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Effect of acute renal failure on the disposition and elimination of [^{3}H]*N*-acetyl procainamide ethobromide in the rat

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The effect of glycerol-induced acute renal failure (ARF) on the disposition and elimination of the organic cation [^{3}H]*N*-acetyl procainamide ethobromide (APAEB) was investigated in the rat. In rats with ARF the plasma clearance, rate constant for the terminal portion of the plasma concentration-time curve and apparent volume of distribution were all decreased ($P < 0.01$). Furthermore, the renal clearance of APAEB and the percentage dose excreted in urine were reduced by 85 and 74%, respectively. Decreased renal excretion probably accounted for the altered kinetics of APAEB in ARF because ligation of the renal pedicles of control rats produced changes in the kinetics of APAEB that were similar to those seen in animals with ARF. No change in either the hepatic content of APAEB or its biliary excretion were detected in rats with ARF. Similarly, the hepatic handling of ouabain and taurocholic acid was previously found to be unaltered in ARF; but by contrast, the hepatic uptake and initial biliary excretion of bromosulphophthalein and indocyanine green were decreased (Bowmer & Yates 1984, Br. J. Pharmacol. 83: 773–782). Together these studies indicate that there is a selective impairment of hepato-biliary transport in ARF.

Hepatic uptake and biliary excretion of organic cations are processes which have been shown in the rat to be saturable, energy-dependent and carrier-mediated (Schanker & Solomon 1963; Hwang & Schanker 1973; Eaton & Klaassen 1978; Nakae et al 1980; Neef et al 1984a). In addition, transport pathways for cations appear to be separate from those for anions both at the level of hepatic uptake and biliary excretion (Schanker & Solomon 1963; Hwang & Schanker 1973). Recent studies have shown hepatic uptake to be the rate-determining step in the transfer of cations from plasma to bile, whereas for organic anions biliary excretion is thought to be rate-limiting (Vonk et al 1978; Nakae et al 1980). Hence any change in the rate of catiqn uptake could have a substantial effect upon the plasma clearance, hepatic content and biliary excretion of these substances.

Impaired hepatic uptake and delayed biliary excretion of the cholephilic anions bromosulphophthalein (BSP) and indocyanine green (ICG) have been previously demonstrated in rats with acute renal failure (ARF) (Bowmer et al 1982; Bowmer & Yates 1984). These changes could not be ascribed to a gross derangement of hepatic function because similar studies using the anion taurocholic acid (TCA) and the neutral substance ouabain, which have transport pathways separate from BSP and ICG (Schwenk et al 1976), have shown the hepatic handling of these compounds to be unaltered in rats with ARF (Bowmer & Yates 1984). It is also likely that organic cations have different hepatic transport routes from BSP and ICG (Schanker & Solomon 1963; Hwang & Schanker 1973) so, to characterize further the effect of ARF on liver function we have investigated the kinetics, hepatic content and biliary excretion of the organic cation *N*-acetyl procainamide ethobromide (APAEB).

APAEB has been used previously to study the hepatic handling of organic cations (Hwang & Schanker 1973; Vonk et al 1978; Nakae et al 1980) and it was chosen for use in this study because it undergoes no appreciable biotransformation in the rat (Hwang & Schanker 1973; Neef et al 1984b) and it is thought to be subject to the same hepatic uptake and biliary excretion mechanism as other cations (Hwang & Schanker 1973). In addition, it is relatively easy to label APAEB with tritium.

MATERIALS AND METHODS

Materials

Procainamide ethobromide (PAEB) was a gift from E. R. Squibb, Princeton NJ. [^{3}H]Acetic anhydride (500 mCi mmol^{-1}) and [^{14}C]methoxyinulin (32·9 μCi mg^{-1}) with radioactive purities >98% were purchased from Amersham International PLC and New

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England Nuclear Ltd, respectively. All other chemicals and reagents were available commercially and were of analytical grade.

Synthesis of APAEB and [^{3}H]APAEB

Both labelled and unlabelled APAEB were synthesized from PAEB by acetylation with acetic anhydride (Fieser 1964). Assay of APAEB by the method of Bratton & Marshall (1939) gave no colour reaction indicating complete acetylation of the amine group of PAEB. This observation was further supported by (i) examination of the infrared spectra of samples of PAEB and APAEB and (ii), after hydrolysis of APAEB (Hwang et al 1971), assay by the method of Bratton & Marshall (1939) indicated that >98% of the PAEB had been acetylated.

The specific activity of [^{3}H]APAEB was 31·4 μCi μmol^{-1} and its radioactive purity was found to be >95% by thin layer chromatography using the two solvent systems described later.

Induction of ARF

Methods for the production and assessment of ARF have been described elsewhere (Bowmer et al 1982). Briefly, male Wistar albino rats (270–350 g) were denied access to water for 24 h and ARF was induced by intramuscular injection of 50% v/v glycerol in sterile saline (0·9% w/v NaCl solution), 10 ml kg^{-1}. Control rats were given saline, 10 ml kg^{-1}, and both groups of rats were studied 48 h after their respective injections. Plasma creatinine and urea were used to monitor the severity of ARF.

Experimental procedure

Rats were anaesthetized with pentobarbitone (60 mg kg^{-1} i.p.) and cannulae were inserted into the trachea, left jugular vein, right carotid artery and common bile duct. In studies to estimate the extent of renal excretion and to determine renal clearance the bladder was cannulated instead of the bile duct. Body temperature (rectal) was maintained at 37 °C with a heating lamp.

APAEB was dissolved in saline, mixed with [^{3}H]APAEB and administered i.v. at a dose of 10 mg kg^{-1}, 14·2 μCi kg^{-1}. Heparinized blood samples (0·1 ml) were taken at appropriate times over 90 min and bile was collected over 5 min periods for 20 min and subsequently over 10 min periods for up to 90 min. To estimate the extent of renal excretion, the total output of urine was collected over 90 min.

In some experiments [^{3}H]APAEB was infused over 2 min into the hepatic portal vein. Drug was administered through a cannula attached to the shaft of a 23 gauge needle which was inserted into the portal vein for the duration of the experiment. A separate series of experiments was conducted in which the kinetics of [^{3}H]APAEB, given via the jugular vein, were characterized in the absence of 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]APAEB.

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

To study the initial hepatic uptake of [^{3}H]APAEB, small slices of liver (30–60 mg) were cut from one of the three major lobes at 2·5, 5, 7·5, 10 and 20 min after injection of [^{3}H]APAEB (Meijer et al 1975). Slices were solubilized in 0·7 ml of FisoSolve (Fisons Ltd) and the cumulative amount of tissue taken constituted less than 2% of total liver weight. Preliminary experiments showed: (i) that [^{3}H]APAEB was uniformly taken up into each of the three lobes thus uptake measured in one slice was assumed to represent uptake in whole liver and (ii) that removal of liver slices did not significantly alter either plasma disappearance or biliary excretion of [^{3}H]APAEB over the 20 min duration of the experiment.

Renal clearance studies

Inulin clearance (CL_{IN}) was determined by giving a priming dose of inulin (40 mg i.v.) followed by a constant rate infusion (57 μl min^{-1}) of inulin (4 mg ml^{-1}) and mannitol (6% w/v) dissolved in saline. Non-radioactive inulin was traced with [^{14}C]methoxyinulin such that 1·5 μCi were infused over 90 min. A stabilization period of 40 to 60 min was allowed, then several clearances were measured over 10 min intervals. Urine flow was estimated gravimetrically assuming unit density and arterial blood samples (0·1 ml) were taken at the midpoint of each urine collection period.

The clearance of [^{3}H]APAEB (CL_{APAEB}) was measured in separate experiments. Mannitol alone was infused (57 μl min^{-1}) and when a constant diuresis had been obtained a bolus i.v. dose of [^{3}H]APAEB (10 mg kg^{-1}; 14·2 μCi kg^{-1}) was given. Diuresis was considered constant when urine flow was the same in three successive 10 min collections and this was obtained about 60 min after starting the mannitol infusion. Several clearance values were obtained over 10 min periods.

Measurement of radioactivity

Bile, plasma and urine (50 μl) were assayed for radioactivity using plastic insert vials and 5 ml of

FisoFluor I liquid scintillator (Fisons Ltd). Radioactivity in digested pieces of liver was measured as described previously (Bowmer & Yates 1984). All samples were counted in a Packard 300 C scintillation spectrometer and quench correction was made by external standard channels ratio and where necessary with internal standards.

Thin layer chromatography

Samples of bile and urine were spotted on silica gel plates (0·25 mm thick) and developed using two solvent systems (Hwang et al 1971). System A consisted of n-butanol–acetic acid–water (200:60:170 v/v) and system B contained ethanol–ammonia–water (722:40:380 v/v). Chromatograms were dried, sprayed with 5M HCl and heated at 100 °C for 90 min before spraying with Bratton & Marshall reagents to detect aromatic amino groups.

Pharmacokinetic calculations and statistics

Concentration-time data were initially analysed using the 'CSTRIP' program (Sedman & Wagner 1976) to determine the number of exponential phases present. Subsequently, areas under the plasma concentration-time curves from zero to infinity (AUC) were calculated using the trapezoid rule with appropriate extrapolations using k, the rate constant for the terminal part of the concentration-time curve (Benet & Galeazzi 1979). Plasma clearance (CLp) and apparent volume of distribution (Vd) were calculated from the AUC (Rowland & Tozer 1980).

Results are expressed as mean ± s.e.m. and statistical comparison was made using the non-paired Student's *t*-test. The hepatic content of [^{3}H]APAEB was analysed by one-way analysis of variance and means were compared by the method of least significant difference (Snedecor & Cochran 1967).

RESULTS

Forty-eight hours after injection of glycerol, mean plasma creatinine and urea levels were increased by about 5- and 10-fold, respectively. There were no significant differences between any group of control and uraemic rats in either body weight or liver weight.

In rats with intact renal pedicles

(i) Jugular vein administration. Fig. 1 shows mean concentrations in plasma obtained after i.v. injection of [^{3}H]APAEB to control and uraemic rats. Analysis of these data with the 'CSTRIP' program suggested the presence of three exponential phases in each

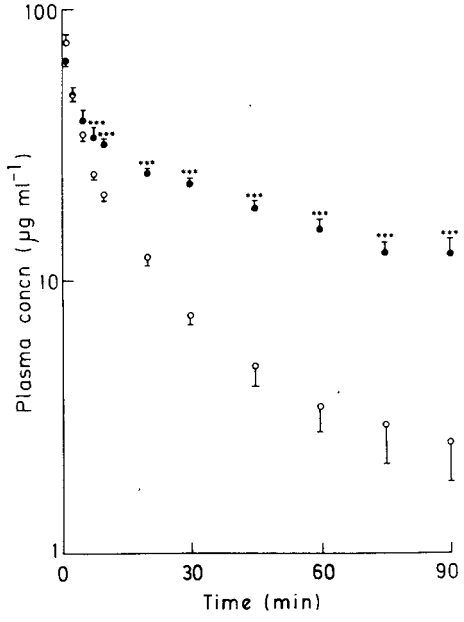

FIG. 1. Plasma concentrations of APAEB after jugular vein administration (10 mg kg^{-1}; 14·2 μCi kg^{-1}) in control rats (○) and rats with acute renal failure (●). Values are mean ± s.e.m. (n = 7). Significantly different from control values, *** $P < 0·001$.

group. Levels of [^{3}H]APAEB in the uraemic group were significantly greater ($P < 0·001$) from 7·5 to 90 min after dosing. The AUC was increased ($P < 0·01$) and there were significant reductions ($P < 0·01$) in CLp, k, and Vd (Table 1). The hepatic content of [^{3}H]APAEB in control and uraemic rats is given in Table 2. From 2·5 to 20 min, accumulation of radioactivity in both control and uraemic livers was slow and within each group, only for the control rats at 20 min was the amount of radioactivity greater ($P < 0·01$) than at 2·5 min. No significant differences were found in the percentage injected dose present in livers from uraemic rats compared to controls up to 20 min after dosing. Biliary excretion rates too were not different up to 30 min (Fig. 2), but thereafter excretion rates in uraemic rats tended to be greater than in controls. However, only from 55 to 90 min were the elevated excretion rates significantly greater ($P < 0·05$) than in control rats. Maximum biliary excretion rates for the controls were achieved between 15 to 25 min after dosing. In the uraemics, excretion rates reached a maximum at about the same time as in controls, but were maintained up to 90 min. Over 90 min the percentage recovery of radioactivity in bile from uraemic rats (16·6 ± 2·0; n= 7) tended to be greater than that in controls (12·1

Table 1. Effect of glycerol-induced acute renal failure on the pharmacokinetics of [^{3}H]APAEB (10 mg kg^{-1}; 14·2 μCi kg^{-1}) in non-ligated (after either jugular or portal administration) and renal pedicle-ligated rats (after jugular administration).

	AUC (μg min ml^{-1})	k (min^{-1})	CLp (ml min^{-1}/100 g b.w.)	Vd (ml/100 g b.w.)
Jugular vein admin.				
Control (n = 7)	1056 ± 160	0·021 ± 0·003	1·34 ± 0·11	72 ± 9·1
Uraemic (n = 7)	3841 ± 672**	0·009 ± 0·002**	0·39 ± 0·04***	41 ± 1·9**
Portal vein admin.				
Control (n = 5)	928 ± 64	0·025 ± 0·004	1·13 ± 0·08	49 ± 6·7
Uraemic (n = 5)	2881 ± 320***	0·011 ± 0·001**	0·37 ± 0·04***	33 ± 2·7†
Pedicle-ligated jugular vein admin.				
Control (n = 6)	3073 ± 224‡‡‡	0·013 ± 0·001‡	0·40 ± 0·02‡‡‡	31 ± 1·7‡‡‡
Uraemic (n = 6)	4481 ± 480*	0·008 ± 0·002*	0·29 ± 0·02**	36 ± 3·3

Results are given as mean ± s.e. mean.
* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$ relative to respective controls.
† $P < 0·05$ relative to non-ligated uraemic rats after jugular vein administration.
‡ $P < 0·05$; ‡‡ $P < 0·01$; ‡‡‡ $P < 0·001$ relative to non-ligated controls after jugular vein administration.

Table 2. Hepatic content of [^{3}H]APAEB after jugular vein administration (10 mg kg^{-1}; 14·2 μCi kg^{-1}) in control and uraemic rats.

Time (min)	Control % dose g^{-1} liver (n = 5)	Uraemic % dose g^{-1} liver (n = 5)
2·5	0·75 ± 0·11†	0·77 ± 0·16
5·0	0·78 ± 0·08	0·76 ± 0·13
7·5	0·82 ± 0·10	0·91 ± 0·21
10·0	0·89 ± 0·07	0·82 ± 0·09
20·0	1·13 ± 0·08*	1·22 ± 0·24

† Results are given as mean ± s.e.m. and the number of animals in parentheses.
* $P < 0·01$ relative to 2·5 min.

± 1·4; n = 7) but the difference was not statistically significant. Bile flow rates in uraemic rats (18 ± 1·5 μl min^{-1}; n = 7) were not significantly different from those in controls (20 ± 1·9 μl min^{-1}; n = 7).

(ii) Portal vein administration. A comparison of kinetic parameters obtained after jugular and portal vein dosage in both control and uraemic rats revealed no change in AUC, CLp and k (Table 1). However, Vd was reduced after portal vein administration in both control and uraemics but only in the latter group was this change statistically significant ($P < 0·05$). In both groups of rats biliary excretion rate was unaffected by portal injection of [^{3}H]APAEB.

Similar differences in kinetic parameters between control and uraemic rats were obtained following portal administration as were seen with these two groups after dosage via the jugular vein (Table 1). There were no significant changes in biliary excretion rates, but the percentage dose excreted, over

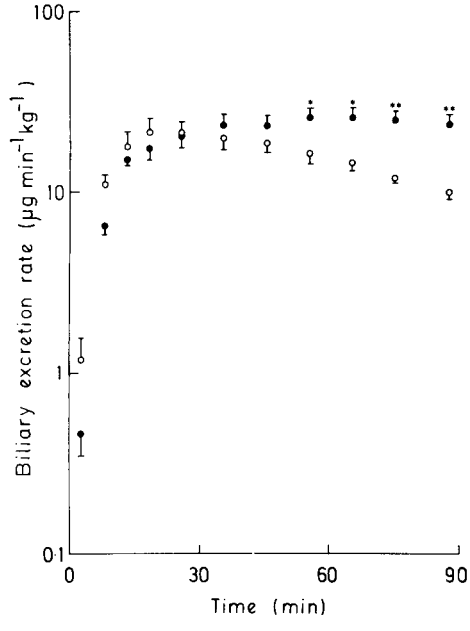

FIG. 2. Biliary excretion profile of APAEB after jugular vein administration (10 mg kg^{-1}; 14·2 μCi kg^{-1}) in control rats (○) and rats with acute renal failure (●). Values are mean ± s.e.m. (n = 7). Significantly different from control values, * $P < 0·05$; ** $P < 0·01$.

90 min, in the bile of uraemic rats (17·4 ± 2·1; n = 5) was greater ($P < 0·05$) than that in the controls (11·7 ± 0·05; n = 5). Again there was no difference in bile flow rates between control (18 ± 1·1 μl min^{-1}; n = 5) and uraemic rats (17 ± 0·8 μl min^{-1}; n = 5).

In rats with ligated renal pedicles

Table 1 shows that ligation of the renal pedicles had a pronounced effect on the pharmacokinetics of

[^{3}H]APAEB. In ligated control rats the AUC increased almost 3-fold and there were decreases ($P < 0.05$) of 70 and 38% in CLp and k, respectively, when compared with non-ligated controls. Vd was reduced by about 57% and this change was also statistically significant ($P < 0.001$). A compensatory rise in the percentage dose excreted into bile over 90 min was observed in control ligated rats (19·7 ± 1·3; n = 6) compared to the non-ligated group (12·1 ± 1·4; n = 7; $P < 0.01$).

No significant changes in kinetic parameters were observed between non-ligated uraemic rats and ligated uraemics (Table 1). Biliary excretion also appeared to be unaffected by renal pedicle ligation. In non-ligated uraemics 16·6 ± 2·0% (n = 7) of the dose entered the bile in 90 min whereas in ligated uraemics 16·0 ± 1·2% (n = 6) of the dose was excreted.

Renal pedicle ligation substantially reduced the differences in kinetic parameters between control and uraemic rats; but CLp and k were still significantly reduced (Table 1). Furthermore, no differences in either percentage dose excreted into bile or biliary excretion rates were found between ligated control and ligated uraemic rats. Bile flow rates estimated over 90 min were not significantly different between these groups of rats. Flow in ligated controls was 20 ± 1·5 μl min^{-1} (n = 6) and 17 ± 2·0 μl min^{-1} (n = 6) in ligated uraemics.

Urinary excretion and renal clearance

The percentage of the dose of [^{3}H]APAEB excreted in urine over 90 min was significantly lower ($P < 0.001$) in uraemic rats (12 ± 5·5; n = 4) than in control rats (46 ± 1·5; n = 4). In addition, Table 3 shows that the mean renal clearances of [^{3}H]APAEB and [^{14}C]methoxyinulin were reduced by about 85 and 95%, respectively, in uraemic animals. Urine flow rate under mannitol diuresis, however, was not significantly different between these groups of rats. The clearance ratio of CL_{APAEB} to CL_{IN} was about 1·6 in control rats, but in uraemic rats this ratio increased to 4·6.

Table 3. Effect of glycerol-induced acute renal failure on the renal clearances of [^{14}C]methoxyinulin (CL_{IN}) and [^{3}H]APAEB (CL_{APAEB}).

	Control (n = 5)	Uraemic (n = 5)
CL_{IN} (ml min^{-1}/100 g b.w.)	1·00 ± 0·05†	0·05 ± 0·01***
Urine flow (ml min^{-1})	0·03 ± 0·01	0·03 ± 0·01
CL_{APAEB} (ml min^{-1}/100 g b.w.)	1·56 ± 0·12	0·23 ± 0·12***
Urine flow (ml min^{-1})	0·04 ± 0·01	0·03 ± 0·01

† Results are given as mean ± s.e.m. CL_{IN} and CL_{APAEB} were determined in separate groups of rats after jugular vein administration.
*** $P < 0.001$ relative to respective controls.

Metabolic stability of [^{3}H]APAEB

When samples of urine or bile from rats which had been injected with [^{3}H]APAEB were chromatographed, single violet spots with R_F values of 0·32 (Solvent A) and 0·14 (Solvent B) were obtained. These R_Fs were identical to those of authentic [^{3}H]APAEB which had been dissolved in these biological fluids.

DISCUSSION

The disposition and elimination of [^{3}H]APAEB appears to be a complex process in both control and uraemic rats. Analysis of the concentration-time data by the method of Sedman & Wagner (1976) suggested that three exponential components were present. The initial disposition phase in controls was fast (Fig. 1) and distribution appeared to be extensive as the Vd (0·72 litre kg^{-1}; Table 1) was similar to the total body water of the rats used (0·69 litre kg^{-1}; Spector 1956). The relatively large Vd probably results from accumulation of [^{3}H]APAEB by the excretory organs, particularly by the kidneys (Neef et al 1984b). This is further supported by the decrease in Vd seen in the uraemic rats and pedicle-ligated control rats. Administration of [^{3}H]APAEB via the portal vein had little effect upon its kinetics and the AUCs obtained after both jugular and portal injection were similar suggesting that the hepatic extraction ratio of this compound is small. Thus it is unlikely that the rate of removal by the liver is limited by hepatic blood flow (Rowland & Tozer 1980).

[^{3}H]APAEB is excreted into both bile and urine and like others (Hwang & Schanker 1973; Neef et al 1984b) we were unable to detect the presence of any metabolites in these body fluids. Renal excretion was the main route of elimination since in control rats 46% of the dose was cleared by this route in 90 min whereas only 12% appeared in the bile during the same time. Furthermore, ligation of the renal pedicles had a pronounced effect on the kinetics of [^{3}H]APAEB in control animals. The renal clearance of [^{3}H]APAEB exceeded that of [^{14}C]methoxyinulin in control rats which, in the absence of significant plasma protein binding (Hwang & Schanker 1973),

suggests that active transport is involved in its renal elimination. Neef et al (1984b) have also implicated the involvement of active transport in the renal excretion of monoquaternary ammonium compounds such as APAEB.

In uraemic rats, CLp, k and Vd were all decreased. These changes are unlikely to be explained by reduced hepatic elimination because firstly, the hepatic content of [^{3}H]APAEB was not significantly altered, secondly, there was no decrement of biliary excretion rate in rats with ARF and thirdly, ligation of the renal pedicles of control rats produced changes in the kinetics of [^{3}H]APAEB that were similar to those observed between control and uraemic non-ligated animals. The lack of any effect of ARF on both hepatic content and biliary excretion is significant because hepatic uptake is thought to be the rate-limiting step in the overall transport of APAEB from plasma to bile (Vonk et al 1978; Nakae et al 1980). Changes, therefore, in the rate of uptake should induce alterations of both hepatic content and biliary excretion rate. In view of the evidence presented above it would seem more unlikely that changes in renal excretion were responsible for the altered kinetics of [^{3}H]APAEB in rats with ARF.

The renal clearance of both [^{3}H]APAEB and [^{14}C]methoxyinulin were both substantially reduced in uraemic animals. Mean urine flow rates, during mannitol diuresis, were not significantly different between control and uraemic rats and, in addition, flow rates in both groups of rats were compatible with those required to elicit flow rate independent values of CL_{IN} (Barber & Bourne 1971). The reduction of CL_{IN} suggests a marked fall in glomerular filtration rate (GFR). In ARF however, significant backleakage of [^{14}C]methoxyinulin across damaged renal tubules could occur (Stein et al 1978) so that CL_{IN} may underestimate GFR. It is possible that both glomerular filtration and tubular secretion of [^{3}H]APAEB were reduced in uraemic rats. However, the clearance ratio increased from 1·6 in controls to 4·6 in uraemics which suggests that filtration was diminished more in ARF than the secretory component.

The quantity of [^{3}H]APAEB excreted in urine over 90 min was markedly reduced in rats with ARF, yet in uraemic rats after both jugular and portal dosage the recovery of radioactivity from bile was only 1·4- and 1·5-fold greater than the respective recoveries in control animals. Even in controls prevention of urinary excretion by ligation of renal pedicles produced only a 1·6-fold increase in overall biliary excretion. Thus in spite of increased availability of APAEB to the liver in rats with ARF and in rats with ligated pedicles, little compensatory increase occurred, over 90 min, in the amount excreted in bile. This is consistent with the work of Hirom et al (1976) who found that for compounds with molecular weights less than 350 (APAEB is 308) and which were eliminated unchanged mainly in urine, prevention of renal excretion by ligating the renal pedicles resulted in only a small increase in biliary excretion.

Previous work with the organic anions BSP and ICG showed that there was a defect in the hepatic uptake of these cholephiles in rats with renal failure and that this resulted in a delay in their appearance in bile (Bowmer et al 1982; Bowmer & Yates 1984). By contrast, the hepatic handling of the neutral glycoside ouabain and the bile acid TCA was unaltered in ARF (Bowmer & Yates 1984). Both these compounds are believed to have hepatic transport systems that are discrete from that shared by BSP and ICG (Schwenk et al 1976). Indeed, ouabain and TCA may have transport routes separate from each other (Meijer et al 1976; Klaassen 1978). The present study suggests that the hepatic transport of the organic cation [^{3}H]APAEB is not perturbed in ARF and it too seems to be handled by the liver in a manner different from that of organic anions such as BSP (Hwang & Schanker 1973). This lends additional support to the conclusion reached by Bowmer & Yates (1984) that the excretory function of the liver is not grossly impaired in renal failure, but rather some transport pathways, for example that utilized by BSP and ICG, seem susceptible to derangement in renal failure. The biochemical basis for this selectivity is unknown but it is currently under investigation.

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