

Tissue Distribution of [^{14}C]Cholyglycine in Rats and Hamsters with a Bile Fistula or Bile Duct Ligation¹ (39621)PETER Y. NG AND ALAN F. HOFMANN²*Gastroenterology Unit, Mayo Clinic, Rochester, Minnesota 55901*

Plasma disappearance of substances taken up largely by the liver, such as bromosulphothalein (BSP) (1-3) or indocyanine green (4, 5), has been used widely as a test for liver function. Recently, the plasma disappearance of a bile acid, cholyglycine, has been proposed as a test for hepatic injury (6, 7). This bile acid clearance is based on the assumption that the plasma disappearance of cholyglycine may be equated with hepatic uptake. To test this assumption, we have defined the tissue distribution of intravenously injected cholyglycine in rats and hamsters; we have used animals with an acute bile fistula, as well as animals with a bile duct obstruction.

Materials and methods. Experimental design. A mixture of [^{14}C]cholyglycine and [^{99}Tc]albumin was injected intravenously as a bolus. Ten minutes later, animals were killed and tissues were removed for determination of radioactivity. The ^{99}Tc was used to estimate the amount of blood contaminating each tissue sample. The sampling time of 10 min was chosen since this time interval corresponds to about three half-lives for plasma disappearance of cholyglycine (in man) (8) and was judged to be a sufficiently long interval for nearly complete tissue uptake to occur.

Chemicals. [^{14}C]Cholyglycine (sp act 51.7 mCi/mmole) was purchased from Amersham/Searle (Arlington Heights, Ill.) and had a radiopurity greater than 96% by zonal scanning (9) using a thin-layer system for bile acid conjugates (10). [^{99}Tc]Albumin was prepared using a commercial kit (New England Nuclear Radiopharmaceutical Division, North Billerica, Mass.). A solution

of [^{14}C]cholyglycine was mixed with [^{99}Tc]albumin to give a concentration of 5 μCi of ^{14}C and 15 μCi of ^{99}Tc radioactivity per milliliter.

Animal preparation. Male Sprague-Dawley rats weighing 250-350 g (Mayo Clinic stock) and adult golden Syrian hamsters (Engel Laboratory, Farmersburg, Ind.) were used. Animals were fed a commercially available diet (Purina Rat Chow, Purina Company, St. Louis, Mo.) *ad libitum*, and were allowed free access to water.

Bile fistula animals were prepared immediately prior to injection of the isotope mixture. The animal was anesthetized with diethyl ether. The abdomen was opened with a small midline incision. The common bile duct was isolated and cannulated near its entrance into the duodenum with polyethylene tubing (PE 60, Clay Adams, Parsippany, N.J.).

Bile duct ligation was performed 4 days prior to the experiment. The animal was anesthetized similarly and the abdominal cavity was opened. The common bile duct was isolated, doubly ligated with silk, and divided between ligatures. The abdomen was then closed with sutures and the animal was allowed to recover, during which time he was given free access to food and water.

Injection of isotope. Animals were anesthetized lightly with diethyl ether, and the solution containing the two isotopes was injected intravenously into the femoral vein. Ten minutes later, animals were killed by exsanguination with cardiac puncture; a laparotomy was performed and tissues were removed. A sample of about 200 mg was taken in duplicate from each tissue and immediately weighed on a tissue balance, before estimation of radioactivity.

Determination of radioactivity. The ^{99}Tc activity in each tissue sample was determined using a Beckman 310 gamma counter (Beckman Instruments, Inc., Scientific In-

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struments Division, Irvine, Calif.). Samples were then kept until the ^{99}Tc radioactivity had decayed for at least 10 half-life times. Samples were then oxidized using a wet combustion technique (11), and ^{14}C radioactivity was determined by liquid scintillation spectroscopy.

In order to validate the wet combustion procedure used for tissues, samples of some tissue were digested in 10% KOH in order to permit liquid scintillation counting. Digestion in KOH gave higher radioactivity for several tissues than the perchloric acid-catalyzed wet oxidation technique, but differences were sufficiently small that the wet oxidation procedure was judged to afford quantitative recovery of radioactivity. In any case, for major organs, results obtained from both methods were in good agreement.

In order to determine radioactivity present in the carcass, the carcass was suspended in 10% KOH with a homogeneous solution occurring in several days. Aliquots were taken, bleached with 30% hydrogen peroxide at 80° for 1 hr, and radioactivity was determined in a toluene-based scintillation mixture containing a solubilizer.

Calculations. From the ratio of ^{14}C to ^{99}Tc in the blood sample, the true tissue radioactivity was calculated. Weights of individual tissues were determined on a number of animals (Table I) and were used to calculate radioactivity present in the entire tissue.

In preliminary experiments it was found that the [^{99}Tc]albumin contained free ^{99}Tc which was taken up excessively by some tissues (liver, spleen, kidneys), resulting in a falsely high value for entrapped blood. To correct for this, each batch of [^{99}Tc]albumin was mixed with [^{125}I]albumin (Mallinckrodt Nuclear, Mallinckrodt, Inc., St. Louis, Mo.), and the mixture was injected into rats and hamsters. The ratio of ^{99}Tc to ^{125}I in each tissue was obtained 10 min later and was used to correct observed ^{99}Tc counts.

Statistical analyses were done using the Student's *t* test.

Results. Healthy bile fistula animals. The tissue distribution of the intravenously administered [$1\text{-}^{14}\text{C}$]cholyglycine in healthy bile fistula and bile duct-obstructed rats and hamsters is shown in Tables II and III, respectively. It is obvious that in the healthy

TABLE I. ORGANS AND TISSUE WEIGHT OF GOLDEN SYRIAN HAMSTERS AND SPRAGUE-DAWLEY RATS.^a

Organs	Hamster	Rat
Blood	7.32 ± 0.04^b	7.29 ± 0.32^b
Enterohepatic		
Liver	4.36 ± 0.38	4.08 ± 0.16
Stomach	0.77 ± 0.03	0.46 ± 0.02
Small intestine	1.44 ± 0.02	2.03 ± 0.11
Colon	0.86 ± 0.01	0.47 ± 0.01
Cecum	0.56 ± 0.01	0.29 ± 0.02
Cardiovascular-renal		
Heart	0.33 ± 0.01	0.30 ± 0.01
Lungs	0.60 ± 0.07	0.61 ± 0.02
Kidneys	1.07 ± 0.22	0.76 ± 0.01
Others		
Pancreas	0.13 ± 0.01	0.20 ± 0.03
Spleen	0.14 ± 0.02	0.33 ± 0.03
Testes	2.89 ± 0.10	1.15 ± 0.07
Uterus	0.39 ± 0.02	—
Brain	0.74 ± 0.02	0.64 ± 0.03

^a Weights are expressed as g/100 g animal \pm SE. Averages are of four animals each.

^b ml/100 g animal.

bile fistula animals, the liver is the organ responsible for the removal and excretion of serum bile acid. More than 50% of the injected ^{14}C radioactivity was excreted into the fistula bile within 10 min, while less than 1% remained in the circulation. The other organs did not pick up any appreciable amount of radioactivity.

Bile duct-obstructed animals. In the bile duct-obstructed animals, much more radioactivity was retained in the blood. In the bile duct-obstructed rats, all the major organs, except the liver, testes, brain, and the upper small intestine, contained a significantly larger amount of injected ^{14}C radioactivity when compared with the corresponding organs of the bile fistula rats (Table II). In the bile duct-obstructed hamsters, a similar increase in the uptake of ^{14}C radioactivity was seen in most major organs with the exceptions of the pancreas, the upper small intestine, and the colon. Uptake of ^{14}C -label by the liver was significantly greater in the bile duct-obstructed hamsters than in the bile fistula hamsters ($P < 0.05$), but there was no significant difference in liver uptake in the two groups of rats (Table II).

The total recovery of injected ^{14}C radioactivity from the bile fistula animals was between 72–78%. The ^{14}C radioactivities contained in the bones, skin, and fat were not included. Because in the bile duct ob-

TABLE II. TISSUE DISTRIBUTION OF INTRAVENOUSLY ADMINISTERED [1-¹⁴C]CHOLYGLYCINE AT 10 MIN IN RATS.^a

Organs	Healthy acute bile fistula rats (N = 9)	4-Day biliary obstructed rats (N = 9)	
Blood	0.89 ± 0.1	19.69 ± 0.73	
Enterohepatic			
Liver ^b	12.48 ± 1.40	10.07 ± 1.02	N.S.
Bile	57.68 ± 2.76	—	
Stomach	0.17 ± 0.02	0.04 ± 0.03	
Upper intestine	2.21 ± 0.44	1.30 ± 0.5	N.S.
Lower intestine	0.71 ± 0.08	1.35 ± 0.7	
Colon	0.17 ± 0.03	0.34 ± 0.02	
Cecum	0.13 ± 0.02	0.24 ± 0.01	
Cardiovascular-renal			
Heart	0.02 ± 0.01	0.12 ± 0.01	
Lungs	0.10 ± 0.01	0.66 ± 0.03	
Kidneys	0.49 ± 0.04	2.75 ± 0.20	
Others			
Pancreas	0.09 ± 0.01	0.23 ± 0.02	
Spleen	0.05 ± 0.01	0.11 ± 0.01	
Genitals	0.20 ± 0.02	0.24 ± 0.02	N.S.
Muscle ^c	4.26 ± 0.62	14.96 ± 0.78	
Brain	0.02 ± 0.01	0.04 ± 0.01	N.S.
Total recovery	78.21 ± 2.56	51.28 ± 1.35	

^a All values are significant at 0.1% level ($P < 0.001$), except when denoted N.S. (nonsignificant). Results are expressed as percentage dose administered per organ (mean ± SE).

^b Liver includes intrahepatic biliary tract.

^c Muscle mass is taken to account for 40% of the total body weight of the animal (20).

TABLE III. TISSUE DISTRIBUTION OF INTRAVENOUSLY ADMINISTERED [1-¹⁴C]CHOLYGLYCINE AT 10 MIN IN HAMSTERS.^a

Organs	Healthy acute bile fistula hamsters (N = 5)	4-Day biliary-obstructed hamsters (N = 7)	P
Blood	0.41 ± 0.05	16.43 ± 1.17	<0.001
Enterohepatic			
Liver ^a	12.79 ± 1.53	19.80 ± 2.40	<0.05
Gallbladder	0.07 ± 0.02	0.1 ± 0.04	<0.001
Bile	55.29 ± 2.54	—	
Stomach	0.14 ± 0.01	0.45 ± 0.04	<0.001
Upper intestine	0.56 ± 0.13	0.82 ± 0.07	N.S.
Lower intestine	0.21 ± 0.02	0.91 ± 0.13	<0.001
Colon	0.33 ± 0.05	0.45 ± 0.04	N.S.
Cecum	0.09 ± 0.01	0.29 ± 0.04	<0.001
Cardiovascular-renal			
Heart	0.01 ± 0.01	0.11 ± 0.01	<0.001
Lungs	0.07 ± 0.01	0.43 ± 0.04	<0.001
Kidneys	0.18 ± 0.01	0.96 ± 0.06	<0.001
Others			
Pancreas	0.05 ± 0.01	0.06 ± 0.01	N.S.
Spleen	0.03 ± 0.01	0.02 ± 0.01	<0.001
Genitals	0.11 ± 0.02	0.42 ± 0.05	<0.001
Muscle	2.28 ± 0.22	11.48 ± 0.93	<0.001
Brain	0.01 ± 0.01	0.02 ± 0.01	<0.05
Total recovery	72.30 ± 3.68	51.78 ± 0.96	<0.001

^a Results are expressed as percentage dose administered per organ (mean ± SE).

^b Liver includes intrahepatic biliary tract.

structed hamsters the recovery, as calculated, was only slightly better than 50%, the carcass of several such animals were dissolved in 10% KOH solution. The total recovery thus obtained ranged from 91-95%. Similar recovery studies were carried out in two bile fistula hamsters; total recovery was 90 and 94%.

It is of interest to note that in bile duct-obstructed hamsters, about 10% of the injected ^{14}C label resided in the skin.

Discussion. In the healthy rat and hamster, radioactivity was taken up entirely by the liver and promptly excreted into bile. The hepatic capacity for bile acid uptake is known to be extraordinarily high (12, 13), but it is not known whether the absence of appreciable uptake by other tissues is because bile acids are largely albumin bound, or whether the membrane of the hepatocyte has a particular affinity for the bile acid molecule, or both. Single pass hepatic clearance of cholyglycine is nearly complete, based on studies in the dog (14), so that hepatic removal appears to be blood flow limited.

In the bile duct-obstructed rat and hamster, there was striking plasma retention. Tissue concentrations were significantly higher, but there was no particular organ that showed an especially enhanced uptake.

All in all, these data support the report of Bergstrom *et al.* (15) that in the healthy rat, bile acids are largely localized to the enterohepatic circulation. In bile duct obstruction, it is now recognized that there is extensive sulfation of bile acids (16, 17) promoting renal excretion. The anatomical distribution of injected bile acids in the chronically jaundiced rat was recently reported by Hanitkevych *et al.* (18) who observed that 2 weeks after bile duct ligation, bile acids were present largely in plasma and kidney and not in other tissues. The data thus imply that in healthy man, plasma disappearance of injected cholyglycine may be equated with hepatic uptake. Nonetheless, this point cannot be tested directly in man until a bile acid tagged with a gamma-emitting isotope is synthesized.

Recently Klaassen (19) examined the tissue distribution of intravenously administered bromosulphophthalein in the rat. He obtained tissues at 10 min as in our studies

and corrected for trapped plasma. His data cannot be compared directly with ours, but he found a greater fraction of bromosulphophthalein was present in the kidney than we observed for cholyglycine.

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