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Assessment of the plasma disappearance of cholyl-1^{14}C-glycine as a test of hepatocellular disease

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SUMMARY The plasma disappearance of a tracer dose of cholyl-1^{14}C-glycine has been examined in 12 control subjects and in 32 patients with hepatocellular dysfunction. Simple analysis of the data did not detect hepatic dysfunction except in severe hepatocellular disease. The greatest degree of discrimination between normal subjects and patients with mild liver disease was obtained by taking the ratio of the plasma retention at 60 minutes to that at 10 minutes; it was similar to that obtained with serum γ-glutamyl transferase. The two hour post-prandial plasma 'total' bile acid concentration gave complete separation between the control subjects and patients with liver disease.

Recent studies have shown that the plasma disappearance of intravenously administered cholylglycine could be used as a sensitive test of hepatic dysfunction (Korman *et al.*, 1975; La Russo *et al.*, 1975). Since it was shown that the fraction of the initial dose remaining in the plasma over the first 10 minutes was independent of the dose (2-15 μmol/kg body weight), this suggested that the disappearance of a radioactive tracer dose might be studied. Such an approach would eliminate the need for radioimmunoassay measurements (Simmonds *et al.*, 1973) and raised the possibility of a test based on a single time point measurement (Calcraft *et al.*, 1975).

In this study we have assessed the value of the plasma disappearance of cholyl-1^{14}C-glycine for the detection of hepatic dysfunction, and compared it with the two hour post-prandial plasma 'total' bile acid concentration (Kaplowitz *et al.*, 1973; Barnes *et al.*, 1975), and also serum γ-glutamyl transferase activity, which is claimed to be a sensitive indicator of hepatic dysfunction (Rosalki, 1975).

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Methods

SUBJECTS

The control subjects (12) were laboratory staff and patients with no history of liver disease and with normal liver function tests. The patients with biopsy proven liver disease (32) were classified into three groups with minimal (11), definite (12), or severe (nine) hepatocellular disease, with or without cholestasis. The classification, made by S.S., was based on clinical data, conventional liver function tests (bilirubin, alkaline phosphatase, aspartate transaminase, albumin, and prothrombin time) and liver biopsy, without knowledge of the bile acid test or serum γ-glutamyl transferase activity (Table).

PROCEDURES

Cholyl-1^{14}C-glycine was purchased from the Radiochemical Centre, Amersham, Bucks and purified by thin-layer chromatography (Gregg, 1966), 99% of which migrated as a single spot coincident with authentic cholylglycine. Cholyl-1^{14}C-glycine (10 μCi, 0·2 μmol), which was dissolved in 5 ml saline (0·154 mol/l) and sterilised, was injected into a cubital vein in subjects who had fasted overnight. Blood samples (5 ml) were taken at frequent intervals (zero, two, four, six, eight, 10, 12, 15, 20, 30, 45, and 60 minutes) from the opposite arm through an indwelling catheter. A further blood sample was also taken two hours after lunch for the 'post-prandial' determination of the 'total' bile acid concentration.

In addition, Evans blue (2 mg/kg body weight) was administered simultaneously with the isotope in

Table *Details of liver function tests and clinical diagnoses in patients used in study*

No.	*Sex*	*Age (yr)*	*Wt. (kg)*	*Total bilirubin (μmol/l)*	*Alkaline phosphatase (KA/dl)*	*Aspartate transaminase (IU/l)*	*Albumin (g/l)*	*Diagnosis*
Controls								
1	M	30	64	5	5	11	50	Syphilis
2	F	43	67	17	6	10	45	Normal
3	F	39	57	7	6	14	74	Gastric ulcer
4	F	77	48	5	6	15	41	Angina
5	F	56	69	9	7	9	45	Normal
6	F	35	55	7	4	8	42	Normal
7	M	66	82	12	5	11	50	Normal
8	M	48	60	9	5	12	50	Adrenal tumour
9	M	39	65	7	10	14	51	Normal
10	F	34	55	5	11	6	55	Normal
11	M	28	60	18	5	16	45	Normal
12	M	36	72	15	10	11	—	Normal
Minimal liver disease								
13	F	51	88	5	20	16	47	Primary biliary cirrhosis
14	M	44	74	5	21	7	42	Non-specific hepatitis
15	M	35	61	12	8	15	45	Schistosomal liver disease
16	M	36	58	12	11	23	46	Alcoholic hepatitis (resolving)
17	M	53	73	10	5	12	47	Fatty change
18	F	83	52	14	36	26	35	Pancreatitis, gallstones
19	M	52	79	10	7	31	34	Hydatid disease
20	M	22	68	7	13	9	46	Liver abscesses
21	M	59	67	34	7	16	52	Alcoholic hepatitis
22	M	56	70	17	14	22	42	Intralobular cholestasis, siderosis
23	M	50	60	23	5	11	48	Alcoholic liver disease
Definite liver disease								
24	M	39	60	20	20	54	38	Alcoholic liver disease
25	M	51	89	127	6	16	44	Dubin Johnson/cholestasis
26	F	72	46	5	8	8	37	Polycystic liver disease
27	M	56	59	26	10	72	39	Haemochromatosis
28	M	55	83	22	12	16	52	Budd Chiari
29	M	40	76	3	13	14	50	Alcoholic liver disease
30	M	52	85	9	8	23	47	Alcoholic liver disease
31	M	46	65	68	26	49	—	Primary biliary cirrhosis
32	M	23	71	9	9	71	48	Resolving hepatitis
33	M	38	71	87	110	42	—	Biliary stricture
34	M	37	89	12	28	30	38	Cirrhosis
35	M	68	60	9	12	13	44	Haemochromatosis
Severe liver disease								
36	F	53	59	27	160	106	44	Primary biliary cirrhosis
37	F	49	51	15	9	12	39	Haemochromatosis
38	M	67	59	53	14	57	25	Cryptogenic cirrhosis, shunt
39	M	30	65	15	38	25	40	Cryptogenic cirrhosis
40	M	47	89	39	23	89	44	Active chronic hepatitis
41	M	27	69	63	25	33	35	Alcoholic hepatitis
42	M	41	84	29	14	48	31	Cirrhosis
43	M	46	55	19	9	36	45	Alcoholic cirrhosis
44	M	49	50	10	14	59	46	Haemochromatosis
Normal range				5-17	3-13	4-15	35-50	

four control subjects and in nine patients with liver disease in order to obtain an accurate measure of plasma volume. In a further six subjects the test was carried out twice: in two patients 'cold' cholylglycine was given with the isotope, and in four patients a study was performed 90 minutes after a fatty breakfast. These studies were performed at least one week before the standard study in the fasting state.

Measurement of radioactivity in plasma (1 ml) after its addition to 12 ml NE-260 scintillation fluid (Nuclear Enterprises, Edinburgh, Scotland) was made in a Philips liquid scintillation spectrometer. Smaller amounts of plasma (0·2-0·5 ml) from icteric patients were used to minimise quenching effects. The concentration of Evans blue was determined spectrophotometrically at 610 nm (Gibson and Evans, 1937): a fasting plasma sample obtained before injection served as the plasma blank.

Bile acids were extracted from plasma (1 ml) using the resin XAD-7 (Rohm and Haas (UK) Ltd., Croydon, Surrey) by a batch method (Barnes and Chitranukroh, 1977). The plasma 'total' bile acid concentration was determined by a modification of the fluorimetric 3-hydroxysteroid dehydrogenase method of Murphy *et al.* (1970). Semicarbazide hydrochloride was used instead of hydrazine hydrate as the ketone trapping agent, methanol replaced ethanol, and a freeze dried enzyme (Inter-

national Enzymes Ltd., Windsor, Berks) was employed.

CALCULATIONS

The 'zero time' Evans blue concentration was calculated by extrapolation of the concentration values over the period of the test. The plasma volume was then determined by the ratio of the dose given (mg), to the 'zero time' concentration of Evans blue (mg/l). The mean value (±SEM) determined in the subjects in this study was 51·7 ± 3·2 ml/kg body weight, slightly higher than previously determined values (Lawson, 1962) in control subjects.

The initial volume of distribution (V_1) of cholyl-l^{14}C-glycine was determined after computer fitting of the plasma disappearance data to a double exponential equation, $y = A\ e^{-\alpha t} + B\ e^{-\beta t}$, by applying the formula, $V_1 = \frac{\text{Dose}}{A + B}$ where A and B are the intercepts of each exponential at time (t) = 0.

Results

PLASMA CHOLYLGLYCINE DISAPPEARANCE

The disappearance of cholyl-l^{14}C-glycine from the plasma was very rapid in control subjects and in all groups of patients (Fig. 1). When the results were expressed per litre of plasma only 3-4% of the initial dose remained after the first 10 minutes in control subjects and in patients with minimal and definite hepatic dysfunction. By 60 minutes less than 1% remained. Although increased retention was observed in the patients with severe hepatic dysfunction, nonetheless the 10 minute and 60 minute values were 5·5% and 2·0% respectively.

Since the concentration of radioactivity per litre of plasma is influenced by the total plasma volume, results have been expressed as total plasma retention in order to see if this improved the discrimination. In 13 subjects (four controls, nine patients) the results for total plasma retention at 10 minutes were calculated using the individual's plasma volume, obtained with Evans blue, and compared with those in which the mean plasma volume (51·7 ml/kg body weight) for the group was used. No improvement in discrimination was obtained (Fig. 2). The factor (51·7 ml/kg) has therefore been used to calculate total plasma retention in all subsequent studies.

After 10 minutes the mean total percentage plasma retention was 9·6% for control subjects and 12·9, 11·5, and 17·7% in the three patient groups (minimal, definite, and severe hepatic disease). There was almost complete overlap of data from each of the groups (Fig. 3a). The separation was improved at 60 minutes, the mean total percentage plasma retention for control subjects was 0·52%, and 1·5, 2·8, and

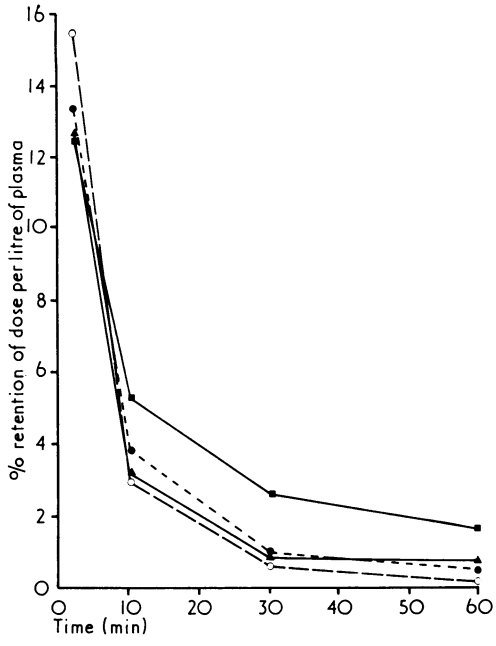

Fig. 1 *Plasma disappearance of intravenously administered cholyl-l^{14}C-glycine (10μCi) expressed as the mean percentage of the initial dose per litre.* ○ - - - ○ *Control subjects. Hepatocellular dysfunction:* ● - - - ● *minimal,* ▲——▲ *definite,* ■——■ *severe.*

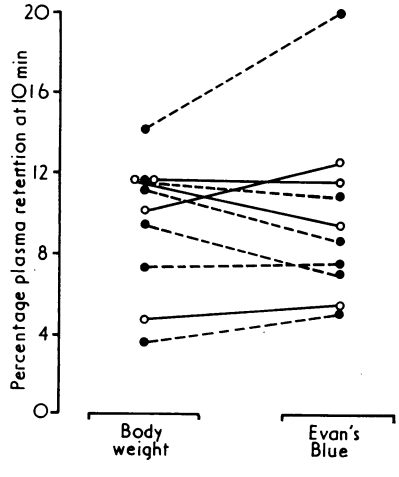

Fig. 2 *Comparison of the results obtained for plasma retention data at 10 minutes after intravenous administration of 10μCi cholyl-l^{14}C-glycine using plasma volume values calculated with a factor based on body weight (51·7 ml/kg); and direct determination using Evans blue. Control subjects* (○), *patients with hepatocellular dysfunction* (●).

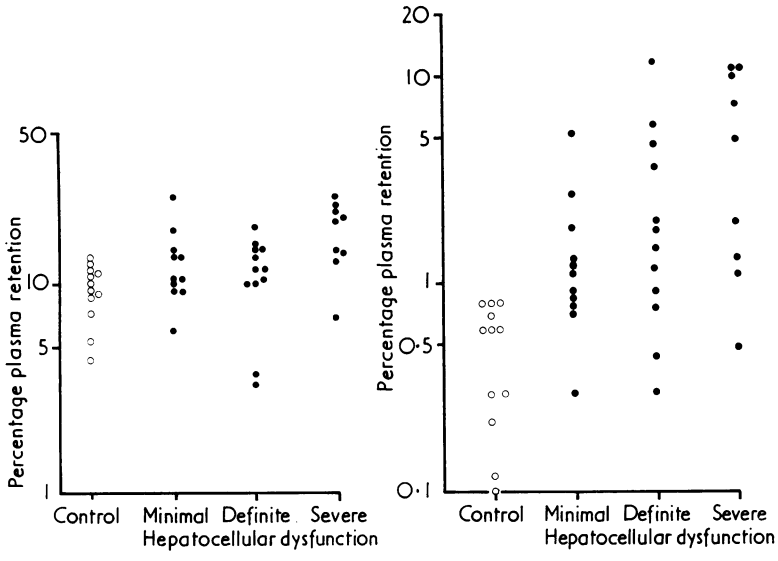

Fig. 3 *Percentage total plasma retention of cholyl-l^{14}C-glycine 10 minutes (a) and 60 minutes (b) after its intravenous administration.*

5·2% for the three groups of patients (Fig. 3b).

Analysis of the plasma disappearance curves indicated a mean initial volume of distribution (V_1) 1·54 times (range 1·00-2·62) greater than the plasma volume. In order to minimise the individual variations of the volume of distribution and deviations in the calculated plasma volume on the basis of body weight, the ratio of the 60 minute value to the 10 minute value was expressed as a percentage. This calculation decreased the overlap of the data from control subjects and patients with liver disease (Fig. 4). In the minimal disease group data from four out of 11 patients fell inside the normal range. A further two in the definite group and one in the severe group were within the normal range.

The effect of a large load of bile acids on the plasma disappearance of cholyl-l^{14}C-glycine was studied in six patients who were also studied with the tracer alone. In two patients the simultaneous administration of cholylglycine (5 μmol/kg body weight) increased plasma retention at 10 minutes (patient 1, 6·0% → 8·3%, patient 2, 10·8% → 11·9%). Studies in four patients 90 minutes after a fatty breakfast, which caused gallbladder contraction, showed a small but significantly decreased retention (mean 8·3%) at 10 minutes compared with results obtained in the fasting state (mean 9·8%, $P < 0.05$, paired Student's *t* test).

PLASMA BILE ACID CONCENTRATIONS

The mean two hour post-prandial concentration was 12·6 μmol/l in control subjects, and 24·2, 44·4, and 112·5 μmol/l in the three groups of patients (Fig. 5). All the patients had values falling outside the normal range. In 16 of 32 patients with hepatocellular disease fasting plasma 'total' bile acid concentrations were within the normal range, al-

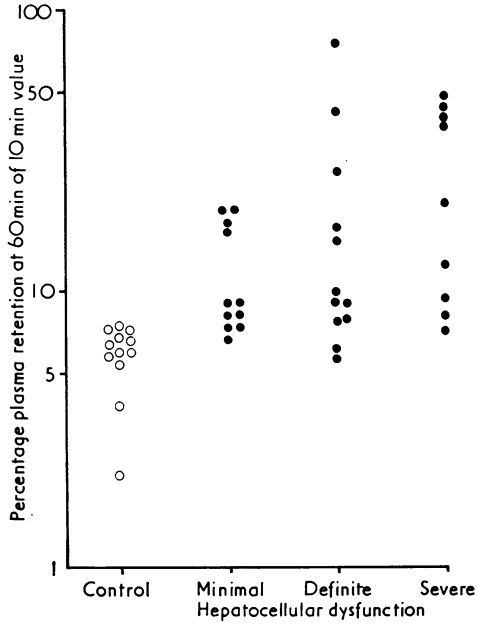

Fig. 4 *Plasma retention of intravenously administered cholyl-l^{14}C-glycine after 60 minutes expressed as a percentage of the retention at 10 minutes.*

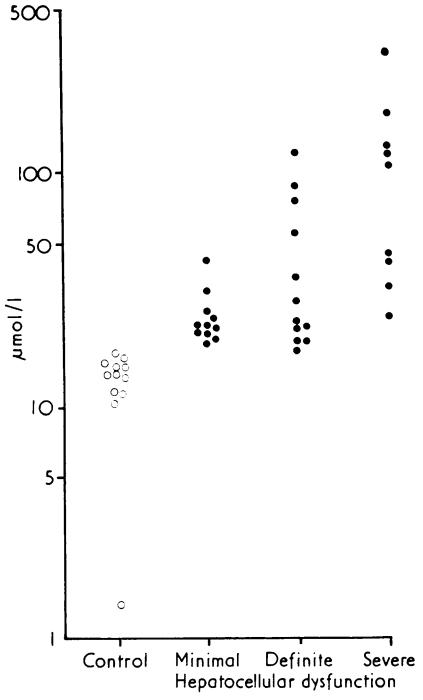

Fig. 5 *Two hour post-prandial total plasma bile acid concentrations.*

though their two hour post-prandial concentrations were abnormal.

SERUM γ-GLUTAMYL TRANSFERASE

The γ-glutamyl transferase activity in the serum was normal (4-18 IU/l females, 6-28 IU/l males) for all the control subjects except one. Of the patients with hepatocellular disease there were three false negatives in the minimal disease group, three in the definite disease group, and two in the severe disease group.

Discussion

This study has shown that the plasma disappearance of a tracer dose of cholyl-l^{14}C-glycine is not a sensitive test of hepatic dysfunction. This is in contrast with the work of LaRusso *et al.* (1975) and Cowen *et al.* (1975a) who showed that the fractional plasma disappearance retention 10 minutes after intravenous administration of 'cold' cholylglycine completely discriminated patients with hepatocellular disease (chronic active hepatitis, primary biliary cirrhosis, and Wilson's disease) from normal subjects.

Since the plasma disappearance of a tracer dose (0·2 μmol), 3·5% of initial dose per litre of plasma remaining after 10 minutes, appears not to be the same as the clearance of the 'cold' cholylglycine (140-1050 μmol/70 kg), less than 0·1% of initial dose per litre of plasma remaining after 10 minutes, studies were repeated in a few patients where additional bile acid loads were received by the liver. In two patients, the simultaneous administration of 'cold' cholylglycine (5 μmol/kg body weight) caused a slightly increased retention in the first 20 minutes. Thus variation in the load (0·2 → 350 μmol/70 kg body weight) did not lead to much alteration of the percentage of the initial dose retained per litre of plasma, as was noted by Korman *et al.* (1975), using the radioimmunoassay method, and by Calcraft *et al.* (1975a) who studied the plasma disappearance of cholyl-^{35}S-taurine. A more likely explanation for the difference in the results obtained in the present investigation and Korman *et al.* (1975) and Cowen *et al.* (1975a) is methodological; radioimmunoassay does not distinguish between endogenous cholylglycine and the exogenous load, whereas use of ^{14}C-labelled cholylglycine does permit this distinction. It is noteworthy that, in a separate study on plasma bile acid disappearance in healthy control subjects (Cowen *et al.*, 1975b), when 24^{14}C-cholylglycine was used, the mean percentage plasma retention of the initial dose (3·4%) after 10 minutes was the same as that observed in the current study.

There is no obvious explanation for the fact that the two hour post-prandial bile acid concentration is a better test for the detection of liver disease (Kaplowitz *et al.*, 1973; Barnes *et al.*, 1975, Schwarz *et al.*, 1975) than measurements based on the plasma disappearance of cholyl-l^{14}C-glycine. As noted by Cowen *et al.* (1975b), the plasma disappearance of cholyl-l^{14}C-glycine is influenced by mixing in the plasma compartment, initial tissue distribution, as well as hepatic uptake. In addition, consideration has to be given to the fact that hepatic blood flow is an important factor in the rapid elimination of cholyl-l^{14}C-glycine from plasma (Gillette, 1971) and may be reduced in liver disease (Leevy and Kiernan, 1973). Indeed, it has been shown by Kaye *et al.* (1973) that portosystemic shunting of blood leads to delayed plasma elimination of 24^{14}C-cholic acid. A possible explanation for the slight increases in the plasma elimination of cholyl-l^{14}C-glycine after a fatty meal compared with fasting state could be increases in portal flow.

Deviation of plasma volume from that predicted on the basis of body weight was potentially a factor affecting the degree of separation between data from control subjects and patients with liver disease. However, correction for this, using individual plasma volumes determined with Evans blue, was not helpful. In order to overcome the problem of the volume

of distribution of the isotope, the fraction of the radioactivity in the plasma after 60 minutes to that which was in the plasma after 10 minutes was calculated. It was assumed that after 10 minutes initial mixing and distribution of the label among the extravascular space, as well as in the plasma, was complete, as the plasma disappearance of cholyl-l^{14}C-glycine over the period from 10 to 60 minutes was monoexponential. This method led to the greatest discrimination between the data from control subjects and patients with liver disease. Nonetheless, four subjects out of 11 in the minimal disease group were in the control range, which made it similar to the data obtained from serum γ-glutamyl transferase activities. Furthermore, the need for precise sampling with respect to time on two occasions and the low level of radioactivity in samples taken at 60 minutes (less than 100 dpm per ml plasma) prevent its use as a test of hepatic dysfunction.

This study has again demonstrated the high degree of sensitivity for detection of hepatic dysfunction of the two hour post-prandial measurement of blood bile acids by 3α-hydroxysteroid dehydrogenase (Barnes *et al.*, 1975; Schwarz *et al.*, 1975); this remains the method of choice in this laboratory for detecting minimal liver disease.

Addendum

Since this study was completed, Ferguson *et al.* (1976) have confirmed our findings.

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