of the liver on the Ga-68 BP-IDA uptake, four rats with an initial body weight of 165 g received a 0.1% solution of phenobarbital as drinking water for a 10-day period. Animals receiving normal drinking water served as controls. Six min after i.v. injection of 10 mg Ga BP-IDA labeled with 100 μCi Ga-68 (1.5 ml solution, pH 8.0, containing Ga^{3+} and BP-IDA in a stoichiometric ratio of 1:1), each animal was killed under ether anesthesia and liver and duodenum were removed and measured on a Ge/Li detector. Gluthatione S-transferase was determined in the 100,000 *g* supernatant of the liver homogenate with 1-chloro-2,4-dinitrobenzene as the substrate, according to a method of Habig (*10*).

Preliminary toxicity studies on BP-IDA were carried out with three groups of female mice, each group consisting of seven animals. Solutions of BP-IDA in physiological saline were injected intravenously, corresponding to dosages of 200, 250, and 300 mg/kg. None of the animals died during an observation of more than 4 wk. The highest dose of 300 mg/kg given to mice corresponds to 9000 times the proposed human dose.

Two healthy volunteers were examined following i.v. injection of 500 μCi Ga-68 complexed with 2.5 mg BP-IDA. A multicrystal positron scanner (2) was used for absorption-corrected scintigraphy, Ga-68 in the liver was determined quantitatively.

RESULTS

Chemistry. The chemical structure of BP-IDA was fully confirmed by data from C-13 nuclear magnetic resonance spectra, listed in Table 1. The rapid and complete complexing of ionic Ga-68 by BP-IDA was demonstrated by paper chromatography (Fig. 2). R_f values for Ga-68 BP-IDA, as determined with a radiochromatogram scanner, and for BP-IDA (violet spot after fuming with NH_3), were identical. The complex proved to be stable through a pH range from 4.0 to 8.5. A further increase in pH slowly broke down the complex with release of gallium hydroxide and gallate. The partition coefficient of 2.1 (activity in n-octanol divided by activity in the buffer) for Ga-68 BP-IDA at a pH of 7.4 indicates that lipophilic groups are predominant in the

TABLE 1. C-13 NMR SPECTROSCOPY OF BP-IDA IN DMSO-d_6

Carbon no.*	Shift (ppm)	Carbon no.	Shift (ppm)
C_1	93.5 s†	$C_{7'}$	16.6 q
C_2	166.6 s	$C_{7''}$	16.5 q
C_3	139.0 s	$C_{8'}$	56.5 t
C_8	154.2 s	$C_{9'}$, $C_{10'}$	56.3 t
$C_{4'}$	157.7 s	$C_{11'}$, $C_{12'}$	172.2 s
$C_{4''}$	157.2 s		

* For numbering of carbon atoms see Fig. 1.

† Indicates multiplicity determined by means of off-resonance decoupled spectra. s, d, t, and q = singlet, duplet, triplet, and quartet. The remaining 14 aromatic carbon atoms showed shifts from 133.9 to 115.2 ppm. Assignment could not be clearly given.

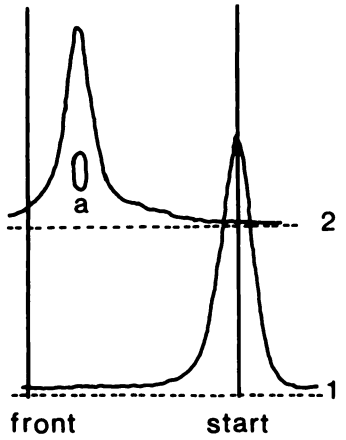

FIG. 2. Paper chromatogram of $^{68}Ga(OH)_3$ (lower) and 2. Ga-68 BP-IDA (upper). Ten μl of a 0.25 *N* NaCl solution (pH 6) containing 1.3 μg BP-IDA were spotted. Support: Whatman No. 1 chromatographic paper. Solvent: ethylacetate/methanol/H_2O (6:3:3). a = zone pf BP-IDA.

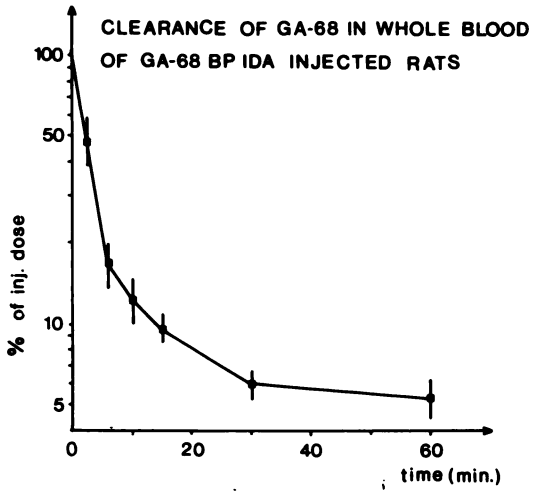

FIG. 4. Blood clearance of Ga-68 activity in rats following injection of Ga-68 BP-IDA. Each data point represents mean value + s.d. for seven animals. Blood was assumed to be 6% of body weight.

complex. The electrophoretic migration of the complex towards the anode shows that Ga-68 BP-IDA is negatively charged (Fig. 3).

Organ distribution. The organ distribution of Ga-68 BP-IDA in rats showed that the maximum liver uptake reached 50–60% of injected dose during the first 10 min (Table 2). Cumulative excretion into the duodenum, 60 min after administration, was 75% of injected dose. The highest hepatic elimination rate into the duodenum was observed between 6 and 10 min, while total urinary excretion (urine + kidneys) remained below 0.75% of injected dose. Blood activity declined with an initial $t_{1/2}$ of 2 min (Fig. 4).

Severe hepatic malfunction followed ligation of the common bile duct. The 1-hr urinary excretion of the positron-emitting radiotracer, however, was nearly unaffected, rising from an average value of 0.15% in animals without obstruction (Table 2) to a maximum of 0.9% during hepatic failure (Table 3).

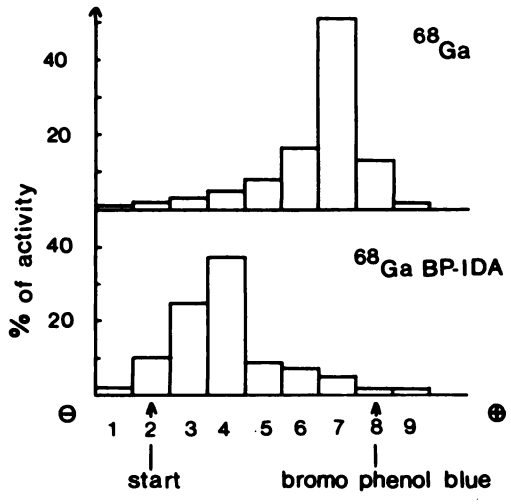

FIG. 3. Agarose gel electrophoresis of Ga-68 BP-IDA and ionic Ga-68. Fifty μl of 0.25 *N* NaCl containing 50 μCi of ionic Ga-68, or 50 μCi of Ga-68 complexed by 6.5 μg BP-IDA, were spotted together with 50-μl solution of 1.5% agarose.

Biliary excretion. Hepatobiliary excretion of Ga-68 BP-IDA in anesthetized animals is shown in Fig. 5, curve 1. When results were compared with those obtained in unanesthetized animals (Table 2), it was evident that the elimination rate of the radiopharmaceutical is reduced during the first 30 min. The total biliary excretion at 2 hr also exceeded 70% of the injected dose. Average bile flow was 82 ± 25 mg/15 min per 100 g body weight. The same amount of Ga-68 activity was found in the duodenum and in isolated bile, suggesting that there is no reabsorption of bile-excreted activity from the duodenum. This is confirmed by the results of an oral administration of Ga-68 BP-IDA in rats (n = 3), which showed less than 0.02% of administered activity in the whole blood at 1 hr after administration.

Bilirubin and BSP competition. Following infusion of unconjugated bilirubin at a rate of 0.12 μmole/min per 100 g body weight, serum levels of bilirubin rose from 0.5 mg/dl (before infusion) to 14 mg/dl (after priming dose and 15 min infusion) up to 21.5 mg/dl (after 75 min infusion). Bilirubin excretion in bile, per min and per 100 g body weight, increased from 0.8 ± 0.3 nmole before infusion to an average of 75 ± 7 nmole during the entire infusion period. The latter value corresponds to 80% of the bilirubin transport maximum of the rat liver (*11*). Average bile flow per 15-min interval was 95 ± 20 mg/100 g throughout the experiment. The 1-hr cumulative excretion of Ga-68 BP-IDA in bile during bilirubin infusion was about 20% less than in uninfused animals (Fig. 5). The organ distribution 1 hr after injection of Ga-68 BP-IDA indicates that its urinary excretion was not affected by bilirubin infusion (Table 4). Bile excretion of Ga-68 BP-IDA in the second experiment, where animals were infused with 0.09 μmole bilirubin/min per

TABLE 2. ORGAN DISTRIBUTION OF Ga-68 IN RATS* AFTER I.V. INJECTION OF Ga-68 BP-IDA

Organ	Percent of injected dose†					
	2.5 min	6.0 min	10 min	15 min	30 min	60 min
Blood	48.4 ± 11.1	16.7 ± 3	12.3 ± 2.4	9.7 ± 1.2	6.0 ± 0.7	5.3 ± 0.9
Liver	51.4 ± 9.6	60.3 ± 3.6	53.9 ± 5.6	44.0 ± 3.6	17.4 ± 1.8	11.2 ± 1.7
Kidneys	1.5 ± 0.5	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	0.4 ± 0.05	0.4 ± 0.01
Spleen	0.3 ± 0.05	0.2 ± 0.03	0.15 ± 0.02	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.02
Muscle	8.0 ± 1.6	8.0 ± 3.2	4.8 ± 1.6	4.8 ± 1.6	4.8 ± 1.6	4.8 ± 1.6
Bone	7.7 ± 1.6	3.6 ± 0.8	3.2 ± 0.8	3.2 ± 0.8	2.0 ± 0.2	2.8 ± 0.4
Duodenum	3.6 ± 0.9	13.4 ± 2.3	31.0 ± 6.0	43.2 ± 5.1	68.5 ± 3.8	74.9 ± 2.7
Urine	—	—	—	0.15 ± 0.11	0.16 ± 0.11	0.14 ± 0.07

* Average weights (n = 42): whole animal ±s.d. 403 ± 18 g; liver 13.1 ± 2.0 g; kidneys 2.4 ± 0.2 g; spleen 0.8 ± 0.08 g. Blood, bone, and muscle were assumed to be 6%, 10%, and 40% respectively of body weight.

† Data are mean ± s.d. for 7 animals.

100 g, is shown in Fig. 5, curve 3. The activity excretion was delayed only slightly during the first hour after injection. The 2-hr cumulative excretion, however, was identical with that of uninfused animals. Bile flow during this experiment was 70 ± 17 mg/15 min per 100 g, with a bilirubin excretion rate of 56 ± 13 nmole per min and 100 g. Serum levels of bilirubin reached 23.5 mg/dl (after priming dose and 195 min infusion).

BSP infusion of 155 nmole/min per 100 g body weight led to a severe reduction of Ga-68 BP-IDA excretion into the bile (Fig. 5, curve 4), indicating that infusions of similar molar amounts of BSP and bilirubin result in different competitive actions on Ga-68 BP-IDA excretion. Serum levels of BSP at the end of the infusion were not uniform, ranging from 10.2 to 21.0 mg/dl. Bile flow during the whole experiment was 115 ± 19 mg/15 min per 100 g, with a BSP excretion rate of 129 ± 19 nmole per min and 100 g.

Metabolism. The extraction rate of Ga-68 from liver homogenates from animals injected with Ga-68 BP-IDA decreased with time (Table 5). Blank values indicate that the extraction of Ga-68 BP-IDA added to a liver homogenate is not complete, while ionic Ga-68 is nearly

TABLE 3. ORGAN DISTRIBUTION OF Ga-68 IN RATS* WITH LIGATED COMMON BILE DUCT†, AT 1 HR AFTER INJECTION OF Ga-68 BP-IDA

Organ	% inj. dose‡
Blood	44.6 ± 5.0
Liver	10.0 ± 1.0
Kidney	1.9 ± 0.1
Spleen	0.5 ± 0.05
Muscle	19.4 ± 1.0
Bone	15.8 ± 1.8
Duodenum	3.6 ± 0.3§
Urine	0.6 ± 0.3

* Average weights (n = 7): whole animal ± s.d. 255 ± 12 g; liver 10.2 ± 0.6 g; kidneys 1.8 ± 0.1 g; spleen 0.7 ± 0.07; duodenum (with contents) 8.7 ± 0.9 g. Blood, bone, and muscle were assumed to be 7%, 10%, and 40% of body weight.

† Serum bilirubin was 2.4 ± 0.7 mg/dl (in unligated animals 0.5 mg/dl).

‡ Mean from 7 animals ± s.d.

§ Mainly Ga-68 activity from blood.

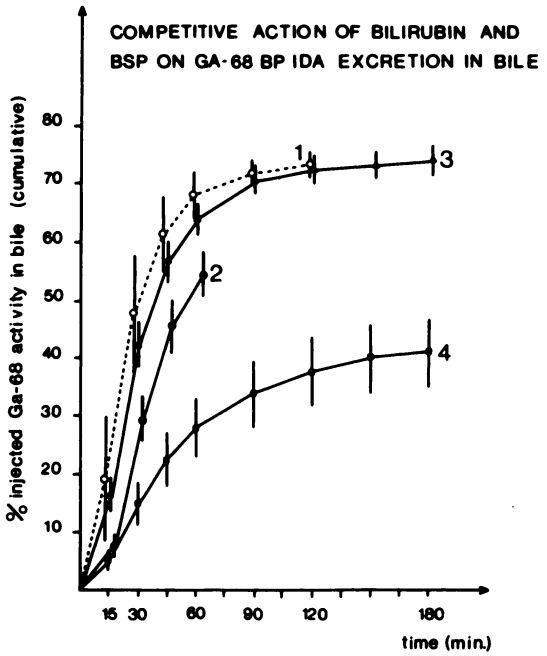

FIG. 5. Competition of bilirubin and sulfobromophthalein (BSP) on Ga-68 BP-IDA excretion. Data represent mean ±s.d. for six animals: 1, Uninfused animals; 2, animals infused with bilirubin at a rate of 0.12 μmole/min per 100 g body weight; 3, animals infused with bilirubin at a rate of 0.09 μmole/min per 100 g body weight; 4, animals infused with BSP at rate of 0.155 μmole/min per 100 g body weight.

TABLE 4. ORGAN DISTRIBUTION OF Ga-68 IN BILIRUBIN-INFUSED* RATS† AT 1 HR AFTER INJECTION OF Ga-68 BP-IDA

Organ	% inj. dose‡
Blood	8.4 ± 2.1
Liver	22.2 ± 3.4
Kidneys	0.5 ± 0.1
Spleen	0.15 ± 0.03
Muscle	6.6 ± 1.4
Bone	3.9 ± 0.4
Bile	54.5 ± 4.0
Urine	0.11 ± 0.06

* Bilirubin was delivered at a rate of 0.12 μmole/min per 100 g body weight.

† Average weights (n = 6): whole animal ± s.d. 358 ± 16 g; liver 15.9 ± 0.9 g; kidneys 2.5 ± 0.2 g; spleen 1.0 ± 0.15 g; total urine 1.6 ± 0.6 g. Blood, bone, and muscle were assumed to be 6%, 10%, and 40% of body weight.

‡ Mean for 6 animals ± s.d.

completely absorbed by the precipitate during centrifugation.

The chromatographic behavior of Ga-68 activity, immediately after its excretion in bile, is shown in Fig. 6. The major part of the activity migrated like unchanged Ga-68 BP-IDA, but the tailing off of activity indicated a beginning release of Ga-68 from the complex. Cleavage of the complex was much more evident in bile analyzed 2 hr after excretion. Hydrolysis seems to be responsible for the breakdown of the complex, because the pH of bile increased from 7.9 during excretion up to 9.1 after standing for several hours at 37°. The metabolite of BP-IDA, visualized on the chromatogram by means of NH_3 vapor, contained no Ga-68 activity.

The influence of different pH and of β-glucuronidase on the bile-excreted metabolite of Ga BP-IDA is shown in Fig. 7. Chromatograms of bile show the predominant spot of unaltered Ga BP-IDA, and a metabolite of higher hydrophilicity (lower R_f). This metabolite remained stable in normal and pH 7.2-buffered bile (Fig. 7, spots 1, 2). Acidifying of bile to pH 5.1 (Fig. 7, spot 3), or adding β-glucuronidase to bile buffered at pH 7.2 (Fig. 7, spot 4) or pH 5.1 (Fig. 7, spot 5), revealed appreciable amounts of an additional derivative with an intermediate R_f. This derivative may be the result of a partial release of glucuronic acid from the primary metabolite. Identical results were obtained when this study was repeated with BP-IDA.

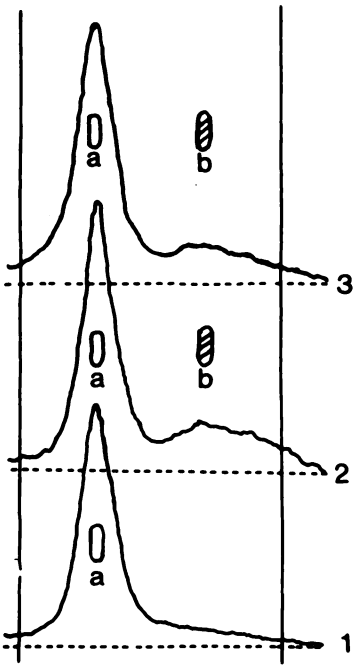

FIG. 6. Paper chromatograms of 1. Ga-68 BP-IDA; a 10-μl 0.25 *N* NaCl solution (pH 6.5) containing 1.3 μg BP-IDA was spotted. 2. 10 μl bile excreted within 15 min after i.v. injection of 66 μg Ga-68 BP-IDA was spotted immediately after excretion. 3. Procedure as in 2, with one exception: animal was given 330 μg Ga-68 BP-IDA. Support and solvent as in Fig. 2. a = zone of BP-IDA. b = zone of metabolite.

TABLE 5. EXTRACTION OF Ga-68 FROM THE LIVER HOMOGENATE OF RATS* INJECTED WITH Ga-68 BP-IDA

Min. after injection	% inj. dose† in liver	% activity extracted† from homogenate
5	61.8(60.0–64.0)	74.4(71.3–75.4)
60	9.4(9.0–10.1)	25.6(24.1–28.2)

* Average weight of animal ± s.d. 230 ± 8 g; liver 10.5 ± 0.5 g (n = 8)

† Mean of 4 animals (range in parentheses).

Blank values: Ga-68 BP-IDA and ionic Ga-68, incubated for 15 min with an inactive liver homogenate, were extractable to 75% and 3% respectively.

Phenobarbital treatment enhances liver weight and ligandin (gluthathione S-transferase) content of the liver (Table 6). Uptake after a single i.v. injection of 10 mg of Ga BP-IDA was also increased. The amount of the increase corresponds well with data for increased BSP uptake in the livers of phenobarbital-treated rats, as reported by Reyes et al. (*12*). Since it is known that ligandin binds BSP (*13*), it is believed that Ga-68 BP-IDA is also stored and transported by liver ligandin. No significant change in the bile excretion rate was observed.

The hepatic uptake of Ga-68 BP-IDA in two healthy volunteers shows that the maximum activity accumulation was 60% of injected dose (Fig. 8). This was comparable to results found in rats. Uptake and excretion in man, however, are much slower than in rats. Figure 9

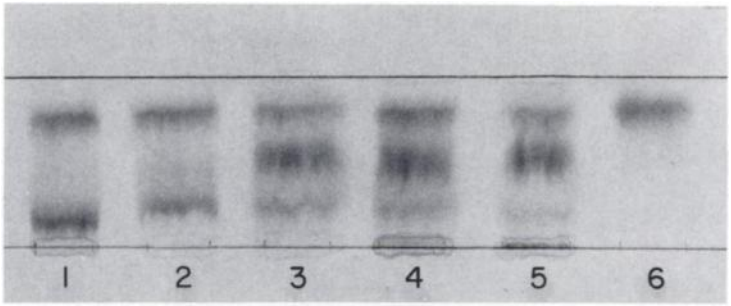

FIG. 7. Paper chromatograms of bile-excreted Ga BP-IDA. Support and solvent as in Fig. 2. 1: Normal bile, 10 μl were spotted. 2: Bile diluted 1:1 with a phosphate buffer (pH 7.2), 20 μl spotted. 3: Bile diluted 1:1 with acetate buffer (pH 5:1), 20 μl spotted. 4: Bile diluted 1:1.5 with phosphate buffer (pH 7.2) mixed with β-glucuronidase (0.04 U); 25 μl were spotted. 5: Bile diluted 1:1.5 with acetate buffer (pH 5:1) mixed with β-glucuronidase (0.04 U); 25 μl spotted. 6: Standard solution of Ga BP-IDA in H_2O; 5 μl containing 8.5 μg Ga BP-IDA were spotted.

shows scintigrams of the liver, gallbladder, and upper gut of a test person after administration of 500 μCi Ga-68 BP-IDA.

DISCUSSION

Substitution of one ortho position in the benzene ring of phenolic compounds by a methyliminodiacetic acid group leads to more effective metal complexing agents than compounds bearing the aminodiacetic acid group alone. This is because the oxygen atom of the hydroxyl group acts as a third dentate, and facilitates formation of octahedral (coordination number of 6) metal complexes (*14*). Thus phthaleins and alizarins bearing a methyl iminodiacetic acid group ortho to a hydroxyl group are useful for photometric determination of a wide variety of metals, especially of rare-earth and group III elements of the periodic table (*6,15*). Investigations by West et al. on the complexing of group III elements with alizarin complexone (1,2-dihydroxyanthraquinone-3-methylimino-N,N-diacetic acid) identified a metal-to-chelate ratio of 1:1. Furthermore, the stability of the Ga(III) complex was greater than that of the corresponding EDTA complex (*15*). The chemical structure, proposed by the above authors, for M^{3+} and methyliminodiacetic-acid-substituted phenols is shown in Fig. 10. The structure seems to be valid for the Ga-68 BP-IDA complex as well.

The electrophoresis of Ga-68 BP-IDA identified a negative charge on the complex. While the 1:1 chelation of the tridentate ligand with Ga^{3+} compensates these negative charges, the remaining negative charge may arise from the lactone,carbinol,quinone equilibrium of the phthalein moiety. Cleavage of the lactone by addition of a hydroxyl group to carbon atom 1 (see Fig. 1) begins at pH 7 and leads to a negatively charged carboxyl group. In spite of the similar chemical structures of BSP (Fig. 11) and Ga-68 BP-IDA, the metabolism of the two compounds differs. BSP is metabolized solely by enzymatic conjugation with SH-groups containing peptides (preferentially gluthathione) and simultaneous release of stoichiometric amounts of bromide (*16–19*). From the chromatograms of glucuronidase-treated bile it is evident, however, that the conjugation of Ga-68 BP-IDA with glucuronic acid is a part of its metabolism. Similar results are reported for the metabolism of monosulfonated tetrabromophenolphthalein (disulfonated tetrabromophenolphthalein = BSP) (*16*). Coupling of glucuronic acid with the Ga complex may occur at the hydroxyl group of the phenolic moieties as well as at the carboxyl groups of the IDA. In both cases the enzymatic attack should lead to a metabolite that is incapable of

TABLE 6. UPTAKE OF Ga-68 BP-IDA BY LIVERS OF PHENOBARBITAL*-TREATED RATS†

	Liver weight (g)	Gluthathione S-transferase (nmole/mg protein per min)	Ga-68 activity, liver	% inj. dose‡ duodenum
Control	9.6(8.9–10.2)	1296(1053–1432)	31.3(28.8–32.8)	5.1(4.6–5.6)
Phenobarbital	13.9(12.8–15.6)	2718(2551–3052)	40.4(36.2–42.3)	3.8(3.1–4.4)

* Mean uptake of phenobarbital during a 10-day treatment: 172 mg per animal (n = 4).
† Average weight of animal ± s.d. at end of phenobarbital treatment: 250 ± 8.5 g (n = 8).
‡ Animals were killed 6 min after injection.
All data represent mean of 4 animals (range in parentheses).

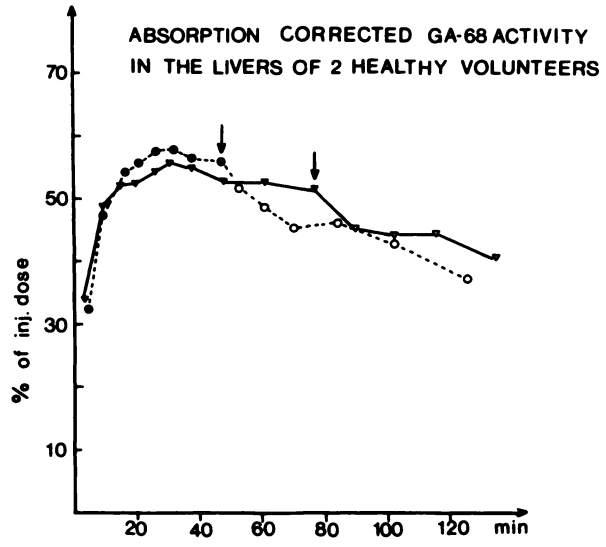

FIG. 8. Hepatic uptake and excretion of Ga-68 in two healthy volunteers. Each was given 500 μCi Ga-68 complexed by 2.5 mg BP-IDA. Closed symbols, activity of liver; open symbols, activity in liver, minus gallbladder activity. ↓ = time of gallbladder visualization.

further complexing with Ga-68. This would result in release of ionic Ga-68 in the liver. The radiochromatograms of bile-excreted activity suggest that the observed metabolite is not labeled with Ga-68. Extraction from liver homogenates with ethanol/isoamylalcohol demonstrates an increase of nonextractable Ga-68 activity with advancing time. These results support the hypothesis of enzymatic attack of the IDA-substituted phenol moiety of the Ga-68 complex.

The competitive action of bilirubin with Ga-68 BP-IDA excretion began at a serum level of 20 mg/dl. Our experiments did not permit conclusions as to whether the competition occurred during uptake or during ligandin binding and transport. This was because the infusion of increasing amounts of bilirubin led to increased serum and intrahepatic bilirubin levels. The serum levels were directly determined. The increased intrahepatic bilirubin content was assessed indirectly from the constant 60% excretion rate during infusion of two different bilirubin concentrations. In comparison, the BSP excretion rate (80% of BSP infused) was higher than that observed for bilirubin, and competitive action with Ga-68 BP-IDA started at lower serum levels (3–4 mg/dl, unpublished data). Assuming a similar mechanism for Ga BP-IDA and BSP binding on ligandin, suggested by their closely related chemical structure, the different competitive action of BSP and bilirubin on the excretion of Ga BP-IDA can be explained by additional competition of BSP during transport. Whereas bilirubin—like most of the other biological substrates, chemical drugs, or ste-

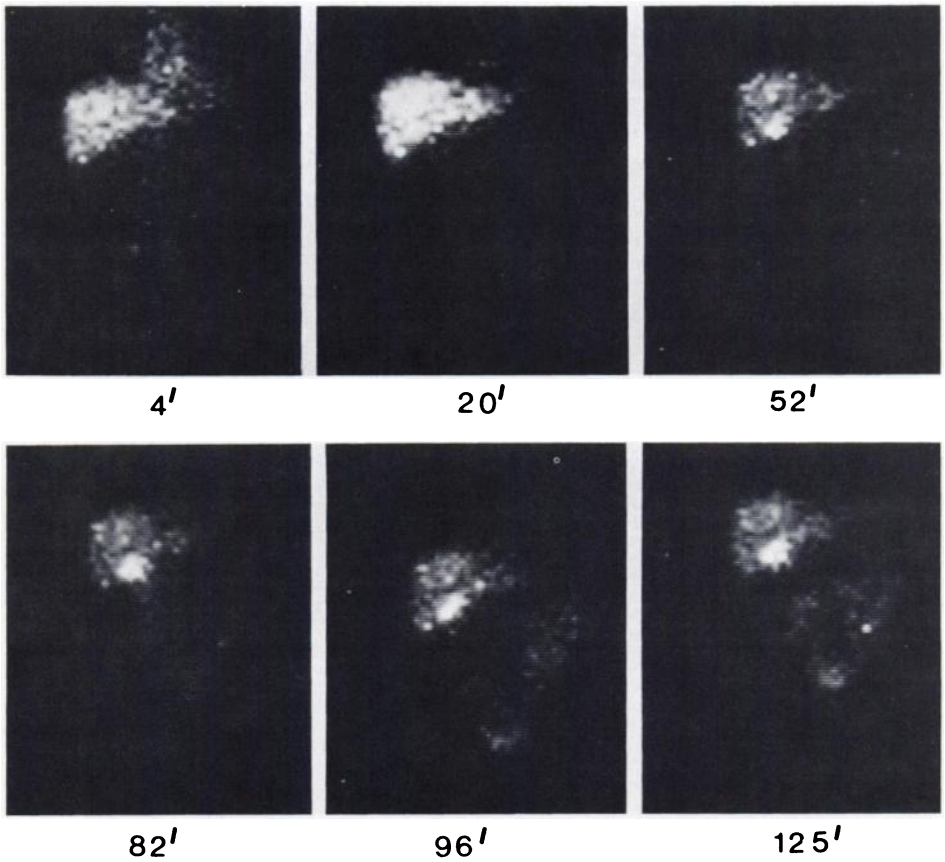

FIG. 9. Absorption-corrected scintigrams of healthy volunteer (4, 20, 52, 82, 96, and 125 min after injection). Activity calculation based on whole-body scans (not shown) differed by 5% from actually administered dose.

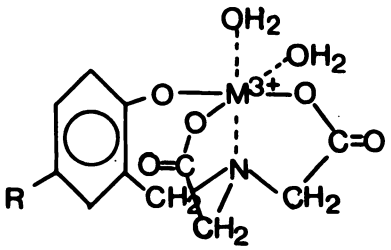

FIG. 10. Ga^{3+} complex with IDA-substituted phenols.

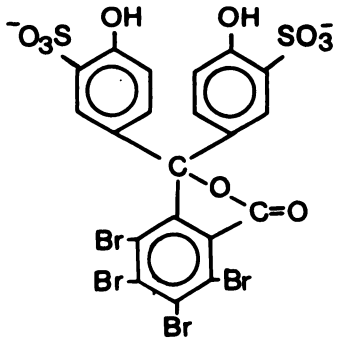

FIG. 11. Chemical structure of bromosulfophthalein.

roids—is bound to ligandin by one hydrophobic binding site, the binding of BSP is unusual and involves two binding sites per molecule, which are neighboring and which have hydrophobic and anionic binding properties (*13*). The competitive action of BSP during transport appears to prevail over the competitive action that bilirubin has on Ga BP-IDA glucuronidation. This is because cellular metabolism in the liver has only minor influence on transport of substrates (*20*).

It is difficult to compare data for Tc-99m HIDA, as reported in the literature, with current results of Ga-68 BP-IDA. Nonetheless, a different pharmacokinetic behavior of the two compounds is evident. The Ga complex shows a less pronounced peaking of activity in bile during the first 15 min after injection, but has 60% hepatic accumulation during this time. Tc-99m HIDA excretion into bile reaches 53% during the first 15 min (*21*), but activity accumulation in the liver of rats never exceeds 12.5% (*22*). The 1-hr cumulative excretion of both radiopharmaceuticals is similar, being 65.7% for Tc-HIDA (*21*) and 67.7% for Ga-68 BP-IDA (Fig. 5, curve 1). The difference in the excretion rate at earlier times may be due to a slower transport of Ga-68 BP-IDA through the hepatocytes.

Both radiopharmaceuticals are negatively charged metal chelates. The molar ratio of Tc to HIDA is 1:2, with Tc being in the 3+ oxidation state. Complex formation with the bidentate HIDA results in one uncompensated negative charge on the ligands (*23*). The negative charge of the Ga complex, however, results from the cleavage of the lactone ring, and is therefore pH-dependent. Competitive action of either bilirubin or BSP decreases biliary excretion of both pharmaceuticals. While competition of these compounds leads to an increased renal excretion of Tc-99m HIDA (*24*), urinary excretion of Ga-68 BP-IDA is unaffected. This might be due to the high lipophilic character of the Ga complex. Finally, there is a difference in metabolic fate. Tc-99m HIDA is thought to pass the liver without enzymatic attack (*25–27*), whereas Ga-68 BP-IDA is partially conjugated, presumably to decrease its lipophilicity for biliary excretion.

Two healthy volunteers were examined with Ga-68 BP-IDA. The decline of hepatic activity was slower than that measured in rats. This appears to be due to a longer tracer transit time in man, and probably to a higher release of ionic Ga-68. The different doses of BP-IDA given to humans (33 μg/kg) and to rats (750 μg/kg) do not appear responsible. Different doses of BP-IDA given to rats (150 μg/kg and 750 μg/kg) failed to influence Ga-68 excretion into bile. Scintigrams of the volunteers demonstrate late gallbladder visualization. The gallbladder activity remained slight, never exceeding 10–15% of injected dose.

These initial results with Ga-68 BP-IDA show that quantitative scintigraphic measurements of the hepatic uptake and excretion of the tracer are feasible. An improved visualization of the hepatobiliary tract with Ga-68 BP-IDA should be possible with tomographic imaging of the liver.

FOOTNOTES

* Kieselgel 60, 0.25 mm, Merck.

† Silica gel, Mallinckrodt, free from heavy metals, Serva, Heidelberg, FRG.

ACKNOWLEDGMENT

We are indebted to Mrs. E. Frei (Institute of Toxicology and Chemotherapy, German Cancer Research Center) for determination of gluthathione S-transferase, and to Mrs. I. Koehler (Institute of Biochemistry, German Cancer Research Center) for C-13 NMR spectroscopy. We thank Mrs. Ellen Bender for her help in preparing the manuscript.

REFERENCES

1. GLIEDMAN ML, WILK PJ: A surgeon's view of hepatobiliary scintigraphy. *Semin Nucl Med* 12:2–4, 1982
2. OSTERTAG H, KÜBLER W, KUBESCH R, et al: A linear whole-body scanner for quantitative positron imaging. IEEE Transactions, Vol. N.S. 28, 174–178, 1981
3. BAEYER A: Über die Verbindungen der Phthalsäure mit den phenolen. *Liebigs Ann Chemie* 202:153–172, 1880
4. COPISAROW M: XXVI Phthaleins and fluorans. *J Chem Soc (London)* 117:209–218, 1920
5. PRATT DS, DOANE FB, HARVEY AW: Phthalic acid derivates; constitution and color. XVI. Phenoltetrabromophthalein. *J Am Chem Soc* 41 II:1289–1293, 1919
6. ANDEREGG G, FLASCHKA H, SALLMANN R, et al: Metallindikatoren VII. Ein auf Erdalkaliionen ansprechendes

Phthalein und seine analytische Verwendung. *Helv Chim Act* 37:113-120, 1954

7. SCHUHMACHER J, MAIER-BORST W: A new $^{68}Ge/^{68}Ga$ radioisotope generator-system for production of ^{68}Ga in dilute HCl. *Int J Appl Rad Isot* 32:31-36, 1981
8. JENDRASSIK L, GRÓF P: Vereinfachte photometrische Methoden zur Bestimmung des Blutbilirubin. *Biochem Z* 297:81-89, 1938
9. RICHTERICH R: Klinische Chemie. Akademische Verlagsgesellschaft Frankfurt/M. 1968, pp 444-445
10. HABIG WH, PABST MJ, JAKOBY WB: Gluthathione S-transferases. *J Biol Chem* 249:7130-7139, 1974
11. WEINBREN K, BILLING BH: Hepatic clearance of bilirubin as an index of cellular function in the regenerating rat liver. *Br J Exp Path* 37:199-204, 1956
12. REYES H, LEVI AJ, GATMAITAN Z, et al: Studies of Y and Z, two hepatic cytoplasmic organic anion-binding proteins: effect of drugs, chemicals, hormones and cholestasis. *J Clin Invest* 50:2242-52, 1971
13. KETTERER E, TIPPING E, BEALE D, et al: Ligandin, Gluthathione Transferase and Carcinogen Binding. In *Gluthathione.* Arias IM, Jakoby WB, Eds. Raven Press, New York 1976, pp 243-257
14. SCHWARZENBACH G, ANDEREGG G, SALLMANN R: Komplexone XXIII. Der Phenolatsauerstoff als Koordinationspartner. *Helv Chim Act* 35:1785-1793, 1952
15. LEONARD MA, WEST TS: Chelating reactions of 1,2 dihydroxyanthraquinone 3-ylmethylamine-N,N diacetic acid with metal cations in aqueous media. *J Chem Soc (London)* 157:4477-4486 1960
16. JAVITT NB: Biochemical Probes for the Study of Binding and Conjugation of Gluthathione S-Transferases. In *Gluthathione.* Arias IM, Jakoby WB, Eds. Raven Press, New York 1976, 309-315
17. COMBES B, STAKELUM GS: A liver enzyme that conjugates sulfobromophthalein sodium with gluthathione. *J Clin Invest* 40:981-988, 1961
18. WHELAN G, COMBES B: Competition by unconjugated and conjugated sulfobromophthalein (BSP) for transport into bile. Evidence for a single excretory system. *J Lab Clin Med* 78: 230-244, 1971
19. SCHUHMACHER J, MAIER-BORST W: Recoil labelling of sulfobromophthalein with ^{82}Br during neutron irradiation. *Int J Appl Rad Isot* 23:519-525, 1972
20. SCHWENK M: Transport systems of isolated hepatocytes. *Arch Toxicol* 44:113-126, 1980
21. SMITH RB, COUPAL J, DELAND FH, et al: Pharmakokinetics of hepatobiliary imaging agents in rats. Concise communication. *J Nucl Med* 20:45-49, 1979
22. ZIVANOVIC MA, TAYLOR DM, MCCREADY VR, et al: The stability and distribution of Tc-HIDA in vivo and in vitro. *Eur J Nucl Med* 4:369-373, 1979
23. LOBERG MD, FIELDS AT: Chemical structure of technetium-99m-labelled N-(2,6-Dimethyl phenyl carbamoyl methyl)-imino diacetic acid (Tc-HIDA). *Int J Appl Rad Isot* 29:167-173, 1978
24. HARVEY E, LOBERG M, RYAN J, et al: Hepatic clearance mechanism of Tc-99m-HIDA and its effect on quantitation of hepatobiliary function. *J Nucl Med* 20:310-313, 1979
25. CHIOTELLIS E, SUBRAMANIAN G, MCAFEE JG: Chemistry of labelling Schiff's base (pyridoxal amino acid complex) with Tc-99m and their use in hepatobiliary scintigraphy. *J Nucl Med* 16:520, 1975
26. CALLERY PS, FAITH WC, LOBERG MD, et al: Tissue distribution of technetium-99m and carbon-14 labelled N (2,6-dimethylphenyl-carbamoylmethyl) iminodiacetic acid. *J Med Chem* 19:962-964, 1976
27. WISTOW BW, SUBRAMANIAN G, VAN HEERTUM RL, et al: An evaluation of ^{99m}Tc-labelled hepatobiliary agents. *J Nucl Med* 18:455-461, 1977