RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

Ruthenium-97 Hepatobiliary Agents for Delayed Studies of the Biliary Tract I: Ru-97 PIPIDA: Concise Communication

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Failure of early diagnosis of biliary atresia results in the development of cirrhosis and death. Commonly used hepatobiliary agents are not ideal for follow-up studies because of their unfavorable physical properties or short half-life. The excellent physical properties of Ru-97 should overcome these limitations. Therefore, Ru-97 PIPIDA (N,α-(*p*-isopropyl acetanilide) iminoacetic acid) is being investigated as a potential hepatobiliary agent that would allow an improved diagnosis of the disease. Ruthenium-97 PIPIDA and Tc-99m PIPIDA showed similar blood clearance rates in dogs. Ru-97 PIPIDA scintigrams in dogs showed early uptake in liver and gallbladder and slow excretion through the gastrointestinal tract. Biodistribution studies were performed in normal rats and rats with biliary obstruction. The findings suggest that Ru-97 PIPIDA should be useful for delayed studies (1–3 days) of the biliary tract.

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The study of the biliary tract using radionuclides started almost 25 years ago when Taplin et al. labeled rose bengal with I-131 (*1*). This tracer has been used in different situations where the determination of function of the biliary tract is required. As a result of the poor physical properties of I-131 (high β energy, long half-life, relatively high radiation dose), the permissible activity is lower than is needed for quality imaging. Further research brought about the development of new compounds labeled with technetium-99m (*2–5*). Although the relatively short half-life, low energy, low radiation dose, and excellent imaging properties seemed to make Tc-99m-labeled compounds the agents of choice, there are situations where delayed studies are required, e.g., severe hepatitis, neonatal hepatitis, choledochal cyst, biliary atresia, etc., where the short half-life of Tc-99m does not permit adequately delayed studies. The excellent physical properties of Ru-97 (primary energy of 216 keV, with γ abundance 85%, and 324 keV with abundance 11%, $T_{1/2}$ = 2.9 d) suggest that Ru-97-labeled hepatobiliary agents should be useful in such cases (*6*). Therefore, investigations were carried out using Ru-97 PIPIDA (N,α-(*p*-isopropyl acetanilide) iminoacetic acid) as a potential hepatobiliary agent that would provide improved diagnostic capabilities in such diseases.

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MATERIALS AND METHODS

Radiopharmaceutical preparation. Most initial investigations were carried out using Ru-103, due to its convenient 39.6-day half-life. Carrier-free Ru-103 chloride was obtained from the Oak Ridge National Laboratory as a solution in 3.5 *N* hydrochloric acid. Ruthenium-97 was prepared as needed at the Brookhaven Linac Isotope Producer from proton spallation of high-purity (>99.9%) rhodium foil (*7*). The target was bombarded with 200-MeV protons from the Linac. After the bombardment, the target was transferred to a processing hot cell and dissolved by ac electrolysis in 6 *N* hydrochloric acid (0.3 A/cm^2, ~15 hr). After evaporating the solution to near dryness, radiochemical sep-

aration of Ru-97 was achieved by distillation of RuO_4 from a sulfuric acid medium in the presence of permanganate. The distillate was collected in ice-cold ethanol-hydrochloric acid (1:1). Recovery of the Ru-97 from the target was almost quantitative.

Analysis of ruthenium-103 chloride, by paper chromatography in methyl ethyl ketone/conc. HCl (70:30 v/v) (*8*), indicated the presence of ruthenium (III) and ruthenium (IV) species in the hydrochloric acid solution. Conversion of the ruthenium (IV) to ruthenium (III) was effected when desired by refluxing the mixture in ethanol overnight. No significant difference (in vitro or in vivo) was observed in the behavior of the PIPIDA complex when prepared with either of the two ruthenium species, perhaps due to the eventual predominance of ruthenium (III) in the final product. Further work will be necessary in order to define the oxidation state of ruthenium in the Ru-PIPIDA complex.

Because of the findings with Ru-103, no effort was made to ensure the absence of ruthenium (IV) in the Ru-97 chloride solutions, even though on occasion 30–50% ruthenium in these solutions was found to be tetravalent.

Various parameters for a successful labeling of PIPIDA with Ru-97 were studied in order to obtain a product that showed in vivo behavior similar to that of Tc-99m PIPIDA. The ligand was dissolved in normal saline (10–15 mg/ml) and the pH adjusted to 7.5. Then the desired amount of Ru-97 in 3.5 *N* HCl was added to 1–5 ml of the ligand solution. The pH was adjusted to 4.0. This solution was then heated for 30 min at 90°C in a boiling-water bath and cooled to room temperature. The final pH was adjusted to 6.5. This product was then passed through a 0.22-μm Millipore filter preparatory to calibration and injection.

Tc-99m PIPIDA was prepared using a kit* that contained 20 mg PIPIDA and 0.2 mg $SnCl_2 \cdot 2H_2O$ per kit.

Biodistribution studies. Several analytical methods were evaluated for establishing the radiochemical purity of the Ru-labeled PIPIDA. Polyamide thin layer chromatography (TLC) in methanol, gel filtration using Biogel P-2 or Sephadex G-15, and cellulose acetate electrophoresis in borate/KCl buffer (pH 8), all provided good separation of the bound from unbound activity. Tc-99m PIPIDA preparations were also analyzed by the above methods.

Female Sprague-Dawley rats weighing 250 ± 25 g were injected i.v. with 15 μCi of Ru-103 PIPIDA or Tc-99m PIPIDA. For convenience in tissue distribution studies that did not involve imaging, the 39.4-day Ru-103 was used instead of Ru-97. Surgical ligation of the common bile duct was performed 1 day before radiopharmaceutical administration. Normal animals were killed at 15, 20, 60, 120 min, and 24 hr after injection while the rats with the obstructed common bile duct were studied at 24 hr.

Blood clearance studies were performed in mongrel dogs weighing 18.2 ± 2.3 kg. The animals were anesthetized with sodium pentobarbital and injected with 500 μCi of Ru-97 PIPIDA or Tc-99m PIPIDA. Blood samples were obtained at fixed intervals. Aliquots of blood were measured in a NaI(Tl) well scintillation counter. Images were obtained with a scintillation camera after 7, 20, 110, and 150 min, and 24, 48, and 72 hr.

RESULTS

Table 1 shows typical TLC results for Ru-PIPIDA. TLC also showed that in our Tc-99m PIPIDA ≥95% of the Tc-99m is bound.

Table 2 shows comparative biodistribution of Ru-103 PIPIDA and Tc-99m PIPIDA in normal rats. Ru-103 PIPIDA showed distribution similar to that of Tc-99m PIPIDA, except that for the former blood activity was slightly higher and kidney activity lower.

Figure 1 plots the blood clearances of Ru-97 PIPIDA and Tc-99m PIPIDA. The two are closely comparable, as shown by the figures on the graphs. The first compo-

TABLE 1. TLC (POLYAMIDE; 100% METHANOL) OF Ru-LABELED PIPIDA

Final ligand conc. mg/ml	R_f*	% ruthenium activity: 10 min incubation, 25°	24 hr incubation, 25°	30 min heating, 90°	60 min heating, 90°
5	0	80	54	9	6
	1.0	20	46	91	94
10	0	71	40	7	4
	1.0	29	60	93	96
15	0	62	35	4	2
	1.0	38	65	96	98

* Unbound ruthenium (ionic or hydrolyzed) stays at the point of origin in this system. Complexed ruthenium moves with the solvent front.

TABLE 2. COMPARATIVE BIODISTRIBUTION OF Ru-103 PIPIDA AND Tc-99m PIPIDA AT VARIOUS TIMES AFTER DOSE

	Control rats (n = 6)			
	Ru-103 PIPIDA		Tc-99m PIPIDA	
Organ	% dose/organ	% dose/g	% dose/organ	% dose/g
		15 min		
Blood	6.55 ± 0.27	0.37 ± 0.02	5.51 ± 0.19	0.29 ± 0.09
Liver	5.47 ± 0.88	0.46 ± 0.07	5.09 ± 0.82	0.42 ± 0.04
Kidneys	0.53 ± 0.03	0.27 ± 0.01	1.71 ± 0.04	0.81 ± 0.01
G.I. tract	28.47 ± 9.40	4.21 ± 0.55	53.80 ± 17.39	2.44 ± 1.32
		30 min		
Blood	2.44 ± 0.34	0.14 ± 0.02	2.08 ± 0.12	0.12 ± 0.01
Liver	3.69 ± 0.21	0.41 ± 0.07	3.42 ± 0.57	0.38 ± 0.14
Kidneys	0.56 ± 0.08	0.34 ± 0.02	1.34 ± 0.06	1.16 ± 0.36
G.I. tract	58.8 ± 2.46	3.52 ± 0.23	52.30 ± 1.36	4.67 ± 2.51
		60 min		
Blood	1.73 ± 0.35	0.09 ± 0.02	1.50 ± 0.42	0.10 ± 0.03
Liver	2.81 ± 0.99	0.31 ± 0.12	2.58 ± 0.94	0.31 ± 0.09
Kidneys	0.45 ± 0.07	0.24 ± 0.02	0.81 ± 0.08	0.46 ± 0.04
G.I. tract	55.00 ± 12.4	3.89 ± 1.50	44.86 ± 7.35	2.98 ± 0.42
		120 min		
Blood	1.37 ± 0.21	0.08 ± 0.01	0.58 ± 0.06	0.04 ± 0.01
Liver	1.70 ± 0.26	0.18 ± 0.03	0.61 ± 0.06	0.07 ± 0.001
Kidneys	0.33 ± 0.07	0.16 ± 0.03	0.84 ± 0.10	0.49 ± 0.04
G.I. tract	46.90 ± 12.80	2.83 ± 1.08	60.30 ± 7.51	3.66 ± 1.55

nent of both compounds represented more than 90% of the administered doses. Figure 2 shows the scintigrams of a normal dog after an i.v. dose of 500 μCi of Ru-97 PIPIDA. The scan after 7 min showed the image of the liver and the lack of activity through the biliary tree and gallbladder. After 20 min the gallbladder concentrates the tracer. The scintigrams at 110 and 150 min showed the excretion of Ru-97 PIPIDA into the gastrointestinal tract. The lower large intestine was well visualized after 24, 48, and 72 hr.

Table 3 shows a 24-hr distribution of Ru-103 PIPIDA in normal rats and others with biliary obstruction. Blood,

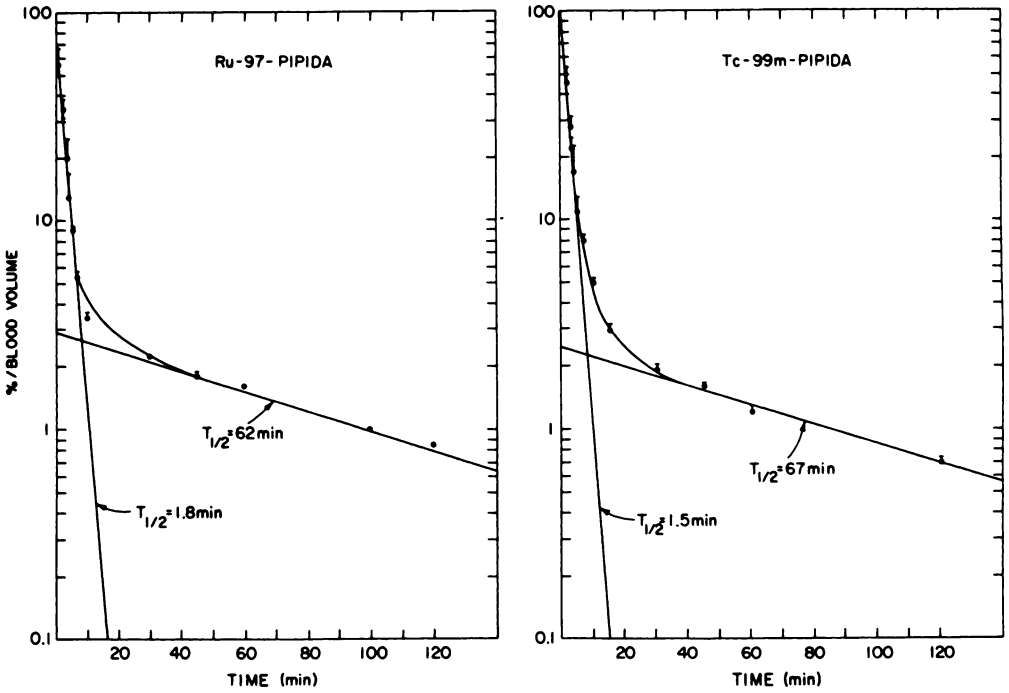

FIG. 1. Blood clearances of Ru-97 PIPIDA and Tc-99m PIPIDA in dogs.

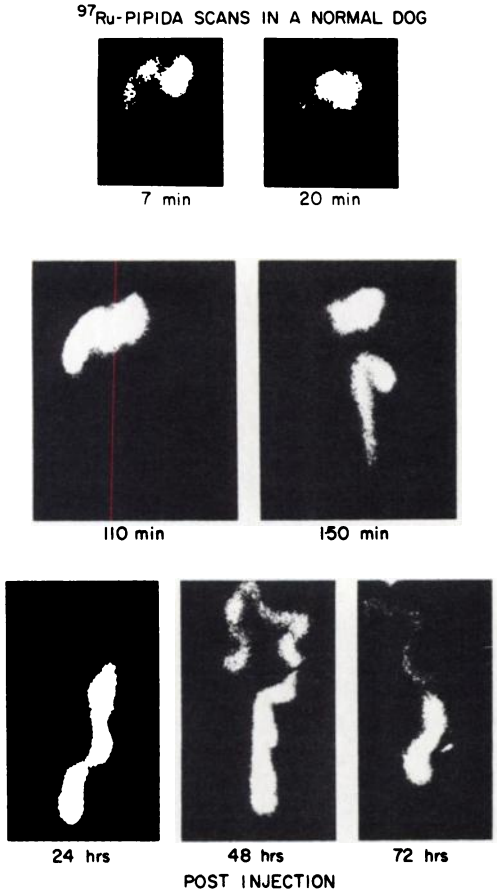

FIG. 2. Scintiphotos, at times indicated, of normal dog given Ru-97 PIPIDA. At 20 min, gallbladder was well visualized; intestines appeared after 150 min and were seen up to 72 hr.

kidneys, liver, and gastrointestinal activity was much higher than normal in rats with obstruction.

Dosimetry for Ru-97 and Tc-99m PIPIDA is compared with I-131 rose bengal (Table 4). The general method for calculation followed the MIRD technique. Specific assumptions for Ru-97 and Tc-99m PIPIDA are outlined below:

1. Three component blood clearance curves;

$$A(t) = 93.8e^{-0.045t} + 4.93e^{-0.056t} + 1.25e^{-0.01t}$$

(half-lives of 1.53, 12.38, and 69.3 min; from dog data)

2. Biological $T_{1/2}$ for clearance from liver = 1.5 hr (rat data).
3. Irreversible catenary. (Blood → liver → SI → ULI → LLI) with one bypass, the gallbladder.
4. Mean residence time in gut = 4, 13, and 24 hr for the SI, ULI, and LLI, respectively (*9*).
5. Gallbladder. Assume 10% of activity appears in gallbladder with biological half-time of 1.5 hr; 75% goes to SI in 3 hr; rest after 9 hr. Absorbed dose = liver dose from penetrating radiations + ½ dose from nonpenetrating radiations, + ½ gallbladder content dose (*9*).

Assumptions for I-131 rose bengal were similar except that the blood removal curve had two components (90% with $T_{1/2}$ = 4 min. and 10% with $T_{1/2}$ = 90 min.). Values for I-131 rose bengal are similar to those given by Freeman et al (*9*).

DISCUSSION

Persistent obstructive jaundice in the first months of life continues to present a perplexing diagnostic problem. Almost 15% of all infants with prolonged jaundice may need surgical treatment. Cholestasis appearing in the first months of life may be caused by many factors, including neonatal hepatitis, biliary atresia, choledochal cyst, toxoplasmosis, herpes simplex, Coxsackie virus, syphilis, drug-induced cholestasis, and a number of congenital syndromes (Gilbert, Crigler-Najjar, partial deficiency of glucoronyl-transferase, Dubin-Johnson, and Rotor). The most common cause of jaundice (85%) is neonatal hepatitis (*10*).

The differential diagnosis between neonatal hepatitis and biliary atresia is often difficult. In biliary atresia, irreversible liver damage often occurs during the first 2 mo of life and death eventually occurs within 2 yr (*11*).

To avoid irreversible damage, a permanent and effective drainage needs to be established surgically between the biliary ductal system and the gastrointestinal tract (*12*). Therefore, the early and accurate diagnosis of the cause of jaundice is imperative.

TABLE 3. COMPARATIVE BIODISTRIBUTION OF Ru-103 PIPIDA IN CONTROLS AND RATS WITH BILIARY OBSTRUCTION

	24 hr (n = 6)			
	Control		Biliary obstruction	
Organ	% dose/organ	% dose/g	% dose/organ	% dose/g
Blood	1.25 ± 0.03	0.07 ± 0.002	7.02 ± 2.17	0.50 ± 0.15
Liver	1.30 ± 0.40	0.09 ± 0.01	4.30 ± 0.63	0.65 ± 0.05
Kidneys	0.21 ± 0.03	0.03 ± 0.01	4.02 ± 0.45	2.20 ± 0.27
G.I.	1.89 ± 0.29	0.17 ± 0.06	9.22 ± 2.85	1.38 ± 0.87

TABLE 4. RADIATION ABSORBED DOSE OF I-131 ROSE BENGAL, Ru-97 PIPIDA, AND Tc-99m PIPIDA

Organ	Absorbed dose* (rads per mCi)		
	I-131 Rose Bengal	Ru-97 PIPIDA	Tc-99m PIPIDA
Whole Body	0.39	0.15	0.026
Blood	0.41	0.15	0.026
Gall Bladder	1.1	0.32	0.20
Liver	0.86	0.24	0.10
Small intestine (SI)	3.6	1.1	0.34
Upper large intestine (ULI)	14.9	2.2	0.64
Lower large intestine (LLI)	41.0	4.1	0.26
Ovaries	1.8	0.98	0.11
Testes	0.16	0.07	0.005

* Retention times in SI, ULI, and LLI were r = 13, and 24 hr, respectively.

Over the years, many tests have been developed in the attempt to differentiate biliary atresia from neonatal hepatitis, in addition to serial bilirubin and enzyme estimations. These include the I-131 rose bengal test (*13*, *14*), I-131 rose bengal sequential scans (*15*), red blood cell peroxidase test (*16*), RBC lipid profile (*17*), serum 5′-nucleotidase level (*18*), lipoprotein-X determination (*19*), serum-alpha-fetoprotein (*20*), needle biopsy of the liver (*21*), and exploratory laparotomy (*22*). The last two procedures are often necessary to make a correct diagnosis. However, invasive procedures often contribute to the development of other complications, resulting in a worsened prognosis. For example, needle biopsy and laparotomy involve the use of general anesthesia, which may result in increased incidence of biochemical abnormalities, development of cirrhosis, and mortality (*23*,*24*). Also the I-131 rose bengal test poses technical difficulties, since it requires separate urine and uncontaminated stool collections. In series of eight neonates with jaundice, Tc-99m *p*-butyl IDA failed to detect extrahepatic biliary tract activity in three out of five cases of hepatitis (*25*). Therefore, to date, I-131 rose bengal is still an agent of choice in neonatal hepatobiliary scintigraphy. However, the relatively high radiation dose from I-131 (because of its beta emissions and long half-life) limits it use (Table 4). Furthermore, the imaging characteristics of Ru-97 are more favorable because of its lower-energy photons, which permit images containing greater information density.

In biliary atresia the stools are, of course, acholic from the beginning. When the jaundice becomes severe, the stools may show a trace of bile on the surface because of some of the abundant bile pigment in the blood is excreted by the intestinal mucosa (*26*). This might explain the GI activity of Ru-103 PIPIDA observed in the rats with biliary obstruction (Table 3). In addition, the high blood activity at 24 hr in the obstructed animals may account in part for the elevated GI activity by excessive blood in the bowel wall.

Because an early correct diagnosis of biliary atresia or neonatal hepatitis is of critical importance, we believe that the development of improved hepatobiliary agents labeled with radionuclides, such as Ru-97 with intermediate half-life and favorable physical characteristics for scanning, will provide physicians with an important diagnostic tool that should lead to improved therapy for children considered to have a poor prognosis.

FOOTNOTE

* Diagnostic Isotope, Inc., Bloomfield, NJ.

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REFERENCES

1. TAPLIN GV, MEREDITH OM, KADE H: The radioactive (I^{131} tagged) rose bengal uptake excretion test for liver function using external gamma ray scintillation counting techniques. *J Lab Clin Med* 45:665-678, 1955
2. KRISHNAMURTHY GT, TUBIS M, ENDOW JS, et al: ^{99m}Tc-penicillamine—a new radiopharmaceutical for cholescintigraphy. *J Nucl Med* 13:447, 1972 (abst)
3. DEWANJEE MK, FLIEGEL C, TREVES S, et al: ^{99m}Tc tetracyclines. Preparation and biological evaluation. *J Nucl Med* 15:176-182, 1974
4. BAKER RJ, BELLEN JC, RONAI PM: Technetium-99m-pyridoxylideneglutamate: A new hepatobiliary radiopharmaceutical. I. Experimental aspects. *J Nucl Med* 16:720-727, 1975
5. HARVEY E, LOBERG M, COOPER M: Tc-99m-HIDA. A new radiopharmaceutical for hepatobiliary imaging. *J Nucl Med* 16:533, 1975 (abst)
6. SRIVASTAVA SC, RICHARDS P, MEINKEN GE, et al: Evaluation of radiolabeled ruthenium compounds as tumor-localizing agents. In *Radiopharmaceuticals II. Proceedings 2nd Int Symp Radiopharmaceuticals*, March 1979, Seattle, Washington, New York, Society of Nuclear Medicine, 1979, p 265-274
7. KU TH, RICHARDS P, SRIVASTAVA SC, et al: Production of ruthenium-97 for medical applications. In *World Fed. of Nucl. Med. Biol. Second Int. Cong.*, Washington, DC, Sept 18-21, 1978, p 17 (abst)
8. BURSTALL FH, DAVIES GR, LINSTEAD RP, et al: Inorganic

chromatography in cellulose. Part II. The separation and detection of metals and acid radicals on strips of absorbent paper. *J Chem Soc* 516-528, 1950

9. FREEMAN LM, PATTON DD, ROSENTHALL L, et al: Summary of current radiation dose estimates to humans from ^{123}I, ^{124}I, ^{126}I, ^{130}I, and ^{131}I as sodium rose bengal. *J Nucl Med* 16:1214-1217, 1975 (MIRD Report No. 7)
10. DELORIMIER AA: Surgical management of neonatal jaundice. *N Engl J Med* 288:1284-1286, 1973
11. OH-I R, KASAI M, TAKAHASHI T: Intrahepatic biliary obstruction in congenital bile duct atresia. *Tohoku J Exp Med* 99:129-149, 1969
12. FONKALSRUD EW, KITAGAWA S, LONGMIRE W: Hepatic lymphatic drainage to the jejunum for congenital biliary atresia. *Am J Surg* 112:188-194, 1966
13. BRENT RL, GEPPERT LJ: Use of radioactive rose bengal. In the evaluation of infantile jaundice. *J. Dis Child* 98:720-730, 1959
14. SHARP HL, KRIVIT W, LOWMAN JT: The diagnosis of complete extrahepatic obstruction by Rose Bengal I^{131}. *J Pediatr* 70:46-53, 1967
15. SILVERBERG M, ROSENTHALL L, FREEMAN LM: Rose bengal excretion studies as an aid in the differential diagnosis of neonatal jaundice. *Semin Nucl Med* 3:69-80, 1973
16. MELHORN DK, GROSS S, IZANT RJ: The red cell hydrogen peroxide hemolysis test and vitamin E absorption in the differential diagnosis of jaundice in infancy. *J Pediatr* 81: 1082-1087, 1972
17. NEERHOUT RC: Abnormalities of erythrocyte stromal lipids in hepatic disease. *J Lab Clin Med* 71:438-447, 1968
18. YEUNG CY: Serum 5'-nucleotidase in neonatal hepatitis and biliary atresia. Preliminary observations. *Pediatrics* 50: 812-814, 1972
19. POLEY JR, ALAUPOVIC P, SEIDEL D, et al: Differential diagnosis between "neonatal hepatitis" and extrahepatic biliary atresia in infants: a new test. *Gastroenterology* 58:983, 1970
20. ZELTZER PM, FONKALSRUD EW, NEERHOUT RC, et al: Differentiation between neonatal hepatitis and biliary atresia by measuring serum-alpha-fetoprotein. *Lancet* 1:373-375, 1974
21. PORTER M, RILEY HD, GRAHAM H: Needle biopsy of the liver in infants and children. *J Pediatr* 65:176-188, 1964
22 HAYS DM, WOOLLEY MM, SNYDER WH, et al: Diagnosis of biliary atresia: Relative accuracy of percutaneous liver biopsy, open liver biopsy, and operative cholangiography. *J Pediatr* 71:598-607, 1967
23. LANDING BH: Changing approach to neonatal hepatitis and biliary atresia. *Pediatrics* 53:647-648, 1974 (commentary)
24. LAWSON EE, BOGGS JD: Long-term follow-up of neonatal hepatitis. Safety and value of surgical exploration. *Pediatrics* 53:650-655, 1974
25. COLLIER BD, TREVES S, DAVIS MA, et al: Simultaneous ^{99m}Tc-p-butyl-IDA and ^{131}I-rose bengal scintigraphy in neonatal jaundice. *Radiology* 134:719-722, 1980
26. ANDERSON W: Ed. *Boyd's Pathology for the Surgeon.* 8th ed. Philadelphia and London, W.B. Saunders Co., 1967, p 315