consistent with the assigned structures. Compounds Ia–Ig gave a strong qualitative test for sulfur using the oxygen flask ignition procedure.

The general procedure for the synthesis of the 3-aryl-3-hydroxypropanoic acids (Va–Vg) is illustrated by the preparation of 3-(3-chloro-4-methylphenyl)-3-hydroxypropanoic acid (Vg). The Reformatsky reaction was carried out as described (6) using 3-chloro-4-methylbenzaldehyde (9) (154 g, 1.0 mole), ethyl bromoacetate (150 ml, 1.3 moles), and zinc dust (90 g, 1.35 g-atoms). The crude ester was treated with a solution of 85% potassium hydroxide (80 g, 1.2 moles) in 95% ethanol (1500 ml). The mixture was refluxed for 4 hr and was then evaporated *in vacuo*.

The residue was dissolved in water (1500 ml) and extracted with ether. The aqueous layer was acidified with concentrated hydrochloric acid and extracted with chloroform (2 × 700 ml). The chloroform extracts were combined and dried over anhydrous magnesium sulfate. The drying agent was removed by filtration, and the solvent was removed *in vacuo* to leave the solid product (Vg).

The general procedure for the synthesis of the *S*-(4-chlorophenyl) 3-aryl-3-hydroxypropanethioates (Ia–Ig) is illustrated by the preparation of *S*-(4-chlorophenyl) 3-(3-chloro-4-methylphenyl)-3-hydroxypropanethioate (Ig). A solution of Vg (43 g, 0.2 mole) and VI (29 g, 0.2 mole) in dichloromethane (~600 ml) was stirred at room temperature. A solution of VII (41 g, 0.2 mole) in a small amount of dichloromethane was added in one portion. There was an immediate, very exothermic, reaction with the formation of a white precipitate. After the initial reaction had subsided, the reaction mixture was stirred for 4 hr at room temperature and then filtered. The filtrate was evaporated to dryness under reduced pressure to leave a white, solid product (Ig).

REFERENCES

(1) G. Scheurbrandt and K. Bloch, *J. Biol. Chem.*, **237**, 2064 (1962).

(2) A. T. Voris, S. Matsumura, and K. Bloch, *ibid.*, **239**, 3653 (1964).

(3) D. Brock, L. Kass, and K. Bloch, *ibid.*, **242**, 4432 (1967).

(4) G. Helmkamp, Jr., R. Rando, D. Brock, and K. Bloch, *ibid.*, **243**, 3229 (1968).

(5) L. Kass, *ibid.*, **243**, 3223 (1968).

(6) R. Adams, "Organic Reactions," vol. I, Wiley, New York, N.Y., 1947, p. 16.

(7) R. Freedman and R. E. Chamberlain, in "Antimicrobial Agents and Chemotherapy," G. L. Hobby, Ed., Ann Arbor, Mich., 1967, p. 502.

(8) H. R. Snyder, Jr., C. F. Spencer, and R. Freedman, *J. Pharm. Sci.*, **66**, 1204 (1977).

(9) R. J. Alaimo and C. J. Hatton, *J. Med. Chem.*, **19**, 349 (1976).

(10) D. S. Noyce, P. A. King, C. A. Lane, and W. L. Reed, *J. Am. Chem. Soc.*, **84**, 1638 (1962).

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Assessment of Enterohepatic Circulation of ^{3}H-Digoxin with a Minimal Interruption Technique

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Abstract □ The biliary excretion of ^{3}H-digoxin in rats prepared for bile sampling with minimal interruption of the enterohepatic circulation was compared with that in rats with complete interruption after intraduodenal or intravenous administration. Following dosage by either route, significantly more radioactivity was recovered from animals with nearly intact enterohepatic circulation. The method described allows direct measurement of this cycle in unanesthetized animals without the consequences of bile depletion.

Keyphrases □ ^{3}H-Digoxin—biliary excretion measured by enterohepatic circulation minimal interruption technique, rats □ Excretion, biliary—^{3}H-digoxin, measured by enterohepatic circulation minimal interruption technique, rats □ Enterohepatic circulation—minimal interruption technique used to measure biliary excretion of ^{3}H-digoxin, rats □ Cardiotonic agents—digoxin, biliary excretion measured by enterohepatic circulation minimal interruption technique, rats

The enterohepatic cycle plays a major role in the biotransformation and disposition of some drugs. This aspect of cardiac glycoside metabolism has received considerable attention since it was suggested (1) that enterohepatic recycling is a major determinant of the half-life of these compounds. Direct measurement of the enterohepatic cycle is not usually done; instead, conclusions are drawn from data on fecal excretion or bile fistula preparations.

While these methods are useful for estimating the hepatobiliary contribution to total drug disposition, they allow only indirect conclusions about the extent of recycling. A model previously used for studies of biliary responses to partial hepatectomy and hypoxia (2–4) was adopted to compare the biliary excretion of digoxin in rats with minimal and total interruption of the enterohepatic circulation.

EXPERIMENTAL

^{3}H-Digoxin[1] (specific activity of 1.0 mCi/1.33 μg) was 96% radiochemically pure. Solutions of a specific activity of 0.25 μCi/μg/0.01 ml were prepared by diluting the stock solution with unlabeled digoxin. Aliquots of standards and samples were assayed for radioactivity in a liquid scintillation spectrometer after addition to 10 ml of scintillation medium[2], using automatic external standardization.

Twenty-four female Wistar rats, 250–300 g, were anesthetized with ether, and a midline incision was made. The proximal bile duct was cannulated with polyethylene No. 50 tubing[3]. A duodenostomy was constructed with polyethylene No. 160 tubing, and both duodenal and biliary cannulas were brought through a skin incision in the right flank. The two tubes were connected with a short length of flexible tubing (1.5 mm i.d., 2.4 mm o.d.), the skin was closed with a single suture, and the midline wound was closed.

The animals were allowed to recover in individual metabolic cages; they were observed and allowed free access to food and water for at least 48

[1] New England Nuclear Corp., Boston, Mass.
[2] Scintisol-Complete, Isolab, Akron, Ohio.
[3] Intramedic, Becton, Dickinson and Co., Parsippany, N.J.

hr. Twenty animals in good condition were then fasted overnight; on the morning of experiment, they were placed in a restraint cage but allowed continued access to water. The flank incision was opened by cutting the single suture, and the bile and duodenal cannulas were disconnected.

Experiments began with the intraduodenal or intravenous administration of ^{3}H-digoxin in a dose of 2.5 μCi/10 μg/100 g. For intraduodenal administration, the dose was instilled into the duodenal cannula and the tubing was clamped. Intravenous doses were given over 1 min into a tail vein. Five rats in each group (minimally or totally interrupted) received drug by either the intraduodenal or intravenous route. Collection of bile began at the same time. Hourly bile volume was measured by collection in a graduated centrifuge tube.

In minimally interrupted enterohepatic cycle experiments (10 animals), duplicate 0.025-ml aliquots of each bile sample were immediately removed for assay, and the remainder of the collected bile was returned to the intestine through the duodenal cannula. Since hourly bile volumes were approximately 1.0 ml or more, the removal of 0.05 ml for assay resulted in no more than a 5% interruption of the cycle. For interrupted enterohepatic cycle experiments (10 animals), similar aliquots were taken for assay, but the total hourly bile volume was replaced by fresh donor rat bile containing no drug. Thus, in the latter group of animals, bile salt depletion was prevented while total removal of drug *via* the bile fistula was achieved.

Collections were continued for 12 hr. Total radioactivity over 12 hr was calculated as the sum of the product of hourly bile volume and disintegrations per minute per milliliter for each sample and expressed as a percent of the dose.

RESULTS

Significantly more radioactivity was recovered in bile from animals with minimally interrupted enterohepatic circulation when compared with those with total biliary drainage. After intravenous dosing, 90.2 ± 18.5 and 60.5 ± 21.1% ($p < 0.05$) were recovered from intact and interrupted animals, respectively. Following intraduodenal dosing, 77.1 ± 22.9 and 42.9 ± 2.9% ($p < 0.025$) were recovered. Since neither group was depleted of bile, the results reflect the recycling of tritium-labeled drug and metabolites during the study period.

Characterization of the recovered radioactivity was not carried out in order not to remove more bile from the intact animals than the minimum required for assay. Previously (5), the majority of the radioactivity recovered from bile fistula rats 12 hr after dosing was unchanged ^{3}H-digoxin plus its principal metabolites.

DISCUSSION

The results of this study are in good agreement with those of other investigations. Rietbrock *et al.* (6) reported 71 and 45% excretion of ^{3}H-digoxin 12 hr after intravenous and intraduodenal administrations, respectively, in anesthetized rats with bile fistulas receiving donor bile replacement. Russell and Klaassen (7), using bile fistula rats without donor bile, found 59% biliary excretion of ^{3}H-digoxin 12 hr after intravenous dosing.

Abshagen *et al.* (5), using a method similar to that described here, found in anesthetized rats that interruption of the enterohepatic circulation reduced the blood half-life of total radioactivity by 50% and that the principal metabolites of ^{3}H-digoxin, bis- and monodigitoxoside, were extensively recycled along with the parent glycoside. Since the percent excretion of dose in intact rats was not compared with that reported for bile fistula animals, the present investigation was conducted to assess, by direct comparison, the contribution of reabsorbed digoxin and metabolites to total hepatobiliary disposition. The principal metabolites of digoxin possess positive inotropic activity (8). Therefore, it seems reasonable to relate the biological duration of action to the retention of both the parent drug and metabolites as estimated by total radioactivity assay.

Several methods are available for estimating the role of biliary excretion and intestinal reabsorption in drug disposition. The determination of fecal excretion does not allow direct measurement of biliary excretion or whether recycling occurs. Bile fistula studies allow measurement of biliary output but do not assess recycling. Refeeding bile from dosed animals to other recipient animals demonstrates that recycling can occur but does not permit its quantitation in the presence of the original dose. Demonstration of a reduced $T_{1/2}$ in bile fistula animals confirms that biliary excretion plays a considerable role in total body drug disposition but does not predict the extent of recycling. The present method allows continuous sampling of biliary excretion while imposing minimal interruption of the enterohepatic circulation in unanesthetized animals.

This principle previously was applied to an existing method for assessing biliary physiology in humans. With double-lumen tubes for duodenal perfusion of a nonabsorbable marker and correction for incomplete recovery of aspirated bile, 30% of a single parenteral dose of ^{3}H-digoxin was found in the bile of normal volunteers in 24 hr (9). This result contrasts with a calculated total percent enterohepatic cycling of 6.5% from studies of postoperative patients with bile fistulas (10). The results of the present study and previous findings suggest that underestimation of the enterohepatic cycle is likely when methods that interrupt this cycle are employed.

REFERENCES

(1) G. T. Okita, *Fed. Proc.*, **26,** 1125 (1967).

(2) N. R. Thomford and P. T. Butterfield, *Surgery,* **66,** 1073 (1969).

(3) P. T. Butterfield, G. M. Penn, and N. R. Thomford, *ibid.*, **68,** 651 (1970).

(4) Y. Kakumoto, R. C. Gardner, K. J. Isselbacher, and R. A. Malt, *Surg. Forum,* **22,** 47 (1971).

(5) U. Abshagen, K. V. Bergmann, and N. Rietbrock, *Naunyn-Schmiedebergs Arch. Pharmacol.,* **275,** 1 (1972).

(6) N. Rietbrock, U. Abshagen, K. V. Bergmann, and H. Kewitz, *ibid.*, **274,** 171 (1972).

(7) J. Q. Russell and C. D. Klaassen, *J. Pharmacol. Exp. Ther.,* **186,** 455 (1973).

(8) P. M. Stephen, S. Dutta, and B. H. Marks, *Naunyn-Schmiedebergs Arch. Pharmacol.,* **292,** 251 (1976).

(9) J. H. Caldwell and C. T. Cline, *Clin. Pharmacol. Ther.,* **19,** 410 (1976).

(10) J. E. Doherty, W. F. Flanigan, M. L. Murphy, R. T. Bullock, G. L. Dalrymple, O. W. Beard, and W. H. Perkins, *Circulation,* **42,** 867 (1970).

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