The Role of Tubular Reabsorption in the Renal Excretion of Bile Acids

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1. The renal excretion of bile acids was studied in an isolated rat kidney preparation perfused with a protein-free medium. 2. Tubular reabsorption exceeded 95% for both non-sulphated and sulphated bile acids at filtered loads of less than 30 nmol/min. 3. At low filtered loads the reabsorption of taurocholate and taurochenodeoxycholate was almost complete. Efficient reabsorption of taurochenodeoxycholate was maintained over a wider range of filtered loads than for taurocholate. These observations suggest that active transport may occur. 4. At high filtered loads saturation of reabsorption of taurocholate and taurochenodeoxycholate did not occur, which indicates that passive diffusion is involved in reabsorption. 5. Active proximal-tubular secretion of bile acids was not demonstrated in competition experiments with *p*-aminohippurate. 6. The fractional reabsorption of taurocholate, chenodeoxycholate 3,7-disulphate and chenodeoxycholate 7-monosulphate was decreased by the addition of taurochenodeoxycholate to the perfusate, so that their renal excretion was enhanced. This interaction between the bile acids for reabsorption may explain the different composition of bile acids in urine compared with that in plasma in cholestasis in man. 7. Conjugated bilirubin decreased the fractional reabsorption of both taurocholate and taurochenodeoxycholate at low filtered loads (less than 30 nmol/min) but not at high filtered loads (400 nmol/min).

Since bile acids are only found in measurable amounts in the urine of patients with liver disease who have markedly elevated plasma bile acid concentrations, it has been thought that the kidney does not play an important role in the metabolism and circulation of bile acids. However, studies in man have shown that the bile acid sulphates (mainly of chenodeoxycholate conjugates) are present in much greater proportion in the urine than in the plasma (Stiehl, 1974; Makino *et al.*, 1975; Stiehl *et al.*, 1975). In addition, Summerfield *et al.* (1977) found that the proportion of cholate conjugates compared with non-sulphated chenodeoxycholate conjugates is seven times as great in the urine as in the plasma. Estimation of the total amount of bile acid filtered by the kidney each day suggested that an efficient tubular reabsorption process occurs, whose properties could well explain the altered bile acid composition in urine.

Since systematic investigations were not possible in man, an animal model was used. Reabsorption of bile acids by the kidney has been reported in dogs (Weiner *et al.*, 1964; Zins & Weiner, 1968). However,

these studies in intact animals had two major limitations; firstly, efficient hepatic extraction of bile acids required continuous intravenous infusion of bile acids, which did not give stable plasma concentrations; secondly, because of extensive plasma protein binding of bile acids, measurement of the non-protein-bound concentration was necessary. To overcome these problems an isolated rat kidney preparation perfused with a recirculating protein-free dextran medium has been used in the present study (Gollan & Billing, 1975). The investigation was designed to examine the renal excretion mechanisms of various bile acids and their interrelation with respect to reabsorption, so as to provide possible explanations for the bile acid composition in human cholestatic urine. Preliminary reports of these studies have been published elsewhere (Barnes *et al.*, 1976a,b).

Materials and Methods

Reagents

The ^{51}Cr -EDTA (specific radioactivity 1 mCi/mg), sodium *o*-[^{125}I]iodohippurate (specific radioactivity 50 $\mu\text{Ci/mg}$) and [^3H]inulin (specific radioactivity 150 $\mu\text{Ci/mg}$) used in the assessment of renal function, and the bile acids, sodium tauro[24- ^{14}C]cholate (51 $\mu\text{Ci}/\mu\text{mol}$) and sodium [24- ^{14}C]chenodeoxycholate (55 $\mu\text{Ci}/\mu\text{mol}$) were purchased from The

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Radiochemical Centre, Amersham, Bucks., U.K. Sodium [24-¹⁴C]lithocholate (54 μ Ci/ μ mol) was purchased from Amersham-Buchler G.m.b.H., Braunschweig, W. Germany. Each bile acid was purified by t.l.c. (on 0.25 mm-thick silica gel CT; Reeve Angel, London S.E.1, U.K.), so that 99% of the radioactivity migrated with authentic bile acid standard. The solvent system 1,2-dichloroethane/acetic acid/water (10:10:1, by vol.) (Gregg, 1966) was used for sodium tauro[24-¹⁴C]cholate and the solvent system 2,2,4-trimethylpentane/di-isopropyl ether/acetic acid/propan-2-ol (10:5:5:2, by vol.) for sodium [24-¹⁴C]-chenodeoxycholate.

Non-radioactive bile acids were purchased from Weddel Pharmaceuticals, Wrexham, Clwyd, Wales, U.K. Taurine was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A., and 20% (w/v) sodium *p*-aminohippurate from Merck, Sharpe and Dohme, Hoddesdon, Herts., U.K. Dicyclohexylcarbodi-imide, tri-*n*-butylamine and ethyl chloroformate were purchased from Aldrich Chemical Co., Wembley, Middx., U.K.

Conjugated bilirubin was prepared from human hepatic bile, by a modification (Gollan *et al.*, 1976) of the method of Lucassen (1961). The preparation used in this study contained 77% (w/v) conjugated bilirubin (78% diconjugates, 22% monoconjugates); bile acids, cholesterol, phospholipid and protein were not detected, and inorganic salts and water were the only contaminants.

Synthesis of [24-¹⁴C]chenodeoxycholate derivatives

Diammonium [24-¹⁴C]chenodeoxycholate 7-mono-sulphate and 3,7-disulphate. An adaptation of the method of Mumma (1966) was used; [24-¹⁴C]chenodeoxycholic acid (5 μ Ci, 20 μ mol) was dissolved in dimethylformamide (0.5 ml), and dicyclohexylcarbodi-imide (200 μ mol) was added and the solution cooled to 0°C. Concentrated H₂SO₄ (50 μ mol) was mixed separately with dimethylformamide (0.5 ml), cooled to 0°C, and then both solutions were mixed. Rapid precipitation of dicyclohexylurea indicated that the reaction had occurred. The mixture was left for 1 h at 0°C. The precipitate was removed by centrifugation (200g, 5 min, 4°C) and the pellet washed with dimethylformamide (1 ml). The supernatant and washings were added to 20 ml of water, and the pH was adjusted to 8.0 with 0.1 M-NaOH. Chloroform (20 ml) was added, the mixture vigorously shaken and then centrifuged (750g, 20 min, 4°C) to separate the phases. The upper aqueous phase was evaporated to dryness *in vacuo* in a rotary evaporator at 50°C. The residue was dissolved in aqueous methanol and the mono- and di-sulphates were separated on t.l.c. plates (0.25 mm-thick silica gel 60, BDH Chemicals, Poole, Dorset, U.K.), with the solvent system chloroform/methanol/acetic acid/

water (65:24:10:5, by vol.), which was a modification of that used by Cass *et al.* (1975). Virtually all the chenodeoxycholic acid monosulphate prepared by this method was the 7-monosulphate (Summerfield *et al.*, 1976). The 7-monosulphate and 3,7-disulphate were eluted from the silica with methanol/water (1:1, v/v): after addition of excess of aq. NH₃ (sp.gr. 0.880) and the removal of methanol by a stream of N₂, they were freeze-dried from aqueous solution.

Sodium tauro[24-¹⁴C]chenodeoxycholate. This bile acid was synthesized from [24-¹⁴C]chenodeoxycholic acid (20 μ Ci, 40 μ mol) by the mixed-anhydride method of Norman (1955). It was purified by preparative t.l.c. on silica gel 60 with the solvent system of Gregg (1966), 1,2-dichloroethane/acetic acid/water (10:10:1, by vol.).

Isolated perfused rat kidney

Before operation, male Sprague-Dawley rats (350–450 g) were fed *ad libitum* on a standard laboratory diet (Modified 41B; Oxoid Ltd., London S.E.1, U.K.). The right kidney was prepared for perfusion by a technique similar to that described by Nishitsutsuji-Uwo *et al.* (1967). Under ether anaesthesia the ureter was cannulated and a venous cannula inserted into the inferior vena cava, distal to the renal vein. A metal arterial cannula was then placed in the mesenteric artery, and, with perfusate flowing, it was advanced across the aorta into the renal artery, so that renal plasma flow was not impaired. The kidney was dissected from the animal and placed in an enclosed tray, and the venous cannula connected into the recirculating perfusion system.

The protein-free medium consisted of Krebs improved Ringer 1 solution (Dawson & Elliott, 1959) modified by the substitution of 6.0% (w/v) dextran (mol.wt. 70000) in 0.154 M-NaCl (Lomodex 70; Fisons Pharmaceuticals, Loughborough, Leics., U.K.) for 0.154 M-NaCl, and also by the addition of 8.4 mm-urea. The final concentration of dextran was 3.7% (w/v). The perfusate (120 ml) was maintained at 38°C and equilibrated at pH 7.4 with O₂/CO₂ (19:1). It was recirculated through the kidney at a flow rate of 32–36 ml/min and was continuously filtered by an in-line Dacron filter (14 μ m pore size, type NC; Millipore, London N.W.10, U.K.). ⁵¹Cr-EDTA (4 μ Ci) was added to the perfusate to determine the glomerular filtration rate throughout each study; this enabled results from different experiments to be combined. In experiments involving ¹⁴C-labelled bile acids [³H]inulin was used instead of ⁵¹Cr-EDTA; similar values for glomerular filtration rate have been obtained with these two markers (Gollan & Billing, 1975). The effective renal plasma flow was determined by addition of sodium *o*-[¹²⁵I]iodohippurate (12 μ Ci).

A 10 min equilibration period was allowed for

mixing of the added bile acid and other test substances, and perfusion of the kidney then continued for a further 60 min. Urine was collected over 5 min intervals for 60 min and a sample of perfusate was taken at the midpoint of each collection period. The filtered bile acid load (nmol/min) in each period was calculated from the product of the perfusate concentration (μM) and the corresponding glomerular filtration rate (ml/min). The reabsorption rate (nmol/min) was obtained from the difference between the filtered load and urinary excretion (nmol/min). The fractional reabsorption of bile acids was calculated by dividing the reabsorption rate by the filtered load. Fractional clearance was calculated by subtracting the fractional reabsorption from unity.

Measurement of radioactivity

^{51}Cr -EDTA and sodium *o*-[^{125}I]iodohippurate were measured in the perfusate (0.2 ml) and in the total urine collection for each 5 min period in a dual-channel gamma-spectrometer (1280 Ultragamma; LKB-Wallac, South Croydon, Surrey, U.K.). Samples of perfusate and urine containing [^3H]inulin and ^{14}C -labelled bile acid were prepared for radioactivity counting by incubation with 0.5 ml of NCS solubilizer (Amersham/Searle Corp., Arlington Heights, IL, U.S.A.) at 37°C for 4 h. Samples containing bile pigments were treated with 0.3 ml of 30% (v/v) H_2O_2 in addition to the NCS solubilizer to eliminate the colour interference. Radioactivity was determined by the addition of 15 ml of a scintillant mixture, consisting of 2,5-diphenyloxazole (4g), 1,4-bis-(5-phenyloxazol-2-yl)benzene (50 mg) and 500 ml of Triton X-100 [Rohm and Haas (U.K.) Ltd., Croydon, Surrey, U.K.] in toluene (1 litre), to solubilized samples and counting in a Phillips liquid-scintillation spectrometer. The vials were kept for 48 h at 4°C after addition of scintillant before being measured. Counting efficiencies were determined by using the external-standard-ratio method, and correction of the count rate (c.p.m.) was performed automatically by the scintillation analyser; efficiency for ^3H was 40% and for ^{14}C was 72%.

Measurement of bile acids

Bile acid concentrations were measured by a modified form of the fluorimetric 3-hydroxy steroid dehydrogenase method (Murphy *et al.*, 1970). In experiments where the perfusate concentration exceeded $400\mu\text{M}$, the samples were diluted into the concentration range of the standards (0 – $200\mu\text{M}$). For all other experiments, 1–2 ml of perfusate or urine was extracted with the non-ionic resin XAD-7 [Rohm and Haas (U.K.) Ltd.] (Barnes & Chitranukroh, 1977). No reaction occurred between chenodeoxycholate 7-sulphate and 3-hydroxy steroid

dehydrogenase (International Enzymes Ltd., Windsor, Berks., U.K.), despite the free 3α -hydroxyl group on this bile acid (Haslewood & Haslewood, 1976). This enabled direct measurement of non-sulphated 3α -hydroxy bile acids in the presence of bile acid sulphates. In competition experiments between non-sulphated 3α -hydroxy bile acids, one of the bile acids was labelled with ^{14}C at C-24. Confirmation that the material in the urine was essentially unchanged (less than 2%) enabled the amounts of each bile acid to be calculated.

Experimental

Function of isolated perfused kidneys. In studies reported here the glomerular filtration rate was 1.11 ± 0.06 ml/min (mean $\pm$ s.e.m., no. of separate studies = 27) after 30 min perfusion which is within the normal range reported for the rat kidney *in vivo* (Harvey & Malvin, 1965). The clearance of sodium *o*-[^{125}I]iodohippurate, 4.33 ± 0.38 ml/min (mean $\pm$ s.e.m., $n = 20$), was also comparable with that in the intact rat (Peters, 1959). It has been previously shown, by using this model, that proximal-tubular function was well maintained, and the glucose reabsorption was 95% of the filtered load (Gollan & Billing, 1975). Na^+ reabsorption was slightly decreased (80–90% of filtered load) in spite of maintenance of oncotic pressure by the replacement of protein by dextran (Lomodex-70).

Tubular reabsorption. In a series of experiments, bile acids were perfused individually [sodium taurocholate (6), sodium taurochenodeoxycholate (10), sodium chenodeoxycholate (4), diammonium [24- ^{14}C]chenodeoxycholate 7-monosulphate (1) and triammonium [24- ^{14}C]chenodeoxycholate 3,7-disulphate (4)]; they were dissolved in 0.154 M-NaCl (1 ml) and added to the perfusate to give a final concentration of 10–500 μM . The low solubility of sodium lithocholate in the protein-free medium precluded the addition of carrier, and hence 5 μCi of sodium [24- ^{14}C]lithocholate, dissolved in 2 ml of 0.45 M- NaHCO_3 , was added to the perfusate (final concentration approx. 0.8 μM in four studies). In none of these experiments did the perfusate concentration change by more than 10% over the 1 h period of study.

The effect of increasing the perfusate concentrations at a constant rate on the reabsorption of taurocholate and taurochenodeoxycholate was studied by using a syringe pump (Scientifica and Cook Electronics, London W.3, U.K.). A baseline period of 10 min to study the reabsorption of a bolus amount of each bile acid to the perfusate (concentration 10 μM) preceded each infusion. The final perfusate concentrations after 60 min were approx. 200 μM in two experiments and 1400 μM in one experiment for taurocholate, and 150 μM in three experiments and

800 μM in one experiment for taurochenodeoxycholate.

Inhibition of reabsorption of bile acids. To establish whether the tubular reabsorption of different bile acids involves a common pathway, the effect of sodium taurochenodeoxycholate on the excretion of sodium taurocholate, diammonium chenodeoxycholate 7-monosulphate and triammonium chenodeoxycholate 3,7-disulphate was examined. In two experiments sodium tauro[24- ^{14}C]cholate (2.5 μCi , 3.6 μmol) was added to the perfusate, allowed to equilibrate, and after a 20 min baseline period a bolus of sodium taurochenodeoxycholate (10 μmol) was added (mean total bile acid concentration in perfusate 110 μM). In three experiments triammonium [24- ^{14}C]chenodeoxycholate 3,7-disulphate (0.25 μCi , 1 μmol), and in one experiment diammonium [24- ^{14}C]chenodeoxycholate 7-monosulphate (0.25 μCi , 1 μmol), were dissolved in 0.5 ml of water and added to the perfusate; after a 20 min baseline period, sodium taurochenodeoxycholate was infused at a constant rate for 40 min to give a final perfusate concentration of 80 μM .

Inhibition of reabsorption by conjugated bilirubin. To assess whether conjugated bilirubin influences the renal excretion of bile acids, sodium taurochenodeoxycholate (66 μmol) was added to the perfusate (concentration 500 μM) in two experiments, and, after a 20 min baseline period, conjugated bilirubin, dissolved in 1 ml of water, was added to give perfusate concentrations of 50–85 μM . In two further experiments sodium tauro[24- ^{14}C]cholate (2.5 μCi , 2.5 μmol) and in one experiment sodium tauro[24- ^{14}C]chenodeoxycholate (2.0 μCi , 2.5 μmol) were added to the perfusate to give a concentration of 30 μM , and after a 20 min baseline period conjugated bilirubin was added (35–50 μM). The perfusate bilirubin concentrations were determined by a micro-modification of the method of Michaelsson *et al.* (1965), with caffeine as the accelerator.

Tubular secretion. To see if proximal-tubular secretion of bile acids occurs, sodium taurocholate (66 μmol in two experiments and 8 μmol in one experiment) or sodium taurochenodeoxycholate (66 μmol , two experiments) was added to the perfusate, and after a 20 min baseline period 0.4 ml of *p*-aminohippurate solution (0.92 M) was added. Perfusate *p*-aminohippurate concentrations, determined by the method of Smith *et al.* (1945), ranged from 2.9 to 3.9 mM during the subsequent 40 min perfusion.

Results

Tubular reabsorption

At filtered loads in the range 400–600 nmol/min (corresponding to a perfusate concentration of 500 μM), there was no significant difference in the

fractional reabsorption of taurocholate (0.36 ± 0.04 , mean $\pm$ S.E.M.), taurochenodeoxycholate (0.42 ± 0.01) and chenodeoxycholate (0.40 ± 0.03) (Table 1). At lower filtered loads (80–120 nmol/min), there was a 2-fold increase in the fractional reabsorption of these bile acids (Table 1). Fraction reabsorption approaching 1.0 was observed for lithocholate, [24- ^{14}C]chenodeoxycholate 7-monosulphate and [24- ^{14}C]chenodeoxycholate 3,7-disulphate at low perfusate concentrations (0.8–15 μM).

The infusion experiments demonstrated that both taurocholate and taurochenodeoxycholate were almost completely reabsorbed at low perfusate concentrations (filtered load less than 30 nmol/min) (Fig. 1). As the filtered load increased, the fractional reabsorption of taurocholate decreased and then became constant. The overall reabsorption continued to rise linearly with load, and did not exhibit saturation even when the filtered load was as high as 1400 nmol/min (Fig. 2). The fractional reabsorption of taurochenodeoxycholate remained greater than 0.95 with filtered loads of up to 200 nmol/min. It then decreased and became constant at filtered loads greater than 500 nmol/min, so that, as observed for taurocholate, saturation did not occur (Fig. 3). The slope of the linear increase in reabsorption at high filtered loads determined by least-mean-square analysis was 0.38 ± 0.07 (mean $\pm$ S.D., combined data from three infusion studies) for taurocholate and 0.33 ± 0.04 (combined data from three infusion studies and eight further experiments) for taurochenodeoxycholate.

Table 1. Renal tubular reabsorption of bile acids by the isolated kidney

Bile acids were added to the perfusate and, after a 10 min equilibration period, urine samples were collected over 5 min periods and samples of perfusate were obtained at the midpoint of each period. The number of observations related to several experiments for each bile acid (see under 'Experimental') and results are expressed as mean $\pm$ S.E.M.

Bile acid	No. of observations	Filtered load (nmol/min)	Fractional reabsorption
Taurocholate	9	535 $\pm$ 26	0.36 $\pm$ 0.04
	4	101 $\pm$ 8	0.74 $\pm$ 0.04
Taurochenodeoxycholate	32	498 $\pm$ 10	0.42 $\pm$ 0.01
	5	98 $\pm$ 5	0.94 $\pm$ 0.01
Chenodeoxycholate	5	535 $\pm$ 35	0.40 $\pm$ 0.03
	12	100 $\pm$ 5	0.90 $\pm$ 0.01
Chenodeoxycholate 7-monosulphate	4	9.1 $\pm$ 0.4	0.96 $\pm$ 0.01
Chenodeoxycholate 3,7-disulphate	14	7.9 $\pm$ 0.5	0.96 $\pm$ 0.01
Lithocholic acid	19	0.89 $\pm$ 0.02	0.97 $\pm$ 0.01

Fig. 1. Renal tubular reabsorption of taurocholate and taurochenodeoxycholate in response to increasing filtered loads

After equilibrium (10min) and a baseline period (10min), with an initial perfusate concentration of $10\mu\text{M}$, the bile acid was infused for 50min. Urinary excretion was measured over 5min intervals. Bile acid reabsorption was calculated as the difference between the filtered load and the urinary output. The data shown were combined from five perfused kidneys in which the medium contained either taurocholate (●) (two studies) or taurochenodeoxycholate (○) (three studies). ----, Total reabsorption of the filtered load.

Fig. 2. Tubular reabsorption of taurocholate over a wide range of filtered loads

Data from two infusion studies carried out in the range of filtered loads from 10 to 200nmol/min and one infusion study over the range from 200 to 1400nmol/min have been combined. A limited number of data points from experiments at low filtered loads have been plotted for clarity. ----, Complete reabsorption of the filtered load.

Fig. 3. Tubular reabsorption of taurochenodeoxycholate at high filtered loads

Grouped data from the three infusion studies (●) and from experiments (○) carried out at constant perfusate concentration ($350\text{--}550\mu\text{M}$) have been combined. The straight line drawn through the data above a filtered load of $150\mu\text{M}$ was calculated by least-mean-square analysis. ----, Complete reabsorption of the filtered load.

Inhibition of reabsorption by bile acids

The effect of taurochenodeoxycholate ($80\mu\text{M}$) on the reabsorption of tauro[$24\text{-}^{14}\text{C}$]cholate ($30\mu\text{M}$) was to decrease the initial mean fractional reabsorption from 0.93 ± 0.01 to 0.48 ± 0.08 ($P < 0.001$) (Table 2). The mean fractional reabsorption of taurochenodeoxycholate was 0.93 ± 0.01 , a value similar to that observed when it was studied alone (Table 1).

Addition of taurochenodeoxycholate to the perfusate to give an increasing filtered load (maximum 100nmol/min) caused a non-linear decrease in the fractional reabsorption of [$24\text{-}^{14}\text{C}$]chenodeoxycholate 3,7-disulphate in each of three experiments (Fig. 4). The fractional reabsorption of taurochenodeoxycholate also fell from 0.95 to 0.88. A similar result was obtained for [$24\text{-}^{14}\text{C}$]chenodeoxycholate 7-monosulphate. At the highest filtered load of taurochenodeoxycholate (100nmol/min), the fractional reabsorption of both bile acid sulphates was less than 0.4.

Inhibition of reabsorption by conjugated bilirubin

Conjugated bilirubin caused a marked decrease in the fractional reabsorption of both taurocholate and taurochenodeoxycholate at low filtered loads ($<30\text{nmol/min}$). The mean fractional reabsorption of taurocholate fell significantly from 0.67 ± 0.02 to 0.27 ± 0.03 and that of taurochenodeoxycholate from 0.85 ± 0.02 to 0.56 ± 0.02 (Table 3). In contrast, at

Table 2. *Effect of taurochenodeoxycholate on the renal tubular reabsorption of taurocholate*
 Tauro[24-¹⁴C]cholate (30 μ M) was added to the perfusate, and after equilibration was studied for a baseline period of 20 min. Taurochenodeoxycholate (80 μ M) was then added and the urinary excretion of both bile acids examined for a further 20 min. Results are means $\pm$ S.E.M. The data from two experiments have been combined.

Bile acids in perfusate	No. of observations	Taurocholate		Taurochenodeoxycholate	
		Filtered load (nmol/min)	Fractional reabsorption	Filtered load (nmol/min)	Fractional reabsorption
Taurocholate (30 μ M)	8	26.4 $\pm$ 1.0	0.93 $\pm$ 0.01	—	—
Taurocholate (30 μ M) + taurochenodeoxycholate (80 μ M)	6	24.6 $\pm$ 2.0	0.48 $\pm$ 0.08*	62.3 $\pm$ 5.5	0.93 $\pm$ 0.01

* Significantly different ($P < 0.001$) (unpaired Student's t test) from the mean fractional reabsorption of taurocholate when perfused without taurochenodeoxycholate.

Fig. 4. *Effect of taurochenodeoxycholate on the renal tubular reabsorption of [24-¹⁴C]chenodeoxycholate 3,7-disulphate*

[24-¹⁴C]Chenodeoxycholate 3,7-disulphate (1 μ Mol) was added to the perfusate and, after equilibration (10 min) and a baseline period (10 min), taurochenodeoxycholate was infused for 50 min. Urinary output was measured over 5 min intervals and filtered bile acid load and fractional reabsorption were calculated as described in Table 1. The mean values of fractional reabsorption of chenodeoxycholate 3,7-disulphate from three experiments are plotted.

high filtered loads (400 nmol) the fractional reabsorption of taurochenodeoxycholate was unchanged by the addition of conjugated bilirubin (50–80 μ M) to the perfusate (Table 3).

Tubular secretion

In two experiments the mean fractional reabsorption of taurochenodeoxycholate (filtered load 500 nmol/min) was not significantly changed by the addition of *p*-aminohippurate to the perfusate (0.33 $\pm$ 0.03 to 0.38 $\pm$ 0.04, $P > 0.03$). Similar results were obtained in experiments with taurocholate at filtered loads of 500 nmol/min (0.43 $\pm$ 0.01 to 0.40 $\pm$ 0.02, $P > 0.1$) and 70 nmol/min (0.48 $\pm$ 0.04 to

0.52 $\pm$ 0.01, $P > 0.3$). The perfusate concentration of *p*-aminohippurate exceeded its tubular maximum excretory capacity as indicated by the 60% decrease in *o*-[¹²⁵I]iodohippurate excretion, within 5 min of its addition to the perfusate.

Discussion

Tubular reabsorption

These studies in the isolated perfused rat kidney have demonstrated the importance of renal tubular reabsorption of bile acids, without which considerable amounts of bile acids would normally be excreted in urine. At low filtered loads the fractional reabsorption of all the non-sulphated and sulphated bile acids studied was close to 1.0, indicating the existence of an efficient mechanism.

The absence of overall saturation of tubular reabsorption and the pattern of the reabsorption rates with respect to increased filtered loads of both taurocholate and taurochenodeoxycholate is similar to the observations made on bile acid absorption in the distal small intestine (Schiff *et al.*, 1972; Krag & Phillips, 1974), where both active transport and passive diffusion of bile acids co-exist. The linear increase in reabsorption rate observed at filtered loads above 80 nmol/min for taurocholate and 300 nmol/min for taurochenodeoxycholate may be taken to represent passive diffusion. At lower filtered loads the greater fractional reabsorption may be interpreted as being the result of a second process which becomes saturated at high filtered loads. Although the slopes of the linear increase in reabsorption were similar for both taurocholate (0.38) and taurochenodeoxycholate (0.33), the capacity of the underlying saturable process was far greater for taurochenodeoxycholate than for taurocholate. This resulted in greater differences in mean fractional reabsorption between taurocholate (0.74) and taurochenodeoxycholate (0.94) at filtered loads of 100 nmol/min than at higher filtered loads (400–600 nmol/min), when the values were 0.36 and 0.42 respectively.

Table 3. *Effect of conjugated bilirubin on the tubular reabsorption of taurocholate and taurochenodeoxycholate in the isolated kidney*

The reabsorption of taurocholate (30 μ M, two experiments) and taurochenodeoxycholate (30 μ M, one experiment, and 400 μ M, two experiments) was studied for a 20min baseline period. Conjugated bilirubin was then added and reabsorption of the bile acids reassessed for a further 20min. The mean fractional reabsorption ($\pm$ S.E.M.) is given for each period.

Perfusate	No. of observations	Mean filtered load (nmol/min)		Mean fractional reabsorption of bile acid
		Bile acid	Conjugated bilirubin	
Taurocholate	8	30.7 $\pm$ 2.6	—	0.67 $\pm$ 0.02
Taurocholate+conjugated bilirubin	12	40.7 $\pm$ 1.1†	44.2 $\pm$ 3.3	0.27 $\pm$ 0.03*
Taurochenodeoxycholate	4	29.3 $\pm$ 4.0	—	0.85 $\pm$ 0.02
Taurochenodeoxycholate +conjugated bilirubin	6	37.6 $\pm$ 1.0†	27.0 $\pm$ 3.3	0.56 $\pm$ 0.02*
Taurochenodeoxycholate	8	390 $\pm$ 26	—	0.53 $\pm$ 0.02
Taurochenodeoxycholate +conjugated bilirubin	12	414 $\pm$ 42	70.8 $\pm$ 10.3	0.52 $\pm$ 0.01

* Significantly different (unpaired Student's *t* test; *P* < 0.001) from the mean fractional reabsorption measured in baseline period.

† There was no increase in perfusate concentration; this rise was due to a higher glomerular filtration rate in the second study period.

Although taurochenodeoxycholate and chenodeoxycholate have a different pK_a (1.9 and 5.2 respectively; Small, 1971), this did not influence their fractional reabsorption (0.42 and 0.40 respectively) at high filtered loads (400–600 nmol/min). It is unlikely therefore that passive non-ionic diffusion contributes greatly to reabsorption, since the concentration of non-ionized molecules will be small (less than 5%) at the pH of the urine (mean pH 6.5) in the isolated kidney. Weiner *et al.* (1964) observed no difference in reabsorption between taurocholate, glycocholate and cholate in the intact dog and demonstrated that changing the urinary pH to 8.0 had no effect on reabsorption. On the basis of their findings, they concluded that reabsorption only occurs by active transport, which is at variance with the data in the present study.

The mathematical separation of the reabsorption data into passive and saturable components, as has been done in studies of bile acid absorption in the intestine (Schiff *et al.*, 1972; Krag & Phillips, 1974), cannot be made, since the changes in concentration of bile acids at different points along the tubules are unknown, and it is only possible to determine the overall reabsorption rate. Estimation of the passive diffusion component from the filtered load and the passive diffusion coefficient (determined at high filtered loads) leads to an overestimation at low filtered loads, where reabsorption is nearly complete, because of the marked fall in concentration which must occur in the tubules. Although this problem has been overcome in studies on glucose

absorption in the small intestine (Sladen & Dawson, 1969), their method was not appropriate to the kidney because the geometry of the kidney tubules cannot be equated with the small intestine, and because of the variation along the tubules with regard to salt and water transport.

The apparent saturation of one component of reabsorption, the inhibition of reabsorption by other bile acids (see below) and the observation of Na⁺-dependent reabsorption in the proximal tubules in stop-flow studies (Zins & Weiner, 1968) are all consistent with the hypothesis that active transport occurs. However, it is necessary to show that the tubules can transport bile acids against a concentration gradient, and that reabsorption is sensitive to metabolic poisons, in order to establish this.

Inhibition of tubular reabsorption

Bile acids. The observation that taurochenodeoxycholate had a greater affinity for the saturable mechanism than taurocholate suggested that these bile acids might mutually inhibit each other's reabsorption. Indeed, experiments when two bile acids were perfused together demonstrated inhibition of the reabsorption of taurocholate, chenodeoxycholate 7-monosulphate and chenodeoxycholate 3,7-disulphate by taurochenodeoxycholate, the inhibition of the reabsorption of the bile acid sulphates being greater than that for taurocholate. However, these experiments do not provide conclusive evidence that the inhibition observed was competitive.

Conjugated bilirubin. It has been shown previously, by using the isolated rat kidney preparation, that conjugated bilirubin undergoes renal tubular reabsorption (Gollan & Billing, 1975), although no information is available on the mechanism of its reabsorption. In the present study, conjugated bilirubin markedly inhibited the renal tubular reabsorption of both taurocholate and taurochenodeoxycholate at low filtered loads (40 nmol/min), but not at high filtered loads (400 nmol/min); the latter observation may be partly explained by the comparatively smaller ratio of conjugated bilirubin to bile acid, and that at high filtered loads taurochenodeoxycholate is largely absorbed by passive diffusion, which is not inhibited by conjugated bilirubin. These results suggest that the reabsorption of bile acids and conjugated bilirubin by the renal tubules occurs by the same mechanisms; this is in contrast with their uptake by the liver which is thought to occur by separate processes (Paumgartner & Reichen, 1976).

Tubular secretion. Proximal-tubular secretion of bile acids does not appear to be an important process, since *p*-aminohippurate did not affect the reabsorption of taurocholate and taurochenodeoxycholate at high filtered loads (500 nmol/min) or taurocholate at lower filtered loads (70 nmol/min), although it effectively inhibited the proximal-tubular secretion of its analogue *o*-[¹²⁵I]iodohippurate. These observations in the isolated rat kidney are in contrast with the results obtained in stop-flow studies in the dog kidney (Zins & Weiner, 1968).

Relation of perfusion studies to renal function in vivo

Studies *in vitro* have indicated that the proportion of bile acids in plasma which were not bound to protein ranged from 5 to 20% in man (Rudman & Kendall, 1957; Burke *et al.*, 1971; Cowen *et al.*, 1975). Systematic studies at high plasma concentrations, such as occur in cholestasis, have not been made, so it is not known to what extent differential protein binding might influence urinary excretion. It seems unlikely that the interaction of the bile acids and their sulphates would be qualitatively different from those observed with the isolated kidney perfused with a protein-free medium. Since the bile acid concentrations in plasma in cholestasis in the rat may rise to 400–500 μM (S. Barnes & B. H. Billing, unpublished work), the filtered loads *in vivo* could be as much as 100 nmol/min. Thus the observations made in the present study with the isolated perfused rat kidney over the range of filtered load 0–100 nmol/min may be regarded as relevant to the renal function in the intact animal. Notwithstanding the species difference, such observations may also be used in the interpretation of urinary bile acid composition in man.

Urinary bile acid excretion in man. The total amount of bile acid which is filtered from the blood by the kidney per day in normal man can be estimated to be approx. 100 μmol , if one assumes that the normal renal clearance of inulin is 110 ml/min (i.e. 156 litres/day), and the unbound (ultrafilterable) plasma bile acid concentration is 0.5–1.0 μM [total plasma concentration 5–10 μM (Schwarz *et al.*, 1974); percentage unbound 5–20% (Cowen *et al.*, 1975)]. Efficient tubular reabsorption of bile acids is implied, since only 1–2 μmol appears in the urine per day (Back, 1973; Stiehl, 1974). This is maintained in cholestasis, since, when the plasma concentration rises to 200 μM causing 2000–4000 μmol to be filtered per day, only 40–80 μmol appears in the urine. Thus the degree of reabsorption effected by the renal tubules is comparable with that observed in the small intestine. Further similarity to absorption by the ileum has been reported by Accatino & Simon (1976), who demonstrated that the plasma membranes isolated from kidney homogenates bind cholic acid as avidly as do homogenates of liver and distal ileum.

The greater capacity for the tubular reabsorption of taurochenodeoxycholate compared with taurocholate observed in these studies would suggest that the greater urinary excretion of cholate conjugates relative to chenodeoxycholate conjugates observed in man (Summerfield *et al.*, 1977) could be readily explained by the interaction of these bile acids with the tubular reabsorption processes rather than by inherently different renal clearances. This interaction between the bile acids can also be used to explain the preferential renal excretion of bile acid sulphates. Several authors have demonstrated that, in the presence of marked cholestasis, the renal clearance of bile acid sulphates is 10–100 times that of the non-sulphated bile acids in severe cholestasis (Stiehl, 1974; Makino *et al.*, 1975; Stiehl *et al.*, 1975). Summerfield *et al.* (1977) also showed that the clearance of the bile acid sulphates was linearly related to the total bile acid urinary output, in spite of the fact that the plasma concentration of bile acid sulphates did not vary greatly with the degree of cholestasis; the increased concentration of plasma bile acids was due mainly to the non-sulphated bile acids. They therefore postulated that the increased clearance of bile acid sulphates was due in part to non-sulphated bile acids inhibiting the reabsorption of bile acid sulphates, as had been previously demonstrated in the ileum in man (Low-Beer *et al.*, 1969). To compare the inhibition data from the present study with the observations in man (Summerfield *et al.*, 1977), the fractional clearance of the bile acid sulphates has been plotted against the total urinary output of bile acids (Fig. 5). This showed that indeed there was a strong linear relationship between fractional clearance of bile acid sulphate and total urinary output. Our results therefore support

Fig. 5. Relationship between fractional clearance of $[24-^{14}\text{C}]$ chenodeoxycholate 3,7-disulphate and taurochenodeoxycholate, and total bile acid urinary output

Experimental procedure is described in Fig. 4. Fractional clearances of taurochenodeoxycholate (○) and chenodeoxycholate 3,7-disulphate (●) are plotted against the total bile acid output (sum of chenodeoxycholate 3,7-disulphate and taurochenodeoxycholate excretion). The data shown were obtained from three perfused kidneys.

the hypothesis that the increased renal excretion of bile acid sulphates in human cholestasis can be explained by inhibition of tubular reabsorption of bile acid sulphates by non-sulphated bile acids. The finding of cholyl sulphokinase in rat kidney (Chen *et al.*, 1974) and studies in the isolated perfused rat kidney (Summerfield *et al.*, 1976) have suggested that renal synthesis of bile acid sulphates may be important; its role relative to that of differential tubular reabsorption of bile acid sulphates in determining urinary bile acid composition still remains to be elucidated.

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