Metabolism and choleretic activity of homochenodeoxycholic acid in the hamster

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Abstract The hepatic metabolism and the choleretic effect of homochenodeoxycholic acid, the C_{25} homologue of chenodeoxycholic acid, were investigated in the hamster. After intravenous administration of ^{3}H-labeled homochenodeoxycholic acid into biliary fistula hamsters, more than 80% of the radioactivity was recovered in bile in 4 h. A relatively small proportion of homochenodeoxycholic acid was present in bile as the taurine (22%) or glycine (4%) conjugate. However, more than 70% of the administered compound was biotransformed into C_{23} bile acids. The major C_{23} metabolites in bile were norchenodeoxycholic acid (17%), tauronorchenodeoxycholic acid (33%), and a trihydroxy norbile acid (identified as 3α,5β,7α-trihydroxy-24-nor-5β-cholan-23-oic acid, 19%). Small amounts (<5%) of sulfate(s) and glucuronide(s) were also detected. Homochenodeoxycholic acid, when infused intravenously into the hamster, produced a striking choleresis. The increase in bile flow after infusion of this compound was 6- to 7-times that induced by chenodeoxycholic acid. The apparent choleretic activity of homochenodeoxycholic acid, 181 μl/μmol, was much greater than that of chenodeoxycholic acid, 11 μl/μmol. In conclusion, homochenodeoxycholic acid induced a hypercholeresis of the same order of magnitude as norchenodeoxycholic acid, presumably because considerable proportions of this compound were degraded to the hypercholeretic norchenodeoxycholic acid via β-oxidation in the liver.—**Miki, S., B. I. Cohen, T. Mikami, and E. H. Mosbach.** Metabolism and choleretic activity of homochenodeoxycholic acid in the hamster. *J. Lipid Res.* 1993. **34:** 915–921.

Supplementary key words norchenodeoxycholic acid • hypercholeresis • *Mesocricetus auratus* • biotransformation • apparent choleretic activity • cholehepatic shunt

It has been shown that decreasing the length of the side chain of the natural bile acids by a single carbon atom causes a remarkable change in their biological properties (1–5). In the rat, norcholic acid (norCA), the C_{23} homologue of cholic acid (CA), exerts a greater choleretic effect than cholic acid (1). Norchenodeoxycholic acid (norCDCA) and norursodeoxycholic acid (norUDCA) infused intravenously caused a striking bicarbonate-rich hypercholeresis in the hamster and the rat (3, 4). "Cholehepatic shunting" has been proposed as a possible mechanism responsible for the hypercholeretic effect (1, 3–8). The hepatic biotransformation of norCDCA and norUDCA is complex. Little conjugation with glycine or taurine occurred, but in the rat, hamster, and guinea pig these dihydroxy norbile acids were recovered in bile either unchanged, in the form of hydroxylated derivatives, or as sulfates and glucuronides (3, 4).

The C_{25} homologues of CA and ursodeoxycholic acid (UDCA), namely, homocholic acid (homoCA), and homoursodeoxycholic acid (homoUDCA), undergo β-oxidation to form norbile acids in the rat (9, 10). Therefore, we postulated that homodihydroxy bile acids would induce a hypercholeretic effect if considerable amounts underwent hepatic degradation to C_{23} bile acids. However, detailed studies on the choleretic effect of homobile acids have not been carried out previously. This paper deals with the hepatic metabolism and choleretic effect of homochenodeoxycholic acid (homoCDCA), the C_{25} homologue of chenodeoxycholic acid (CDCA), in the biliary fistula hamster.

MATERIALS AND METHODS

Labeled and reference compounds

[24-^{14}C]CDCA (sp act 50.0 μCi/μmol) was purchased from NEN Research Products (Boston, MA). [11,12-^{3}H]homoCDCA (sp act 5.5 × 10^5 dpm/mg) was synthesized by the Arndt-Eistert reaction (11), from [11,12-^{3}H]norCDCA. [11,12-^{3}H]norCDCA was a gift from Dr.

Abbreviations: CA, cholic acid; CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; MS, mass spectrometry.

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A. F. Hofmann. Radiochemical purity of the labeled compounds was better than 99% as determined by radio-TLC. Each labeled compound was dissolved in aqueous 1% $NaHCO_3$ solution.

Unlabeled homoCDCA, norCDCA, and norCA were synthesized as described previously (11, 12). Glycine and taurine conjugates of CA, CDCA, homoCDCA, norCDCA, and norCA were prepared by published procedures (13).

Liquid scintillation counting

Radioactivity was determined in a Beckman LS 3801 liquid scintillation system (Beckman Instruments, Fullerton, CA) with automatic quench correction, using Aquasol-2 (NEN Research Products) as the scintillation fluid.

Animal experiments

Male golden Syrian hamsters (Sasco, Omaha, NE), weighing between 105 and 120 g, were given chow and water ad libitum, and kept under a controlled 12-h light/dark cycle. The animals were anesthetized by intramuscular injection of ketamine (Ketaset, Bristol Labs, Syracuse, NY) with a dose of 20 mg/animal. Intramuscular injections of ketamine (5–10 mg/dose) were used to maintain anesthesia as required.

A polyethylene catheter (PE-10, 0.28 mm ID, Clay Adams, Parsippany, NJ) was inserted into the left femoral vein and 0.9% NaCl solution was infused at a rate of 1.5 ml/h using a Harvard syringe pump (Harvard Apparatus, Mills, MA). The abdomen was opened by a midline incision, the cystic duct was ligated with a hemostatic clip (Hemo Clip, Edward Weck & Co., Inc., Research Triangle Park, NC), and an external biliary fistula was constructed using PE-10 polyethylene tubing. The urethra was ligated with a hemostatic clip to allow urine to accumulate in the bladder. In experiments dealing with the metabolism of homoCDCA, saline was infused into the femoral vein for 60 min prior to the administration of the labeled compound; [11,12-^{3}H]homoCDCA was then infused for 20 min at a dose of 1 μmol/min per kg; the infusion of saline was then resumed until the end of the experimental period. In control experiments, saline was infused throughout the experimental period. Bile samples were collected in weighed tubes at 20-min intervals for a total period of 5 h. At the end of the experiments, blood was obtained by cardiac puncture and urine by aspiration from the urinary bladder.

In order to compare the effects of homoCDCA and CDCA on bile flow, [11,12-^{3}H]homoCDCA or [24-^{14}C]CDCA were infused intravenously into biliary fistula hamsters at a dose of 1 μmol/min per kg for 60 min. Saline was infused for 120 min during an initial control period and at the end of the bile acid infusion. Bile samples were collected every 20 min for 6 h.

Analytical techniques

In the metabolic experiments, bile samples were collected every 20 min. Four aliquots were taken from each bile sample to determine the metabolites of the administered labeled homoCDCA as follows. The radioactivity of the first set of aliquots (10 μl) was determined by liquid scintillation counting. The second and third sets of aliquots were analyzed by thin-layer chromatography (TLC) to check the conjugation pattern using solvent system A, chloroform–methanol–acetic acid–water 13:4:2:1 (v/v/v/v), and solvent system B, isooctane–ethyl acetate–acetic acid 5:5:1 (v/v/v). TLC was performed on precoated silica gel 60 F_{254} plates (0.2 mm thickness, EM Science, Darmstadt, Germany) using a solution of phosphomolybdic acid in ethanol, 10 g/dl, to detect the spots. Five μl of bile was applied directly to a TLC plate along with reference compounds. After development (16 cm) and detection of spots, each TLC plate was cut into 5-mm segments from origin to the solvent front and each segment was put into a scintillation vial. After addition of 1 ml of methanol, radioactivities were determined by liquid scintillation counting. The fourth set of aliquots (50 μl) was hydrolyzed with cholylglycine hydrolase (EC 3.5.1.24; Sigma) (14); bile salts were extracted with Sep-Pak C_{18} cartridges (Waters Associates, Milford, MA) and analyzed by TLC using solvent systems A and B.

Aliquots of urine and serum were checked for radioactivity. Bile salts in the liver were analyzed according to the method of Yanagisawa et al. (15) and their radioactivity was measured as described above. All biological specimens were stored at −20°C.

Gas–liquid chromatography (GLC) was carried out on a Hewlett-Packard 5890A gas chromatograph equipped with flame ionization detectors and capillary column inlet. Separations of methyl ester trimethylsilylether derivatives were carried out on an SE-30 column (30 meters, 0.32 mm I.D.) from Supelco (Bellefonte, PA). Conditions for analyses were: injector, 280°C; detector, 285°C; oven, 260°C; flow, 36 ml/min. Integration of peaks was carried out on a Hewlett-Packard 3396B integrator. Gas–liquid chromatography–mass spectrometry (GLC–MS) was carried out on a Hewlett-Packard 5992B spectrometer under the following conditions: column, 3% SP-2250; column temperature, 260°C; injection port temperature, 265°C; source pressure, 2×10^{-6} torr; electron energy, 70 eV.

Statistical methods

The numerical data are expressed as mean ± SEM. Differences between the experimental groups were calculated using analysis of variance to determine the F statistic. When the F statistic was significant, Student's *t*-test was used to determine the level of significance (16).

RESULTS

In order to estimate whether homoCDCA is extracted effectively by the liver and secreted into bile, ^{3}H-labeled homoCDCA was infused intravenously into bile fistula hamsters for 20 min and the bile was collected. The recoveries of the administered radioactivity in bile, urine, liver, and blood were (average % ± SEM): bile, 83.3 ± 4.6; urine, 0.3 ± 0.1; liver, 4.6 ± 1.4; blood, not detected. Total recovery averaged 88.2% ± 3.5. **Fig. 1** shows the cumulative recovery of the radioactivity secreted in bile. Radioactivity appeared rapidly in bile and more than 55% was recovered within 1 h.

The bile samples containing the highest radioactivity were analyzed by radio-TLC for each hamster. **Fig. 2** illustrates the qualitative analysis by radio-TLC of the bile collected after infusion of [11,12-^{3}H]homoCDCA using solvent system A. Fig. 2 shows that some of the administered homoCDCA was amidated with taurine (22%) and glycine (4%). However, considerable amounts of homoCDCA were degraded to C_{23} bile acids. About one-third of the total radioactivity was found in the band corresponding to taurine-conjugated norCDCA (norCDC-tau). Glycine-conjugated norCDCA was not secreted into bile (< 0.05%). A new biliary metabolite was detected that had an R_f value smaller than that of CA and similar to that of norCA in solvent systems A and B (Fig. 2 and **Fig. 3**). This unknown metabolite was purified by TLC, eluted with methanol, and derivatized as the methyl ester trimethylsilylether derivative. GLC on SE-30 revealed a single peak (RT = 19.1 min). GLC–MS was carried out using 3% SP-2250 (RT = 6.9 min). The mass spectrum obtained by us was identical with the spectrum of a trihydroxy-norbile acid, 3α,5β,7α-trihydroxy-24-nor-5β-cholan-23-oic acid, in the literature (17). The major fragment ion which supports the structure determination was: *m/z* 444, M–(2 × 90; % abundance = 100%). The nine

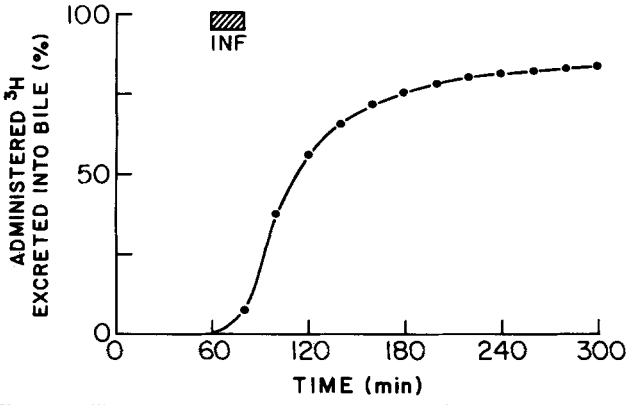

Fig. 1. Biliary secretion of radioactivity in bile fistula hamsters after intravenous infusion of ^{3}H-labeled homoCDCA. After a 60-min control period, the bile acid was infused at a dose of 1 μmol/min · kg for 20 min. Each value represents the average of three male hamsters.

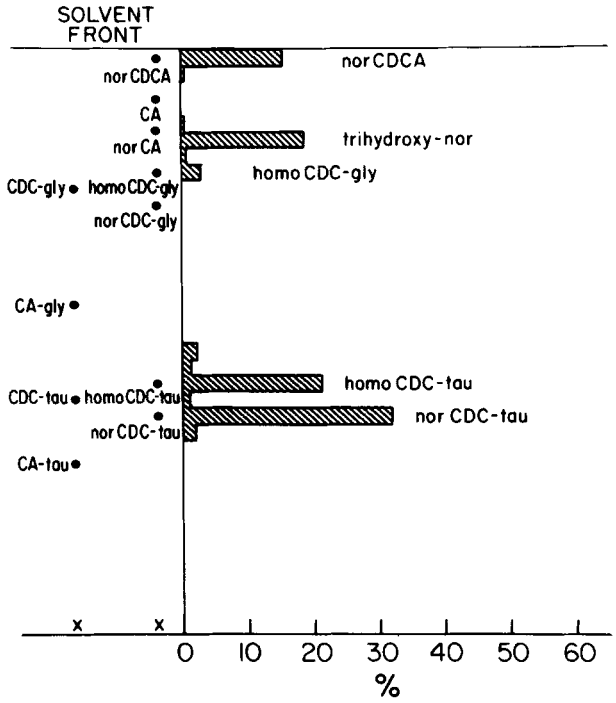

Fig. 2. Thin-layer chromatographic analysis of radioactivity recovered in bile of a bile fistula hamster infused with labeled homoCDCA. The bile sample was analyzed by TLC using solvent system A (chloroform–methanol–acetic acid–water 13:4:2:1, v/v/v/v) before hydrolysis. Reference compounds are as follows: CA-tau, taurocholate; CDC-tau, taurochenodeoxycholate; CA-gly, glycocholate; CDC-gly, glycochenodeoxycholate; norCDC-tau, tauronorchenodeoxycholate; homoCDC-tau, taurohomochenodeoxycholate; norCDC-gly, glyconorchenodeoxycholate; homoCDC-gly, glycohomochenodeoxycholate; norCA, norcholic acid; CA, cholic acid; norCDCA, norchenodeoxycholic acid.

most abundant peaks that were identical to those of the published spectrum of 3α,5β,7α-trihydroxy-24-nor-5β-cholan-23-oic acid (methyl ester trimethylsilylether) were: *m/z* (abundance); 121, 24%; 143, 19%; 196, 10%; 259, 14%; 355, 4%; 372, 4%; 445, 32%; 446, 9%, and 447, 2%. **Table 1** summarizes the quantitative analysis of the labeled metabolites of homoCDCA. Fig. 3 (solvent system B) reveals that the unconjugated dihydroxy bile acid in bile was exclusively norCDCA (17% of total radioactivity); unconjugated homoCDCA was not detected in bile (<0.5%). **Fig. 4** and **Fig. 5** depict the results of radio-TLC (using solvent systems A and B, respectively) of the bile acid mixture from a bile sample after enzymatic hydrolysis with cholylglycine hydrolase. More than 90% of the radioactivity was found in the bands corresponding to homoCDCA (26%), norCDCA (50%) and "trihydroxy norbile acid" (19%). Small amounts (<5%) of sulfate(s) and glucuronide(s) of dihydroxy bile acid(s) which were resistant to hydrolysis with cholylglycine hydrolase were also detected (Figs. 4 and 5).

For the purpose of investigating the choleretic effect of homoCDCA in comparison with that of CDCA, labeled

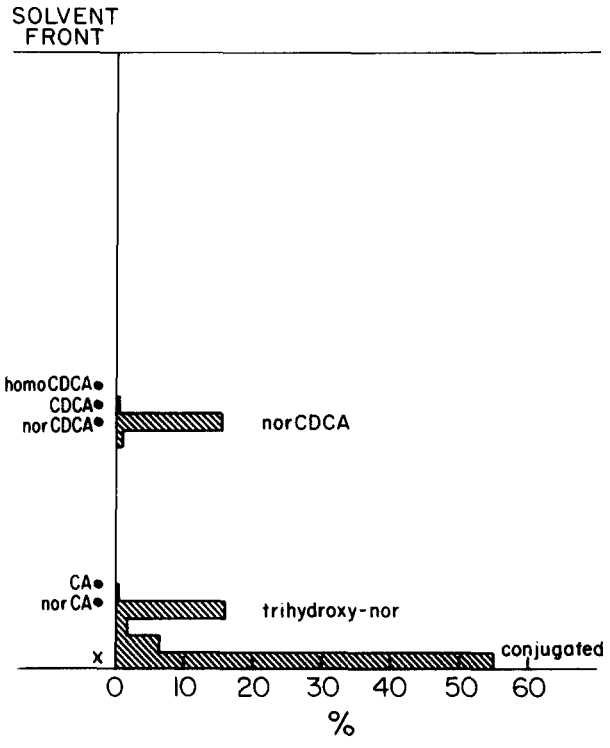

Fig. 3. Thin-layer chromatographic analysis of radioactivity recovered in bile of a bile fistula hamster infused with labeled homoCDCA. The bile sample was analyzed by TLC using solvent system B (isooctane–ethyl acetate–acetic acid 5:5:1, v/v/v) before hydrolysis. Reference compounds are as follows: norCA, norcholic acid; CA, cholic acid; norCDCA, norchenodeoxycholic acid; CDCA, chenodeoxycholic acid; homoCDCA, homochenodeoxycholic acid.

homoCDCA or CDCA was infused into biliary fistula hamsters at a rate of 1 μmol/min · kg for 60 min after a 120-min control period. **Fig. 6** summarizes bile flow and secretion of radioactivity in three animals infused with homoCDCA and three animals infused with CDCA. After the infusion of homoCDCA was started, bile flow increased rapidly and continued to increase even after the infusion was stopped; it rose to more than twice the basal level. Bile flow did not return to the preinfusion level during the experimental period. On the other hand, after CDCA infusion, bile flow increased only during the infusion period and decreased quickly when the infusion was stopped. The increase in bile flow after homoCDCA infusion was 6- to 7-times that of the animals infused with CDCA. After infusion of homoCDCA, bile flow was significantly greater than after infusion of CDCA throughout the experimental period ($P < 0.05$). The apparent choleretic activity was determined as follows (4, 18). The increment in bile flow (μl/min per kg), i.e., the observed value for bile flow, minus the preinfusion (basal) value, was divided by the recovery of radioactivity (μmol/min per kg). The value for homoCDCA, 181 ± 6 μl/μmol (n = 22) was significantly higher than that for CDCA, 11 ± 1 μl/μmol (n = 12) ($P < 0.01$). The quantities of the biliary metabolites of homoCDCA and CDCA were estimated from the radioactivity in bile after infusion of [11,12-^{3}H]homoCDCA and [24-^{14}C]CDCA, respectively. The rate of secretion of radioactivity into bile paralleled the rate of bile flow in both groups (Fig. 6). Less than 0.5% of the total radioactivity was excreted in the urine. After infusion of CDCA, radioactive compounds appeared rapidly in bile and 73.5 ± 1.5% of the radioactivity was recovered during the infusion period. In contrast, after the infusion of labeled homoCDCA recovery of radioactivity was slower ($P < 0.01$); only 27.4 ± 3.6% of the activity was secreted during the infusion period.

DISCUSSION

When homoCDCA was administered intravenously into biliary fistula hamsters, none of the unconjugated compound was recovered in the bile. A relatively small proportion of the infused compound was secreted into bile as the taurine (22%) or glycine (4%) conjugates (Fig. 2 and Table 1). A considerable amount (>70%) of homoCDCA was degraded to C_{23} bile acids via β-oxidation during passage through the liver. Seventeen percent was excreted as unconjugated norCDCA; the remainder underwent various modifications, such as hydroxylation, aminoacyl amidation with taurine, sulfation, and glucuronidation. The

TABLE 1. Biotransformation profile of homoCDCA after intravenous administration into three bile fistula hamsters[a]

Unconjugated			Conjugated				
Trihydroxy Norbile Acid[b]	NorCDCA	HomoCDCA	NorCDC-tau	NorCDC-gly	HomoCDC-tau	HomoCDC-gly	Sulfate(s) and Glucuronide(s)
19.4 ± 3.9	17.3 ± 3.3	0	32.6 ± 3.5	0	22.0 ± 2.7	3.8 ± 0.7	4.9 ± 0.4

[a]Bile samples and their enzymatic hydrolysates were analyzed by thin-layer chromatography using solvent system A (chloroform–methanol–acetic acid–water 13:4:2:1, v/v/v/v), and B (isooctane–ethyl acetate–acetic acid 5:5:1, v/v/v). NorCDCA, norchenodeoxycholic acid; homoCDCA, homochenodeoxycholic acid; norCDC-tau, tauronorchenodeoxycholate; norCDC-gly, glyconorchenodeoxycholate; homoCDC-tau, taurohomochenodeoxycholate; homoCDC-gly, glycohomochenodeoxycholate.

[b]Identified as 3α,5β,7α-trihydroxy-24-nor-5β-cholan-23-oic acid (see text).

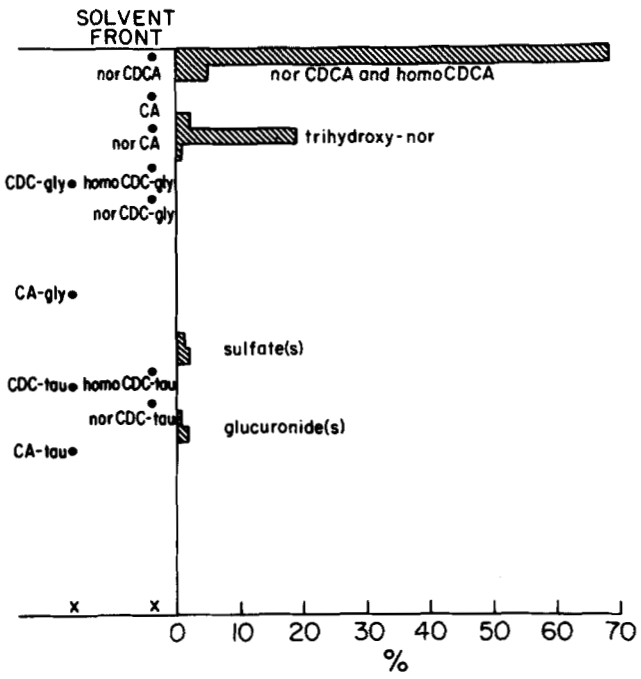

Fig. 4. Thin-layer chromatographic analysis of radioactivity from an enzymatically hydrolyzed bile sample of a bile fistula hamster infused intravenously with [^{3}H]homoCDCA. The bile sample was analyzed with solvent system A (see text). Reference compounds are as follows: CA-tau, taurocholate; CDC-tau, taurochenodeoxycholate; CA-gly, glycocholate; CDC-gly, glycochenodeoxycholate; norCDC-tau, taronorchenodeoxycholate; homoCDC-tau, taurohomochenodeoxycholate; norCDC-gly, glyconorchenodeoxycholate; homoCDC-gly, glycohomochenodeoxycholate; norCA, norcholic acid; CA, cholic acid; norCDCA, norchenodeoxycholic acid.

biliary metabolite of norCDCA that has a R_f value similar to that of norCA (in solvent systems A and B) was identified by GLC–MS as 3α,5β,7α-trihydroxy-24-nor-5β-cholan-23-oic acid. Recently, Schteingart et al. (17) and Yoshii et al. (18) showed that 5β-hydroxylation of norCDCA, 7-methyl norCDCA, and norUDCA was a major biotransformation pathway for norbile acids in the hamster in vivo. Similarly, when norCDCA was injected into the perfusate of an isolated hamster liver, it was 5β-hydroxylated (17). As homoCDCA was substantially degraded to norCDCA in hamster liver, we consider that the most likely structure of this metabolite is 5β-hydroxy-norCDCA, namely, 3α,5β,7α-trihydroxy-24-nor-5β-cholan-23-oic acid.

Bile acid structure has a definite effect on the susceptibility of the side chain to β-oxidation (9, 10). Lindstedt and Tryding (9) showed the gradual increase in the case of β-oxidation of the side chain with increasing chain length. In the rat, bishomocholic acid, the C_{26} homologue of CA, was efficiently degraded to CA, whereas less than 10% of administered homoCA (C_{25}) was degraded to the corresponding C_{23} bile acid, norCA; CA (C_{24}) and norCA (C_{23}) did not undergo β-oxidation of the side chain. Kuramoto et al. (10) demonstrated that more than 95% of homoUDCA was degraded to norbile acids by β-oxidation in the rat liver. HomoUDCA, which has a low relative conjugation efficiency, is poorly converted to the conjugated form and a large proportion of this bile acid is susceptible to β-oxidation (10). In contrast, homoCA, which is readily conjugated by hepatic enzymes, underwent β-oxidation much less readily. In the experiments cited above, only 10% of administered homoCA was transformed into norCA (9).

In order to evaluate the choleretic effect of homoCDCA, it was infused intravenously at a rate of 1 μmol/min per kg for 60 min. Bile flow increased more than twofold above the basal level. The apparent choleretic activity of homoCDCA and its metabolites (181 μl/μmol) was much greater than that of CDCA (11 μl/μmol), demonstrating that infusion of homoCDCA produced hypercholeresis.

It is well known that the 23-nor homologues of the natural dihydroxy bile acids induce a striking bicarbonate-rich choleresis, called hypercholeresis, in the rat and the hamster (3, 4). They undergo little aminoacyl amidation

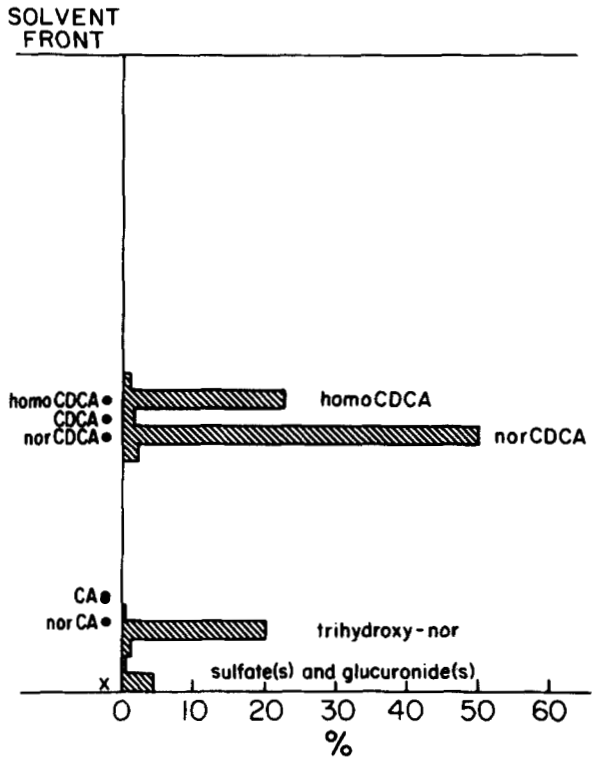

Fig. 5. Thin-layer chromatographic analysis of radioactivity from an enzymatically hydrolyzed bile sample of a bile fistula hamster infused intravenously with [^{3}H]homoCDCA. The bile sample was analyzed with solvent system B (see text). Reference compounds are as follows: norCA, norcholic acid; CA, cholic acid; norCDCA, norchenodeoxycholic acid; CDCA, chenodeoxycholic acid; homoCDCA, homochenodeoxycholic acid.

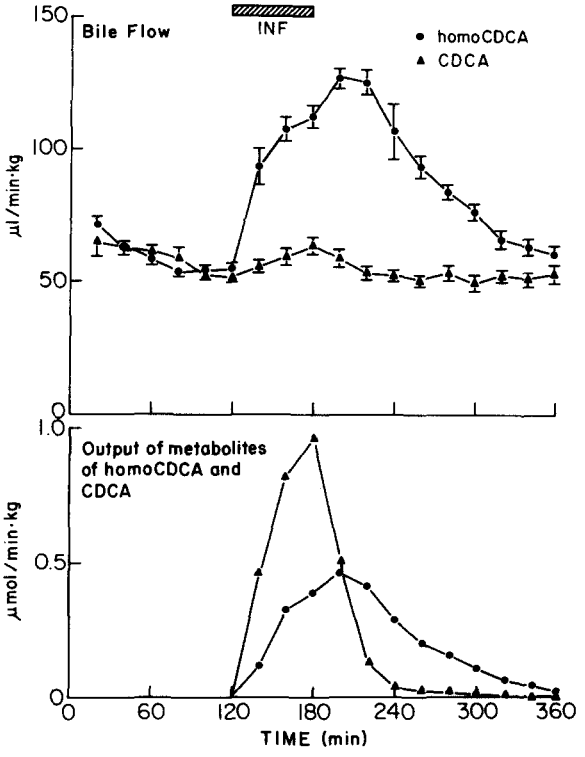

Fig. 6. Bile flow and radioactivity secreted into the bile of bile fistula hamsters (three animals) infused intravenously with [^{3}H]homoCDCA or [^{14}C]CDCA. After a 120-min control period, the bile acid was infused at a dose of 1 µmol/min per kg for 60 min.

with glycine or taurine, and are secreted in bile in the unconjugated form as well as in the form of sulfates and glucuronates (3, 4). Although the mechanism responsible for the hypercholeresis of nordihydroxy bile acids is unknown, "cholehepatic shunting" of the unconjugated molecule has been proposed (1, 3–8). In the present study, more than 70% of the administered homoCDCA was biotransformed to norbile acids and about half of the norbile acids were unconjugated, while homoCDCA excreted into bile was almost completely conjugated. Gurantz et al. (19) have reported that the magnitude of hypercholeresis was directly proportional to the recovery in bile of the unconjugated form of the bile acid; the conjugated biotransformation products of the administered bile acids did not induce hypercholeresis. Therefore, we propose that the striking choleretic activity of homoCDCA was caused indirectly by norCDCA produced from homoCDCA in the liver.

Biliary recovery of the metabolites of homoCDCA (27.4% within 1 h) was significantly slower than that of CDCA (73.5% within 1 h, Fig. 6). Because urinary excretion of homoCDCA and its metabolites was negligible, delayed recovery of the metabolites of homoCDCA might be attributed to inefficient hepatic uptake of homoCDCA, impaired canalicular transport into bile of the metabolites of homoCDCA, or reabsorption of norCDCA (derived from homoCDCA) in the biliary epithelium. Nordihydroxy bile acids are known to be recovered slowly in bile compared to most other bile acids, because these compounds are presumably reabsorbed from the biliary epithelium and returned to the hepatocyte to be secreted again in bile (3, 4).

It is concluded that more than 70% of homoCDCA infused in the hamster was degraded to C_{23} bile acids by β-oxidation. This transformation was associated with a pronounced choleresis. The calculated apparent choleretic activity of homoCDCA was much higher than that of CDCA, suggesting that the hypercholeresis was associated with the observed biotransformation of homoCDCA to norCDCA.

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REFERENCES

1. O'Maille, E. R. L., S. V. Kozmary, A. F. Hofmann, and D. Gurantz. 1984. Differing effects of norcholate and cholate on bile flow and biliary lipid secretion in the rat. *Am. J. Physiol.* **246:** G67–G71.
2. Nakatomi, F., K. Kihira, T. Kuramoto, and T. Hoshita. 1985. Intestinal absorption and metabolism of norcholic acid in rats. *J. Pharmacobio-Dyn.* **8:** 557–563.
3. Yoon, Y. B., L. R. Hagey, A. F. Hofmann, D. Gurantz, E. L. Michelotti, and J. H. Steinbach. 1986. Effect of side-chain shortening on the physiological properties of bile acids: hepatic transport and effect on biliary secretion of 23-norursodeoxycholate in rodents. *Gastroenterology.* **90:** 837–852.
4. Palmer, K. R., D. Gurantz, A. F. Hofmann, L. M. Clayton, L. R. Hagey, and S. Cecchetti. 1987. Hypercholeresis induced by nor-chenodeoxycholate in the biliary fistula rodent. *Am. J. Physiol.* **252:** G219–G228.
5. Gurantz, D., C. D. Schteingart, L. R. Hagey, J. H. Steinbach, T. Grotmol, and A. F. Hofmann. 1991. Hypercholeresis induced by unconjugated bile acid infusion is mediated by biliary secretion and not hepatocyte retention of unconjugated bile acids. *Hepatology.* **13:** 540–550.
6. Lake, J. R., E. L. Renner, B. F. Scharschmidt, E. J. Cragoe, Jr., L. R. Hagey, K. J. Lambert, D. Gurantz, and A. F. Hofmann. 1988. Inhibition of Na^+/H^+ exchange in the rat is associated with decreased ursodeoxycholate hypercholeresis, decreased secretion of unconjugated ursodeoxycholate, and increased ursodeoxycholate glucuronidation. *Gastroenterology.* **95:** 454–463.
7. Hofmann, A. F., D. Gurantz, L. R. Hagey, C. Schteingart, Y. B. Yoon, L. M. Clayton, K. R. Palmer, J. P. Neoptolemos, S. S. Rossi, J. H. Steinbach, and C. Clerici. 1987. The relationship between bile acid biotransformation and bile acid-dependent bile flow. *In* Bile Acids and the

Liver. G. Paumgartner, A. Stiehl, and W. Gerok, editors. MTP Press, Lancaster. 183–193.
8. Gurantz, D., and A. F. Hofmann. 1984. Influence of bile acid structure on bile flow and biliary lipid secretion in the hamster. *Am. J. Physiol.* **247:** G736–G748.
9. Lindstedt, S., and N. Tryding. 1957. On the metabolism of bishomocholic, homocholic, and norcholic in the rat. *Ark. Kemi.* **11:** 137–144.
10. Kuramoto, T., S. Moriwaki, K. Kawamoto, and T. Hoshita. 1987. Intestinal absorption and metabolism of homoursodeoxycholic acid in rats. *J. Pharmacobio-Dyn.* **10:** 309–316.
11. Cohen, B. I., G. S. Tint, T. Kuramoto, and E. H. Mosbach. 1975. New bile alcohols—synthesis of 5β-cholestane-3α,7α-25-triol and 5β-cholestane-3α,7α,25-24(^{14}C)-triol. *Steroids.* **25:** 365–378.
12. Schteingart, C. D., and A. F. Hofmann. 1988. Synthesis of 24-nor-5β-cholan-23-oic acid derivatives: a convenient and efficient one-carbon degradation of the side chain of natural bile acids. *J. Lipid Res.* **29:** 1387–1395.
13. Tserng, K-Y., D. L. Hachey, and P. D. Klein. 1977. An improved procedure for the synthesis of glycine and taurine conjugates of bile acids. *J. Lipid Res.* **18:** 404–407.
14. Kuroki, S., E. H. Mosbach, B. I. Cohen, and C. K. McSherry. 1987. Metabolism of the bile acid analogues, 7β-methyl-cholic acid and 7α-methyl-ursocholic acid. *Gastroenterology.* **92:** 876–884.
15. Yanagisawa, J., M. Ito, M. Ishibashi, H. Miyazaki, and F. Nakayama. 1980. Microanalysis of bile acids in human liver tissue by selected ion monitoring. *Anal. Biochem.* **104:** 75–86.
16. Snedecor, G. W. 1948. Statistical Methods. Iowa State College Press, Ames, IA. 54–81; 182–184.
17. Schteingart, C. D., L. R. Hagey, K. D. R. Setchell, and A. F. Hofmann. 1993. 5β-Hydroxylation by the liver: identification of 3,5,7-trihydroxy bile acids as new major biotransformation products of 3,7-dihydroxy nor-bile acids in rodents. *J. Biol. Chem.* In press.
18. Yoshii, M., E. H. Mosbach, C. D. Schteingart, L. R. Hagey, A. F. Hofmann, B. I. Cohen, and C. K. McSherry. 1991. Chemical synthesis and hepatic biotransformation of 3α,7α-dihydroxy-7β-methyl-24-nor-5β-cholan-23-oic acid, a 7-methyl derivative of norchenodeoxycholic acid: studies in the hamster. *J. Lipid Res.* **32:** 1729–1740.
19. Gurantz, D., C. D. Schteingart, L. R. Hagey, J. H. Steinbach, T. Grotmol, and A. F. Hofmann. 1990. Hypercholeresis induced by unconjugated bile acid infusion correlates with recovery in bile of unconjugated bile acids. *Hepatology.* **13:** 540–550.