THE FORMATION OF BILE ACID SULFATES: A NEW PATHWAY OF BILE ACID METABOLISM IN HUMANS

By Robert H. Palmer

DEPARTMENT OF MEDICINE, UNIVERSITY OF CHICAGO, AND ARGONNE CANCER RESEARCH HOSPITAL*

Communicated by L. T. Coggeshall, July 5, 1967

Bile acids are normally conjugated at the C24 carboxyl group with the amino acids taurine and glycine prior to their excretion into the bile of man and most higher species. In previous studies on biliary metabolites of C^{14}-labeled lithocholic acid in humans,[1] the expected taurine and glycine conjugates were observed, but approximately half of the labeled material occurred as more polar metabolites. In this report, data are presented which identify these compounds as the 3α-sulfate esters of glycolithocholic and taurolithocholic acids. The formation of these sulfate esters delineates a new pathway of bile acid metabolism in man, and has important implications for the clinical significance of lithocholic acid, an endogenous steroid with extensive biological toxicity.

Methods.—Lithocholic acid-24-C^{14} (50 μc, New England Nuclear Corp.) was given orally to two patients with functioning gallbladders, 36 hours prior to cholecystectomy for cholelithiasis. At operation, bile was obtained from the gallbladder.

Thin-layer chromatography was performed using Silica Gel H (E. Merck A. G., Darmstadt, Germany) and the following phase systems: Butanol 1:[2] butanol 50, acetic acid 5, water 5 (pH 1); Butanol 3: butanol 50, 0.01 *M* Tris buffer 9.25, propionic acid 0.75 (pH 3.0); Propionic acid:[3] propionic acid 15, isoamyl acetate 20, water 5, *n*-propanol 10; S15[4]: trimethylpentane 25, ethyl acetate 25, acetic acid 0.25.

Radioactive spots on chromatoplates were detected with a Vanguard glass plate scanner (Vanguard Instrument Co., LaGrange, Ill.).

Sulfate esters of glycolithocholic and taurolithocholic acids were prepared using pyridine–sulfur trioxide[5] and crystallized as the ammonium salts. Details of the synthesis and properties of the compounds will be described separately.

Solvolysis was performed using a modification of the method of Burstein and Lieberman.[6] The steroid sulfate was dissolved in ethanol, acidified to pH 1 or less with 2 *N* HCl, and diluted with 9 volumes of acetone. The solvolysis mixture was then incubated at room temperature for one to three days. Some esterification occurred, and the resulting ethyl esters were hydrolyzed by refluxing for two hours in 5 per cent methanolic KOH.

Results.—In the previous study, labeled biliary metabolites of orally administered lithocholic acid-24-C^{14} were shown to include glycolithocholic acid, taurolithocholic acid, and two polar compounds, I and II.[1] In this study, essentially similar results were obtained in both patients; four corresponding labeled biliary metabolites were observed on thin-layer chromatography using the Butanol 1 system. When bile was chromatographed with the Butanol 3 system, the labeled metabolites separated into two areas, *A* and *B* (Fig. 1). The compounds in area *A*, with the same mobility as glycolithocholic and taurolithocholic acid standards, were eluted and rechromatographed with the Butanol 1 system. Two radioactive spots, with mobilities corresponding to glycolithocholic acid and taurolithocholic acid, were identified. The

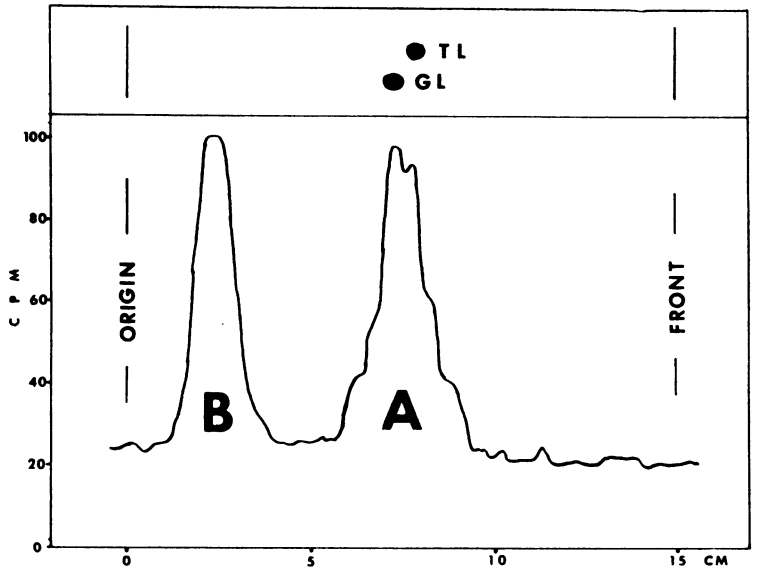

FIG. 1.—Group separation of bile acid sulfates. Thin-layer chromatography of whole bile with the Butanol 3 system. The upper part of the figure shows the mobility of glycolithocholic (*GL*) and taurolithocholic (*TL*) standards, revealed by spraying the plate with concentrated sulfuric acid, and charring. The lower part shows the distribution of labeled biliary metabolites of lithocholic acid-24-C^{14}, as detected by the plate scanner. Peak *A*, at 7–8 cm, represents glycolithocholic and taurolithocholic acids, and peak *B*, at 2–3 cm, indicates the position of their sulfate esters. The area corresponding to each peak was eluted and the components were identified as described in the text.

spots were eluted individually, subjected to strong hydrolysis,[1] and chromatographed with the S15 system. All of the radioactivity from both spots had the same mobility as authentic lithocholic acid.

Labeled compounds from area *B* were eluted and also chromatographed with the Butanol 1 system. Two radioactive spots, presumed to represent compounds I and II, were observed and shown to have the same mobilities as synthetic glycolithocholic acid sulfate and taurolithocholic acid sulfate, respectively. Elution of each spot and solvolysis resulted in labeled compounds with the mobility of glycolithocholic and taurolithocholic acids. Strong hydrolysis of these labeled compounds gave only labeled material having the mobility of authentic lithocholic acid with system S15. Further identification of compounds I and II was obtained by combining compound I with synthetic glycolithocholic acid sulfate, compound II with synthetic taurolithocholic acid sulfate, and crystallizing each as the ammonium salt to constant specific activity.

The proportion of C^{14}-labeled lithocholic acid conjugates that had been esterified with sulfate was estimated from the radioactivity present in areas *A* and *B*. The sulfate ester area (*B*) from the two patients in this study contained 44 and 61 per cent of the total biliary radioactivity, while the two previous patients[1] excreted 59 and 60 per cent of the biliary radioactivity as compounds I and II combined.

Discussion.—These studies describe the identification of two new endogenous steroid sulfates in human bile: 3α-sulfates of glycolithocholic and taurolithocholic

acids. These compounds are the first sulfate esters of bile acids to be described, and constitute evidence for a hitherto unrecognized pathway of bile acid metabolism in man. Sulfate esters of bile acids might have been anticipated, since a number of hydroxylated steroids of the 18, 19, 21, and 27 carbon series have been shown to occur as sulfates in man, and bile alcohol sulfates are found in several lower vertebrates.[7] Sulfation may occur with 3-phenolic hydroxyl groups (estrogens), 3α-hydroxyl groups (etiocholanolone, androsterone, pregnanolone), 3β-hydroxyl groups (dehydroepiandrosterone, cholesterol, myxinol), 24β-hydroxyl groups (ranol), or 26β-hydroxyl groups (latimerol, scymnol, myxinol).

In the previous study[1] on metabolites of lithocholic acid-24-C^{14}, three unknown compounds were observed, whereas in the present experiments only compounds I and II, or glycolithocholic acid sulfate and taurolithocholic acid sulfate, could be detected. However, compound III had the approximate chromatographic mobility of lithocholic acid sulfate, and it is reasonable to assume that it was lithocholic acid sulfate. Small amounts of bile acids can be excreted without being conjugated with amino acids,[8] and it may be that the longer period of enterohepatic circulation in the present experiments (36 hr rather than 20 hr) resulted in complete amino acid conjugation of the labeled free bile acid.

The site of bile acid sulfation remains to be determined. Steroid sulfation occurs in a variety of mammaliam tissues, and there is evidence for at least two sulfate-synthesizing enzyme systems.[9] The liver is a major source of biological sulfates, and furthermore, it is exposed to bile salts during their enterohepatic circulation. However, Carey administered lithocholic acid-24-C^{14} parenterally to a patient with a bile fistula and showed that almost of all the radioactivity appearing in bile was in the form of taurine and glycine conjugates,[10] suggesting that one passage through the liver did not result in significant sulfate formation. Sulfate formation cannot be entirely excluded, since 12–30 per cent of the radioactivity was not recovered, but it appears unlikely. Steroid sulfates can also be synthesized in the intestine,[11] although no great importance has been attributed to this function. Bile acid sulfates may therefore be formed during intestinal reabsorption, or possibly elsewhere during their enterohepatic circulation.

The physiological significance of bile acid sulfation is probably related primarily to the extensive toxic effects of lithocholic acid and its taurine and glycine conjugates, which are quantitatively important products of cholesterol metabolism in man. Most of the cholesterol in humans is catabolized in the liver to the two primary bile acids, cholic and chenodeoxycholic acids. These two steroids, normally formed in roughly equal amounts, then undergo C7 dehydroxylation as the result of bacterial enzyme activity in the intestine, and are converted to the two main secondary bile acids, deoxycholic and lithocholic acids. Therefore, roughly half of the cholesterol catabolized daily is converted to lithocholic acid. The biological toxicities of lithocholic acid and its physiological taurine and glycine conjugates are protean, and include the induction of intense pyrogenic reactions following their intramuscular injection into man,[12] marked local inflammation following their injection into man and experimental animals,[13] lysis of human erythrocytes *in vitro*,[14] and derangements of liver structure and function following their oral administration to a wide variety of species.[15] Many of these toxic effects are shared by other 5β-H steroids, such as etiocholanolone, and sulfate esterification has been shown to abolish the toxicity[16] of

neutral steroids. Biological sulfation of lithocholic acid and its conjugates might therefore be expected to modify their toxic effects, and may serve as an important physiological method of protecting the organism from the deleterious effects of these endogenous compounds. Studies on the pharmacological properties of these sulfate esters are in progress.

It is also possible that bile acid sulfates have physiological functions that are unrelated to detoxification of the parent steroid. Neutral steroid sulfates, for example, serve as biosynthetic intermediates in the formation of other steroids[5] and sulfatides.[17] Free steroids, steroid sulfates, and steroid sulfatides all differ markedly in polarity, and have distinctly different tissue distributions and biological half lives.[17] Thus sulfation of bile acids, as described in this report, may be expected to affect various recognized parameters of bile salt metabolism, such as pool size, turnover, tissue distribution, and elimination. It is conceivable that such alterations could then influence other metabolic processes, such as the feedback control of cholesterol synthesis.

Summary.—This report describes a new pattern of bile acid metabolism in humans. Carbon-14-labeled glycolithocholic acid sulfate and taurolithocholic acid sulfate were recovered from bile following the oral administration of lithocholic acid-24-C^{14} to humans, and shown to represent approximately half of the labeled compounds present in bile. Sulfation may have important effects on the physiological and pharmacological properties of lithocholic acid and its taurine and glycine conjugates.

Systematic names of bile acids referred to by trivial names are as follows: lithocholic acid, 3α-hydroxy-5β-cholanic acid; glycolithocholic acid, 3α-hydroxy-5β-cholanoyl-glycine; taurolithocholic acid, 3α-hydroxy-5β-cholanoyl-taurine; glycolithocholic sulfate, 3α-hydroxy-5β-cholanoyl-glycine-3-sulfate; taurolithocholic sulfate, 3α-hydroxy-5β-cholanoyl-taurine-3-sulfate; cholic acid, 3α, 7α, 12α-trihydroxy-5β-cholanic acid; chenodeoxycholic acid, 3α,7α-dehydroxy-5β-cholanic acid; deoxycholic acid, 3α,12α-dihydroxy-5β-cholanic acid.

* Operated by the University of Chicago for the United States Atomic Energy Commission.

[1] Norman, A., and R. H. Palmer, *J. Lab. Clin. Med.*, **63**, 986 (1964).
[2] Gänshirt, H., F. W. Koss, and K. Morianz, *Arzniemittel-Forsch.*, **10**, 943 (1960).
[3] Hofmann, A. F., *J. Lipid Res.*, **3**, 127 (1962).
[4] Eneroth, P., *J. Lipid Res.*, **4**, 11 (1963).
[5] Calvin, H. I., R. L. VandeWiele, and S. Lieberman, *Biochemistry*, **2**, 648 (1963).
[6] Burstein, S., and S. Lieberman, *J. Am. Chem. Soc.*, **80**, 5235 (1958).
[7] Haslewood, G. A. D., *Biol. Rev. Cambridge Phil. Soc.*, **39**, 537 (1964).
[8] Sjövall, J., *Clin. Chim. Acta*, **5**, 33 (1960).
[9] Roy, A. B., *Biochem. J.*, **74**, 49 (1960).
[10] Carey, J. B., Jr., and G. Williams, *J. Clin. Invest.*, **42**, 450 (1963).
[11] Baulieu, E., *Recent Progr. Hormone Res.*, **19**, 306 (1963).
[12] Palmer, R. H., P. B. Glickman, and A. Kappas, *J. Clin. Invest.*, **41**, 1573 (1962).
[13] Palmer, R. H., *Proc. Soc. Exptl. Biol. Med.*, **119**, 108 (1965).
[14] Palmer, R. H., *Nature*, **201**, 1134 (1964).
[15] Hunt, R. D., *Federation Proc.*, **24**, 431 (1965).
[16] Baulieu, E. E., and G. Michaud, *Bull. Soc. Chim. Biol.*, **43**, 885 (1961).
[17] Oertel, G. W., K. Groot, and D. Wenzel, *Acta Endocrinol.*, **49**, 525 (1965).