Kinetics of ^{14}C-glycocholic acid clearance in normal man and in patients with liver disease

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SUMMARY   The plasma clearance of a tracer dose of ^{14}C-glycocholic acid, and fasting total serum bile acid concentrations were measured in 14 control subjects and in 38 patients with acute and chronic liver disease. In controls plasma clearance was 415 $\pm$ 24 ml min^{-1} m^{-2} (mean $\pm$ SEM), equivalent to a 'first-pass' extraction by the liver of 85 %. Clearance was not significantly different from controls in patients with acute hepatitis or active chronic hepatitis, nor in anicteric patients with primary biliary or alcoholic cirrhosis. Thus bile acid clearance was impaired only in icteric chronic liver disease. In contrast, serum bile acid concentrations were abnormal in all but seven patients, six of whom had active chronic hepatitis in complete biochemical remission. The pattern of plasma disappearance of injected ^{14}C-glycocholic acid was biexponential in controls and patients with liver disease, and computer analysis of the curves suggested that there was significant distribution of bile acid outside the vascular space. The preservation of bile acid clearance in anicteric chronic liver disease confirms that it is dependent more on liver blood flow than on liver cell function.

Up to 50 mmol of bile acids are each day returned to the liver in portal blood in man (La Russo *et al.*, 1974), and yet their concentration in peripheral blood is less than 10-15 μmol l^{-1} (Murphy *et al.*, 1972; Barnes *et al.*, 1975). This suggests that there is an efficient 'first-pass' extraction of bile acids from portal blood. The concentration of bile acids in peripheral blood may be raised in patients with anicteric chronic liver disease (Korman *et al.*, 1974), particularly after a meal (Kaplowitz *et al.*, 1973), suggesting that the hepatic uptake process is impaired early in the progress of liver disease. It has, for instance, been reported that the plasma disappearance rate of an intravenously administered bile acid, either a tracer ^{14}C-nuclide or a small 'cold' dose (La Russo *et al.*, 1975; Hofmann, 1977), may detect this impairment of hepatic bile acid transport and be more sensitive to minor degrees of liver dysfunction than are conventional tests of liver function, including even bromsulphthalein retention. However, it would be surprising if the plasma clearance rate of a tracer dose of a bile acid were a sensitive liver function test when the kinetics of

substances with a similarly high hepatic extraction, such as lignocaine, ethanol, and galactose, are examined. The clearance of these substances, when they are given in small doses so that their elimination follows first-order kinetics, is limited much more by liver blood flow than by liver cell function (Wilkinson and Schenker, 1975; Keiding, 1976).

The aims of this study, therefore, were to determine the kinetics of removal of glycocholic acid from plasma in normal man, to investigate how these may be altered by various acute and chronic liver diseases, and to compare them with concentrations of bile acids in fasting serum.

Methods

All studies were approved by the Ethical Committee of the hospital and by the Medical Research Council Isotopes Advisory Committee.

SUBJECTS

Control subjects
Kinetic studies were performed on six healthy volunteers, aged 23-36 years, and nine control patients, aged 55-81 years, who had no evidence of liver disease nor were taking treatment likely to influence liver function. Fasting serum bile acid concentrations were measured in these 15 and in eight additional control subjects.

Patients with liver disease

Thirty-eight patients with histologically proven liver disease were studied. Their details are shown in Table 1. Two patients with active chronic hepatitis were studied both during biochemical remission (serum aspartate aminotransferase (SGOT) < 2 × raised) and relapse (SGOT > 3 × raised).

TECHNIQUES

Plasma disappearance curves

1-^{14}C-glycine glycocholic acid, specific activity 51 mCi/mmol (Radiochemical Centre, Amersham) was dissolved in normal saline to give a concentration of 1 μCi/ml, passed through a 0·22 μ pore membrane filter, and stored in sealed ampoules. Thin layer chromatography on 0·25 mm silica gel plates (E. Merck, Darmstadt) using n-butanol: acetic acid:water (12:3:5, v/v) solvent confirmed radiopurity > 98%.

Subjects were studied supine after an overnight fast. After an initial blood sample, 5 μCi glycocholic acid was injected intravenously over 15 seconds, and venous blood samples drawn from an indwelling 19G needle in the other arm at 2, 4, 6, 8, 10, 12, 15, 20, 25, 30, 40, 50, 60, 70, 80, and 90 minutes. Samples were centrifuged and 1 ml plasma added to 10 ml liquid scintillator (Unisolve, Koch-Light Laboratories) and counted in an ICN Tracerlab liquid scintillation counter for 30-60 minutes. Correction for sample quenching was by the internal channels ratio method, and results expressed as dpm/ml plasma.

Serum bile acids

Bile acids were extracted from serum on Amberlite XAD-7 (Rohm and Haas [UK] Ltd) using a batch extraction technique (van Berge Henegouwen and Hofmann, 1976). After elution with methanol and evaporation to dryness, the extract was redissolved in 50% aqueous methanol and the bile acid concentration measured fluorometrically after addition of 3αhydroxysteroid dehydrogenase (Sigma Laboratories). Results were expressed in μmol l^{-1}.

ANALYSIS OF DATA

The plasma clearance rate of ^{14}C-glycocholic acid was calculated from the equation

$$\text{Clearance} = \frac{\text{Dose}}{(\text{AUC})_0^\infty} \qquad Eq.\ 1$$

where $(\text{AUC})_0^\infty$ is the area under the graph of concentration (dpm/ml) against time (minutes), with the initial part of the curve extrapolated to time zero and the terminal part to infinity. This area was calculated using the trapezoid rule. The initial volume of distribution (V_d) was calculated from:

$$V_d = \frac{\text{Dose}}{\text{conc}^n \text{ at t} = 0} \qquad Eq.\ 2$$

The concentration/time curve was also analysed by an unweighted iterative non-linear least squares fit programme to fit an equation with two exponential components, of the form

$$C_t = Ae^{-at} + Be^{-bt} \qquad Eq.\ 3$$

where C_t = concentration at time t, A, B are constants and a, b are the slopes or fractional disappearance rates of the two exponentials. Clearance can then be expressed as:

$$\text{Clearance} = \frac{\text{Dose}}{A/a + B/b} \qquad Eq.\ 4$$

Table 1 *Diagnoses and biochemical findings in 38 patients with liver disease*

Histologically confirmed diagnosis	No. of pts.	Sex ratio	Age* (yr)	Albumin g.l^{-1}*	Bilirubin μmol.l^{-1}*	Alk. phos. KAU.dl^{-1}*	SGOT IU l^{-1}*
Acute hepatitis		M F					
Viral	2	2:0	39	43	24	9·5	150
			(27-51)	(39-47)	(14-34)	(6-13)	(110-190)
Alcoholic	4	3:1	45	39	18	13	344
			(31-63)	(34-42)	(14-22)	(8-24)	(155-650)
Active chronic hepatitis							
Without cirrhosis (HBsAg +ve −0)	6	3:3	33	39	16	11	184
			(23-56)	(38-42)	(6-30)	(6-20)	(45-600)
With cirrhosis (HBsAg +ve −3)	7	3:4	39	41	11	14	212
			(23-68)	(36-45)	(6-24)	(7-26)	(30-875)
Alcoholic or cryptogenic cirrhosis							
Alcoholic	7	3:4	55	35	58	16	173
			(49-62)	(29-42)	(10-290)	(6-38)	(50-575)
Cryptogenic	1	0:1	56	40	76	20	90
Primary biliary cirrhosis	11	0:11	59	36	66	67	145
			(37-70)	(31-47)	(10-222)	(18-135)	(60-450)
Normal range				>40	<17	<14	<50

*Mean and range.

These were standardised for body surface area, calculated from height and weight by the formula of Du Bois and Du Bois (1916).

The unpaired Student's *t* test was used to test the significance of any difference between groups of patients.

Results

PLASMA CLEARANCE

Control subjects

The plasma clearance rate of glycocholic acid in healthy volunteers, calculated from Eq. 1, was 451 ± 31 ml.min^{-1}m^{-2} (mean $\pm$ SEM) and in the patient controls 377 ± 33 ml.min^{-1}m^{-2}. These were not significantly different (P > 0.1) and have been combined as the control group (415 ± 24 ml. min^{-1}m^{-2}) in subsequent comparisons with patients with liver disease. Assuming a liver blood flow of 800 ml.min^{-1}m^{-2} and a haematocrit of 0.4, these clearances are equivalent to an hepatic extraction ratio of 0.85 ± 0.19.

The initial volume of distribution of glycocholic acid for the combined control subjects was 2570 ± 218 ml.m^{-2}, there being no significant difference between healthy volunteers and control patients.

Patients with liver disease

The clearances for patients with acute hepatitis, cirrhosis, active chronic hepatitis (ACH), and primary biliary cirrhosis (PBC) are shown in Fig. 1.

Those for patients with acute hepatitis (339 ± 25 ml.min^{-1}m^{-2}) and active chronic hepatitis $\pm$ cirrhosis (375 ± 39 ml.min^{-1}m^{-2}) were not significantly different from control subjects. Clearance rates for patients with alcoholic or cryptogenic cirrhosis (206 ± 45 ml.min^{-1}m^{-2}) and PBC (190 ± 45 ml.min^{-1}m^{-2}) were significantly reduced compared with controls (P < 0.001). However, in patients with anicteric alcoholic and cryptogenic cirrhosis and PBC (serum bilirubin < 20 μmol.l^{-1}) the clearance rates were not significantly different from control subjects (360 ± 47 and 333 ± 33 ml.min^{-1}m^{-2} respectively). There was no significant difference in initial volume of distribution between control subjects and patients with liver disease (Table 2).

Table 2 *Volume of distribution of ^{14}C-glycocholic acid*

Diagnosis	Mean $\pm$ SEM (ml.m^{-2})
Control subjects	2570 ± 218
Acute hepatitis	2420 ± 101*
Active chronic hepatitis	3001 ± 206*
Alcoholic or cryptogenic cirrhosis	2850 ± 135*
Primary biliary cirrhosis	2726 ± 198*

*Not significantly different from control subjects.

COMPUTER ANALYSIS OF DISAPPEARANCE CURVES

In control subjects and in patients with liver disease, the plasma disappearance curve of glycocholic acid was satisfactorily fitted by an equation with two
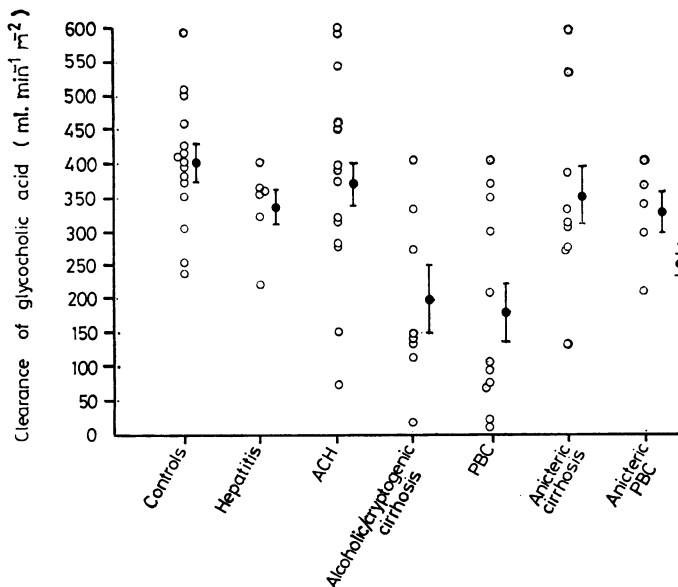

Fig. 1 *Glycocholic acid clearance in control subjects and patients with liver disease.* $\bar{\mathbf{I}}$: *mean $\pm$ SEM.*

exponential components, and was not significantly improved when a third exponential was added. The computer-derived clearances (Eq. 4) correlated closely with those obtained empirically from the area under the curve (Eq. 1) as shown in Fig. 2 ($r = 0.99$).
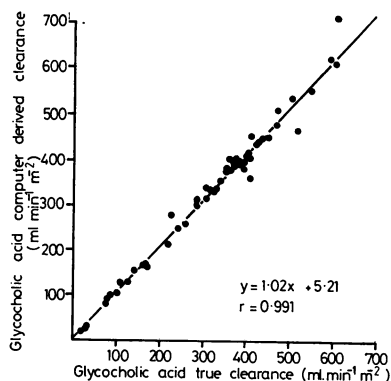

Fig. 2 Comparison between 'true' glycocholic acid clearance, calculated from the area under the time-concentration curve, and the computer-derived clearance.

The fractional disappearance rates of the first and second exponentials are shown in Table 3. In the two groups of patients with impaired plasma clearances—namely, with cirrhosis and PBC—the second exponential component alone was prolonged. The first exponential fractional disappearance rate was never prolonged, even when overall clearance was impaired.

Table 3 *Fractional disappearance rates in control subjects and patients with liver disease*

Diagnosis	Fractional disappearance rate (min^{-1})	
	1st exponential mean ± SEM	2nd exponential mean ± SEM
Control subjects	0·379 ± 0·029	0·058 ± 0·005
Acute hepatitis	0·346 ± 0·033	0·039 ± 0·004
Active chronic hepatitis	0·329 ± 0·034	0·044 ± 0·005
Alcoholic or cryptogenic cirrhosis	0·392 ± 0·094	0·035 ± 0·010*
Primary biliary cirrhosis	0·347 ± 0·038	0·025 ± 0·008†
Anicteric cirrhosis	0·379 ± 0·058	0·044 ± 0·007
Anicteric PBC	0·422 ± 0·060	0·046 ± 0·011
Icteric chronic liver disease	0·330 ± 0·053	0·022 ± 0·007‡

Significantly different from control subjects: *$p < 0.05$
†$p < 0.01$
‡$p < 0.001$

FASTING SERUM BILE ACID CONCENTRATIONS (Fig. 3)

These were measured in 23 control subjects and in 35 of the 38 patients with liver disease. Normal values in control subjects ranged from 1·8-12·3 μmol.l^{-1}. All but seven of the 35 patients with liver disease had raised serum bile acid concentrations, including all patients with PBC and alcoholic/cryptogenic cirrhosis. Six of the seven patients with normal values had ACH in biochemical remission with normal LFTs, and only two of these had progressed to cirrhosis.
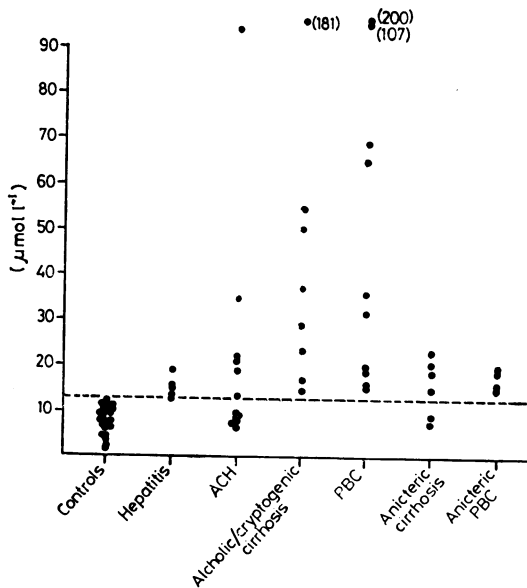

Fig. 3 Total fasting serum bile acid concentration in control subjects and patients with liver disease.

Discussion

A rapid rate of disappearance from plasma of both ^{14}C-labelled and 'cold' glycocholic acid has been previously reported (Cowen et al., 1975; Korman et al., 1975). However, measurements of plasma disappearance rate such as half-life time and percentage retention are influenced by intra- and extravascular distribution, as well as by irreversible hepatic elimination; the use of plasma clearance rate is preferable, as this is influenced neither by changes in blood volume nor by reversible distribution outside the vascular space, factors of particular importance in liver disease. Also, if liver blood flow is known or assumed, the hepatic extraction ratio can be derived from the plasma clearance rate. A rapid clearance of glycocholate with an accompanying

high hepatic extraction has been demonstrated in animals (O'Maille et al., 1967; Hoffman et al., 1975).

Our finding of a normal clearance of glycocholic acid in patients with acute hepatitis and anicteric chronic liver disease is contrary to the view that its plasma disappearance rate is a sensitive test of liver function (La Russo et al., 1975; Hofmann, 1977), but our results are consistent with pharmacokinetic principles (Wilkinson and Schenker, 1975; Keiding, 1976). The clearance of a substance with a high hepatic extraction (or, in enzyme kinetic terms, high V max/Km ratio) is more sensitive to changes in liver blood flow than in parenchymal function. It is difficult to obtain precise measurements of liver blood flow in man, particularly in the presence of liver disease, but it is likely that portal-systemic shunting of blood contributes to the impairment of clearance rate seen in icteric chronic liver disease. Alterations in liver blood flow or in distribution might explain why some investigators have found the test to be discriminating, although Ferguson et al. (1976) and Thjodleifsson et al. (1977) have also recently reported that it is less sensitive than previously thought. In the absence of liver disease the hepatic extraction of glycocholic acid is almost complete in a single passage through the liver, and so its peripheral clearance approximates to liver blood flow and could be used to measure it. When there is severe liver disease, hepatic extraction must also be known before clearance can be used to estimate liver blood flow with certainty, but our results suggest that, at least in anicteric chronic liver disease, peripheral clearance of glycocholic acid does approximate to liver blood flow.

The biexponential shape of the bile acid plasma disappearance curve has been previously noted, but not adequately explained (Kaye et al., 1973; Cowen et al., 1975; Horak et al., 1976). All subjects were fasted, and so enterohepatic recirculation of bile acid during the 90 minute test period is unlikely, and the shape of the curve is unaffected by duodenal intubation and aspiration of bile (Cowen et al., 1975). Two features suggest that the rapid first exponential component is not the result of hepatic uptake alone. Firstly, the initial fractional disappearance rate is normal even in patients with icteric chronic liver disease, in whom plasma clearance and the second exponential component are greatly impaired. Secondly, the initial fractional disappearance rate of 0.38 ± 0.03 in control subjects (equivalent to a half-life time of 1.82 ± 0.17 minutes, in close agreement with 1.67 ± 0.11 minutes found by Cowen et al.) is too rapid to result from hepatic uptake alone. This is because the theoretical upper limit for the initial fractional disappearance rate is when there is complete hepatic extraction and is then equal to the

ratio of liver blood flow to blood volume, approximately 0.30, equivalent to a half-life time of 2.3 minutes. By analogy with the plasma disappearance of many drugs it is likely that the biexponential pattern is produced by simultaneous hepatic uptake and reversible distribution into a peripheral compartment outside the vascular space. Thus bile acid elimination conforms to a pharmacokinetic open two-compartment model.

Our results show that total serum bile acid concentration is a more sensitive test for detecting liver disease than is ^{14}C-glycocholic acid clearance, confirming the recent report of Thjodleifsson et al. (1977). This is also in keeping with pharmacokinetic principles because, in the absence of portal-systemic shunting of blood, the apparent systemic clearance after administration into the *portal* rather than the systemic circulation is independent of liver blood flow and depends solely on liver function. This is surprising, but arises because all portal blood must pass through the liver before reaching the systemic circulation, the site of blood sampling. Clearly, however, factors other than liver cell function also contribute to the concentration of bile acids in blood, including the rate of enterohepatic recirculation and absorption from the intestine, but at present the serum bile acid concentration, particularly after a meal, is probably the most sensitive test for mild liver disease (Kaplowitz et al., 1973). Our results fail to confirm hopes that an intravenous ^{14}C-glycocholic acid clearance test might provide a sensitive test of liver function, but they demonstrate the great efficiency of the hepatic uptake of bile acids in the enterohepatic circulation and show how this function is often well maintained in parenchymal liver disease.

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