Lithocholate metabolism during chenotherapy for gallstone dissolution[1]

1. Serum levels of sulphated and unsulphated lithocholates

R. N. ALLAN[2], J. L. THISTLE, A. F. HOFMANN[3], J. A. CARTER, AND P. Y. S. YU

From the Gastroenterology Unit, Mayo Clinic, Rochester, Minnesota 55901, U.S.A.

SUMMARY   Serum levels of total sulphated and total unsulphated lithocholates were each measured by a specific radioimmunoassay in 66 patients ingesting chenodeoxycholic (chenic) acid for gallstone dissolution and in 35 gallstone patients ingesting either cholic acid or placebo. No changes occurred in serum lithocholate levels in the control groups. In patients ingesting chenic acid, there was a twofold increase in serum levels of total lithocholate, but the percent sulphation ($>75\%$) remained unchanged during chenotherapy. There was no correlation in the chenic acid treated group between serum lithocholate levels and the proportion of lithocholate in biliary bile acids or changes in serum SGOT. The data suggest that there is effective sulphation of lithocholate in such patients; this may explain the lack of hepatotoxicity observed during ingestion of chenic acid.

Chenodeoxycholic (chenic)[4] acid decreases the cholesterol saturation of bile and induces gallstone dissolution in the majority of patients with radiolucent gallstones and radiologically visualising gallbladders (Thistle and Hofmann, 1973; Coyne *et al.*, 1975; Iser *et al.*, 1975; James *et al.*, 1975; Goldstein, 1976). Despite this clear-cut efficacy, clinical testing of this potent agent has expanded only slowly because of dose-related hepatotoxicity observed in the rabbit (Fischer *et al.*, 1974), rhesus monkey (Webster *et al.*, 1975; Dyrszka *et al.*, 1976), and baboon (Morrissey *et al.*, 1975). There is now strong evidence implicating lithocholic acid, the principal bacterial metabolite of chenic acid, as the major factor in chenic acid induced liver injury (Palmer, 1972; Fischer *et al.*, 1974; Morrissey *et al.*, 1975; Salen *et al.*, 1975; Webster *et al.*, 1975; Dyrszka *et al.*, 1976; Gadacz *et al.*, 1976).

In healthy man about one-fifth of the lithocholate newly formed in the colon is absorbed (Allan *et al.*,

1976). The absorbed lithocholate is not only conjugated with glycine or taurine but is also mostly sulphated at the 3 position to form sulphated lithocholyl conjugates, sulpholithocholylglycine, and sulpholithocholyltaurine (Cowen *et al.*, 1975a). These end products of hepatic metabolism, in contrast with other biliary bile acids, are poorly absorbed from the small intestine and rapidly excreted in the faeces (Cowen *et al.*, 1975b). As a result, lithocholate is a very minor constituent of biliary bile acids in healthy man.

In patients receiving chenotherapy for gallstone dissolution, most of the administered chenic acid is ultimately converted to lithocholate before excretion (Danzinger *et al.*, 1973). Since the doses now used for gallstone dissolution (6 to 25 mg/kg) are many times the endogenous synthesis rate of chenic acid (1 to 2 mg/kg-day) (Vlahcevic *et al.*, 1971; Danzinger *et al.*, 1973), we thought it likely that lithocholate absorption from the intestine would be increased in such patients but that sulphation would continue to be effective, since published analyses of biliary bile acid composition (Thistle and Schoenfield, 1971; Danzinger *et al.*, 1973; Coyne *et al.*, 1975; James *et al.*, 1975) had shown that the proportion of lithocholate in biliary bile acids in gallstone patients receiving chenic acid remains quite low. To test these hypotheses, we have carried out two groups of

studies. In the first group, reported herein, we measured levels of sulphated and unsulphated lithocholates in blood and bile and related these to liver tests and liver morphology. In the second group of studies, reported in the accompanying paper (Allan et al., 1976), we measured, using isotope dilution, the amount of lithocholate entering the bile acid pool as well as the size of the exchangeable lithocholate pool in patients ingesting chenic acid for gallstone dissolution and in healthy subjects. We then carried out experiments aimed at quantitating the extent of sulphation of the absorbed lithocholate by defining the biotransformation of intravenously administered radiolabelled lithocholate and its glycine or taurine conjugates.

Methods

PATIENTS

The patients included 53 patients (30 men, 23 women) with radiolucent cholesterol gallstones treated for 12 months with either placebo (17), cholic acid (18), or chenic acid (18) and 13 patients with radio-opaque gallstones treated for 12 months with chenic acid. A progress report of this controlled study has been published previously (Thistle and Hofmann, 1973). The patients treated initially with either placebo or cholic acid subsequently received chenic acid for 12 months and were studied during both phases of their treatment. The dose of chenic acid ingested per day in this group of 66 patients was 13 ± 6 mg/kg (mean $\pm$ SD), and the range of doses was 6 to 25 mg/kg. Three patients did not complete the study, two of their own accord and one after surgery for peptic ulcer. There was no clinical or biochemical evidence of hepatotoxicity in these patients.

PROCEDURE

Fasting-state serum samples were obtained before and at the end of each 12-month course of treatment and were stored at $-20°C$ until assayed. Bile samples after intravenous administration of cholecystokinin were obtained in most patients at the same visit, diluted 1:9 in isopropanol, and stored at 4°C. Serum levels of sulphated and unsulphated lithocholates were determined by specific radioimmunoassays; biliary bile acids were determined by gas/liquid chromatography.

Serum sulphated lithocholate was determined by a radioimmunoassay using antisera at 1:1000 dilution which had been raised in rabbits to sulpholithocholylglycine coupled to bovine serum albumin. The methodology is similar to that published previously for radioimmunoassay of cholyl conjugates (Simmonds et al., 1973). The antibody reacted equally to

sulpholithocholylglycine, sulpholithocholyltaurine, and sulpholithocholate and thus measured all sulphated lithocholate species in serum. However, since sulpholithocholate—that is, sulphate ester of unconjugated lithocholate—is unlikely to be present in appreciable amounts in serum, the assay probably measured sulphated lithocholyl conjugates. The antibody showed little affinity for unconjugated lithocholate or its glycine conjugate (more than a 200-fold excess of these bile acids was required to displace 50% of bound tracer) and virtually no affinity for lithocholyltaurine, sulphocholate, or sulphochenodeoxycholate, or any of the conjugated bile acids predominating in human bile. The within assay coefficient of variation was 2%; the between assay 8%. Details of the method are to be published.

For unsulphated lithocholate species, a second specific radioimmunoassay was developed. Antisera was raised in rabbits by injecting lithocholylglycine coupled to bovine serum albumin, and an antiserum capable of measuring 40-120 pmol at 1:400 dilution was obtained. The antibody reacted equally to lithocholylglycine and lithocholyltaurine but showed some affinity for unconjugated lithocholate. Hence, the values reported here are probably slightly higher than that of unsulphated lithocholate conjugates, depending on the amount of unconjugated lithocholate present in serum. However, since unconjugated lithocholate is rapidly conjugated and excreted in bile (Cowen et al., 1975a), we think it likely that the concentration of unsulphated, unconjugated lithocholate present in serum is quite low. The affinity of the antibody for other major primary and secondary bile acids was extremely low. The within assay coefficient of variation was 3%; between assay, 9%. Details of the method will be published. In 50 healthy volunteers, the mean level ($\pm$ SEM) of immunoreactive fasting serum sulphated of lithocholyl conjugates was $1.6 \pm 0.2 \mu M$ and that serum unsulphated lithocholyl conjugates was $0.3 \pm 0.02 \mu M$.

BILE

For determining biliary bile acid composition, 1 ml of diluted bile sample was evaporated to dryness and the residue saponified with 2 N NaOH and 50% methanol (1:1, v:v) for four hours at 115°C. After acidification, the bile acids were extracted into ether. Sulphated bile acids undergo rapid, complete solvolysis in the ether phase (van Berge Henegouwen, Allan, Hofmann, and Yu, unpublished data). The unconjugated, unsulphated bile acids were esterified using diazomethane; acetate derivatives were made and gas chromatography was carried out using a 3% cyanosilicone stationary phase (AN 600 Analabs) as described (Hofmann and Poley, 1972).

The lithocholate quantitated by this method represents the sum of both sulphated and unsulphated bile acids.

Results

SERUM LITHOCHOLATE LEVEL

In patients ingesting chenic acid the mean ($\pm$ SEM) total serum lithocholates increased from 1·34 ($\pm$ 0·61) μM to 2·33 ($\pm$ 1·06) μM ($p < 0.001$). In a small number of patients the lithocholate levels remained unchanged and in a few levels actually fell during treatment.

In patients ingesting placebo the mean total serum lithocholates were 1·29 ($\pm$ 0·61) μM initially and 1·43 ($\pm$ 0·74 μM) after 12 months of treatment ($p > 0.05$). In patients ingesting cholic acid the mean total serum lithocholates were 1·25 ($\pm$ 0·36) μM initially and, as with placebo, there was no change after 12 months of treatment (1·05 $\pm$ 0·32 μM) ($p > 0.05$) (Table 1, Fig. 1).

In patients ingesting chenic acid the percent sulphation of serum lithocholates was initially high (74·4%) and changed little with treatment (79·2%).

In patients ingesting placebo the mean sulphation of serum lithocholates was similar (78·1%) and also did not change (78·2%) during treatment ($p > 0.05$). In patients ingesting cholic acid the mean sulphation of serum lithocholates fell slightly from 76·2% to 68·1% ($p < 0.001$) (Table 1, Fig. 2).

BILIARY LITHOCHOLATES

In patients ingesting chenic acid the proportion of lithocholate in biliary bile acids increased from 1·2%

	During chenic acid treatment (N = 63)			During placebo treatment (N = 17)			During cholic acid treatment (N = 18)		
	Before	After 12/12	P	Before	After 12/12	P	Before	After 12/12	P
1. *Serum* (μmol/l)									
i-unsul-lithocholates	0·31 $\pm$ 0·11	0·42 $\pm$ 0·15	< 0·001	0·24 $\pm$ 0·05	0·28 $\pm$ 0·08	> 0·05	0·30 $\pm$ 0·11	0·32 $\pm$ 0·08	> 0·05
i-sul-lithocholates	1·02 $\pm$ 0·58	1·90 $\pm$ 1·01	< 0·001	1·05 $\pm$ 0·62	1·14 $\pm$ 0·66	> 0·05	0·95 $\pm$ 0·29	0·73 $\pm$ 0·33	> 0·05
Total lithocholates	1·34 $\pm$ 0·61	2·33 $\pm$ 1·06	< 0·001	1·29 $\pm$ 0·61	1·43 $\pm$ 0·74	> 0·05	1·25 $\pm$ 0·36	1·05 $\pm$ 0·32	> 0·05
Sulphation (%)	74·4 $\pm$ 10·1	79·2 $\pm$ 8·3	< 0·001	78·1 $\pm$ 8·8	78·2 $\pm$ 7·7	> 0·05	76·2 $\pm$ 5·1	68·1 $\pm$ 9·3	< 0·001
2. *Bile* (%)									
Lithocholic	1·2 $\pm$ 1·2	3·2 $\pm$ 2·2	< 0·001	1·3 $\pm$ 1·2	1·9 $\pm$ 1·0	> 0·05	2·1 $\pm$ 1·1	0·8 $\pm$ 0·6	< 0·001
Chenic	38·4 $\pm$ 2·1	86·4 $\pm$ 1·8	< 0·001	40·5 $\pm$ 1·5	39·1 $\pm$ 1·8	> 0·05	39·7 $\pm$ 2·1	13·7 $\pm$ 2·1	< 0·001
Cholic	30·8 $\pm$ 2·0	2·0 $\pm$ 0·7	< 0·001	30·7 $\pm$ 2·7	28·5 $\pm$ 2·0	> 0·05	28·5 $\pm$ 2·5	37·4 $\pm$ 3·1	< 0·05
Deoxycholic	28·4 $\pm$ 2·5	4·0 $\pm$ 1·1	< 0·001	27·0 $\pm$ 2·7	27·0 $\pm$ 2·5	> 0·05	24·7 $\pm$ 3·9	44·9 $\pm$ 3·6	< 0·001

Table 1 *Serum and biliary bile acids in gallstone patients (mean $\pm$ SEM): (1) serum immunoreactive lithocholates, and (2) biliary bile acids as measured by gas liquid chromatography*

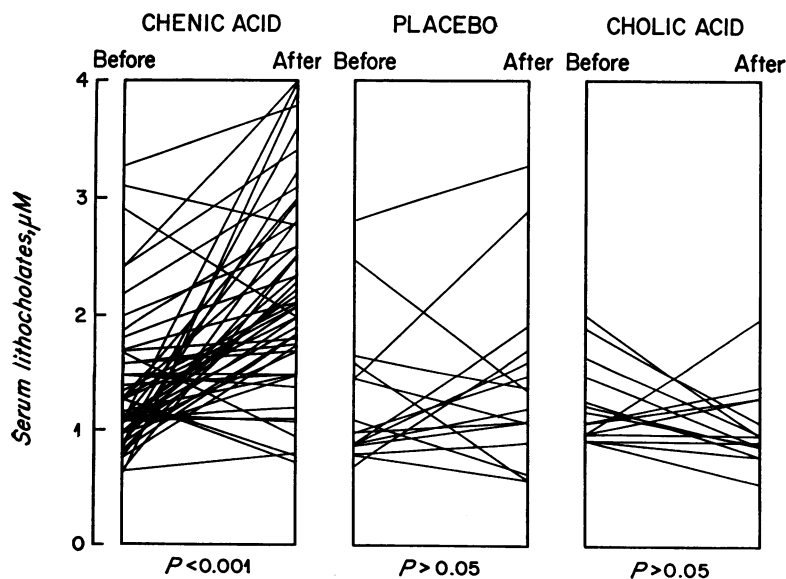i = immunoreactive; unsul = unsulphated; sul = sulphated.

Fig. 1 *Total serum immunoreactive lithocholates before and after 12 months' treatment with chenic acid, placebo, or cholic acid.*

CHENIC ACID   PLACEBO   CHOLIC ACID

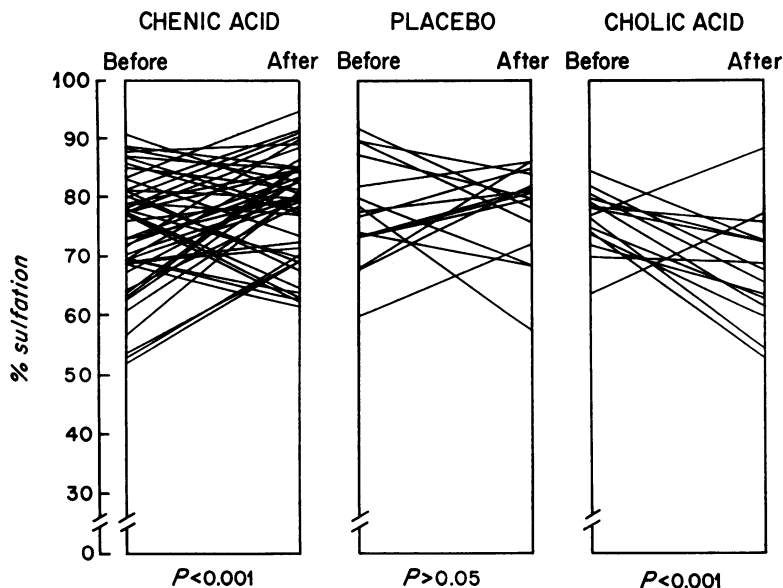**Fig. 2** *Sulphation (%) of serum immunoreactive lithocholates before and after 12 months' treatment with chenic acid, placebo, or cholic acid.*

to 3·2% during chenotherapy (P < 0·001). A typical chromatogram is shown in Fig. 3. However, in patients ingesting placebo there was no significant change in biliary lithocholates. In the patients ingesting cholic acid, the lithocholate fraction in bile fell from 2·1% to 0·8% (Table 1).

Correlations

SERUM LITHOCHOLATES VS BILIARY LITHOCHOLATES

There was little correlation between the total serum lithocholates and the proportion of lithocholate in
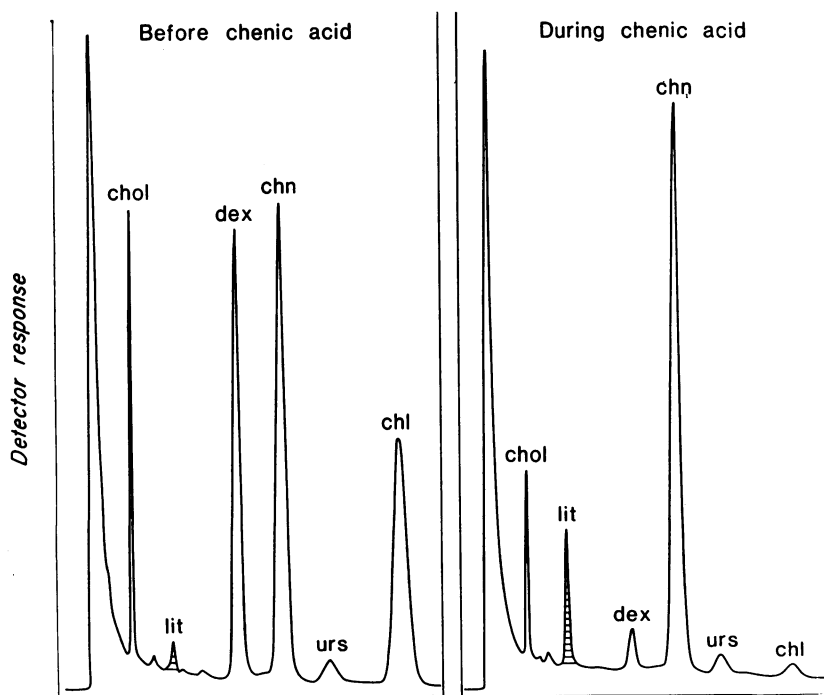

Fig. 3 *Effect of chenotherapy on biliary bile acids as measured by gas liquid chromatography. Abbreviations: chol—cholesterol; lit—lithocholic acid; dex—deoxycholic acid; chn—chenic acid; urs—ursodeoxycholic acid; chl—cholic acid.*

bile (Fig. 4). There was no increase in the correlation coefficient when the serum sulphated and un-sulphated lithocholate fractions were considered separately. We did not determine the degree of sulphation of biliary lithocholate, but in patients ingesting chenic acid, biliary lithocholate is mostly sulphated (Allan *et al.*, 1976).

LITHOCHOLATES AND CHENIC ACID DOSE

There was also little correlation between the prescribed dose of chenic acid and either the total serum lithocholates (Fig. 5) or the proportion of lithocholate in bile. The correlation coefficient did not increase when the sulphated and unsulphated serum fractions were considered separately or when
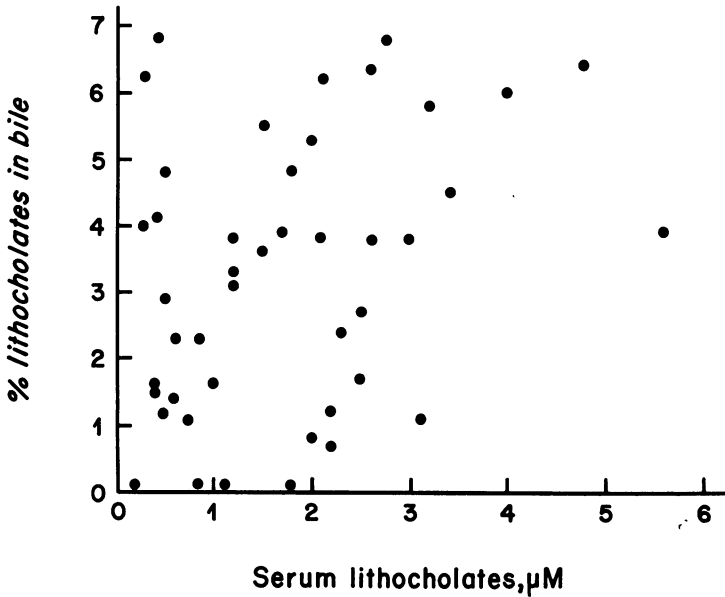

Fig. 4 *Lithocholate in bile (%) vs total serum lithocholates in gallstone patients during cheno-therapy.*
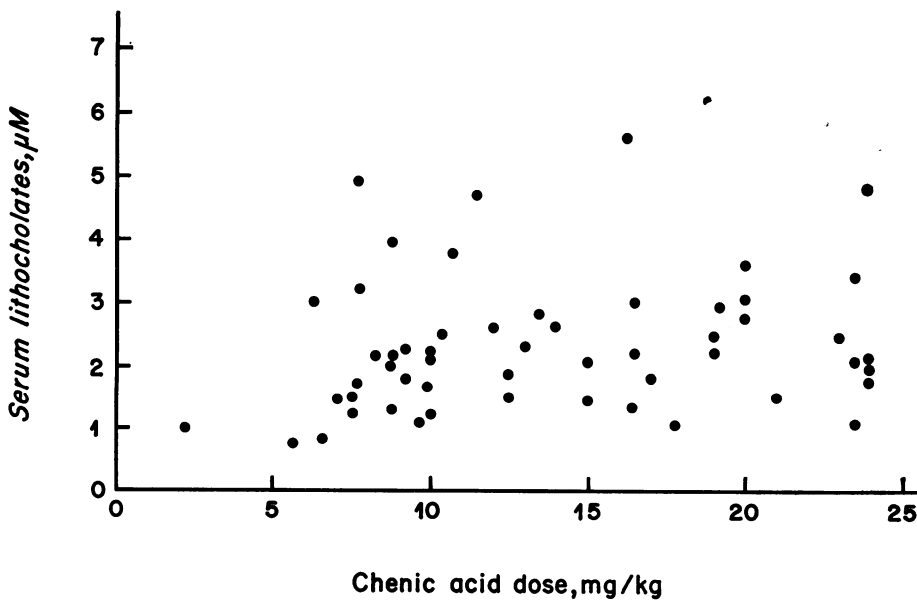

Fig. 5 *Total serum litho-cholates vs chenic acid dose in gallstone patients during chenotherapy.*

the absolute dose of chenic acid ingested was considered rather than dose relative to body weight.

LIVER FUNCTION

Serum bilirubin, alkaline phosphatase, and serum proteins remained normal in all patients throughout the study. In patients ingesting chenic acid, the sharp, transient elevation in SGOT previously reported for nearly a quarter of the patients one to three months after taking chenic acid has not recurred (Thistle and Hofmann, 1973). After 12 months of chenic acid therapy only 11 of the 66 patients had an SGOT outside 95% confidence limits (> 24 u/l serum), only four were more than 30 u/l, and none greater than 50 u/l. Clinically all these patients remained well. In this group of 11 patients the serum SGOT level was not related to the dose of chenic acid ingested, the total serum lithocholates, or the sulphated fraction of serum lithocholates (Table 2). In the 66 patients ingesting chenic acid, the change in SGOT was not related to either the total serum lithocholates or the fraction of lithocholate in bile.

HEPATIC MORPHOLOGY

Thirteen liver biopsies were taken in 11 of these patients ingesting chenic acid. There were no histological differences when compared with an age, sex-matched group of liver biopsies previously reported as normal.

Discussion

SERUM LITHOCHOLATES

These analyses show an increase in serum lithocholate levels in gallstone patients ingesting chenic acid in agreement with the preliminary report of

Stiehl et al. (1975a). We have previously reported that lithocholic acid is a major faecal bile acid in such patients (Danzinger et al., 1973), and, as shown in the accompanying paper (Allan et al., 1976), isotope dilution studies indicated increased absorption of lithocholate in such patients. The origin of serum sulphated lithocholates is not known, but if unsulphated lithocholate is injected intravenously, sulphated lithocholates appear in serum (Cowen et al., 1975c), suggesting reflux from the hepatocyte or extrahepatic sulphation. The hepatic clearance of sulphated lithocholate is considerably slower than that of the other major primary and secondary bile acids (Cowen et al., 1975b).

LITHOCHOLATE IN BILE

Using gas chromatography we observed a parallel increase in the proportion of lithocholate in biliary bile acids in patients ingesting chenic acid as previously observed (Danzinger et al., 1973) and as has been reported also by Coyne et al. (1975) and Stiehl et al. (1975b). This finding has also recently been confirmed by a sensitive mass spectrometric technique (Szczepanik et al., 1975).

SULPHATION OF LITHOCHOLATE

In man during chenic acid ingestion, the serum lithocholate fraction both before and during treatment remained largely sulphated. These data agree with the biotransformation studies reported in the accompanying paper (Allan et al., 1976) which document effective sulphation of absorbed lithocholate in gallstone patients on chenotherapy. We have previously shown in healthy subjects that lithocholate is rapidly sulphated and excreted in bile after intravenous injection and that sulphation causes rapid faecal excretion (Cowen et al., 1975b).

Patient	Chenic acid (mg/kg/day)	SGOT* (IU)			Serum lithocholate Total (uM)		% Sulphation	
		Pretreatment	1 year	2 years†	Pretreatment	1 year‡	Pretreatment	1 year‡
R.H.	8·8	16	25	14	2·9	2·0	86	78
J.C.	9·9	16	25		1·0	1·7	73	82
W.H.	10·3	20	26	21	0·7		50	
C.D.	16·3	22	27	24		5·6		90
C.B.	9·3	16	27	20	0·8	2·8	76	90
G.S.	16·5	16	29	15	1·1	2·2	78	65
J.H.	10·0	30	30	14	1·1	2·1	82	91
P.L.	15·0	30	30	31	1·8	2·1	86	78
W.B.	10·0	16	32		1·1	1·2	86	73
C.B.	9·3	16	35	19				
A.C.	20·0	13	50	51	2·2	3·1	91	82

Table 2   Relationship between chenic acid dosage and SGOT and serum lithocholate levels in gallstone patients with elevated SGOT after one year of chenotherapy

*Highest value for upper limit of normal in Mayo Laboratories is 24 IU/l.
†Chenotherapy continued at same dose.
‡Initial and one year values for serum lithocholate and % sulphation were not significantly different.

LIVER FUNCTION

Despite the observed hepatotoxicity in non-human primates during chenic acid ingestion (Goldstein, 1976), we have not observed significant hepatotoxicity thus far in man and global experience with chenic acid now involves some 1600 patients (Hofmann and Paumgartner, 1975). Indeed, the lack of correlation between levels of SGOT, dose of chenic acid, and levels of serum lithocholates suggests that transient SGOT elevation may not be caused by increases in lithocholate. Indeed, since the SGOT elevations do not correlate with morphological changes, they appear to be 'false positives'. Elevated SGOT has also been observed in two healthy subjects ingesting deoxycholic acid, but biopsies were not obtained (LaRusso and Hofmann, 1975).

MAN VS NON-HUMAN PRIMATE

In the rhesus monkey and baboon ingesting chenic acid there is a striking increase in the proportion of lithocholate in biliary bile acids with lithocholate levels averaging 10% with a dose of 20-40 mg/kg-day (Goldstein, 1976; Webster *et al.*, 1975). Defective sulphation of lithocholate in the rhesus monkey has recently been demonstrated (Gadacz *et al.*, 1976), and the impaired detoxification of lithocholate now provides a simple explanation for the consistent toxicity of chenic acid in the rhesus monkey and its apparent safety in man.

In conclusion, although the serum and biliary lithocholates increased after chenic acid therapy, the lithocholates remained largely sulphated and it is probably this effective sulphation which protects the liver from the hepatotoxic effects of lithocholic acid. The lack of correlation between serum and biliary lithocholate levels precludes its use as a screening test, though to date, because of the lack of observed hepatotoxicity in man, there is no clear evidence that such a test is required. A genetic defect in sulphation in man should be signalled by an increased proportion of lithocholate in biliary bile acids in early childhood since secondary bile acid formation, specifically bacterial dehydroxylation of cholic acid to deoxycholate, begins during the first year of life (Poley *et al.*, 1964) and the same bacterial flora degrade chenic acid to lithocholate (Aries and Hill, 1970). We have previously proposed an algorithm for management of patients during chenotherapy based on serum enzyme levels (Hofmann and Thistle, 1974) and these recommendations still seem sound.

Finally, it might be noted that in the rhesus monkey ingesting chenic acid at toxic dosages, serum enzyme levels show a characteristic time course. To specify, serum SGOT levels peak and rapidly return to near normal values; serum SGPT levels peak and return to about three times normal values; only leucine aminopeptidase (LAP) remains continuously raised (Dyrszka *et al.*, 1976). If these observations may be applied to man, the most useful enzyme for detecting lithocholate related hepatotoxicity in man would be LAP. Yet in 200 patient years of experience with chenic acid at the Mayo Clinic, no elevation of LAP has yet been observed (Hofmann and Paumgartner, 1975).

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