Direct measurement of hepatic extraction of bile acids in subjects with and without liver disease

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Summary

1. The hepatic extraction ratio of 14C-labelled bile acids has been measured directly by hepatic vein catheterization in five patients without liver disease (glycocholic acid, three; cholic acid, two) and in 16 patients with histologically confirmed liver disease (glycocholic acid, seven; cholic acid, nine).

2. After intravenous administration of [14C]-glycocholic acid by bolus injection (two control subjects) or constant infusion (one control subject), directly measured hepatic extraction ratio was 0.91, 0.84 and 0.88, greater than that for indocyanine green. The extraction ratio of [14C]cholic acid in two subjects was 0.72 and 0.70, confirming a lower extraction of the unconjugated bile acid.

3. The hepatic extraction ratio of both bile acids was reduced in patients with chronic liver disease (range 0.07-0.69), although the extraction ratio of glycocholic acid remained normal in one patient with viral hepatitis.

4. Estimates of liver flow calculated from the extraction of [14C]glycocholic acid, but not cholic acid, correlated with those calculated from indocyanine green kinetics, although numbers were small.

5. Measurement of the hepatic extraction of individual bile acids, not previously reported in man, allows a more accurate description of the enterohepatic circulation.

Key words: bile acids, cholic acids, glycocholic acid, liver physiology.

Introduction

Josephson (1939) described the pattern of plasma disappearance of intravenously administered bile acid, which has been used in many reports to detect liver disease (Blum & Spritz, 1966; Kaye, Struthers, Tidball, DeNiro & Kern, 1973; LaRusso, Hoffman, Hofmann & Korman, 1975; Thjodleifsson, Barnes, Chitranukroh, Billing & Sherlock, 1977). Plasma half-life times or percentage retention of the injected bile acid have been used as estimates of hepatic bile acid uptake, but these are at best approximations. More recently, the total volume of plasma cleared per unit time has been measured, and this use of clearance is preferable as it is uninfluenced by changes in distribution volume; with this measurement bile acid uptake has been found to be insensitive for the detection of anicteric liver disease (Gilmore & Thompson, 1978). Even plasma clearance, however, is not a direct reflection of hepatic bile acid uptake; it is influenced by extrahepatic removal, although this is usually small (Ng & Hofmann, 1977).

The present study was designed to measure directly the hepatic extractions of radiolabelled cholic acid and glycocholic acid in normal man, to assess the influence of liver disease on them and to compare them with the extraction of the dye indocyanine green. From plasma disappearance rates in man (Cowen, Korman, Hofmann & Thomas, 1975) and from direct measurements in animals (O'Maille, Richards & Short, 1967, 1969; Hoffman, Donald & Hofmann, 1975; Glasinovic, Dumont, Duval & Erlinger, 1975; Reichen & Paumgartner, 1976; Pries, Sherman, Williams & Hanson, 1979) it has been predicted that bile acids are almost completely extracted from blood in a single passage.
through the liver in healthy man and that extraction is greater for conjugated than unconjugated bile acids. Because of the need for hepatic vein catheterization, the number of subjects without liver disease in the present study is small; nonetheless the study allows these predictions to be tested directly for the first time.

Methods

Preparation of chemicals

\[1^{-14}\text{C}-\text{glycine}\]} Glycocholic acid (specific radioactivity 51 mCi/mmol) and \[24^{-14}\text{C}\}cholic acid (specific radioactivity >50 mCi/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.) were the radionuclides used. Each was dissolved in sodium chloride solution (154 mmol/l saline) to a concentration of 1 $\mu$Ci/ml, passed through a 22 $\mu$m membrane filter and stored in sterile, sealed ampoules. Before use each batch was tested for radiopurity by chromatography of 5 $\mu$l of the above dilution on 0.25 mm silica gel plates (Schleicher and Schüll, Dassel, W. Germany) with butan-1-ol/acetic acid/water solvent (12:3:5, by vol.). All samples were greater than 97% pure and therefore further chromatographic separation was not performed. Indocyanine green was supplied as a freeze-dried powder in sterile ampoules of 25 or 50 mg, and immediately before use it was dissolved in the solvent provided (Hynson, Westcott and Dunning, Baltimore, U.S.A.).

Patients (Tables 1–4)

The studies were approved by the hospital Ethical Committee and, where appropriate, the Department of Health and Social Security Isotopes Advisory Committee, and all patients gave written consent.

Hepatic extraction of \(^{14}\text{C}\}-\text{labelled bile acid was measured in five patients undergoing upper abdominal surgery (Table 2). Three received \([1^{14}\text{C}]\}glycocholic acid and two \([1^{14}\text{C}]\}cholic acid; three of the five also received indocyanine green. None had clinical or biochemical evidence of liver disease, except for one patient with Gilbert's syndrome. She had mild unconjugated hyperbilirubinaemia (25 $\mu$mol/l), but other routine liver function tests, serum total bile acids and liver histology were normal. Four were studied before premedication and surgery, and patient 4 was studied during operation.

Sixteen patients with histologically confirmed liver disease were studied, of whom seven received \([1^{14}\text{C}]\}glycocholic acid and indocyanine green (Table 3) and nine \([1^{14}\text{C}]\}cholic acid and indocyanine green (Table 4). All received 5–20 mg of diazepam intravenously before the pro-

### Table 1. Concentrations of radioactivity of exogenous tracer bile acid and of indocyanine green in a peripheral vein, mesenteric vein, and the aorta

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sampling time (min)</th>
<th>Glycocholic acid (d.p.m./ml)</th>
<th>Indocyanine green (mg/100 ml)</th>
<th>Sampling time (min)</th>
<th>Glycocholic acid (d.p.m./ml)</th>
<th>Indocyanine green (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Peripheral vein</td>
<td>Mesenteric vein</td>
<td></td>
<td>Peripheral vein</td>
<td>Mesenteric vein</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>497</td>
<td>537</td>
<td>*</td>
<td>25</td>
<td>437</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>965</td>
<td>1064</td>
<td>0.253</td>
<td>0.265</td>
<td>22</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>1167</td>
<td>1281</td>
<td>0.372</td>
<td>0.364</td>
<td>21</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>976</td>
<td>988</td>
<td>0.279</td>
<td>0.252</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>672</td>
<td>679</td>
<td>*</td>
<td>*</td>
<td>17</td>
</tr>
</tbody>
</table>

* Not done.

### Table 2. Direct measurement of hepatic bile acid and indocyanine green extraction in patients without liver disease

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Bile acid</th>
<th>Mode of administration</th>
<th>Bile acid extraction ratio (mean ± SD)</th>
<th>Indocyanine green extraction ratio (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>54</td>
<td>M</td>
<td>Gastric ulcer</td>
<td>Glycocholate</td>
<td>Infusion</td>
<td>0.88 ± 0.01</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>38</td>
<td>M</td>
<td>Duodenal ulcer</td>
<td>Glycocholate</td>
<td>Bolus</td>
<td>0.84 ± 0.07</td>
<td>0.71 ± 0.08</td>
</tr>
<tr>
<td>3</td>
<td>65</td>
<td>M</td>
<td>Gallstones</td>
<td>Glycocholate</td>
<td>Bolus</td>
<td>0.91 ± 0.02</td>
<td>0.80 ± 0.02</td>
</tr>
<tr>
<td>4*</td>
<td>36</td>
<td>M</td>
<td>Duodenal ulcer</td>
<td>Cholate</td>
<td>Bolus</td>
<td>0.72 ± 0.04</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>F</td>
<td>Gilbert's syndrome</td>
<td>Cholate</td>
<td>Bolus</td>
<td>0.70 ± 0.04</td>
<td>0.75 ± 0.05</td>
</tr>
</tbody>
</table>

* Studied during laparotomy.
Hepatic bile acid extraction

TABLE 3. Extraction ratio and plasma clearance of glycocholate and indocyanine green in patients with liver disease

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Serum bilirubin (N &lt; 18 µmol/l)</th>
<th>Fasting serum bile acids (N &lt; 12.5 µmol/l)</th>
<th>Glycocholate</th>
<th>Indocyanine green</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Plasma clearance (ml min⁻¹ m⁻²)</td>
<td>Extraction ratio</td>
</tr>
<tr>
<td>1</td>
<td>49</td>
<td>M</td>
<td>Alcoholic cirrhosis</td>
<td>18</td>
<td>15.4</td>
<td>343</td>
<td>0.65</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>F</td>
<td>Alcoholic cirrhosis</td>
<td>20</td>
<td>50.0</td>
<td>158</td>
<td>0.24</td>
</tr>
<tr>
<td>3</td>
<td>53</td>
<td>M</td>
<td>Alcoholic cirrhosis</td>
<td>10</td>
<td>23.2</td>
<td>140</td>
<td>0.36</td>
</tr>
<tr>
<td>4</td>
<td>57</td>
<td>F</td>
<td>Alcoholic cirrhosis</td>
<td>22</td>
<td>29.0</td>
<td>284</td>
<td>0.44</td>
</tr>
<tr>
<td>5</td>
<td>49</td>
<td>M</td>
<td>Active chronic hepatitis</td>
<td>28</td>
<td>28.1</td>
<td>165</td>
<td>0.45</td>
</tr>
<tr>
<td>6</td>
<td>51</td>
<td>M</td>
<td>Viral hepatitis</td>
<td>34</td>
<td>13.3</td>
<td>423</td>
<td>0.87</td>
</tr>
<tr>
<td>7</td>
<td>53</td>
<td>M</td>
<td>Leukaemic infiltration</td>
<td>38</td>
<td>28.7</td>
<td>195</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Procedure and had hepatic venography performed; these may influence liver blood flow (Williams, Zimmon, Thompson & Sherlock, 1964).

Experimental design

Patients were studied supine after an overnight fast. A 7F Cournand catheter was positioned under fluoroscopic guidance in a branch of the right hepatic vein. In patients without liver disease the catheter was introduced percutaneously into the right femoral vein, and in those with liver disease into the right internal jugular vein as part of a diagnostic transvenous liver biopsy (Gilmore, Bradley & Thompson, 1977). Care was taken to position the catheter in the hepatic vein 3-4 cm from the inferior vena cava, to obtain a mixed hepatic venous blood sample, and blood was drawn during quiet respiration to reduce reflux of caval blood into the hepatic veins. Peripheral venous samples were taken from a 19G indwelling needle in an antecubital fossa. After a baseline sample, 10 µCi of [¹⁴C]cholic or [¹⁴C]glycocholic acid and indocyanine green (20 mg/m²) were given by bolus intravenous injection over 15 s into the other arm. In one control subject a constant-rate intravenous infusion was used; [¹⁴C]glycocholic acid was infused (0-23 µCi/min) for 45 min to a total of 10 µCi. Blood samples (5 ml) were drawn simultaneously from the peripheral and hepatic veins at 2, 4, 6, 8, 10, 12 and 15 min. Hepatic vein sampling was discontinued 15-30 min after bolus injection as concentrations became too low to measure accurately, but peripheral samples were continued at 10 min intervals until 90 min, so that plasma clearance could be calculated. The extraction ratio remained constant over the 15 min after injection, with a coefficient of variance always less than 11% and usually less than 6%. Peripheral and hepatic venous blood samples were taken during the last 15 min of the 45 min constant-rate intravenous infusion, and approximation to steady-state concentrations was confirmed by less than 10% variation in plasma concentrations during this time.

Sample analysis

Blood samples were centrifuged, and 1 ml of plasma was added to 10 ml of liquid scintillator (NE 260, Nuclear Enterprises, Edinburgh, Scotland, U.K.). Radioactivity in each sample was measured for 30-60 min in a liquid scintillation counter (NE 8132, Nuclear Enterprises), by using an external standard channel ratio quench correction method. Jaundiced-plasma samples were bleached by exposure to light before radioactivity counting. Counting efficiency was 70-90%, and the relative standard error 0.05-5.6%. Concentration was expressed as d.p.m./ml of plasma. The absorbance of plasma was measured at 800 nm in a spectrophotometer (Unicam SP.600, Pye, Cambridge, U.K.), and the indocyanine green concentration expressed as mg/100 ml of plasma. Serum bile acids were measured fluorimetrically (Murphy, Billing & Baron, 1970).
Bile acid and indocyanine green concentrations in different vessels

Preliminary experiments were undertaken to confirm that the peripheral venous concentration of radiolabelled bile acid approximated to that in the hepatic artery and portal vein. This is necessary to justify the calculation of extraction ratio from concentrations in peripheral and hepatic venous blood.

Five further patients aged 21–73 years (three men and two women) undergoing elective abdominal surgery were studied (Table 1). None had clinical or biochemical evidence of liver disease, and the liver appeared normal at surgery. The reasons for operation were gastrectomy for peptic ulcer (three), closure of colostomy (one) and hemicolectomy for carcinoma of the colon (one). A constant-rate intravenous infusion approximately to steady state was used to avoid differences in concentration resulting from circulation time. At the start of the operation an infusion of \([^{14}\text{C} ]\text{glycocholic acid (0-23–0-33 \mu Ci/min) was started, and in three patients this also contained indocyanine green (0-8 mg min}^{-1} \text{m}^{-2}\). About 15 min later the surgeon took a blood sample (2 ml) from a small mesenteric vein whilst a synchronous sample was taken from a peripheral vein in the arm opposite the infusion. A few minutes later the surgeon took a sample from the aorta just below the coeliac axis, using a fine 23G needle; again a synchronous sample was taken from a peripheral vein. Blood samples were analysed as described above. The concentrations of indocyanine green were almost identical in the three sites. The bile acid concentration in the aortic samples was a mean 5.8% higher than in peripheral venous blood, but this difference was not statistically significant \((P > 0.05)\). The mesenteric vein concentration was 6.1% higher than in the peripheral vein \((P < 0.05)\). Because these differences were small, the peripheral venous concentrations of bile acid and indocyanine green have been taken to approximate the hepatic input concentrations in the hepatic artery and portal vein in subsequent calculations (see the Discussion section).

Calculations

The hepatic extraction ratio \((E)\) was calculated from the plasma concentrations in the hepatic vein and peripheral vein (eqn. 1 and eqn. 2)

\[
E = \frac{\text{inflow concentration} - \text{outflow concentration}}{\text{inflow concentration}}
\]

(1)

\[
E = \frac{\text{peripheral vein} - \text{hepatic vein concentration}}{\text{peripheral vein concentration}}
\]

(2)

Plasma clearance \((C)\) was calculated from \(\text{dose}/(\text{AUC})_{0}^{\infty}\), where \((\text{AUC})_{0}^{\infty}\) is the area under the peripheral concentration–time curve extrapolated to zero and infinity, calculated by the trapezoid rule.

Estimated hepatic blood flow was calculated from:

\[
C = \frac{E(1 - \text{packed cell volume})}{(1 - \text{packed cell volume})}
\]

(3)

Clearances and estimated hepatic blood flow were standardized for body surface area, calculated from height and weight (DuBois & DuBois, 1916). Statistical comparisons were by Student’s \(t\)-test.

Results

Patients without liver disease

The hepatic extraction ratio of \([^{14}\text{C} ]\text{glycocholic acid varied from 0.84 to 0.91 in the three patients studied (Table 2), and an example of the extraction curves is shown in Fig. 1. In patients nos. 2 and 3, extraction of indocyanine green was measured simultaneously and was 11–14% lower. In these two patients the plasma clearances of glycocholic acid and indocyanine green were almost identical in the three sites. The bile acid concentration in the aortic samples was a mean 5.8% higher than in peripheral venous blood, but this difference was not statistically significant \((P > 0.05)\). The mesenteric vein concentration was 6.1% higher than in the peripheral vein \((P < 0.05)\). Because these differences were small, the peripheral venous concentrations of bile acid and indocyanine green have been taken to approximate the hepatic input concentrations in the hepatic artery and portal vein in subsequent calculations (see the Discussion section).

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\[
E = \frac{\text{peripheral vein} - \text{hepatic vein concentration}}{\text{peripheral vein concentration}}
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(2)

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\]

(1)

\[
E = \frac{\text{peripheral vein} - \text{hepatic vein concentration}}{\text{peripheral vein concentration}}
\]

(2)

Plasma clearance \((C)\) was calculated from \(\text{dose}/(\text{AUC})_{0}^{\infty}\), where \((\text{AUC})_{0}^{\infty}\) is the area under the peripheral concentration–time curve extrapolated to zero and infinity, calculated by the trapezoid rule.

Estimated hepatic blood flow was calculated from:

\[
C = \frac{E(1 - \text{packed cell volume})}{(1 - \text{packed cell volume})}
\]

(3)
Hepatic bile acid extraction

Table 4: Extraction ratio and plasma clearance of cholic acid and indocyanine green in patients with liver disease

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Serum bilirubin (N &lt; 17 μmol/l)</th>
<th>Fasting serum bile acids (N &lt; 12.5 μmol/l)</th>
<th>Cholic acid Plasma clearance (ml min⁻¹ m⁻²)</th>
<th>Extraction ratio</th>
<th>Estimated hepatic blood flow (ml min⁻¹ m⁻²)</th>
<th>Indocyanine green Plasma clearance (ml min⁻²)</th>
<th>Extraction ratio</th>
<th>Estimated hepatic blood flow (ml min⁻¹ m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>F</td>
<td>Active chronic hepatitis plus cirrhosis</td>
<td>18</td>
<td>12.6</td>
<td>133</td>
<td>0.37</td>
<td>599</td>
<td>127</td>
<td>0.50</td>
<td>423</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>M</td>
<td>Active chronic hepatitis plus cirrhosis</td>
<td>10</td>
<td>†</td>
<td>185</td>
<td>0.58</td>
<td>541</td>
<td>164</td>
<td>0.78</td>
<td>356</td>
</tr>
<tr>
<td>3</td>
<td>61</td>
<td>F</td>
<td>Active chronic hepatitis plus cirrhosis</td>
<td>270</td>
<td>86</td>
<td>42</td>
<td>0.07</td>
<td>*</td>
<td>38</td>
<td>0.08</td>
<td>*</td>
</tr>
<tr>
<td>4</td>
<td>48</td>
<td>M</td>
<td>Alcoholic cirrhosis</td>
<td>32</td>
<td>19</td>
<td>147</td>
<td>0.29</td>
<td>874</td>
<td>207</td>
<td>0.41</td>
<td>871</td>
</tr>
<tr>
<td>5</td>
<td>52</td>
<td>M</td>
<td>Alcoholic cirrhosis</td>
<td>14</td>
<td>18.8</td>
<td>221</td>
<td>0.54</td>
<td>744</td>
<td>172</td>
<td>0.47</td>
<td>665</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>M</td>
<td>Alcoholic cirrhosis</td>
<td>20</td>
<td>24</td>
<td>266</td>
<td>0.69</td>
<td>622</td>
<td>292</td>
<td>0.71</td>
<td>663</td>
</tr>
<tr>
<td>7</td>
<td>25</td>
<td>F</td>
<td>Active chronic hepatitis plus cirrhosis</td>
<td>9</td>
<td>15.5</td>
<td>134</td>
<td>0.29</td>
<td>770</td>
<td>130</td>
<td>0.38</td>
<td>570</td>
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<tr>
<td>8</td>
<td>37</td>
<td>F</td>
<td>Active chronic hepatitis plus cirrhosis</td>
<td>14</td>
<td>30</td>
<td>140</td>
<td>0.44</td>
<td>550</td>
<td>228</td>
<td>0.52</td>
<td>696</td>
</tr>
<tr>
<td>9</td>
<td>56</td>
<td>M</td>
<td>Alcoholic cirrhosis</td>
<td>9</td>
<td>13.2</td>
<td>199</td>
<td>0.51</td>
<td>685</td>
<td>170</td>
<td>0.58</td>
<td>514</td>
</tr>
</tbody>
</table>

* Estimated hepatic blood flow not calculated, as extraction was < 0.20.
† Not done.

The hepatic extraction ratio of [14C]glucocortic acid during a continuous infusion to steady state (patient no. 1) was similar to that measured after intravenous bolus (patients nos. 2 and 3), [14C]Cholic acid extraction ratio in two patients was 0.72 and 0.70.

Patients with liver disease

The hepatic extraction ratio of [14C]glucocortic acid varied from 0.24 to 0.65 in patients with alcoholic cirrhosis, and was in the normal range at 0.87 in the patient with viral hepatitis (Table 3). In five of seven patients, glucocortic acid extraction was greater than that of indocyanine green, and hepatic blood flows, separately estimated from the extractions and clearances of the two substances, were positively correlated (r = 0.85, P < 0.02).

The hepatic extraction ratio of unconjugated cholic acid varied from 0.07 to 0.69 in nine patients with chronic liver disease (Table 4). Although the hepatic extraction ratios of cholic acid and indocyanine green were positively correlated (r = 0.92, P < 0.01), the correlation between liver blood flows calculated from the kinetics of removal of cholic acid and indocyanine green did not reach statistical significance (r = 0.56, P = 0.14).

Discussion

The results confirm directly in subjects without liver disease what has been previously inferred...
from rapid plasma disappearance curves, namely that glycocholate is almost completely extracted in a single passage through the liver. It was reassuring that hepatic extraction measured in one subject during continuous infusion of $^{14}$C-glycocholate to steady state was similar to that after bolus injection in the other two subjects, because the rapid fall in concentration after a bolus injection could produce errors if the relative timing of the peripheral and hepatic venous samples were not correct because of differences in circulation time. The extraction of unconjugated cholate is lower, as previously reported in the dog (O’Maille et al., 1967) and in keeping with the slower peripheral plasma half-life time in man (Cowen et al., 1975). The extraction of indocyanine green is less than that of glycocholate and similar to that of cholate. Because of the need for hepatic vein catheterization, the numbers of subjects are necessary small, and so a precise ‘normal range’ has not been defined. Therefore caution should be used in drawing general conclusions. Nonetheless, the figures for hepatic extraction ratio agree well with measurements in the dog (O’Maille et al., 1967). Furthermore, the data are important as they have not been reported previously in subjects without liver disease. The two subjects without liver disease in whom cholate extraction was measured were not ideal ‘normals’, as one was studied under general anaesthesia during a laparotomy and the other has constitutional hyperbilirubinaemia (Gilbert’s syndrome); however, the extraction ratios of 0.70 and 0.72 agree with the indirect estimates of a mean cholic acid extraction ratio of 0.77 ± SEM 0.02 previously reported (Gilmore & Thompson, 1980). Indocyanine green extraction was normal in the patient with Gilbert’s syndrome; some of these patients may have prolonged plasma half-life times of indocyanine green (Martin, Vierling, Wolkoff, Scharschmidt, Vergalla, Waggoner & Berk, 1976), although extraction ratios of this dye have not been reported.

The extraction ratios of both cholic acid and glycocholic acid were reduced in patients with chronic liver disease. Extraction was in the normal range, however, in the single patient with viral hepatitis, and was little altered in some of the patients with anicteric chronic liver disease. The elimination of drugs with high hepatic extraction depends more on blood flow than on hepatocellular function (Wilkinson & Shand, 1975), and the plasma clearance of $^{14}$C-glycocholic acid is known to be insensitive for the detection of liver disease (Ferguson, Calcraf, Hofmann & Belobaba, 1976; Gilmore & Thompson, 1978).

Paumgartner, Reichen & Preisig (1974) found the hepatic extraction ratio of $^{14}$C-taurocholate to be 0.12–0.79 in patients with cirrhosis studied before portacaval shunt surgery, but could not determine the extraction in some because of fluctuations in the extraction ratio. They attributed this difficulty to enterohpatic recirculation, although it may have been due to variation in portal–systemic shunting or variable reflux of caval blood into the hepatic veins.

The method used to measure extraction directly by hepatic vein catheterization assumes that the bile acid concentration in peripheral venous blood is equal to the concentration reaching the liver in the hepatic artery and portal vein. The finding that the concentration of tracer bile acid in peripheral venous blood slightly underestimates the concentration in the aorta and mesenteric vein by 5–6% was unexpected. It is unlikely to result from enterohepatic recirculation as it was present within 15 min of the injection, and it affected the aortic as well as the mesenteric vein samples. No occlusion was used to draw the peripheral venous samples; however, the packed cell volume was not measured and so some differences in haemoconcentration of the samples from different sites cannot be excluded. Also, the slightly lower peripheral venous concentration may result from peripheral stagnation or forearm tissue uptake, as has been described for galactose (Tygstrup, 1977). The concentrations of endogenous bile acid are, of course, quite different in hepatic artery and portal vein because of intestinal absorption, but the concentrations encountered in portal venous blood are still many orders of magnitude below those likely to saturate hepatic uptake (Ahlberg, Angelin, Bjorkhem & Einarssson, 1977; Lindblad, Lundholm & Schersten, 1977); therefore uptake is likely to follow first-order kinetics. As the radioisotopic concentrations in the three vessels sampled differ by only 5–6%, the hepatic extraction ratio is reasonably defined by eqn. (1). The simultaneous measurement of bile acid and indocyanine green extraction is justified because indocyanine green does not compete for bile acid uptake (Paumgartner & Reichen, 1975). It is uncertain whether bile acids interfere with indocyanine green transport, but only trace amounts of bile acid have been used.

Although there was a significant correlation between liver blood flows measured by $^{14}$C-glycocholate and indocyanine green extraction, there was sometimes a marked discrepancy in an individual subject. Radioactive exposure from $^{14}$C precludes the repeated use of $^{14}$C-glycocholate, and enterohepatic recirculation prevents its being repeated within a few days. For these reasons, the
Hepatic bile acid extraction


