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Pharmacokinetics and biliary excretion of bromosulphophthalein, [^{3}H]-ouabain and [^{3}H]-taurocholic acid in rats with glycerol-induced acute renal failure

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1 The pharmacokinetics and biliary excretion of bromosulphophthalein (BSP), ouabain and taurocholic acid (TChA) have been studied in rats with glycerol-induced acute renal failure (ARF).

2 In rats with ARF, the hepatic uptake and initial biliary excretion of BSP were decreased. In addition, the rate of BSP conjugation with glutathione by rat liver homogenates was also decreased. This latter change may contribute to the initial decrease in the biliary excretion of BSP.

3 No change was found in the hepatic uptake and biliary excretion of ouabain, but the area under the concentration-time curve was increased and the plasma clearance (Clp) decreased in rats with ARF. This decrease in Clp was not due to reduced renal excretion.

4 The decreased Clp of ouabain in rats with ARF may come from reduced tissue binding and a concomitant decrease in its volume of distribution (Vd).

5 The hepatic handling of TChA appeared unaltered in ARF, but the rate constant for the terminal part of the concentration-time curve (β) was decreased. This change probably resulted from a large increase in Vd in rats with ARF.

6 It is concluded that the decreased uptake of BSP was not due to a non-specific disturbance of hepatocyte function in ARF because the hepatic handling of ouabain and TChA were unaltered.

Introduction

The hepatic transport of endogenous and exogenous substances probably involves a multiplicity of routes both at the initial uptake and final biliary excretion steps. Evidence for this comes largely from studies of competition for transport between pairs of substances. These studies have revealed a number of different routes for organic anions (Alpert *et al.*, 1969; Scharschmidt *et al.*, 1975; Schwenk *et al.*, 1976), cations (Solomon & Schanker, 1963) and uncharged molecules (Kupferberg & Schanker, 1968; Klassen, 1978).

Experimental studies have established that the hepatic uptake of indocyanine green (ICG), an organic anion used to study liver function, is decreased in rats with acute and chronic renal failure (Bowmer *et al.*, 1982a; Yates *et al.*, 1983a,b,c). Moreover, the initial biliary excretion of ICG is decreased, resulting in a delay in the excretion of ICG into bile (Bowmer *et al.*, 1983a). However, little is known about whether these changes in hepatic function are restricted to ICG, or if uptake and biliary excretion of other substances are similarly affected in renal failure.

The purpose of this study was to investigate the effect of glycerol-induced acute renal failure (ARF) on the hepatic uptake and biliary excretion of bromosuphophthalein (BSP), ouabain and taurocholic acid (TChA). BSP and ICG appear to have a common transport route (Scharschmidt *et al.*, 1975; Schwenk *et al.*, 1976); but ouabain and TChA have transport routes separate from each other (Meijer *et al.*, 1976; Klassen, 1978) and from BSP (Schwenk *et al.*, 1976). Ouabain and TChA were also chosen because they are not biotransformed in the rat (Cox *et al.*, 1959; Hoffman *et al.*, 1975). A preliminary account of some of this work has been given (Bowmer *et al.*, 1982b; 1983b).

Methods

Induction of acute renal failure

The method for the production of glycerol-induced ARF has been described in detail elsewhere (Bowmer *et al.*, 1982a). Male Wistar albino rats (250–350 g) were denied access to water for 24 h and ARF was produced by intramuscular injection of 50% v/v glycerol in sterile saline (0.9%w/v NaCl solution), 10 ml kg^{-1}. Control rats were injected with saline, 10 ml kg^{-1}. Both groups of rats were studied 48 h after their respective injections.

Experimental protocol

Rats were anaesthetized with pentobarbitone (60 mg kg^{-1} i.p.): a tracheal cannula was inserted and artificial respiration maintained with a Miniature Ideal Pump (BioScience) (ventilation rate 80 strokes min^{-1}; stroke volume 10 ml kg^{-1}). Cannulae were also inserted into the left jugular vein, right carotid artery and common bile duct. Rectal temperature was maintained at 37°C by means of a heating lamp.

All compounds were dissolved in saline and injected i.v. over 15–20 s. The dose of BSP was 25 mg kg^{-1}. Ouabain was mixed with [^{3}H]-ouabain and administered at a dose of 0.1 mg kg^{-1}; 15 μCi kg^{-1}. Similarly, TChA was mixed with [^{3}H]-TChA and given at 5 mg kg^{-1}; 10 μCi kg^{-1}. Heparinized blood samples (0.1 ml) were removed at suitable times for 70 min with BSP and for 60 min with ouabain and TChA. After each sample was collected, blood was replaced with an equal volume of saline. Bile was collected over 5 or 10 min intervals for 1 h; over 20 min intervals for the second hour and over 30 min intervals for the third hour. Bile volume was measured gravimetrically assuming a density of 1.0 for rat bile.

The urinary excretion of [^{3}H]-ouabain was estimated by collecting urine directly from the bladder of anaesthetized rats as described by Hirom *et al.* (1976). In a separate series of experiments the kinetics of [^{3}H]-ouabain were determined in the absence of any urinary excretion. These experiments were performed in rats whose renal pedicles (renal artery, vein and ureter) were ligated 10 to 15 min before administration of [^{3}H]-ouabain.

Hepatic uptake of [^{3}H]-ouabain in vivo

Slices of liver (30–65 mg) were removed from the left, median and right lobes of anaesthetized rats at 2.5, 5, 7.5, 10 and 20 min after injection of [^{3}H]-ouabain (Meijer *et al.*, 1975). Each slice was blotted, weighed and solubilized in 0.7 ml FisoSolve (Fisons Ltd). The cumulative amount of tissue removed as a percentage of total liver weight was $1.8 \pm 0.2\%$ ($n = 6$) for control rats and $1.8 \pm 0.4\%$ ($n = 6$) for rats with ARF. Meijer *et al.* (1975) found that [^{3}H]-ouabain was taken up uniformly into slices taken at random from different liver lobes. Preliminary experiments confirmed this, so it was assumed that uptake measured in one slice was representative of uptake into the entire liver.

Analysis of bromosulphophthalein in plasma and bile

Samples (50 μl) of plasma and bile were diluted with an appropriate volume of 0.1 M NaOH and the absorbance measured at 575 nm. Plasma samples were also read at 395 nm to correct for haemoglobin contamination ($E_{395} \times 0.093$ = haemoglobin contribution to extinction at 575 nm). The absorption spectrum of the glutathione conjugate of BSP in alkali is almost identical to that of BSP between 500 to 620 nm (Goldstein & Combes, 1966) so the total amount of BSP in bile was measured.

Hepatic glutathione (GSH)

Liver homogenates were prepared by the procedure of Akerboom & Sies (1981) and the fluorometric method of Cohn & Lyle (1966) was used to determine the the GSH content of rat liver.

Hepatic glutathione-S-transferase activity

The spectrophotometric assay of Goldstein & Combes (1966) was used to measure the rate of BSP conjugation with GSH by rat liver homogenates.

Measurement of radioactivity

Radioactivity in aliquots (50 μl) of plasma and bile was measured in a Beckman LS 330 scintillation counter. Samples were counted in plastic insert vials (Sterilin Ltd) using 5 ml of FisoFluor I liquid scintillator (Fisons Ltd). Urine samples (20–200 μl) were counted in plastic scintillation vials (LIP Ltd) with 15 ml of scintillator. Radioactivity in digested pieces of liver was measured after addition of 15 ml of scintillator followed by 0.5 ml 5 M acetic acid. Counting efficiency was assessed by automatic external standard channels ratio and, where appropriate, with internal standards of [^{3}H]-*n*-hexadecane (Amersham International PLC).

Pharmacokinetic calculations

The plasma concentration-time data for BSP were fitted to a biexponential equation by non-linear least squares regression analysis (Snedecor & Cochran, 1967). Data were analysed using a two compartment

model with elimination of BSP from the peripheral compartment (Richards *et al.*, 1959). In this model k_{12} is the apparent first order rate constant for transfer of dye from plasma to liver; k_{21} the rate constant for return of BSP to plasma and k_{23} the rate constant for excretion into bile. These rate constants together with the apparent volume of the central compartment (Vc); the area under the plasma concentration-time curve ($AUC_{0\to\infty}$) and plasma clearance (Clp) were calculated using the equations given by Gibaldi & Perrier (1975). The apparent volume of distribution at steady-state (Vdss) was calculated as:-

$$Vdss = Vc\left(\frac{k_{12}+k_{21}+k_{23}}{k_{21}+k_{23}}\right) \text{ (Bowmer } et\ al.\text{, 1982a)}$$

The disappearance of [^{3}H]-ouabain and [^{3}H]-TChA from plasma was analysed using the 'CSTRIP' programme (Sedman & Wagner, 1976). This indicated that their decay was at least triexponential and so these data were not subjected to compartmental analysis. Instead the kinetics of [^{3}H]-ouabain and [^{3}H]-TChA were described in terms of (1) the rate constant for the terminal part of the concentration-time curve (β); (2) the apparent volume of distribution (Vd) which was calculated from:-

$$Vd = \frac{\text{Dose}}{AUC_{0\to\infty}\beta} \quad \text{(Gibaldi \& Perrier, 1975)}$$

and (3) the plasma clearance (Clp). The area under the plasma concentration-time curve from 0 to 60 min was calculated using the trapezoidal rule and the area under the plasma concentration-time profile from the last observation (Cp_{60}) to infinity was estimated by:-

$$AUC_{60\to\infty} = \frac{Cp_{60}}{\beta}: \quad \text{(Benet \& Galeazzi, 1979)}$$

The $AUC_{0\to\infty}$ was the sum of the two areas.

Results are expressed as mean ± s.d. and statistical comparison was made by the non-paired Student's *t* test.

Materials

BSP and ouabain were obtained from Sigma Chemical Co. and TChA was bought from CP Laboratories Ltd (Bishop Stortford, U.K.). GSH and *o*-phthalaldehyde were obtained from BDH Ltd. [G-^{3}H]-ouabain (37 Ci/mmol) and [G-^{3}H]-TChA (6.6 Ci/mmol), all of stated radioactive purity >97%, were purchased from Amersham International PLC and New England Nuclear Ltd respectively, and were used without further purification.

Results

Intramuscular injection of glycerol resulted in a uraemic state characterized by at least a four fold increase in plasma urea concentration. Mean body weight, wet liver weight and liver weight to body weight ratio were not significantly different between any group of control or uraemic rats used. These results are similar to those previously obtained in our laboratory (Bowmer *et al.*, 1982a; Yates *et al.*, 1983a,b).

Bromosulphophthalein kinetics

Figure 1 shows the mean plasma concentration-time data obtained after i.v. administration of BSP (25 mg kg^{-1}) to control and uraemic rats. Plasma concentrations between 5 to 15 min were significantly elevated ($P<0.05$) in the rats with ARF which suggests that the initial disappearance of BSP was delayed in the uraemic rats. The half-life of this initial disappearance phase, $T_{0.5}\alpha$, was significantly prolonged ($P<0.01$) and the rate constants k_{12} and k_{21} were decreased ($P<0.05$) in uraemic rats (Table 1). There was no significant change in the half-life of the terminal elimination phase, $T_{0.5}\beta$, k_{23}; Vdss or Clp, but Vc was significantly larger ($P<0.05$) in the uraemic rats.

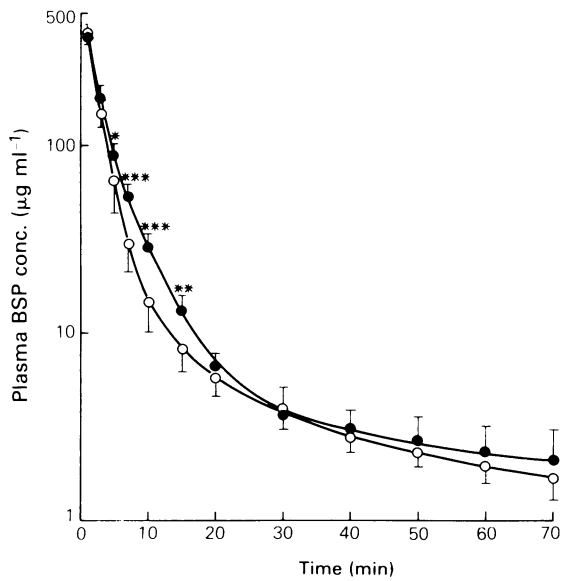

Figure 1 Plasma concentrations of bromosulphophthalein (BSP, 25 mg kg^{-1} i.v.) in control rats (○) and rats with acute renal failure (●). Values are mean ($n=7$); s.d. shown by vertical lines. Significantly different from control vaues: $^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

Table 1 Effect of glycerol-induced acute renal failure on the pharmacokinetics of bromosulphophthalein (BSP, 25 mg kg^{-1} i.v.)

Pharmacokinetic parameters	*Control rats* ($n=7$)	*Uraemic rats* ($n=7$)
$T_{0.5}\alpha$ (min)	1.4 ± 0.2	2.4 ± 0.7**
$T_{0.5}\beta$ (min)	27 ± 10	53 ± 34
k_{12} (min^{-1})	0.49 ± 0.08	0.30 ± 0.07***
k_{21} (min^{-1})	0.0073 ± 0.0033	0.0043 ± 0.0013*
k_{23} (min^{-1})	0.029 ± 0.009	0.020 ± 0.014
Vc (1 kg^{-1})	0.039 ± 0.003	0.066 ± 0.031*
Vp (1 kg^{-1})	0.58 ± 0.18	1.0 ± 0.6
Vd_{ss} (1 kg^{-1})	0.61 ± 0.18	1.1 ± 0.6
Clp (ml min^{-1} kg^{-1} body wt)	15 ± 2	14 ± 3

Results are given as mean ± s.d.
*$P<0.05$; **$P<0.01$; ***$P<0.001$ relative to respective control group.
k_{12} = rate constant for transfer from plasma to liver; k_{21} = rate constant for return of BSP to plasma; k_{23} = rate constant for excretion into bile; Vc = apparent volume of the central compartment; Vp = apparent volume of the peripheral compartment and Vd_{ss} = apparent volume of distribution at steady-state; Clp = plasma clearance.

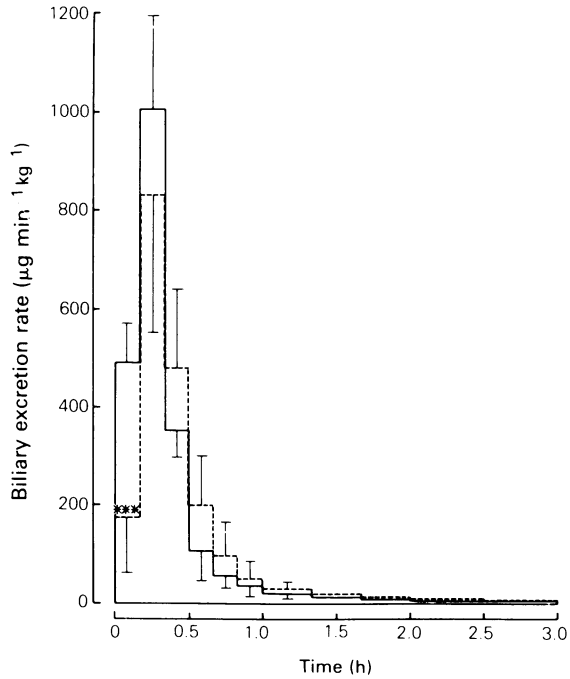

Figure 2 Biliary excretion profile of bromosulphophthalein (BSP, 25 mg kg^{-1} i.v.) in control rats (unbroken line ——) and rats with acute renal failure (broken line----). Values are mean ($n=6$); s.d. shown by vertical lines. Significantly different from control values: ***$P<0.001$.

In uraemic rats the percentage recovery of BSP from bile over 3 h ($80 \pm 9\%$; $n=6$) and overall bile flow rate (3.9 ± 0.9 ml h^{-1} kg^{-1}; $n=6$) were not significantly different from control values ($87 \pm 6\%$ and 4.5 ± 0.9 ml h^{-1} kg^{-1}; $n=6$ respectively). However, the biliary excretion rate during the first 10 min after injection of BSP in uraemic rats (175 ± 115 μg min^{-1} kg^{-1}; $n=6$) was significantly slower ($P<0.001$) than in controls (493 ± 75 μg min^{-1} kg^{-1}; $n=6$) (Figure 2). This initial delay in the biliary excretion of BSP was not caused by decreased bile flow rate at this particular time, because flow rate in rats with ARF (3.3 ± 1.8 ml h^{-1} kg^{-1}; $n=6$) was not significantly different from that in controls (4.2 ± 0.5 ml h^{-1} kg^{-1}; $n=6$). At all other intervals there was no difference in biliary excretion rates between the two groups of rats (Figure 2).

Bromosulphophthalein conjugation

The ability of livers from uraemic rats to conjugate BSP with exogenous GSH was significantly decreased ($P<0.01$). In uraemic rats the *in vitro* glutathione-S-transferase activity was 3.3 ± 0.5 μmol g^{-1} min^{-1} ($n=7$) whereas enzyme activity in controls was 3.9 ± 0.3 μmol g^{-1} min^{-1} ($n=7$). In a separate series of experiments the endogenous GSH content of livers from the uraemic group (3.2 ± 0.5 μmol g^{-1}; $n=8$) tended to be smaller than that in controls (3.7 ± 0.5 μmol g^{-1}; $n=8$). However, this difference was not statistically significant.

Ouabain kinetics

In rats with intact renal pedicles. The disappearance of [^{3}H]-ouabain (0.1 mg kg^{-1} i.v.) from plasma in both control and uraemic rats is shown in Figure 3. At all sample times, mean radioactivity was greater ($P<0.05$) in uraemic than in control plasma. As a result, the $AUC_{0\to\infty}$ was larger ($P<0.02$) in the uraemic group and there was a concomitant decrease ($P<0.02$) in Clp (Table 2). By contrast, no significant change in either β or Vd was observed (Table 2).

Figure 4 shows that between 5 and 20 min following injection of [^{3}H]-ouabain, a significantly greater ($P<0.01$) percentage of the injected dose was found in livers of uraemic rats. However, the percentage of [^{3}H]-ouabain excreted into bile after 1 h was not significantly different between control ($43 \pm 7\%$; $n=6$) and uraemic ($49 \pm 7\%$; $n=6$) rats. In addition,

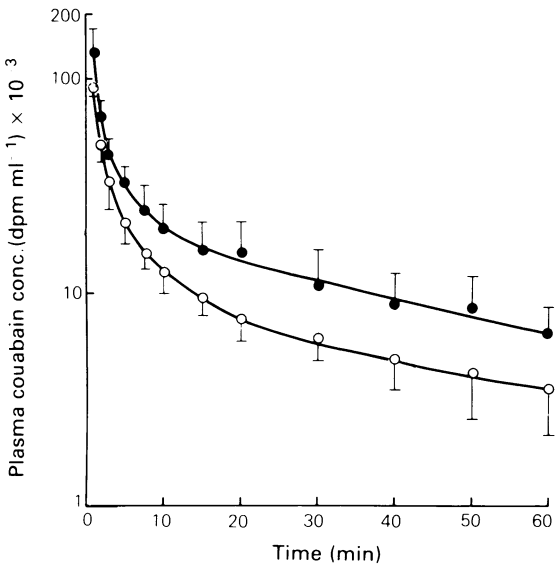

Figure 3 Plasma concentrations of ouabain (0.1 mg kg^{-1}; 15 μCi kg^{-1}) in control rats (○) and rats with acute renal failure (●). Values are mean ($n = 6$); s.d. shown by vertical lines. All concentrations in plasma from uraemic rats were significantly different from control values: $P < 0.05$.

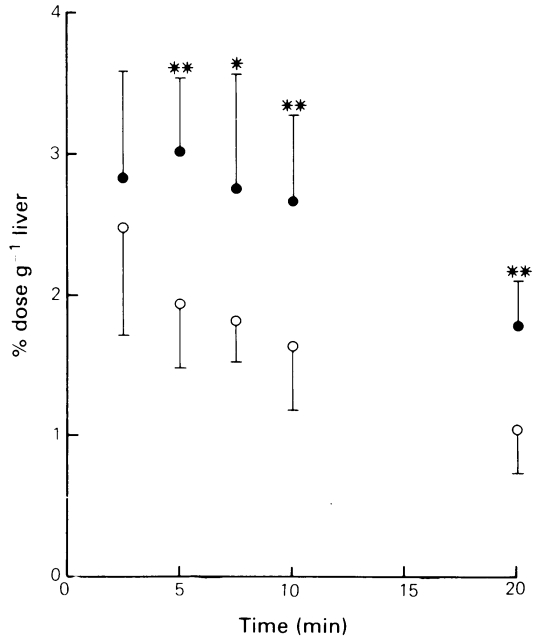

Figure 4 Comparison of the hepatic content of ouabain (0.1 mg kg^{-1}; 15 μCi kg^{-1}; i.v.) in control rats (○) and rats with acute renal failure (●). Values are mean ($n = 6$); s.d. shown by vertical lines. Significantly different from control values: $^{*}P < 0.05$; $^{**}P < 0.01$.

there was no difference in either biliary excretion rate, over any collection interval, or overall bile flow rate between the two groups of animals, so decreased or delayed biliary excretion cannot account for the greater portion of [^{3}H]-ouabain in uraemic livers. In control rats the mean liver to plasma ratio (dpm g^{-1}/dpm ml^{-1}) of [^{3}H]-ouabain between 5 to 20 min was 12 ± 2 ($n = 4$) whilst in uraemic rats its value was 11 ± 2 ($n = 4$). It seems likely, therefore, that the increased levels of ouabain in uraemic livers were a result of correspondingly higher plasma levels.

The percentage of the dose excreted into urine over 1 h was very variable in both groups. In controls the median was 8.0% with a range from 2.4 to 13% and in the uraemic group the median was 1.6% and range from 0.01 to 5.2%. Using the Wilcoxon rank test, the renal excretion of [^{3}H]-ouabain was found to be significantly less ($P < 0.01$) in uraemic rats.

In rats with ligated renal pedicles. These experiments were done to investigate the possibility that decreased renal excretion of ouabain was the cause of

Table 2 Effect of glycerol-induced acute renal failure on the pharmacokinetics of [^{3}H]-ouabain (0.1 mg kg^{-1}; 15 μCi kg^{-1}) in non-ligated and renal pedicle-ligated rats

	$AUC_{0\to\infty}$ (dpm min ml^{-1}) $\times 10^{-6}$	β (min^{-1})	Clp (ml min^{-1} kg^{-1})	Vd (l kg^{-1})
Non-ligated				
Control ($n = 6$)	0.89 ± 0.23	0.020 ± 0.007	35 ± 10	1.8 ± 0.6
Uraemic ($n = 6$)	1.6 ± 0.5**	0.017 ± 0.006	21 ± 6**	1.4 ± 0.4
Ligated				
Control ($n = 4$)	1.5 ± 0.2‡	0.0071 ± 0.0017‡	21 ± 3†	3.0 ± 0.3‡
Uraemic ($n = 4$)	5.2 ± 2.1**‡	0.0035 ± 0.0023*‡	7.6 ± 4.0***‡	2.4 ± 0.3*‡

Results are given as mean ± s.d.
$^{*}P < 0.05$; $^{**}P < 0.02$; $^{***}P < 0.01$ relative to respective control group.
$^{\dagger}P < 0.05$; $^{\ddagger}P < 0.01$ relative to control and uraemic non-ligated rats.

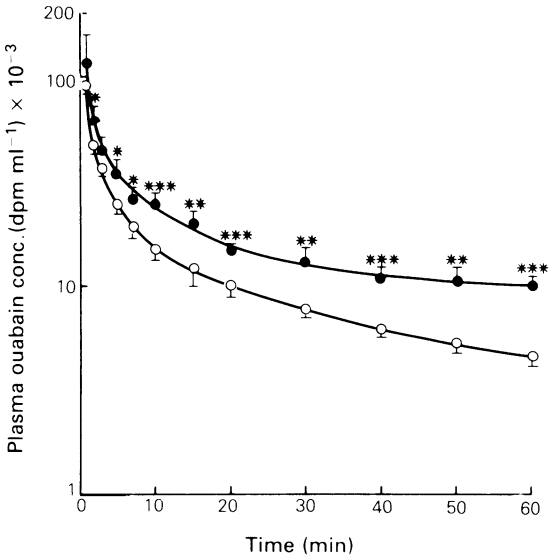

Figure 5 Plasma concentrations of ouabain (0.1 mg kg^{-1}; 15 μCi kg^{-1}) in renal pedicle-ligated control rats (○) and renal pedicle-ligated rats with acute renal failure (●). Values are mean ($n=4$); s.d. shown by vertical lines. With the exception of the 1 and 3 min samples, all concentrations in plasma from rats with acute renal failure were significantly different from control values: $*P<0.05$; $**P<0.01$; $***P<0.001$.

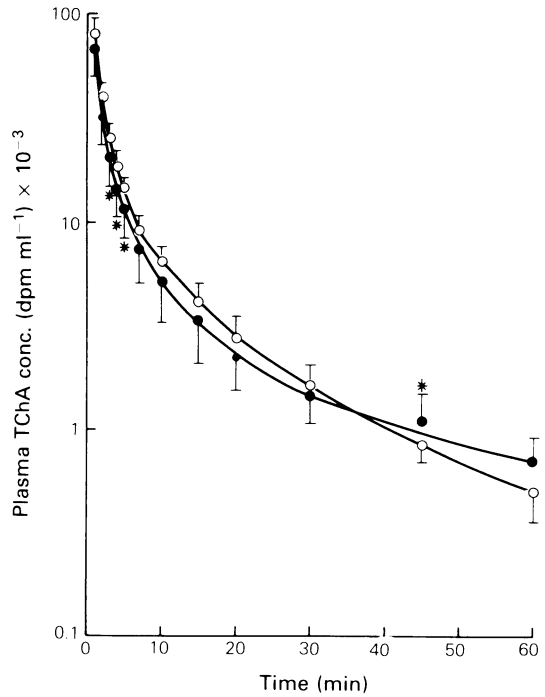

Figure 6 Plasma concentrations of taurocholic acid (TChA, 5 mg kg^{-1}; 10 μCi kg^{-1}) in seven control rats (○) and eight rats with acute renal failure (●). Values are mean; s.d. shown by vertical lines. Significantly different from control values; $*P<0.05$.

increased plasma levels and decreased Clp in uraemic rats. Figure 5 shows the mean radioactivity in plasma from both ligated control and ligated uraemic rats. Except for the 1 and 3 min samples, plasma radioactivity was significantly greater ($P<0.05$) in the ligated uraemic group. The $AUC_{0\to\infty}$ was greater ($P<0.02$) in ligated uraemics; while β, Clp and Vd were all significantly less ($P<0.05$; Table 2). The percentage dose of [^{3}H]-ouabain excreted into bile after 1 h was 52 ± 7 ($n=4$) in ligated controls and 54 ± 5 ($n=4$) in ligated uraemic rats. These recoveries were not significantly different and there was no difference in biliary excretion rates at any time period between ligated control and ligated uraemic rats.

Table 3 Effect of glycerol-induced acute renal failure on the pharmacokinetics of [^{3}H]-taurocholic acid (5 mg kg^{-1}; 10 μCi kg^{-1})

Pharmacokinetic parameters	*Control rats* ($n=7$)	*Uraemic rats* ($n=8$)
$AUC_{0\to\infty}$ (dpm min ml^{-1}) × 10^{-5}	4.1 ± 0.5	3.2 ± 0.8
β (min^{-1})	0.018 ± 0.004	0.012 ± 0.002*
Vd (l kg^{-1})	2.8 ± 0.8	4.4 ± 1.1*
Clp (ml min^{-1} kg^{-1})	48 ± 6	55 ± 18

Results are given as mean ± s.d.
*$P<0.01$ relative to respective control group.

Pedicle ligation itself had a marked effect upon the kinetics of [^{3}H]-ouabain in both control and uraemic rats. In both ligated control and ligated uraemic rats there were significant increases ($P<0.01$) in $AUC_{0\to\infty}$ and Vd and decreases ($P<0.05$) in β and Clp when compared to their respective non-ligated counterparts. It might be anticipated that with negligible renal excretion more [^{3}H]-ouabain would be excreted into bile in both ligated control and ligated uraemic rats. Although the percentages were higher (52 and 54%, respectively) than the corresponding values for non-ligated rats (43 and 49%, respectively), they were not statistically different. Moreover, no differences were found in biliary excretion rates between the respective groups of ligated and non-ligated rats.

Taurocholic acid kinetics

The decline of radioactivity following injection of [^{3}H]-TChA (5 mg kg^{-1}) to control and uraemic rats is

shown in Figure 6. Mean plasma levels of radioactivity were significantly lower ($P<0.05$) between 3 to 5 min in the uraemic group and higher ($P<0.05$) at 45 min than in controls. In the uraemic rats, β was significantly lower ($P<0.01$) and Vd greater ($P<0.01$); but there was no change in Clp (Table 3). There was no difference in biliary excretion rates between control and uraemic rats and no difference after 1 h, in the percentage recovery of [^{3}H]-TChA from bile of control rats ($91\pm5\%$; $n=6$) and uraemic rats ($84\pm6\%$; $n=7$).

Discussion

Compartmental analysis of the disappearance of BSP from plasma demonstrated that in uraemic rats the initial removal of the dye was slowed. As BSP is largely removed from plasma by the liver, slowed removal suggests decreased hepatic uptake in the uraemic group. Furthermore, decreased uptake appears to be associated with an initial delay in the biliary excretion of BSP. These changes in the way in which the liver handles BSP are qualitatively similar to those found previously with ICG (Bowmer *et al.*, 1982a; Yates *et al.*, 1983a,b,c). This is not surprising because there is evidence to suggest that both dyes share the same pathway for hepatic uptake (Scharschmidt *et al.*, 1975; Schwenk *et al.*, 1976). Although uptake was decreased, no change was found in the Clp of BSP in rats with ARF. This may be due to the increase in Vc which would tend to attenuate the effect of the decrease in k_{12} so that no change of Clp occurred in uraemic rats.

By contrast to ICG, BSP is partially conjugated with GSH prior to excretion into bile. Whelan *et al.*, (1970) have shown that the major metabolite, BSP-glutathione, is more efficiently excreted than the parent dye. In addition, conjugation may be a rate limiting step in the overall transfer of BSP from plasma to bile (Whelan *et al.*, 1970). Interference with conjugation either by feeding rats on protein-free diets (Combes, 1965) or by pretreatment with the glutathione-S-transferase inhibitor, benziodarone (Priestley & Plaa, 1969) results in a marked impairment of biliary excretion. In homogenates of livers from uraemic rats, the rate of BSP conjugation with GSH was decreased by about 15%. It is possible, therefore, that part of the initial delay in the biliary excretion of BSP was due to decreased rate of conjugate formation.

Factors other than altered hepatic function are unlikely to have contributed to the decreased uptake of BSP. For example, Vc was increased by about 69% in the uraemic rats, but this change may not have reduced the quantity of dye reaching the liver per unit time because plasma levels of BSP were higher in the uraemic group for a substantial proportion of the initial disappearance phase and total liver blood flow is increased by about 38% in rats 48 h after induction of ARF (Hiley *et al.*, 1980). In common with other highly bound anions (Bowmer & Lindup, 1979) the plasma-protein binding of BSP is decreased in uraemic rats. At an initial concentration of 750 μM, the fraction of BSP bound in diluted uraemic plasma (1:3) is increased almost three fold when compared to diluted plasma from control rats (Bowmer & Yates, unpublished results). Grausz & Schmid (1971) have provided evidence that for BSP, the rate of hepatic uptake is inversely related to the extent of albumin binding, so decreased binding may not contribute to reduced hepatic uptake. However, decreased binding may have contributed to the increase in Vc seen in the uraemic rats. Recent work by Inoue *et al.* (1983) showed that the hepatic uptake of BSP was not impaired in analbuminaemic rats. In these rats, the Clp of BSP was increased which, the authors concluded, was due to a large increase in the Vd of BSP resulting from an absence of albumin binding capacity.

The present study shows that the hepatic uptake and biliary excretion of ouabain are unaltered in uraemic rats. A greater proportion of the dose was present in uraemic livers but this was probably due to increased plasma concentrations of ouabain in the uraemic group. The Clp of ouabain was decreased by about 40% in uraemic rats. Clearly this change was not brought about by altered hepatic elimination and although renal excretion was reduced, this too cannot fully explain the decrease in Clp because Clp was still altered when uraemic rats with ligated pedicles were compared to their respective controls. The most likely explanation is that the apparent Vd of ouabain was smaller in the uraemic rats. Although Vd was some 22% lower in uraemics it was not statistically different from that determined in the control group. However, Vd was significantly lower in the ligated uraemic rats compared to ligated controls.

In patients with renal failure the Vd of digoxin is decreased and is associated with relatively higher plasma concentrations of this drug (Reuning *et al.*, 1973). This change in Vd seems to be related to decreased tissue binding as Jusko & Weintraub (1974) showed that less digoxin accumulated in the myocardium of uraemic patients. It is possible, therefore, that decreased tissue binding was responsible for the higher plasma levels of ouabain in uraemic rats.

There was no change in the Clp of TChA in uraemic rats and this together with the lack of any change in biliary excretion suggests that the hepatic handling of the bile acid is unchanged in ARF. Although β was decreased by about 33% there was a concomitant increase (57%) in Vd which probably accounts for the decrease in β in rats with ARF.

Overall the results suggest that the hepatic uptake and biliary excretion of ouabain and TChA are unaltered in uraemic rats. By contrast, the uptake of BSP was impaired and previous work (Bowmer *et al.*, 1982a;Yates *et al.*, 1983a,b,c) has shown that uptake of ICG is also decreased. TChA and ouabain may be handled differently by the liver from BSP or ICG because there is evidence to suggest that the former have different uptake pathways from the latter (Schwenk *et al.*, 1976). Furthermore, TChA and ouabain may themselves have separate uptake pathways (Meijer *et al.*, 1976; Klassen, 1978). Because there is no general impairment in the hepatic uptake of these compounds it would seem that decreased uptake of BSP and ICG cannot be explained by a non-specific alteration of hepatocyte function in renal failure. Isolated perfused livers from uraemic rats have a decreased ability to remove ICG from the perfusion medium (Yates *et al.*, 1984) which suggests that the metabolites which accumulate in uraemic plasma do not inhibit uptake of ICG. As BSP shares a common uptake pathway (Scharschmidt *et al.*, 1975; Schwenk *et al.*, 1976), this would suggest that the impaired removal of BSP was also not due to inhibition by retained metabolites.

BSP and ICG are known to bind avidly to hepatic cytosol proteins and in particular to ligandin (Levi *et al.*, 1969; Kamisaka *et al.*, 1975; Ketley *et al.*, 1975; Ketterer *et al.*, 1976). A decrease in the binding capacity of these proteins, brought about by either a change in affinity and/or quantity of protein present, might possibly explain the decreased uptake of these dyes. In addition, changes in regard to ligandin may also explain the decreased biotransformation of BSP by homogenates of uraemic livers because ligandin behaves as a glutathione-S-transferase (Habig *et al.*, 1974). If decreased binding capacity of liver cytosol proteins were responsible for impaired uptake of BSP and ICG then it could be predicted that hepatic uptake will be similarly impaired for other substances that bind to these proteins whereas no change in uptake should occur when they do not interact. Ouabain appears to have little affinity for these proteins (Kupferberg & Schanker, 1968; Klassen, 1975) which would be consistent with the lack of any change in the uptake of this drug; but TChA interacts with ligandin (Kamisaka *et al.*, 1975) yet there was no change in the uptake of this bile acid. Furthermore, a number of studies have shown that there is no correlation between the amount of binding proteins in liver cytosol and the rate of hepatic uptake of BSP (Gregus & Klassen, 1982), its non-metabolized analogue dibromosulphophthalein (Meijer *et al.*, 1977) and ICG (Fischer *et al.*, 1978) in the rat. Clearly this evidence, albeit indirect, does not support the idea that impaired uptake results from a decreased binding capacity of liver cytosol proteins in uraemic rats. In order to elucidate the role of cytosol binding proteins in the impaired hepatic uptake of BSP and ICG in uraemic rats, it will be necessary to determine the quantity of these proteins in uraemic livers and to study their binding properties.

The present study adds to our knowledge of liver function in renal failure by demonstrating that the hepatic uptake and initial biliary excretion of BSP are decreased in ARF. The cause of decreased uptake is unclear but it would seem that this change is not due to a gross disturbance of hepatocyte function because the uptake and biliary excretion of ouabain and TChA were unaffected. In addition, these alterations of liver function are not confined to rats with ARF as impaired uptake of BSP has been observed in patients with chronic renal failure (Wernze & Spech, 1971).

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References

AKERBOOM, T.P.M. & SIES, H. (1981). Assay of glutathione, glutathione disulfide and glutathione mixed disulfides in biological samples. In *Methods in Enzymology*, Vol. 77. ed. Jakoby, W.B. pp. 374–375. New York: Academic Press, Inc.

ALPERT, S., MOSHER, M., SHANSKE, A. & ARIAS, I.M. (1969). Multiplicity of hepatic excretory mechanisms for organic anions. *J. gen. Physiol.*, **53,** 238–247.

BENET, L.Z. & GALEAZZI, R.L. (1979). Noncompartmental determination of the steady-state volume of distribution. *J. Pharmac. Sci.*, **68,** 1071–1074.

BOWMER, C.J., EMMERSON, J. & YATES, M.S. (1983a). Delayed biliary excretion of indocyanine green in rats with glycerol-induced acute renal failure. *Biochem. Pharmac.*, **32,** 1641–1642.

BOWMER, C.J., EMMERSON, J. & YATES, M.S. (1983b). Effect of acute renal failure on the elimination of [^{3}H]-taurocholic acid in the rat. *Br. J. Pharmac., Proc. Suppl.*, **79,** 337P.

BOWMER, C.J., HALL, S.T. & YATES, M.S. (1982b). The effect of acute renal failure on the pharmacokinetics of bromosulphophthalein in the rat. *Br. J. Pharmac.*, **75,** 110P.

BOWMER, C.J. & LINDUP, W.E. (1979). Decreased binding of drugs and dyes to plasma proteins from rats with acute renal failure: effects of ureter ligation and intramuscular injection of glycerol. *Br. J. Pharmac.*, **66,** 275–281.

BOWMER, C.J., YATES, M.S. & EMMERSON, J. (1982a). The effect of acute renal failure on the pharmacokinetics of indocyanine green in the rat. *Biochem. Pharmac.*, **31,** 2531–2538.

COMBES, B. (1965). The importance of conjugation with

glutathione for sulfobromophthalein sodium (BSP) transfer from blood to bile. *J. clin. Invest.*, **44,** 1214–1224.

COHN, V.H. & LYLE, J. (1966). A fluorometric assay for glutathione. *Anal. Biochem.* **14,** 434–440.

COX, E., ROXBURGH, G. & WRIGHT, S.E. (1959). The metabolism of ouabain in the rat. *J. Pharm. Pharmac.*, **11,** 535–539.

FISCHER, E., VARGA, F., GREGUS, Z. & GOGL, A. (1978). Bile flow and biliary excretion rate of some organic anions in phenobarbital-pretreated rats. *Digestion*, **17,** 211–220.

GIBALDI, M. & PERRIER, D. (1975). *Pharmacokinetics.* New York: Marcel Dekker Inc.

GOLDSTEIN, J. & COMBES, B. (1966). Spectrophotometric assay of the liver enzyme that catalyzes sulfobromophthalein-glutathione conjugation. *J. lab. Clin. Med.*, **67,** 863–872.

GRAUSZ, H. & SCHMID, R. (1971). Reciprocal relation between plasma albumin level and hepatic sulfobromophthalein removal. *N. Engl. J. Med.*, **284,** 1403–1406.

GREGUS, Z. & KLASSEN, C.D. (1982). Role of ligandin as a binding protein and as an enzyme in the biliary excretion of sulfobromophthalein. *J. Pharmac. exp. Ther.*, **221,** 242–246.

HABIG, W.H., PABST, M.J., FLEISCHNER, G., GATMAITAN, Z., ARIAS, I.M. & JAKOBY, W.B. (1974). The identity of glutathione-S-transferase B with ligandin, a major binding protein of liver. *Proc. natn. Acad. Sci. U.S.A.*, **71,** 3879–3882.

HILEY, C.R., YATES, M.S., ROBERTS, P.J. & BLOOM, A.E. (1980). Alterations in liver blood flow during glycerol-induced acute renal failure in the rat. *Nephron*, **26,** 244–248.

HIROM, P.C., MILBURN, P. & SMITH, R.L. (1976). Bile and urine as complementary pathways for the excretion of foreign organic compounds. *Xenobiotica*, **6,** 55–64.

HOFFMAN, N.E., ISER, J.H. & SMALLWOOD, R.A. (1975). Hepatic bile acid transport: effect of conjugation and position of hydroxyl groups. *Am. J. Physiol.*, **229,** 298–302.

INOUE, M., OKAJIMA, K., NAGASE, S. & MORINO, Y. (1983). Plasma clearance of sulfobromophthalein and its interaction with hepatic binding proteins in normal and analbuminemic rats: Is plasma albumin essential for vectorial transport of organic anions in the liver? *Proc. natn. Acad. Sci. U.S.A.*, **80,** 7654–7658.

JUSKO, W.J. & WEINTRAUB, M. (1974). Myocardial distribution of digoxin and renal function. *Clin. Pharmac. Ther.*, **16,** 449–454.

KAMISAKA, K., LISTOWSKY, I., GATMAITAN, Z. & ARIAS, I.M. (1975). Interaction of bilirubin and other ligands with ligandin. *Biochemistry*, **14,** 2175–2180.

KETLEY, J.N., HABIG, W.H. & JAKOBY, W.B. (1975). Binding of nonsubstrate ligands to the glutathione-S-transferases. *J. biol. Chem.*, **250,** 8670–8673.

KETTERER, B., TIPPING, E., HACKNEY, J.F. & BEALE, D. (1976). A low-molecular-weight protein from rat liver that resembles ligandin in its binding properties. *Biochem. J.*, **155,** 511–521.

KLASSEN, C.D. (1975). Biliary excretion of drugs: Role of ligandin in newborn immaturity and in the action of microsomal enzyme inducers. *J. Pharmac. exp. Ther.*, **195,** 311–319.

KLASSEN, C.D. (1978). Independence of bile acid and ouabain hepatic uptake: studies in the newborn rat. *Proc. Soc. exp. Biol. Med.*, **157,** 66–69.

KUPFERBERG, H.J. & SCHANKER, L.S. (1968). Biliary secretion of ouabain-^{3}H and its uptake by liver slices in the rat. *Am. J. Physiol.*, **214,** 1048–1053.

LEVI, A.J., GATMAITAN, Z. & ARIAS, I.M. (1969). Two hepatic cytoplasmic protein fractions, Y and Z, and their possible role in the hepatic uptake of bilirubin, sulfobromophthalein, and other anions. *J. clin. Invest.*, **48,** 2156–2167.

MEIJER, D.K.F., BOGNAKI, J. & LEVINE, W.G. (1975). Effect of nafenopin (SU-13,437) on liver function. Hepatic uptake and biliary excretion of ouabain in the rat. *Drug Metab. Dispos.*, **3,** 220–225.

MEIJER, D.K.F., VONK, R.J., SCHOLTENS, E.J. & LEVINE, W.G. (1976). The influence of dihydrocholate on hepatic uptake and biliary excretion of ^{3}H-taurocholate and ^{3}H-ouabain. *Drug. Metab. Dispos.*, **4,** 1–7.

MEIJER, D.K.F., VONK, R.J., KEULEMANS, K. & WEITERING, J.G. (1977). Hepatic uptake and biliary excretion of dibromosulfophthalein. Albumin dependence, influence of phenobarbital and nafenopin pretreatment and the role of Y and Z protein. *J. Pharmac. exp. Ther.*, **202,** 8–21.

PRIESTLEY, B.J. AND PLAA, G.L. (1969). Effects of benziodarone on the metabolism and biliary excretion of sulfobromophthalein and related dyes. *Proc. Soc. exp. Biol. Med.*, **132,** 881–885.

REUNING, R.H., SAMS, R.A. & NOTARI, R.E. (1973). Role of pharmacokinetics in drug dosage adjustment.I Pharmacologic effect kinetics and apparent volume of distribution of digoxin *J. Clin. Pharmac.*, **13,** 127–141.

RICHARDS, T.G., TINDALL, V.R. & YOUNG, A. (1959). A modification of the bromosulphthalein liver function test to predict the dye content of the liver and bile. *Clin. Sci.*, **18,** 499–511.

SCHARSCHMIDT, B.F., WAGGONER, J.G. & BERK, P.D. (1975). Hepatic organic anion uptake in the rat. *J. Clin. Invest.*, **56,** 1280–1292.

SCHWENK, M., BURR, R., SCHWARZ, L. & PFAFF, E. (1976). Uptake of bromosulfophthalein by isolated liver cells. *Eur. J. Biochem.*, **64,** 189–197.

SEDMAN, A.J. & WAGNER, J.G. (1976). CSTRIP, a fortran IV computer program for obtaining initial polyexponential parameter estimates. *J. Pharmac. Sci.*, **65,** 1006–1010.

SNEDECOR, G.W. & COCHRAN, W.G. (1967). *Statistical Methods.* 6th ed., pp. 465–467. Ames Iowa, U.S.A.: Iowa State University Press.

SOLOMON, M. & SCHANKER, L.S. (1963). Hepatic transport of organic cations: active uptake of a quaternary ammonium compound procaine amide ethobromide by rat liver slices. *Biochem. Pharmac.*, **12,** 621–626.

WERNZE, H. & SPECH, H.J. (1971). Untersuchungen über Speicherkapazität und Transportmaximum der Leber für Bromosulphthalein bei chronischer Niereninsuffizienz. *Klin. Wschr.*, **49,** 1318–1322.

WHELAN, G., HOCH, J. & COMBES, B. (1970). A direct assessment of the importance of conjugation for biliary transport of sulfobromophthalein sodium. *J. lab. Clin. Med.*, **75,** 542–557.

YATES, M.S., EMMERSON, J. & BOWMER, C.J. (1983a).

Hepatic clearance of indocyanine green during the course of glycerol-induced acute renal failure in the rat. *J. Pharm. Pharmac.*, **35,** 335–338.

YATES, M.S., BOWMER, C.J. & EMMERSON, J. (1983b). The plasma clearance of indocyanine green in rats with acute renal failure: effect of dose and route of administration. *Biochem. Pharmac.*, **32,** 3109–3114.

YATES, M.S., EMMERSON, J. & BOWMER, C. (1983c). Pharmacokinetics of indocyanine green in rats with chronic renal failure. *J. Pharm. Pharmac.*, **35,** 593–594.

YATES, M.S., BOWMER, C.J. & EMMERSON, J. (1984). Effect of acute renal failure on the clearance and biliary excretion of indocyanine green in perfused rat liver. *Biochem. Pharmac.*, **33,** 1695–1696.

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