Gut, 1979, **20**, 1083–1087

Bile acid clearance in liver disease

K. L. LUEY,[1] AND K. W. HEATON[2]

From the University Department of Medicine, Bristol Royal Infirmary, Bristol

SUMMARY The disappearance of intravenously administered cholylglycine-^{14}C was studied in the fasting and postprandial states in seven subjects with healthy livers and 10 patients with liver disease. In neither group was there any significant difference in the pattern of ^{14}C disappearance. In another 10 patients with liver disease there was no significant change when a loading dose of cholylglycine was given orally two hours beforehand. Clearance of bile acids seems to be unimpaired in all except severe liver disease. The apparent deterioration in endogenous bile acid removal after meals may be due simply to the increased amount of bile acids which are in circulation and available for portosystemic shunting.

Despite theoretical attractions and initially promising reports (Josephson, 1941; LaRusso *et al.*, 1975; Calcraft *et al.*, 1975; Hofmann, 1977), the so-called intravenous bile acid tolerance test, which is normally performed in the fasting state, is now known to discriminate poorly between normal subjects and hepato-biliary disease (Ferguson *et al.*, 1976; Isaacs *et al.*, 1976; Thjodleifsson *et al.*, 1977; Gilmore and Thompson, 1977). In contrast, several studies have shown that the postprandial serum bile acid concentration, which has been called the endogenous bile acid tolerance test (van Blankenstein *et al.*, 1977), discriminates excellently and does so better than the same measurement in the fasting state (Kaplowitz *et al.*, 1973; Barnes *et al.*, 1975; Fausa and Gjone, 1976; Thjodleifsson *et al.*, 1977), though not all workers agree on this point (Pennington *et al.*, 1977; van Blankenstein *et al.*, 1977). If the postprandial serum concentration is indeed a better discriminator, this may be related to the finding that the serum bile acid concentration rises more after a meal in patients with liver disease than it does in healthy subjects (Fausa, 1976; Osuga *et al.*, 1977; van Blankenstein *et al.*, 1977). These observations imply that the diseased liver removes endogenous bile acids less completely after a meal than in the fasting state. Possible reasons for this are hepatic overload from the increased bile acids in circulation that follow gallbladder contraction, and increased shunting of absorbed bile acids round the liver. If hepatic overload is the correct explanation, the disappearance of intravenously administered bile acid should, in liver disease but not in health, be slower after a meal and after an artificial load of bile acids than in the fasting state. Furthermore, if clearance were indeed slower in these circumstances, the hope of a sensitive bile acid disappearance test might after all be realised. This study was designed to examine these possibilities.

Methods

Seven hospital patients with no clinical evidence of liver disease and normal routine liver function tests (Table 1) acted as controls. These and 10 patients with biopsy-proven chronic liver disease (Table 2) were each studied twice—after an overnight fast and, on another day, two hours after the ingestion of a Lundh test meal (Casilan, 150 g; corn oil, 18 g; dextrose, 40 g; water 200 ml). Another 10 subjects with biopsy-proven liver disease (Table 3) (one of whom was also a member of the first group) were studied twice—in the fasting state and, on another day, two hours after the oral ingestion of 2·5 g of non-radioactive cholylglycine (Weddell Pharmaceuticals, London) in gelatine capsules. The latter studies were termed 'loaded' tests. In each group half the subjects underwent the fasting study second in order. Subjects were semi-recumbent during the tests. All subjects gave informed consent to the investigation.

Cholyl-1^{14}C-glycine (Radiochemical Centre, Amersham, Bucks.) was dissolved in saline and sterilised by filtration. Ten μCi in 0·19 μmol were given intravenously for each test. Venous blood was taken from an indwelling catheter in the opposite arm before and 2, 4, 6, 9, 12, 15, 20, 30, 40, 50, and 60 minutes after injection. Duplicate 1 ml aliquots of

[1]Present address: Gastrointestinal Unit, Christchurch Public Hospital, Private Bag, Christchurch, New Zealand.
[2]Requests for reprints should be addressed to K. W. Heaton.
Received for publication 16 July 1979

Table 1 *Control subjects—clinical details and kinetics of cholylglycine-^{14}C disappearance*

Age (yr)	Sex	Diagnosis	Clearance (ml. min^{-1})		$T\frac{1}{2}\beta$ (min)	
			Fasting	Postprandial	Fasting	Postprandial
44	F	Irritable bowel	586	771	8·5	8·6
57	F	Deep venous thrombosis	712	707	15·0	21·2
68	M	Sinus bradycardia	491	502	22·0	21·6
69	F	Osteoarthrosis of spine	396	416	18·3	22·6
61	M	Pulmonary infarct	857	824	14·8	7·6
47	F	Psychogenic vomiting	408	519	17·7	14·3
46	M	Gilbert's syndrome (liver biopsy normal)	994	727	16·2	19·1
Mean (SE)			635 (87)	638 (59)	16·1 (1·5)	16·4 (2·4)

$T\frac{1}{2}\beta$ half-time for slow phase of disappearance curve.

Table 2 *Clinical details and kinetics of cholylglycine-^{14}C disappearance of 10 liver disease patients studied fasting and post-prandially*

Age (yr)	Sex	Diagnosis	Evidence for diagnosis	Bilirubin (μmol l^{-1})	Alk. Phos. (KAU dl^{-1})	AsT (IUl^{-1})	Albumin (g dl^{-1})	Globulin (g dl^{-1})	Cholylglycine-^{14}C kinetics			
									Clearance (ml. min^{-1})		$T\frac{1}{2}\beta$ (min)	
									Fasting	Post-prandial	Fasting	Post-prandial
50	F	1° biliary cirrhosis	Biopsy	11	59	29	36	35	467	317	36·5	39·4
68	M	Alocholic cirrhosis	Biopsy	13	32	34	31	51	632	886	25·1	14·6
67	M	Cryptogenic cirrhosis	Biopsy	30	60	46	35	43	291	418	54·5	35·8
†57	F	1° biliary cirrhosis	Biopsy	300	33	55	19	56	101	71	99·5	132·5
50	F	1° biliary cirrhosis	Biopsy, laparotomy	66	54	56	31	42	295	160	26·1	61·8
72	F	Cryptogenic cirrhosis	Laparotomy	6	11	13	39	35	181	229	28·8	33·2
†67	F	1° biliary cirrhosis	Biopsy, laparotomy	60	95	55	29	44	119	75	61·3	125·4
52	M	Chr. active cirrhosis	Biopsy	15	7	8	35	48	1026	924	27·4	31·2
58	M	Alcoholic cirrhosis	Biopsy, laparoscopy	16	13	21	39	48	536	445	29·5	22·0
69	M	Cryptogenic cirrhosis	Biopsy, laparoscopy	19	19	14	39	32	326	268	30·8	29·6
Mean (SE)									397 (89)	379 (96)	30·2*	34·5*
Normal value				≤17	≤13	≤17	≥35	22–36				

*Median. †Died. $T\frac{1}{2}\beta$ half-time for slow phase of disappearance curve.

plasma from each sample were equilibrated with 10 ml NE-260 micellar scintillation fluid (Nuclear Enterprises, Edinburgh, Scotland) and counted in a liquid scintillation counter. Jaundiced sera were diluted if necessary to reduce quenching. Radioactivity was plotted against time giving a biexponential decay curve which was fitted by computer to the equation $y = ae^{-\alpha t} + be^{-\beta t}$, where y is serum concentration, a and b are constants, α and β are the rate constants for the rapid and slow exponentials, and t is time in minutes.

Bile acid clearance was calculated from the formula

$$C = \frac{\text{Dose}}{a/\alpha + b/\beta}$$

The computer-derived half-time for the slow phase of the disappearance curve ($T\frac{1}{2}\beta$) was used as another measure of bile acid removal from the plasma. The fast phase was not used because it is not affected by liver disease (Gilmore and Thompson, 1978b).

Statistical significance of differences was calculated using student's *t* test or the Wilcoxon rank sum test as appropriate for normally or non-normally distributed data.

Results

In the controls (Table 1) bile acid clearance and $T\frac{1}{2}\beta$ were closely similar in the fasting and postprandial states. Clearance was 635±87 (mean±SEM) and

Table 3 *Clinical details and kinetics of cholylglycine-C^{14} disappearance of liver disease patients studied with and without bile acid load*

Age (yr)	*Sex*	*Diagnosis*	*Evidence for diagnosis*	*Bilirubin (μmol l^{-1})*	*Alk. Phos. (KAU dl^{-1})*	*AsT (IUl^{-1})*	*Albumin (g dl^{-1})*	*Globulin (g dl^{-1})*	*Cholylglycine-^{14}C kinetics*			
									Clearance (ml. min^{-1})		*$T\frac{1}{2}\beta$ (min)*	
									Fasting	*After load*	*Fasting*	*After load*
63	F	Cryptogenic cirrhosis	Biopsy, laparotomy	12	32	13	33	39	432	426	20·0	24·1
41	F	Chr. active cirrhosis	Biopsy, laparotomy	18	22	28	39	41	316	442	43·2	21·4
65	M	Alcoholic cirrhosis	Biopsy, laparoscopy	19	17	10	35	38	747	673	22·1	18·7
58	M	Alcoholic cirrhosis	Biopsy, laparoscopy	16	13	21	39	48	553	679	20·0	18·6
67	M	Cryptogenic cirrhosis	Biopsy	36	24	9	46	31	1249	823	29·5	16·6
57	M	Alcoholic hepatitis	Biopsy	12	16	16	26	40	779	754	42·1	16·7
64	F	Post-hepatitic cirrhosis	Biopsy, laparotomy	27	13	30	26	26	392	378	15·2	19·4
60	F	Cryptogenic cirrhosis	Biopsy, laparotomy	64	51	90	29	48	90	79	80·5	91·1
39	F	Alcoholic cirrhosis	Biopsy	33	9	21	33	50	328	230	31·7	42·3
54	F	Cryptogenic cirrhosis	Biopsy	49	37	92	30	36	214	128	40·4	67·4
Mean (SE)									510 (107)	461 (83)	30·6*	20·4*
Normal value				≤17	≤13	≤17	≥35	22–36				

*Median. $T\frac{1}{2}\beta$ half-time for slow phase of disappearance curve.

638±59 ml min^{-1}, respectively, while $T\frac{1}{2}\beta$ was 16·1±1·5 and 16·4±2·4 minutes, respectively.

In the liver disease patients bile acid clearance varied over a wide range in both the fasting and postprandial states (Table 2) and, by the paired *t* test, there was no significant difference between the two sets of values (mean 397±89 and 379±96 ml min^{-1}, respectively). With $T\frac{1}{2}\beta$ the fasting and postprandial values were again not significantly different (median 30·2 and 34·5 minutes, respectively; paired Wilcoxon test NS). The two patients who died from their liver disease, and who also had the lowest clearance and the highest $T\frac{1}{2}\beta$ values, both showed a fall in clearance and a rise in $T\frac{1}{2}\beta$ after the meal.

In the liver disease patients who were studied after a bile acid load (Table 3), cholylglycine clearance was similar in the fasting and loaded states—namely, 510±107 and 461±83 ml min^{-1}, respectively (paired *t* test NS). There was also no difference in $T\frac{1}{2}\beta$ (median 30·6 and 20·4 minutes, respectively; paired Wilcoxon test NS). The two patients who had the lowest clearance values in the fasting state both showed a rise in clearance after the load.

Pooling all 20 fasting studies in the liver disease patients gave a wide scatter of clearance values from 90 to 1249 ml min^{-1} (mean 454±69). Overall, fasting clearance was not significantly different from that in the seven control subjects (unpaired *t* test $0{\cdot}05<P<0{\cdot}10$), though 11 of the patients had values below the lowest of the controls. In the 10 patients who were studied postprandially clearance was significantly less than in the controls studied postprandially (unpaired *t* test $P<0{\cdot}02$). However, there was considerable overlap. $T\frac{1}{2}\beta$ was significantly prolonged in the liver disease patients compared with the controls, both in the fasting state and postprandially (both $P<0{\cdot}01$ by Wilcoxon test), but in each case there was again considerable overlap.

Discussion

The finding that subjects with healthy livers showed no deterioration of bile acid clearance and $T\frac{1}{2}\beta$ after a meal was expected in view of the large reserve capacity of the normal liver and agrees with the report of LaRusso *et al.* (1978). However, there was also no postprandial deterioration in patients with proven chronic liver disease, except in the two patients with the most severe disease. This confirms the preliminary findings of Thjodleifsson *et al.* (1977), who even found a slight improvement in clearance after a meal in their four patients. It suggests that, except in advanced cirrhosis, the liver has considerable reserve capacity for uptake of bile acids. It also implies that the discriminating power of the bile acid disappearance test is not likely to be improved if the test is performed after a meal.

It can normally be assumed that eating a meal markedly increases the amount of bile acids in circulation by causing the expulsion of concentrated bile from the gallbladder. This assumption cannot safely be made in cirrhotic patients, as they are known to have a high frequency of gallstones (Bouchier, 1969; Nicholas *et al.*, 1972) and poor gallbladder contractility (Turnberg and Grahame, 1970). On ethical and practical grounds it was impossible to obtain radiographic data on all the controls and cirrhotic patients studied and three patients were known to have undergone cholecystectomy. It is possible, therefore, that in some patients bile acid kinetics failed to change postprandially partly because there was an impaired or absent gallbladder response to the meal. In this case, a change should have been obtained in the 'loaded' study, which was designed reliably to increase the amount of bile acid absorbed. No such change occurred, except in the two most severely ill patients.

Taken together, these findings imply that the postprandial rise in serum bile acid concentrations which is generally said to occur even in mild liver disease is not due to hepatic overload. The present study does not point specifically to the responsible mechanism but a plausible theory (Kaye *et al.*, 1973) blames it on portosystemic shunting, which after a meal operates on a greater amount of circulating bile acids. Alternatively, when splanchnic blood flow increases after a meal there could be an increased proportion of portal blood bypassing the liver. Consistent with this emphasis on the portal blood flow is a preliminary report that an oral tolerance test using cholic acid was more impaired in anicteric liver disease than its intravenous counterpart (Gilmore and Thompson, 1978a). It is certainly more logical to present a bile acid to the liver by the portal than the systemic route.

Not only does the intravenous bile acid tolerance test have theoretical shortcomings, but the present findings add to the evidence that it is of doubtful clinical value. Similarly, Gilmore and Thompson (1978b) have found intravenous cholylglycine-^{14}C clearance to be often normal in mild liver disease.

We thank Professor A. E. Read for permission to study his patients and for his active encouragement. K.L. was supported by a grant from the Royal Australasian College of Physicians and Roche Products N.Z. Ltd.

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