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PARACETAMOL METABOLISM IN THE ISOLATED PERFUSED RAT LIVER WITH FURTHER METABOLISM OF A BILIARY PARACETAMOL CONJUGATE BY THE SMALL INTESTINE

ROLAND GRAFSTRÖM, KARI ORMSTAD, PETER MOLDÉUS and STEN ORRENIUS
Department of Forensic Medicine, Karolinska Institutet, S-104 01 Stockholm, Sweden

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Abstract—The isolated, perfused rat liver metabolized paracetamol to glucuronide, sulphate and glutathione conjugates. Sulphate conjugation was the preferred route of metabolism at the drug concentrations studied, but formation of glutathione conjugate became increasingly prominent at higher paracetamol concentrations. Sulphate conjugation was saturated at a paracetamol concentration in the perfusate of 5 mM, while formation of glucuronide or glutathione conjugates was not yet saturated at 10 mM. The sulphate conjugate was predominantly excreted into the perfusate whereas the excretion pattern for the glucuronide and glutathione conjugates changed with time. Initially, both these conjugates were almost exclusively excreted in the bile, after 90 min perfusion mainly into the perfusate. Preformed paracetamol conjugates were not transferred from perfusate to bile by the isolated perfused liver.

Freshly isolated intestinal cells rapidly converted paracetamol-*S*-glutathione to paracetamol-*S*-cysteine which was slowly acetylated to the *N*-acetylcysteine derivative. Methionine stimulated, and serine·borate inhibited, the breakdown of paracetamol-*S*-glutathione by intestinal cells, indicating the involvement of γ-glutamyltranspeptidase in the reaction. Biliary paracetamol-*S*-glutathione was metabolized similarly by the small intestine *in situ*; the subsequent appearance of the cysteine conjugate in plasma revealed that breakdown of the glutathione conjugate occurred before or during passage through the intestinal wall. Direct absorption of paracetamol-*S*-cysteine from the intestinal lumen to the portal blood was verified by instillation of this derivative in the intestinal lumen *in situ*. Analysis of plasma also indicated enterohepatic circulation and reabsorption of biliary paracetamol and paracetamol glucuronide from the intestinal lumen.

INTRODUCTION

Paracetamol (*N*-acetyl-para-aminophenol) is a widely used analgesic and antipyretic drug. In therapeutic usage paracetamol is considered nontoxic but when taken in overdose it produces liver damage in man [1] and various animal species [2–4]. Paracetamol is primarily metabolized in the liver by conjugation with glucuronic acid and sulphate but may also undergo cytochrome P-450-dependent *N*-hydroxylation [3, 4]. The *N*-hydroxy derivative can subsequently dehydrate spontaneously to yield acetamidoquinone, which is believed to be the toxic species of the drug [4, 5]. The reactive metabolite is trapped by conjugation with glutathione (GSH), which may lead to depletion of hepatic GSH followed by covalent binding of the electrophilic metabolite to critical cellular macromolecules; this effect is considered to be the ultimate cause of cell damage [4, 6, 7].

In our laboratory paracetamol metabolism has been studied with suspensions of freshly isolated cells from rat liver and kidney [8, 9]. In liver cells three major metabolites are formed, the glucuronide, sulphate and glutathione conjugates. Paracetamol-*S*-glutathione is rapidly metabolized by isolated kidney cells to paracetamol-*S*-cysteine which is slowly acetylated to the *N*-acetylcysteine derivative. Removal of the γ-glutamyl group from paracetamol-*S*-glutathione is catalyzed by γ-glutamyltranspeptidase and that of the glycyl residue by a particulate peptidase [10].

Glucuronide and glutathione conjugates of various drugs are known to appear at high concentrations in bile. However, *in vivo* paracetamol metabolites are almost quantitatively eliminated in the urine [6], suggesting that the bulk of the liver metabolites are excreted into the blood, or that a reabsorption of biliary metabolites from the intestine occurs. Most investigations of metabolism and toxicity of paracetamol have been performed *in vivo* and thus do not provide information relating to this problem. The isolated perfused liver offers an *in vivo*-like model in which the distribution of a drug between perfusate and bile may easily be followed.

In the present study we have used the isolated perfused rat liver to investigate the metabolism of paracetamol and the distribution of paracetamol metabolites between perfusate and bile. The role of the small intestine in the metabolism of paracetamol and biliary paracetamol metabolites in the rat has been studied in the small intestine *in situ* and with isolated intestinal cells. The results support a predominant role of the liver in overall paracetamol metabolism and indicate that although high metabolite concentrations do occur in bile, the bulk of metabolites are released into the perfusate. The small intestine appears to play a minor role in the total metabolism of paracetamol but is active in the further metabolism of the glutathione conjugate.

MATERIALS AND METHODS

Animals and chemicals. Male Sprague–Dawley rats weighing 180–250 g were maintained on pelleted food (Anticimex avelsfoder R3, Astra, Södertälje, Sweden) and tap water *ad lib.* Paracetamol-*S*-cysteine was a generous gift from Sterling-Winthrop, Newcastle, England. Paracetamol and other chemicals were obtained from local commercial sources.

Isolated, perfused liver. Ether anaesthetized rats were given 1000 IE heparin in a tail vein. The abdominal cavity was opened by a midventral incision, the bile duct isolated and a catheter (Intramedic polyethylene tubing PE 10, inner dia. 0.28 mm, outer dia. 0.61 mm) was introduced, pushed up to the liver hilus and secured with a ligature. Bile immediately started to drip out of the distal tip of the catheter, and was collected as "blank". A conical-tipped probe cannula was inserted into the portal vein and secured in place with a ligature close to the liver hilus. Perfusion was started with the liver *in situ* using modified Krebs–Henseleit buffer, pH 7.4, containing 25 mM Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) and 2% bovine serum albumin. The buffer was oxygenated (95% O_2, 5% CO_2) and maintained at 37°C. As soon as the cannula had been inserted, the liver was dissected free and placed on a plastic grid in a glass chamber. The perfusate was allowed to drain freely from the hepatic vein back to the reservoir; thus we had a closed recirculating system with separate collection of bile. The flow of perfusate through the liver was kept at about 15 ml/min and the perfusion pressure at 20 cm H_2O. Bile flow was 600±50 μl/hr during the experiments. As soon as the surgical procedure was completed and bile flow stabilized (10–25 min), drug (paracetamol or preformed conjugates) was added to the perfusate and immediately mixed. From this time point samples were taken from perfusate and bile every 15 min. The samples were deproteinized by dilution with 3 N perchloric acid (1:1.5), centrifuged and the supernatant was analyzed by high performance liquid chromatography (HPLC). Conjugates of paracetamol were obtained by incubating hepatocytes from phenobarbital pretreated rats with 5 mM paracetamol dissolved in the modified Krebs–Henseleit buffer. Hepatocytes were isolated according to the procedure of Moldéus *et al.* [11].

Closed intestinal segment in situ. Ether anaesthetized rats were given 1000 IE heparin in a tail vein. The abdominal cavity was opened by a midventral incision. A 12–15 cm segment of the gut distal of the pyloric sphincter was flushed with Krebs–Henseleit buffer, pH 7.4, filled with 2 ml of paracetamol, paracetamol-*S*-cysteine or preformed paracetamol conjugates in buffer, and closed by two ligatures tied at both ends. Great care was taken to avoid compromising the blood vessels. In the experiments where paracetamol-*S*-cysteine was instilled in the intestinal lumen, the renal vessels were ligated to avoid any metabolic interference from the kidneys. The bile duct was cannulated and the bile collected as well. Samples were taken from the intestinal lumen, portal and caval veins by needle puncture with a syringe after 15 min. The samples were deproteinized by dilution (1:1.5) with 3 N perchloric acid, centrifuged and the supernatant assayed by HPLC.

Preparation of isolated intestinal cells. Intestinal cells were isolated according to the method of Stohs *et al.* [12] as modified by Grafström *et al.* [13]. Villous mucosa from a 40 cm small intestinal segment succeeding pylorus, was incubated for 10 min at 37° in a Hank's medium also containing 4 mM $CaCl_2$, 25 mM $NaHCO_3$, 5 mM glucose, 25 IU heparin/ml, 0.10% collagenase and 0.06% hyaluronidase. The resulting cell suspension was washed twice with a Krebs medium supplemented with 25 mM Hepes, 5 mM glucose, 25 IU heparin/ml and 500 IU penicillin/ml. 95% of the freshly isolated cells excluded trypan blue and NADH. Viability was unchanged during 2–3 hr after cell isolation when the intestinal cells were kept at 4°C in the supplemented Krebs medium.

Assay. Metabolites of paracetamol were analyzed by HPLC as described by Moldéus [8]. Separation of the metabolites was achieved on a reversed phase μ-Bondapak C-18 column (Waters Associates, Inc., Massachusetts, U.S.A.) attached to a Spectra Physics model 3500 liquid chromatograph equipped with a variable UV detector and a Spectra Physics System 1 integrator. Quantification of the metabolites was accomplished using paracetamol as a standard since the molar extinction coefficients of the metabolites and paracetamol are essentially the same [8, 14].

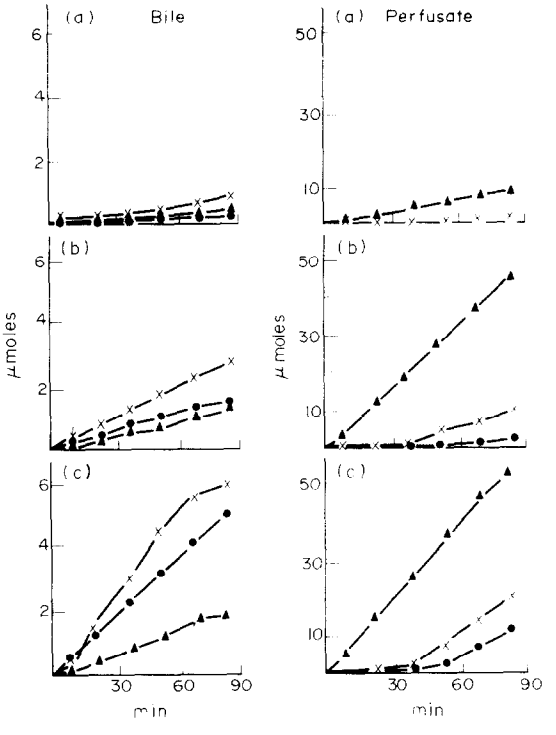

Fig. 1. Total amounts of paracetamol conjugates excreted into the bile and perfusate from a rat liver perfused with a Krebs–Henseleit medium containing 1 (A), 5 (B) and 10 (C) mM paracetamol. All diagrams show one experiment typical of the three performed.
●——●, paracetamol-*S*-glutathione; ▲——▲, paracetamol sulphate; ×——×, paracetamol glucuronide.

Table 1. Disposition of paracetamol metabolites in the isolated perfused liver*

Paracetamol concentration	Metabolites	μmoles of product: perfusate	%	bile	%	perfusate + bile	%	Partition coefficient perfusate / bile	Percent metabolites excreted in bile
1mM	Paracetamol sulphate	10.00	79	0.55	33	10.55	73	18.2	
	Paracetamol glucuronide	2.30	18	0.85	50	3.15	22	2.7	
	Paracetamol-*S*-glutathione	0.37	3	0.30	17	0.67	5	1.2	
	Total	12.67	100	1.70	100	14.37	100		12%
5mM	Paracetamol sulphate	44.50	78	1.30	25	45.90	74	31.8	
	Paracetamol glucuronide	10.20	18	2.50	48	12.70	20	4.1	
	Paracetamol-*S*-glutathione	2.10	4	1.40	27	3.40	6	1.6	
	Total	56.80	100	5.20	100	62.00	100		8%
10mM	Paracetamol sulphate	52.00	64	1.80	14	53.80	57	28.9	
	Paracetamol glucuronide	20.80	25	6.00	47	26.80	29	3.5	
	Paracetamol-*S*-glutathione	7.80	11	4.90	39	12.70	14	1.6	
	Total	80.60	100	12.70	100	93.30	100		14%

* The isolated liver was perfused for 90′ with different concentrations of paracetamol. Bile was collected during the experiment. Bile and perfusate were analyzed for paracetamol metabolites by HPLC. For further details see Materials and Methods.

RESULTS

When paracetamol was dissolved in Krebs–Henseleit buffer, pH 7.4, and recirculated through the isolated rat liver, free paracetamol as well as conjugated metabolites were found in both bile and perfusate. Figure 1 shows the total amount of paracetamol metabolites excreted from the liver via the bile duct and the hepatic vein, respectively.

The perfused rat liver metabolized paracetamol to glucuronide, sulphate and glutathione conjugate (Fig. 1, Table 1). The formation of all conjugates increased with increasing paracetamol concentration and at all concentrations the sulphate conjugate was the major metabolite (Table 1). The sulphate conjugate was predominantly excreted into the perfusate whereas the excretion pattern for the glucuronide and glutathione conjugate changed with time (Fig. 1). Initially these conjugates were almost exclusively excreted in bile and for up to 30 min of perfusion only very small amounts of these metabolites were found in the perfusate. At this time there was a rapid increase in excretion of both the glucuronide and glutathione conjugates into the perfusate and at 90 min this was the major route of excretion for these conjugates (Table 1). Even though at this time the bulk of metabolites was excreted into the perfusate the concentration was 100–1000-fold higher in the bile.

Distribution of preformed paracetamol metabolites between perfusate and bile was investigated by perfusion of the isolated liver with paracetamol and paracetamol conjugates produced in isolated liver cells (Fig. 2). Perfusion with a mixture of preformed conjugates, containing only 0.10 mM free

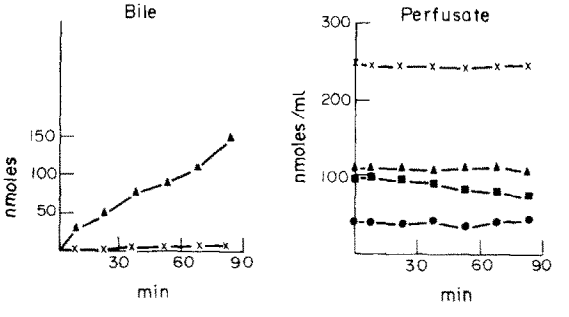

Fig. 2. Total paracetamol conjugate contents in bile and in perfusate recirculated through an isolated rat liver. The perfusate contained preformed conjugates, manufactured by incubating isolated hepatocytes from phenobarbital-treated rats with 5 mM paracetamol for 2 hr. Diagrams show one experiment typical of the three performed. ▲——▲, paracetamol sulphate; ×——×, paracetamol glucuronide; ●——●, paracetamol-*S*-glutathione; ■——■, paracetamol.

paracetamol, showed that paracetamol-*S*-glutathione and paracetamol glucuronide were excreted into the bile only to a very slight degree. The concentration of these metabolites in the perfusate hardly changed during 90 min perfusion. Paracetamol sulphate appeared in the bile in somewhat higher concentration than the other metabolites, but the concentration of paracetamol sulphate in the perfusate was unchanged. Free paracetamol in the perfusate decreased linearly with time. This decrease was only partly explained by biliary excretion of sulphate conjugate, accounting for 60 per cent of the total loss, indicating that the remaining 40 per cent of the conjugate formed were released into the perfusate.

Metabolism of paracetamol conjugates, preformed by incubating paracetamol with hepatocytes isolated from phenobarbital pretreated rats was studied with a suspension of isolated intestinal cells. Paracetamol-*S*-glutathione was rapidly converted to paracetamol-*S*-cysteine by intestinal cells (Fig. 3). No paracetamol-*S*-cysteinylglycine was detected, indicating a rapid hydrolysis of this intermediate. The cysteine conjugate was then acetylated at a low rate (approximately 0.03 nmole per 10^6 cells and min) to paracetamol-*S*-acetylcysteine. The total of the three sulfhydryl conjugates was essentially constant during the incubation, indicating stoichiometric conversion of the GSH conjugate to the cysteine and *N*-acetylcysteine derivatives. The metabolism of free paracetamol by the isolated intestinal cells contributed minimally to the total concentration of sulfhydryl conjugates [13].

Methionine at a concentration of 1 mM stimulated by almost 50 per cent the conversion of paracetamol-*S*-glutathione to paracetamol-*S*-cysteine (Fig. 3). In contrast, serine·borate, a specific inhibitor of γ-glutamyltranspeptidase [15], nearly completely blocked the metabolism of the glutathione conjugate by intestinal cells, which confirms the role of the γ-glutamyltranspeptidase in the reaction sequence.

Paracetamol was instilled in the intestinal segment *in situ* and samples were taken after 15 min from the intestinal lumen, portal and caval veins and from the separately collected bile: the distribution of paracetamol metabolites is shown in Table 2. Glucuronide and sulphate conjugates appeared both in the gut lumen and in plasma. A small proportion of the absorbed paracetamol was probably conjugated with glucuronic acid or sulphate during the passage

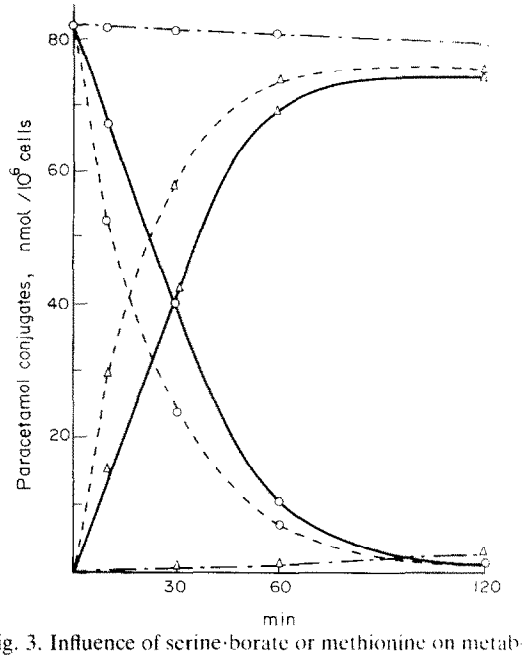

Fig. 3. Influence of serine·borate or methionine on metabolism of glutathione-*S*-paracetamol in isolated intestinal cells. One experiment typical of the three performed. ———, no addition; – – –, 1 mM methionine; –·–·–, 10 mM serine·borate; o, paracetamol-*S*-glutathione; Δ, paracetamol-*S*-cysteine.

through the intestinal wall as has been previously described [16]. However, the major part of these metabolites seemed to be circulating liver metabolites, indicated by the high concentration of conjugates in the caval vein. The luminal fluid consisted only to a minor part of glucuronide and sulphate conjugates. No paracetamol-*S*-glutathione or paracetamol-*S*-cysteine was detected in the intestinal lumen or plasma. The major biliary metabolite was the sulphate conjugate which was present at twice the concentration of the glucuronide. Paracetamol-*S*-glutathione accounted for only 1 per cent of biliary paracetamol metabolites.

The isolated livers of five rats were perfused with 5 mM paracetamol and the bile was collected, pooled and diluted 10-fold with Krebs–Henseleit buffer. The biliary paracetamol metabolite solution was then instilled in the closed intestinal segment. The distribution of paracetamol metabolites in the intestinal

Table 2. Percentual distribution of paracetamol metabolites in intestinal lumen, bile and plasma after instillation of paracetamol into the small intestine*

Metabolites	Intestinal† lumen	Bile‡	Plasma	
			Portal vein†	Caval vein†
Paracetamol	97.8	13.1	15.7	29.2
Paracetamol glucuronide	1.8	23.8	22.9	25.2
Paracetamol sulphate	0.4	62.0	19.4	45.6
Paracetamol-*S*-glutathione	—	1.1	—	—
Total	100.0	100.0	100.0	100.0

* Initial paracetamol concentration was 5 mM. For further details see Materials and Methods.
† Samples were taken 15 min after instillation of paracetamol in the intestinal lumen.
‡ The bile duct was cannulated and bile collected for 15 min.

Table 3. Percentual distribution of paracetamol metabolites in intestinal lumen, bile and plasma after instillation of biliary paracetamol metabolites into the small intestine*

Metabolites	Intestinal lumen		Bile	Plasma	
	0 min	15 min	0–15 min	Portal vein 15 min	Caval vein 15 min
Paracetamol	27.8	10.0	0.5	38.5	12.1
Paracetamol glucuronide	41.9	56.1	28.6	35.2	32.3
Paracetamol sulphate	9.2	12.7	64.0	21.8	53.6
Paracetamol-*S*-glutathione	18.2	4.0	5.9	<1.0	<1.0
Paracetamol-*S*-cysteine	2.9	17.2	1.0	4.5	2.0
Total	100.0	100.0	100.0	100.0	100.0

* The instilled solution of biliary paracetamol metabolites contained 680 μM paracetamol, 1025 μM paracetamol glucuronide conjugate, 225 μM paracetamol sulphate conjugate, 445 μM paracetamol-*S*-glutathione and 71 μM paracetamol-*S*-cysteine. For further details see Table 2 and Materials and Methods.

lumen and in portal plasma is given in Table 3. Paracetamol was more easily absorbed than the glucuronide or sulphate conjugates as suggested by a decreased concentration of paracetamol, and an increased proportion of glucuronide and sulphate conjugates, in the intestinal lumen after 15 min. However, the major change in the intraluminal distribution of metabolites was the conversion of paracetamol-*S*-glutathione to paracetamol-*S*-cysteine. The cysteine conjugate, but only trace amounts of the glutathione conjugate, was found in plasma, which indicates that paracetamol-*S*-glutathione is metabolized to the cysteine derivative before or during the passage through the intestinal wall. The major metabolite in plasma was the glucuronide. The increased proportion of this metabolite in plasma suggests a direct uptake of the biliary paracetamol glucuronide in addition to a small production of glucuronide conjugate by the intestinal epithelial cells.

Instillation of paracetamol-*S*-cysteine into the closed intestinal segment *in situ* resulted in a direct uptake of this conjugate which appeared in portal plasma and in bile (Table 4). After 15 min the concentration was 11 μM in both portal and caval plasma and 30 μM in bile. Paracetamol-*S*-acetylcysteine was not detected in plasma although present in the intestinal lumen and in bile (Table 4). Assuming a plasma volume of 7 ml (in a 200 g rat), the uptake of paracetamol-*S*-cysteine from the intestine may be calculated to approximately 80 nmoles of cysteine conjugate in 15 min. Since bile flow was ≈150 μl in 15 min, only 5–6 nmoles were taken up by the liver and excreted in bile.

Table 4. Concentrations of paracetamol-*S*-cysteine and its acetylated metabolite in intestine, plasma and bile after instillation of cysteine-*S*-paracetamol into the small intestine*

Source	paracetamol-*S*-cysteine (μM)	paracetamol-*S*-acetylcysteine (μM)
Intestinal lumen†	2310	20
Plasma†	11	not detected
Bile‡	30	6

* Paracetamol-*S*-cysteine, at a concentration of 1.6 mM, was instilled into the closed small intestinal segment *in situ* after the renal vessels had been ligated and the bile duct cannulated. One experiment typical of the three performed.

† Samples were taken 15 min after instillation of cysteine-*S*-paracetamol in the intestinal lumen. The same concentration of cysteine-*S*-paracetamol was observed in caval and portal plasma.

‡ Bile was collected for 15 min.

DISCUSSION

Metabolism of paracetamol was studied in the isolated, perfused rat liver at 1, 5 and 10 mM drug concentrations in the perfusate. A therapeutic dose (0.6–1 g) in man leads to a drug concentration of 1–2 mM in portal plasma. A concentration of 10 mM thus represents an overdose which, however, did not damage the isolated perfused liver in regards to the investigated drug metabolic functions.

Our findings of sulphate, glucuronide and glutathione conjugates as the main metabolites of paracetamol confirms clinical-pharmacological studies [5] as well as results obtained with isolated hepatocytes [8]. Paracetamol-*S*-cysteine was not detected in the perfusate, but inconstantly at low concentrations in the bile.

Under our experimental conditions, overall metabolism of paracetamol appeared to be linear up to a drug concentration of 5 mM in the perfusate. The total amount of conjugates formed was close to 5-fold higher with 5 mM than with 1 mM paracetamol. A further increase in drug concentration to 10 mM, however, resulted in only 50 per cent increase in total amount of metabolites excreted; the sulphate conjugate increased by only 17 per cent, the glucuronide conjugate by 111 per cent and the glutathione conjugate by 373 per cent. Thus, sulphate conjugation appeared to become saturated. In isolated hepatocytes saturation of the sulphate conjugation system occurs already at a paracetamol concentration of 1 mM; glucuronidation becomes saturated at 5 mM, whereas GSH conjugation is not saturated even at 25 mM paracetamol concentration [8]. Under our experimental conditions we were not able to saturate either glucuronidation or GSH conjugation in the perfused liver. This difference may

reflect an uneven distribution of paracetamol between the individual hepatocytes in the intact liver.

Even though the bulk of the paracetamol conjugates was recovered in the recirculating perfusate after 90 min of perfusion (cf. Table 1), initially the glucuronide and glutathione conjugates were mainly excreted in bile (cf. Fig. 1). The reason for this change in excretion pattern with time is not clear but a toxic effect of paracetamol on biliary excretion may have been a contributory factor. A predominant excretion of the glutathione conjugate into bile would be in agreement with the observation of Wahlländer and Sies that the *S*-glutathione conjugate of 1-chloro-2,4-dinitrobenzene formed in the perfused liver is exclusively excreted in the bile [17].

On some occasions we saw a transient decrease in bile flow, but this was always accompanied by an increase in biliary metabolite concentrations, denoting that bile flow was not a limiting factor for conjugate excretion in the bile. Wahlländer and Sies recently reported that bile flow was stimulated during biliary excretion of a *S*-glutathione conjugate of 1-chloro-2,4-dinitrobenzene in the isolated perfused liver [17]. In contrast to them we observed no stimulation of bile flow even at the highest rate of paracetamol-*S*-glutathione formation and excretion.

The partition between the hepatic venous and biliary routes of excretion varied between the conjugates. Paracetamol sulphate was mainly excreted in the perfusate and only to a very small extent in bile. Glucuronide and glutathione conjugates appeared at much higher concentrations in bile, as compared to the sulphate conjugate, but still the hepatic vein was the main outflow route for all three metabolites. The excretion rates were linear in perfusate as well as in bile for all three conjugates and the relative proportions remained constant in perfusate and in bile. However, the pattern of conjugates varied with initial paracetamol concentration. At increasing paracetamol concentration sulphate conjugation played a less prominent role, whereas GSH conjugation became quantitatively more important.

Perfusion of the isolated liver with a medium containing preformed paracetamol conjugates, in addition to a very low concentration of free drug, showed that the conjugates were not absorbed. This was most likely due to their low lipid solubility. The only conjugate that appeared in bile under these conditions was paracetamol sulphate. This may be surprising, since the sulphate conjugate is the least prominent of the biliary paracetamol metabolites (cf. Table 1). However, in similarity to the results obtained when paracetamol was instilled in the intestinal lumen (cf. Table 3), it has been shown that at very low paracetamol concentrations (in the micromolar range), the sulphate conjugate is the main metabolite in bile as well as in hepatic venous blood, indicating a preference for this metabolic pathway [18]. The unchanged concentrations of glucuronide and glutathione conjugates in the perfusate, their absence in bile, and the linear decrease of free paracetamol in the perfusate suggest that additional synthesis of paracetamol sulphate was responsible for the appearance of this metabolite in bile.

Using paracetamol as substrate and a combination of isolated rat liver and intestinal cells we have demonstrated that formation of paracetamol-*S*-glutathione by hepatocytes is followed by further metabolism of this conjugate in the intestinal cells. In isolated intestinal cells paracetamol is preferentially conjugated with glucuronic acid and only trace amounts of the sulphate, glutathione and cysteine conjugates can be detected [13]. In contrast, isolated intestinal cells rapidly converted the GSH conjugate to paracetamol-*S*-cysteine which in turn was slowly acetylated to the *N*-acetylcysteine derivative. Hydrolysis of paracetamol-*S*-cysteinylglycine was rapid since this conjugate was only present in trace amounts. An almost stoichiometric relationship occurred between the disappearance of the GSH conjugate and the formation of the cysteine and *N*-acetylcysteine derivatives. The breakdown of paracetamol-*S*-glutathione by the intestinal cells was stimulated by methionine and inhibited by serine·borate. Methionine is known to be an efficient acceptor of the γ-glutamyl moiety in the γ-glutamyltranspeptidase reaction [19], whereas serine·borate has been reported to be a transition state inhibitor of the enzyme [20]. Thus, the increased metabolism of the GSH conjugate found with methionine, and the almost complete inhibition by serine·borate, clearly confirm the role of intestinal γ-glutamyltranspeptidase in the breakdown of paracetamol-*S*-glutathione by the intestinal cells.

When biliary paracetamol metabolites were instilled in the intestinal lumen, a rapid metabolism of the GSH conjugate occurred, which resulted in an increase in the amount of cysteine conjugate in the intraluminal fluid and the appearance of this conjugate in plasma. The enhanced concentration of paracetamol-*S*-cysteine in the intestinal lumen supports the assumption that this metabolite is formed through metabolism of the glutathione conjugate by superficially located γ-glutamyltranspeptidase [21] and dipeptidase in the brush border of the villous tip cells and that these reactions probably occur before absorption of the cysteine conjugate. That the cysteine conjugate is indeed absorbed from the intestine is supported by the fact that paracetamol-*S*-cysteine instilled in a closed intestinal segment *in situ* was found at rather high concentrations in the plasma already after 15 min (cf. Table 4).

When biliary paracetamol metabolites were instilled in the closed intestinal segment, the major metabolite recovered in portal plasma was the glucuronide. The increased proportion of glucuronide could not be explained by its formation in the intestinal wall, but rather to a direct absorption from the intestinal lumen, as have been previously reported for glucuronides of other compounds [22, 23]. Subsequent passage and excretion into the portal blood of unchanged glucuronide and other conjugates may contribute to the enterohepatic circulation of paracetamol and drugs in general. Glucuronides of compounds that undergo enterohepatic circulation are normally thought to be hydrolyzed by the intestinal flora and the aglycone subsequently absorbed [24]. Cleavage of paracetamol glucuronide occurs in the distal region of the gut [25], which exhibits conjugate splitting enzyme activities in the tissue and in the

flora [25, 26], since very little paracetamol is excreted in faeces [27].

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