

Bile acid sulfates. II. Formation, metabolism, and excretion of lithocholic acid sulfates in the rat

ROBERT H. PALMER

Department of Medicine, The University of Chicago, Pritzker School of Medicine, and Argonne Cancer Research Hospital,* Chicago, Illinois 60637

ABSTRACT Sulfate esterification has been shown previously to be a prominent feature of lithocholate metabolism in man. These studies were undertaken to ascertain whether this metabolic pathway is also present in rats, and to investigate the physiological significance of bile acid sulfate formation. Lithocholic acid- $24\text{-}^{14}\text{C}$ was administered to bile fistula rats, and sulfated metabolites were identified in bile by chromatographic and appropriate degradative procedures. They constituted only a small fraction (2–9%) of the total metabolites but a more significant fraction (about 20%) of the secreted monohydroxy bile acids, most of the lithocholate having been hydroxylated by the rat liver. When sulfated glycolithocholate was administered orally, it was absorbed from the intestine without loss of the sulfate, presumably by active transport, and secreted intact into the bile. In comparison with non-sulfated lithocholate, an unusually large fraction (24%) of the sulfated bile acid was excreted in the urine, and fecal excretion took place more rapidly. Both the amino acid and sulfate moieties were extensively removed prior to excretion in the feces. Hydroxylation of bile acid sulfates or sulfation of polyhydroxylated bile acids did not occur to any great extent, if at all.

SUPPLEMENTARY KEY WORDS rat bile · glycolithocholic acid sulfate · absorption · renal excretion of bile acids · fecal excretion of bile acids

LITHOCHOLIC ACID is an endogenous human bile acid that possesses extensive biological toxicity. The implications of this toxicity, which includes the capacity of this steroid and various metabolites to produce fever and inflammation (1, 2), liver damage (1, 3), and tissue injury (1, 3, 4), for human disease have been discussed previously (1, 5–7).

In previous studies, sulfation was shown to be an important aspect of lithocholate metabolism in man (8–10). The present series of experiments was undertaken to answer the following questions: (a) Does sulfation of lithocholate also occur in the rat, and if so, is intestinal absorption a requisite for sulfation? (b) Can sulfated bile salts be absorbed from the rat intestine in vivo? (c) Does sulfation alter the biochemical pathways of lithocholate metabolism in vivo? (d) Does sulfation affect the route and rate of lithocholate excretion? The results demonstrate that sulfation of lithocholates does occur in rats, with consequent alterations in their patterns of metabolism and excretion.

METHODS

Chromatography

TLC was performed with silica gel H (Brinkmann Instruments) and the following phase systems: Butanol 1 (11), *n*-butanol–acetic acid–water 50:5:5 (pH 1); Butanol 3

Address reprint requests to: Dr. Robert H. Palmer, Department of Medicine, University of Chicago, 950 East 59th Street, Chicago, Ill. 60637.

The following trivial names and abbreviations have been employed: TLC, thin-layer chromatography; lithocholic acid (L), 3α -hydroxy- 5β -cholanolic acid; glycolithocholic acid (GL), 3α -hydroxy- 5β -cholanoyl glycine; tauroolithocholic acid (TL), 3α -hydroxy- 5β -cholanoyl taurine; chenodeoxycholic acid, $3\alpha,7\alpha$ -dihydroxy- 5β -cholanolic acid; deoxycholic acid, $3\alpha,12\alpha$ -dihydroxy- 5β -cholanolic acid; cholic acid, $3\alpha,7\alpha,12\alpha$ -trihydroxy- 5β -cholanolic acid; β -muricholic acid, $3\alpha,6\beta,7\beta$ -trihydroxy- 5β -cholanolic acid; GLS, glycolithocholic acid-C-3-sulfate; TLS, tauroolithocholic acid-C-3-sulfate; LS, lithocholic acid-C-3-sulfate; "sulfate" refers to the 3-sulfate ester of the corresponding bile acid unless otherwise indicated.

* Operated by the University of Chicago for the U.S. Atomic Energy Commission.

(9, 10), *n*-butanol–0.01 M Tris buffer–propionic acid 50:9.25:0.75 (pH 3.0); T40 (8, 12), trimethyl pentane–ethyl acetate–acetic acid 40:20:0.5; S15 (12), trimethyl pentane–ethyl acetate–acetic acid 25:25:0.25; and S11 (12), trimethyl pentane–ethyl acetate–acetic acid 25:25:5.

Radioisotopes

Lithocholic acid-24-¹⁴C (7.58 mCi/mmol; New England Nuclear Corp.) was shown to be >99% pure by radioautography after TLC with system T40. The glycine conjugate was prepared (13) and esterified with sulfuric acid as described previously (10), except that the reaction mixture was purified by preparative TLC, Butanol 3 system. Samples of urine, bile, and fecal extracts were counted in a Nuclear-Chicago liquid scintillation counter; quenching was not appreciable, and corrections were not made. Radioautography of chromatograms was performed with RP-54 X-Omat medical X-ray film (Eastman Kodak) and quantitated with a Zeiss PMQ II chromatogram scanner. Curves were prepared by advancing the film in 1-mm increments and recording the optical density, which is linearly related to the radioactivity (14), at each point (e.g., Fig. 1). The distribution of radioactivity was estimated from the relative areas under the peaks, as determined by cutting out and weighing the paper. The exposure was made of sufficient duration to give discrete peaks, so that the results usually agreed within 2–3% of the value for each peak on duplicate determinations.

Miscellaneous

Solvolysis of sulfates and strong alkaline hydrolysis of amino acid conjugated bile acids was performed as described previously (10). Samples of bile, urine, or feces were stored at either 4 or –20°C until analyzed.

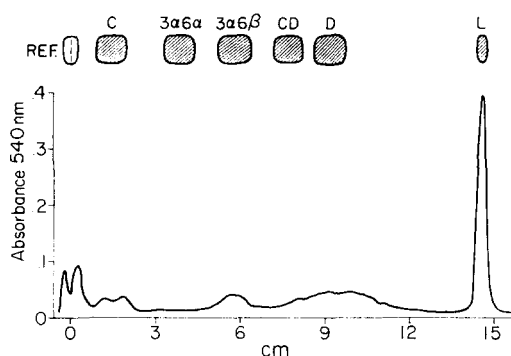


FIG. 1. Sulfated metabolites of lithocholic acid-24-¹⁴C in rat bile. The conjugated sulfate fraction was subjected to solvolysis, strong hydrolysis, and then chromatography with system S11. Radioactivity, determined by radioautography, is quantitated below. Hatched areas are reference bile acids: (C), cholic; (3 α ,6 α), hydrodeoxycholic; (3 α ,6 β), 3 α ,6 β -dihydroxycholic; (CD), chenodeoxycholic; (D), deoxycholic; (L), lithocholic.

Experimental Design

Three experimental approaches were utilized. In the first, the ability of bile fistula rats to form sulfated lithocholates was examined. In the second, bile fistula rats were used to determine whether a sulfated lithocholate could be absorbed from the intestine *in vivo*. In the third, the biological disposition of a sulfated bile salt in intact rats was studied. Details of the experimental design are given below.

A. Sulfation of Lithocholic Acid-24-¹⁴C in Rats. Three bile fistula rats received lithocholic acid-24-¹⁴C. Bile was collected in hourly fractions, the isotope content was determined, and the labeled derivatives were analyzed. Rat F received 10.8×10^6 cpm through a gastric tube, and bile from an initial small peak during the second hour and from a second large peak during the 50th hour was taken for analysis. Rat G received lithocholate-24-¹⁴C intraperitoneally. Most of the isotope appeared in the bile within 3 hr, and bile from the first hour was analyzed. Rat H was prepared with a cannula in the duodenum, so that bile could be removed or infused back into the duodenum at will. The rat was kept in a restraining cage with food and water *ad lib*. After 24 hr with an intact enterohepatic circulation, bile was diverted to a fraction collector and replaced with a constant infusion of saline at an equal rate. The rat then received intraperitoneal injections of lithocholic acid-24-¹⁴C (approximately 1×10^6 cpm) at the beginning of biliary diversion (H-24), after 16 hr of diversion (H-40), and 24 hr after restoration of an intact enterohepatic biliary circulation (H-72). These times were chosen to see whether depletion of the bile salt pool would influence the results. Bile from all six samples was plated directly on silica gel H and chromatographed with the Butanol 3 system. Quantitative radioautography was used to determine the distribution of isotope among four major groups of compounds (see Table 1); there was excellent visual separation of the groups, without tailing. Various groups were then eluted with chloroform–methanol 1:1 and rechromatographed on appropriate systems as indicated.

B. Intestinal Absorption of Glycolithocholic Acid-24-¹⁴C-3-sulfate (GLS). Two bile fistula rats were prepared, and bile was collected and analyzed as under A. The first rat received approximately 7×10^4 cpm of GLS in 0.5 ml of 50% ethanol by injection into the duodenum. In the absence of acid, the presence of water, and the absence of heat, the sulfate can be considered to be stable for these purposes (see Fig. 2). Because of some seepage through the injection site in the first few seconds, the second rat received approximately 11×10^6 cpm of GLS in 3.0 ml of 30% ethanol through a small polyethylene stomach tube. Bile, urine, and feces from the second rat were collected separately, and the total isotope in bile, urine, and 80% ethanol extracts of feces was determined. The

chemical form of the isotope recovered in bile was inferred from its chromatographic behavior on thin-layer chromatography with the Butanol 1 and Butanol 3 systems, and that in fecal extracts with the Butanol 3 and S11 systems.

C. Biological Disposition of Lithocholic Acid-24-¹⁴C and Glycolithocholic Acid-24-¹⁴C-3-sulfate in Intact Rats. A group of three female Sprague-Dawley rats each received lithocholic acid-24-¹⁴C (approximately 2×10^6 cpm) in 1.5 ml of 30% ethanol by intubation. A second group received similar amounts of glycolithocholic acid-24-¹⁴C-3-sulfate. The rats were kept in individual metabolism cages, with food and water ad lib., and urine and feces were collected separately. Feces were extracted by refluxing two times for 2 hr each with 80% ethanol. Excretion of radioactivity was essentially complete after 9 days; the total amount recovered in urine and feces was used to calculate the biological half-life ($t_{1/2}$), assuming that excretion of isotope could be described by the equation $\log Y = -kt$ (Ref. 15), where Y = the fraction of total radioactivity ultimately recovered that was still present in the body at any time (t).¹ While this may not be entirely appropriate when there is significant renal excretion of isotope, the experimental data were, in fact, described by this equation (see Results and Discussion). The chemical form of the isotope in the fecal extracts was identified by its chromatographic behavior, using radioautography for qualitative and quantitative analysis of thin-layer chromatograms.

EXPERIMENTAL RESULTS

A. Sulfation of Lithocholic Acid-24-¹⁴C in Rats

After the administration of labeled lithocholic acid to bile fistula rats, labeled compounds having the chromatographic mobility of glyco- or taurolithocholic acid sulfate were observed in all six samples of bile obtained from three rats. As shown in Table 1, the amounts were small, constituting less than 10% of the labeled metabolites in all cases. However, if only the monohydroxy compounds are considered, i.e., those that had not undergone hydroxylation, sulfated derivatives accounted for an average of 20.6% (19.6, 17.5, 16.1, 29.1, 20.8) of the total secreted in the first or second hours; they accounted for almost all of the lithocholate secreted in the single late sample (F-50). Further confirmation of the presence of sulfated derivatives of lithocholic acid was provided by relatively specific sequential degradation procedures. Compounds in group I (Table 1) were eluted and treated with cholyglycine hydrolase (16). This resulted in conversion of a

¹ Regression lines were calculated for the data from each animal, using the method of least squares and computer programs kindly supplied by Dr. John Skosey.

large fraction of the isotope to a compound with the unusual and distinctive chromatographic mobility (Butanol 3 system) of lithocholic acid sulfate (10). After elution of this spot and solvolysis (10), 73% of the radioactivity had the same mobility as reference lithocholic acid when chromatographed with system S15.²

In another approach, the group I compounds from sample F-50 were subjected first to solvolysis and then to strong alkaline hydrolysis. TLC (system S11) revealed several small peaks, as shown in Fig. 1, but the major peak at the front appeared to be a single compound with the mobility of lithocholic acid when it was rechromatographed with system T40. It is not clear whether the minor peaks represent group II compounds cochromatographing with the group I compounds, or whether they are also derived from sulfated compounds. In either case they account for only a small fraction of the isotope in the group I compounds.

Analysis of group II compounds by elution, strong alkaline hydrolysis, and chromatography with system S11 showed an unremarkable pattern of expected murine metabolites of lithocholic acid (Table 1). Quite clearly, the distribution of metabolites in the group I compounds (sulfates) shown in Fig. 1 differs from that of the group II compounds, the former being primarily monohydroxycholic acids and the latter being primarily di- and trihydroxycholic acids.

B. Intestinal Absorption of Glycolithocholic Acid-24-¹⁴C-3-sulfate

When glycolithocholic acid-24-¹⁴C-3-sulfate was placed in the intestinal tracts of bile fistula rats, approximately 70% of the administered dose was recovered in the bile. The major portion of the isotope was excreted 10–20 hr following its administration (Fig. 2). In the second rat, the total isotope recovered was approximately 80% of the administered dose; of the recovered isotope, 88.9% was from bile, 11.0% from feces, and 0.1% from urine. Chromatographic analysis of the chemical form of the isotope in bile showed that most of it was GLS. Isotope appearing later in bile contained an increasing fraction as LS or TLS. All the of isotope collected from bile had the chromatographic mobility of sulfated monohydroxycholates; no isotope was found in the nonsulfated derivative areas and no polyhydroxylated derivatives were observed.

² The identity of the remaining isotope derived from the sulfated fraction was not established, but it should be noted that conditions for either complete enzymatic hydrolysis of amino acid conjugates or complete solvolysis have not been established, and we have been unable to obtain complete enzymatic hydrolysis of reference compounds (10). Therefore, failure to obtain complete conversion of group I labeled compounds to lithocholic acid need not imply the presence of significant amounts of other compounds

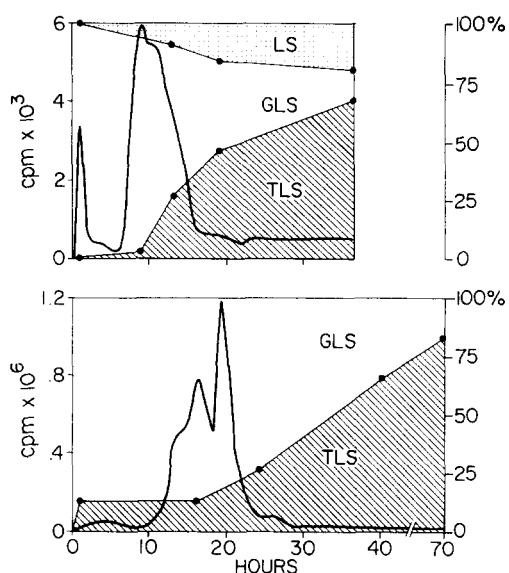


FIG. 2. Absorption and excretion of sulfated bile acids in vivo. Glycolithocholic acid-24- ^{14}C -3-sulfate was administered to bile fistula rats by intraduodenal injection (top) or intragastric instillation (bottom). Solid line, excretion of radioactivity with time. Distribution of labeled metabolites determined at points indicated: LS (lithocholic acid sulfate), stippled area; GLS (glycolithocholic acid sulfate), white area; TLS (tauroolithocholic acid sulfate), hatched area.

The isotope in the feces of the second rat was examined, and approximately 29% was found in the sulfated fraction. Analysis of the nonsulfated fraction showed that 10% of the isotope had the mobility of monohydroxycholates, 10% had the mobility of 3-ketocholates, 3% ran slightly behind (more polar than) cholanic acid, and 48% ran slightly ahead of (less polar than) cholanic acid. Mild alkaline hydrolysis shifted most of the latter (front) peak to the peak just behind cholanic acid, con-

sistent with its being an ethyl ester formed during the extraction process.

C. Biological Disposition of Lithocholic Acid-24- ^{14}C and Glycolithocholic Acid-24- ^{14}C -3-sulfate in Intact Rats

The disposition of sulfated and nonsulfated lithocholates differed in both route and rate of excretion. Rats fed labeled lithocholate excreted an average of only 6% (3.3, 10.5, 4.2) of the administered isotope in the urine, whereas those fed the sulfated glycine conjugate excreted 23.8% (20.2, 24.8, 26.4) of the administered isotope in the urine.

The rate of total isotope excretion in urine and feces was more rapid in those rats fed the sulfated glycine conjugate than in those fed free lithocholate. Excretion of isotope followed first order kinetics during the first 4 days (i.e., the time required for more than 90% excretion), as shown by the high r values in Table 2. After this time, it was felt that the probability of extensive microbial transformation (e.g., loss of the sulfate) and the low levels of radioactivity involved would make kinetic analysis unreliable. However, during the first 4 days, the mean biological half-life ($t_{1/2}$) for GLS was found to be 0.80 days, while the mean half-life for L was 1.26 days (Table 2). Similar values were obtained when fecal excretion of isotope was analyzed separately; these data also followed first order kinetics (Table 2 and Fig. 3).

Finally, two rats from each group were arbitrarily chosen, and the isotopic material excreted on the first and fourth day was analyzed by TLC. During day 1, one rat (G-2) that was fed the sulfate excreted 15% of the labeled material as LS and about 5% as GLS, whereas the other rat (G-1) excreted only a trace of sulfated material, mainly LS. In each case, the remainder of the radioactivity was in the form of free bile salts, mainly lithocholate, isolithocholate, and 3-ketocholates, with traces

TABLE 1 BILIARY METABOLITES OF LITHOCHOLIC ACID-24- ^{14}C IN BILE FISTULA RATS

Rat and time of bile collection in hours	Distribution of Radioactive Metabolites in Bile					
	F-2	F-50	G-1	H-24*	H-40†	H-72‡
Type of injection§	IG	IG	IP	IP	IP	IP
Group I compounds, conjugated and sulfated	9	2	7	2	3	3
Group II compounds, conjugated, not sulfated	87	93	93	98	97	97
β -Muricholic acid	15.5	73.0	18.2	38.0	51.0	56.0
3 α ,6 β -Dihydroxy-5 β -cholanic acid	9.6	14.5	21.1	47.6	26.7	25.6
Chenodeoxycholic acid	29.0	2.1	20.4	2.0	9.0	4.1
Unknown		3.4				
Lithocholic acid	33.0	tr.	33.3	10.4	10.3	11.3
Group III compounds, not conjugated, sulfated (lithocholic acid sulfate)		0.5				
Group IV compounds, not conjugated, not sulfated (free bile acids)	4.0	4.5				

* Injected at hour 24; enterohepatic circulation intact for 24 hr after operation.

† Injected at hour 40; after 16 hr of biliary diversion.

‡ Injected at hour 72; enterohepatic circulation reconstituted 24 hr previously.

§ IG, intragastric instillation; IP, intraperitoneal.

TABLE 2 EXCRETION OF ISOTOPE AFTER ADMINISTRATION OF LITHOCHOLIC ACID-24-¹⁴C AND GLYCOLITHOCHOLIC ACID-24-¹⁴C-3-SULFATE TO INTACT RATS

Compound Administered	Rat No.	Total Excretion*			Fecal Excretion Only†		
		r‡	Slope (k)	t _{1/2} days	r	Slope (k)	t _{1/2} days
L	1	0.992	-0.285	1.06	0.989	-0.272	1.11
L	2	0.991	-0.212	1.42	0.991	-0.203	1.48
L	3	0.975	-0.231	1.30	0.976	-0.230	1.30
Mean ± SD§			-0.243 ± 0.043	1.26 ± 0.18		-0.235 ± 0.035	1.30 ± 0.19
GLS	1	0.944	-0.400	0.75	0.984	-0.401	0.75
GLS	2	0.987	-0.409	0.74	0.992	-0.411	0.73
GLS	3	0.994	-0.335	0.90	0.997	-0.320	0.94
Mean ± SD§			-0.381 ± 0.040	0.80 ± 0.09		-0.377 ± 0.050	0.81 ± 0.11

* The total isotope recovered from urine and feces was used to calculate the rate of total isotope excretion up to day 4.

† The total isotope recovered from feces alone was used to calculate the rate of fecal isotope excretion up to day 4.

‡ Correlation coefficient.

§ The difference between the means of L and GLS in each vertical column is statistically significant at the 0.02 level (Student's *t* test).

of more and less polar metabolites. By day 4, most of the radioactivity was in the di- and trihydroxy areas on TLC.

Rats fed lithocholate excreted a considerably greater proportion of the radioactivity as di- and trihydroxy bile salts during day 1. No sulfated compounds were observed. During day 4, marked differences were seen between the two lithocholate rats. Rat L-1, with the shortest *t*_{1/2} of the three, excreted mostly di- and trihydroxy metabolites and virtually no monohydroxy bile salts, whereas rat L-2, with the longest *t*_{1/2}, excreted lithocholate predominantly,

with only traces of polyhydroxylated compounds. No bile salts less polar than lithocholate were observed in either rat.

DISCUSSION

The many toxic properties of lithocholic acid make it important to understand the metabolism, physiological disposition, and pharmacological properties of this compound and its metabolites. In the preceding communication, the role of sulfation in the metabolism of lithocholic acid in humans was described (10). The present studies (part A) demonstrate that sulfate esters of lithocholic acid can also be formed in the rat and suggest that this experimental animal could be used for further investigations on the site or sites and mechanism of sulfation. The significance of this observation lies not in the extent of sulfation in this species, which is small, but in the fact that it occurs at all, so that the enzymatic mechanism must be present. This metabolic pathway is probably quantitatively less important in the rat than in the human, because hydroxylated derivatives of lithocholic acid, which are the expected products of lithocholate metabolism in rat liver (17) but are not formed in the human liver, do not seem to undergo sulfation to any great extent. Similarly, it is of interest that sulfated lithocholates do not appear to undergo hydroxylation either, since significant amounts of polyhydroxylated bile acids were not isolated from the sulfate fractions in any of the present experiments. Hence, hydroxylation and sulfation may be alternative mechanisms for processing the less water-soluble steroids.

Identification of sulfated derivatives in the bile of rats that had received labeled lithocholate by intraperitoneal injections suggests that sulfation probably occurs in the liver, although other tissues, such as the intestinal mucosa, may also have this capability.

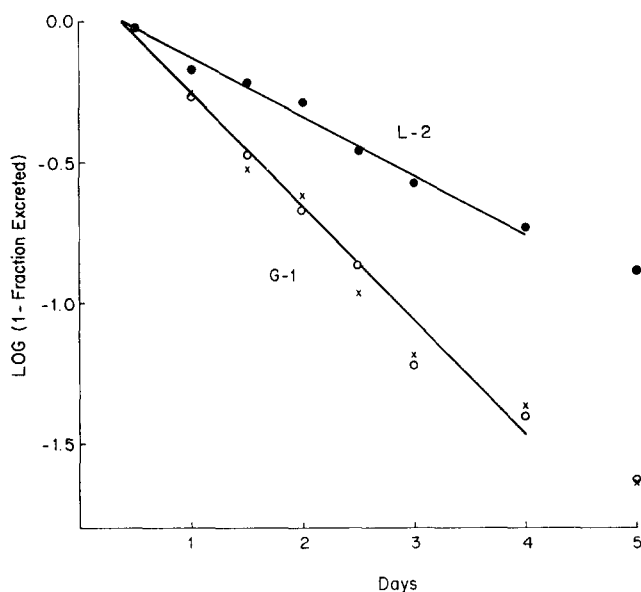


FIG. 3. Excretion of a sulfated or a nonsulfated ¹⁴C-labeled bile salt in two representative rats. The total isotope excreted in urine and feces was used to calculate the rate of excretion of lithocholate-24-¹⁴C (L-2, ●—●) and glycolithocholate-3-sulfate-24-¹⁴C (G-1, O—O). *t*_{1/2} for L-2 was 1.30 days; *t*_{1/2} for G-1 was 0.75 days. Calculations based on fecal excretion of isotope alone, excluding urinary excretion, gave similar results for rat G-1 (x—x).

Current information on the physiological disposition (intestinal absorption and excretion) of lithocholic acid indicates that it can certainly be absorbed from the intestinal tract of man and experimental animals, but the quantitative aspects of the processes involved are poorly documented. Lithocholic acid formed endogenously is considerably less well absorbed than its dihydroxy counterpart, deoxycholic acid, but whether this is a result of the physical properties of these molecules or whether it reflects the further microbial transformation of lithocholate to even less soluble derivatives (8) remains unclear. Any lithocholate, or its taurine or glycine conjugate, that reaches the upper intestine by ingestion or via the enterohepatic circulation can presumably be absorbed by ionic or nonionic monomeric diffusion (18, 19), although its relative water insolubility may limit access to the mucosa. These same compounds can also probably be absorbed by the active transport system in the terminal ileum; however, their water insolubility may also limit this pathway of absorption *in vivo* just as it has complicated attempts to study their affinity for the transport system *in vitro*. The relative contributions of these two pathways to the absorption of lithocholates has not been evaluated, although it is a matter of some importance in considering their biological role. For example, rapid absorption of lithocholates in the upper intestine would short-circuit the normal enterohepatic circulation and effectively increase the exposure of the liver to these compounds.

Sulfation of lithocholic acid and its conjugates alters their physical properties by greatly enhancing their water solubility (20). The sulfate group, constituting a second and fully ionized polar group, would be expected to abolish nonionic absorption from the intestine, and it might inhibit ionic diffusion because of its size and position. Conversely, the increased water solubility of sulfated lithocholates might facilitate access to the mucosa, thus enhancing absorption by ionic diffusion and delivery to the active ileal transport system. There, the rate of absorption might depend on the relative affinity of sulfated lithocholates for the transport system and the extent to which other bile salts compete for the uptake sites.

The experiments described in part *B* demonstrate that a representative sulfate, GLS, can be absorbed from the intestinal tract and excreted in the bile. However, the time lag of 8–12 hr prior to the major peak of isotope excretion in bile suggests that passive absorption in the upper intestine was minimal, and that the major route of absorption was by the active ileal transport mechanism. This hypothesis (active ileal transport but little or no passive jejunal absorption of sulfated lithocholates) has been strongly supported by the detailed studies of Low-Beer, Tyor, and Lack on the *in vivo* and *in vitro* absorp-

tion of sulfated and nonsulfated lithocholates in the guinea pig (21).

The results in part *B* also strongly suggest that the sulfated lithocholates were absorbed without disruption of the sulfate linkage, for had the sulfate moiety been removed, the results from part *A* would indicate that less than 10% of the labeled lithocholates could have been expected to be resulfated, whereas in fact all of the isotope appearing in bile was found in the sulfate fraction.

Conversely, there was a progressively increasing fraction of the excreted isotope that had lost the glycine moiety and was either reconstituted with taurine and excreted as TLS or excreted without amino acid conjugation as LS. This sulfation does not *prevent* microbial deconjugation *in vivo*, although the possibility of altered enzyme kinetics remains. Clearly, sulfated taurine and glycine conjugates of lithocholic acid can serve as substrates for the cholyglycine hydrolase of Nair, Gordon, and Reback (16) *in vitro*, as demonstrated in this and the preceding (10) reports, but no kinetic data are available for sulfated compounds.

The studies described in part *C* were undertaken to obtain information about the disposition of sulfated lithocholates. Since lithocholate is almost completely conjugated with taurine following its absorption, the comparison was really between the excretion of TL and GLS, two compounds of approximately equal polarity and chromatographic mobility (10). Thus, it was surprising to find that the renal excretion of the sulfated lithocholate was larger than would be anticipated in the absence of jaundice, and was of such magnitude (24% of the administered radioactivity) that it should be considered in any discussion of the physiological disposition of lithocholates in general. Bile salts that escape hepatic extraction from portal vein blood pass into the systemic circulation and, if not protein-bound, through the renal glomeruli. They then appear to be actively reabsorbed in the proximal tubules, so that bile salts are not normally present in the urine in large amounts (22). Whether the increased excretion of sulfated lithocholates observed in these experiments was due (*a*) to decreased protein-binding and consequent increased filtration, as seems probable on the basis of solubility studies, (*b*) to decreased reabsorption, as seems likely by analogy with intestinal reabsorption studies, (*c*) to some other unknown mechanism, or (*d*) to some combination of these remains an open question. Increased exposure of renal tissue to sulfated lithocholates could be of pathological importance if studies on the pharmacological properties of these compounds demonstrate appreciable toxicity.

In addition to increased renal excretion of sulfated lithocholates, the results presented here suggest a more rapid fecal excretion. Fecal excretion of radioactive bile acids, including lithocholic acid, by the rat has been

shown to follow first order kinetics, and biological half-lives have been calculated from the rate constants of the lines described by the equation $k = -\frac{1}{t} \log \left(1 - \frac{u_t}{u_{max}} \right)$, where u_t is the total amount of isotope excreted by time t , and u_{max} is the maximum amount recovered from feces (15). However, this method of analysis might not be valid if an appreciable amount were excreted by the kidneys, as is the case with GLS, since the fraction not yet excreted in feces $\left(1 - \frac{u_t}{u_{max}} \right)$ would not remain available for fecal excretion, but would be in part excreted by the kidneys. Therefore, in the present experiments the amount excreted in both urine and feces was substituted for u , and the data was plotted in the usual manner. When the values obtained for k were compared with values obtained using fecal excretion alone for u , they were found to be virtually the same (Table 2).

How can similar values for k and $t_{1/2}$ be reconciled with the observation that an average of 24% of the labeled GLS recovered was present in the urine? The explanation is not entirely clear, but it may be that the earlier excretion in urine as compared with feces diminishes the effect of the urinary excretion on the rate constant calculated for total excretion, since urinary excretion is continually being related to a larger apparent pool. Thus, radioactivity in urine, which was relatively large initially, became small compared with radioactivity in feces during subsequent time periods, so that the slope of the line representing total excretion became essentially the same as that for fecal excretion. This would suggest that the total excretion is underestimated during the initial time periods, and that the biological $t_{1/2}$ is actually shorter than estimated by these methods.

From these considerations, it follows that the calculated rate constants refer primarily to fecal excretion, and that there appears to be an increased rate of fecal excretion of glycolithocholic acid-C-3-sulfate relative to lithocholic acid, or rather its primary derivative, tauro-lithocholic acid (see above). Data in the literature suggest that taurine conjugates are eliminated in feces more rapidly than glycine conjugates (18, 19, 23-26), presumably because the lower pK of taurine prevents non-ionic absorption. However, both the taurine and glycine conjugates of lithocholic acid are absorbed by ionic diffusion in the jejunum, as well as by active transport in the ileum (21). In contrast, the sulfate group effectively abolishes absorption by both ionic and nonionic diffusion, but does not prevent absorption by active transport (21). Hence, diminished passive absorption of GLS is consistent with and may explain the increased fecal excretion observed in these experiments. Sulfation, there-

fore, apparently acts to deplete the circulating pool of lithocholates by enhancing fecal excretion as well as urinary excretion. It may thus constitute a second explanation, in addition to the extensive microbial transformations previously described (8), for the relative paucity of lithocholates in bile.

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