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Metabolism and Excretion of S-Conjugates Derived from Hexachlorobutadiene in the Isolated Perfused Rat Kidney

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SUMMARY

Renal processing of the *S*-conjugates derived from hexachlorobutadiene (HCBD), *S*-(pentachlorobutadienyl)glutathione (PCBG), and *S*-(pentachlorobutadienyl)-L-cysteine (PCBC) was studied in the isolated perfused rat kidney. At an initial perfusate concentration of 20 μM, both conjugates were rapidly eliminated from the perfusate. Calculation of the fractional clearance rates revealed the dominant role of nonfiltering mechanisms in this process. This was confirmed by the strong inhibitory effect of 50 μM probenecid. *S*-(Pentachlorobutadienyl)-*N*-acetyl-L-cysteine (*N*-Ac-PCBC) was detected as the major metabolite of both PCBG and PCBC in urine and perfusate. PCBC and *S*-(pentachlorobutadienyl)cysteinylglycine were minor urinary metabolites formed from PCBG; only *N*-Ac-PCBC and PCBC were detected in the perfusate. At an initial *S*-conjugate concentration of 100 μM in the perfusate, the rate of elimination of both PCBG and PCBC continuously decreased during the perfusion, mainly as the result of a reduced excretion of *N*-Ac-PCBC. This indicates marked disturbance of *N*-acetylation and/or transport under these conditions. Addition of probenecid resulted in a significantly reduced renal elimination of both *S*-conjugates, predominantly due to a reduced rate of mercapturate excretion. In contrast, the nephrotoxicity of PCBC or PCBG was not significantly influenced by probenecid. It is concluded from these experiments that the kidney has the capacity to metabolize HCBD *S*-conjugates and that nonfiltering excretion of the mercapturic acid plays a decisive role. The pathways of HCBD *S*-conjugate metabolism in the kidney were shown to be dependent on their initial concentrations in the perfusate, most probably as a consequence of concentration-dependent toxic disturbances of transport and/or *N*-acetylation.

Conjugation with glutathione in the liver, catalysed by glutathione *S*-transferases, is a major metabolic pathway for electrophilic xenobiotics or their metabolites. The glutathione *S*-conjugates formed are transported to the kidney. Renal proximal tubules possess high activities of GGT and dipeptidase(s), which hydrolyse glutathione conjugates to cysteine *S*-conjugates. *N*-Acetylation of the cysteine moiety by renal *N*-acetyltransferase yields mercapturic acids excreted in urine as metabolites of glutathione conjugates (1). Excretion of the cysteine conjugates has also been reported (2).

Recently, glutathione conjugation has been demonstrated for several haloalkenes such as trichloroethylene (3), tetrachloroethylene (4), and HCBD (5). This metabolic step results in the formation of highly nephrotoxic metabolites (6).

HCBD, a model compound for the investigation of haloalkene-induced nephrotoxicity, is readily conjugated with glutathione to form the nephrotoxic PCBG (7), which is excreted in bile (8). PCBG or its metabolites may be reabsorbed from the gut, transported to the kidney in the bloodstream, and processed to PCBC.

PCBC is cleaved by renal cysteine conjugate β-lyase (β-lyase) to ammonia, pyruvate, and a highly reactive *S*-retaining fragment assumed to cause nephrotoxicity by covalent binding to cellular constituents (9). The reaction of this intermediate with water was suggested to result in the formation of 1,1,2,3-tetrachlorobut-1,2-enoic acid, which has been identified in urine from HCBD-treated mice (10). In addition, PCBC can be acetylated in the kidney to the corresponding mercapturic acid, *N*-Ac-PCBC, another urinary metabolite of HCBD in rodents (5, 10).

Little information is available about the renal uptake and processing of haloalkene *S*-conjugates. *S*-(1,2-dichlorovinyl)-glutathione, a glutathione conjugate derived from trichloroethylene, is concentrated by isolated rat kidney cells (11). The corresponding cysteine conjugate, *S*-(1,2-dichlorovinyl)L-cysteine, can also be actively transported into the kidney cell line LLC-PK$_1$ (12). Active transport was suggested to be a prerequisite for the nephrotoxicity of the *S*-conjugates, as indicated

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ABBREVIATIONS: GGT, γ-glutamyltranspeptidase; FAB, fast atom bombardment; GFR, glomerular filtration rate; HCBD, hexachloro-1,3-butadiene; *N*-Ac-PCBC, *S*-(pentachlorobutadienyl)-*N*-acetyl-L-cysteine; PCBC, *S*-(pentachlorobutadienyl)-L-cysteine; PCBCG, *S*-(pentachlorobutadienyl)-L-cysteinylglycine; PCBG, *S*-(pentachlorobutadienyl)glutathione; HPLC, high performance liquid chromatography.

by the protective effect of probenecid (13). The mercapturic acid *N*-Ac-PCBC is transported very effectively by isolated renal tubules and was suggested to be excreted rapidly into the tubular lumen by the probenecid-sensitive renal organic anion secretory system (14), as demonstrated for other mercapturic acids (15).

The present study was designed to elucidate the mechanisms of the renal processing of both PCBG and PCBC, using the experimental model of the isolated perfused rat kidney. Metabolites occurring in perfusion medium and urine were identified and quantified to provide further information on the role of the kidney in *S*-conjugate metabolism. Insight is obtained into the transport pathways of renal excretion of PCBG and PCBC by estimation of the amounts of both their glomerular filtration and their elimination by nonfiltering pathways.

Materials and Methods

Chemicals: [^{3}H]Inulin was obtained from Amersham Buchler (Braunschweig, FRG); probenecid and other chemicals used for the kidney perfusion were purchased from Sigma Chemical Co. (St. Louis, MO). Starch 2-hydroxyethylether was a generous gift from Pfrimmer (Erlangen, FRG). All other chemicals were at least analytical grade and were obtained locally.

The following *S*-conjugates were synthesized as described previously: PCBG (10), PCBC (16), and *N*-Ac-PCBC (5).

PCBCG. To a solution of 2 mmol of cysteinyl glycine in 100 ml of liquid ammonia, sodium was added until the blue color of the solution persisted for at least 2 min; 3 mmol of HCBD dissolved in 4 ml of dry dimethyl formamide were then added slowly and the reaction mixture was stirred at −30° for 4 hr. After evaporation of the ammonia, the residue was dissolved in water and acidified to pH 2 with 2 N HCl and the PCBCG was purified by preparative HPLC (250 × 8 mm steel column filled with Lichrosorb RP-18, 5 μm; for conditions see Ref. 4). Mass spectrum (FAB): ^{35}Cl, for *m/z* 415 (5 Cl); 401 (5 Cl) $(M + H)^+$; 386 (5 Cl); 330 (3 Cl, $M^+ - 2$ Cl); 298 (5 Cl); 255 (5 Cl, $Cl_5C_5S^+$); 222 (3 Cl); purity; 98%; thin layer chromatography (butanol/acetic acid/water 12:3:5); R_F = 0.53 UV spectrum; λ = 221 (ε = 22 000); λ = 280 (ε = 11 500).

Animals and perfusion. Female Wistar rats weighing 180–200 g were obtained from the Institut für Versuchstierkunde (Hannover, FRG) and were allowed free access to water and food (Altromin, Lage, FRG). Animals were anesthetized with pentobarbital (Nembutal) (50 mg/kg, intraperitoneally). The surgical procedure and recirculating perfusion were performed as described by Newton and Hook (17). Instead of albumin, 6% (w/v) starch 2-hydroxyethylether was added to the perfusate as oncotic agent. Over a period of 90 min after equilibration (15 min), the functional characteristics of the perfused kidney were as follows: perfusion medium flow, 6.5 ± 1.4 ml/min/100 g of body weight; GFR, 1017 ± 62 μl/min/g of kidney; urinary GGT, 30 ± 8 units/ml; fractional excretion of glucose, 0.033 ± 0.010 (values are means ± standard deviations from four independent experiments). After equilibration (perfusion time zero), PCBC or PCBG were added to the perfusate. One-minute fractions of urine were collected at various time points in preweighed vials and stored on ice. Simultaneously, 1-ml samples of perfusate were taken and stored in ice-cooled vials.

Determination of nephrotoxicity and renal function. GGT activity and glucose concentration in urine were determined with commercial test kits (Sigma; Boehringer, Mannheim, FRG). GFR was calculated from the clearance of [^{3}H]inulin added to the perfusion medium (final activity, 74 Bq/ml) containing 2 mg/100 ml unlabeled inulin. Fractional clearance of *S*-conjugates was obtained from their actual clearance and the GFR. The actual clearance of *S*-conjugates was determined by measuring decreases in perfusate concentration of PCBG and PCBC over 1-min clearance periods.

Quantification of metabolites. After collection of urine, vials were weighed and perfusion medium and urine samples were diluted 1:1 with methanol. After storage on ice for 1 hr, precipitates were removed by centrifugation and supernatants (200 μl) were injected into a liquid chromatography system (Waters, Milford, MA) consisting of two M 6000 A pumps, a model 660 solvent programmer, and a U6 K injector. Separations were performed on steel columns (250 × 4 mm) filled with Partisil ODS III (5 μm, Whatman, Maidstone, Great Britian). Metabolites were eluted with a linear gradient (100% H_2O/13 mM trifluoroacetic acid to 100% methanol) in 40 min; flow rate was 1 ml/min. The eluate was monitored by UV spectroscopy (1040 diode array detector; Hewlett Packard, Avondale, PA) at 280 nm, and peak areas were integrated using the standard software provided with the 1040 detector. Concentrations of metabolites were calculated by computerized comparison with standard curves.

Results

Perfusion of PCBC. At an initial concentration of 20 μM, PCBC was rapidly resorbed from the medium used for perfusion by the isolated perfused kidney (Fig. 1). A transient increase in the concentration of the metabolite *N*-Ac-PCBC was observed in the perfusate. It attained maximum perfusate concentration 10–15 min after addition of PCBC; subsequently its concentration decreased below the limit of detection. At a PCBC concentration of 100 μM, appreciable amounts of both PCBC and its metabolite *N*-Ac-PCBC were retained (Fig. 1). At both concentrations of PCBC perfused, the urine contained PCBC and *N*-

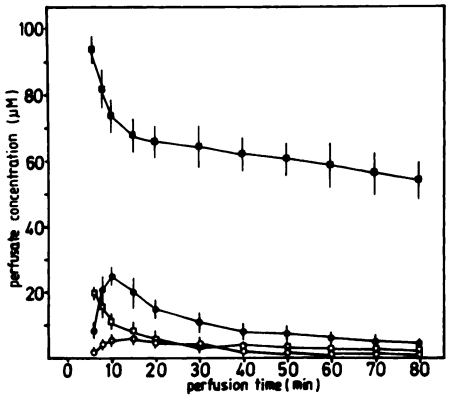

Fig. 1. Perfusate concentration of PCBC (*squares*) and *N*-Ac-PCBC (*circles*) after addition of 20 μM (*open symbols*) or 100 μM (*closed symbols*) PCBC to the perfusion medium. Values are means ± standard deviations from three isolated perfused rat kidneys.

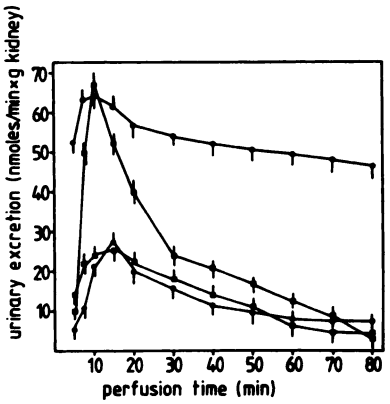

Fig. 2. Urinary excretion of PCBC (*circles*) and *N*-Ac-PCBC (*squares*) after addition of 20 μM (*open symbols*) or 100 μM PCBC (*closed symbols*) to the perfusion medium. Values are means ± standard deviations from three isolated perfused rat kidneys.

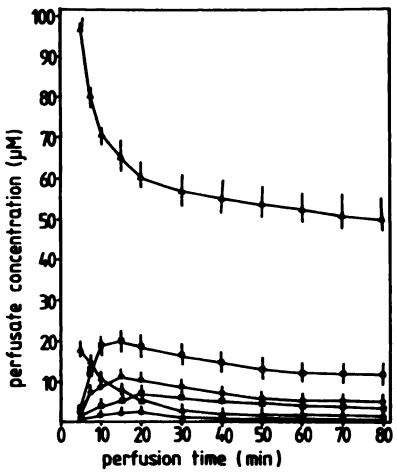

Fig. 3. Perfusate concentration of PCBG (*triangles*), PCBC (*circles*), and *N*-Ac-PCBC (*squares*) after addition of 20 μM (*open symbols*) or 100 μM (*closed symbols*) PCBG to the perfusion medium. Values are means ± standard deviations from three isolated perfused rat kidneys.

Ac-PCBC. At 20 μM, both PCBC and *N*-Ac-PCBC were continuously excreted in an approximate ratio of 1:1 (Fig. 2). Their excretion rate decreased with time, resulting in a metabolite-free urine after 90 min. Ten minutes after an addition of 100 μM PCBC to the perfusion medium, the mercapturic acid represented the major urinary metabolite (Fig. 2). However, its excretion rate declined markedly during the perfusion period, whereas the excretion of unmetabolized PCBC was reduced to only a minor extent.

Perfusion of PCBG. At an initial concentration of 20 μM, PCBG was rapidly eliminated from the perfusion medium and PCBC and *N*-Ac-PCBC appeared as transient metabolites, which, however, were also completely eliminated by the end of the perfusion time. At a concentration of 100 μM PCBG, neither the glutathione *S*-conjugate nor its metabolites were completely eliminated from the perfusion medium (Fig. 3). In urine, in addition to PCBC and *N*-Ac-PCBC, the presence of a third metabolite was indicated by HPLC separations. This metabolite exhibited an UV spectrum typical for *S*-conjugates with a pentachlorobutadienyl moiety (Fig. 4). It was isolated by semipreparative HPLC and subjected to FABMS. The spectrum (Fig. 5) showed several fragments indicating the presence of five chlorine atoms (for ^{35}Cl, *m/z* 401, $(M + H)^+$; 255, $Cl_5C_4S^+$) in the molecule and was identical to the FAB mass spectrum of synthetic PCBCG (not shown). The retention times of the metabolite were identical to those of synthetic PCBCG under various chromatographic conditions. The metabolite was therefore definitively identified as PCBCG.

At an initial concentration of 20 μM PCBG, *N*-Ac-PCBC was the major urinary metabolite present together with low concentrations of PCBC and PCBCG (Fig. 6). Initially, PCBG was excreted at low concentrations. After perfusion periods of 60 min, this increased to about the level of the metabolites. With 100 μM PCBG, N-Ac-PCBC was initially excreted in urine in large amounts but its excretion rate rapidly declined during the perfusion. In samples taken at later time points, PCBC and PCBCG concentrations increased and, finally, unmetabolized PCBG became the major excretion product (Fig. 6).

Studies on the mechanism of *S*-conjugate excretion. To determine the effects of the HCBD *S*-conjugates on the GFR, clearance of [^{3}H]inulin was determined in the presence of PCBG or PCBC. PCBC (100 μM) reduced GFR by 22% at the end of the perfusion (not shown). PCBG (100 μM) also caused a moderate (10%) reduction of GFR, whereas neither *S*-conjugate influenced GFR at concentrations of 20 μM in the perfusion medium. The fractional clearance of both PCBC and PCBG was about 6-fold greater than unity, whereas in the presence of probenecid (50 μM), the fractional clearance of both *S*-conjugates was equal to unity (Fig. 7).

Perfusions in the presence of 50 μM probenecid revealed a corresponding suppression of total urinary excretion of HCBD *S*-conjugates, mainly due to a marked decrease of mercapturic acid excretion. Under these conditions, PCBC and PCBCG were the major urinary metabolites from 20 μM PCBG (Fig. 8), whereas unmetabolized PCBC was the main excretion product when probenecid was coperfused with 20 μM PCBC (not shown).

Nephrotoxicity was measured as release of GGT into urine. PCBC (20 μM) produced pronounced toxicity as demonstrated in Fig. 8. PCBG (20 μM) was also nephrotoxic, producing, however, a smaller and later increase in urinary GGT. In both cases probenecid was ineffective in protecting the kidney against the *S*-conjugate-induced toxicity.

Discussion

PCBG and PCBC are quickly taken up by the isolated perfused rat kidney. Inasmuch as the fractional clearances of

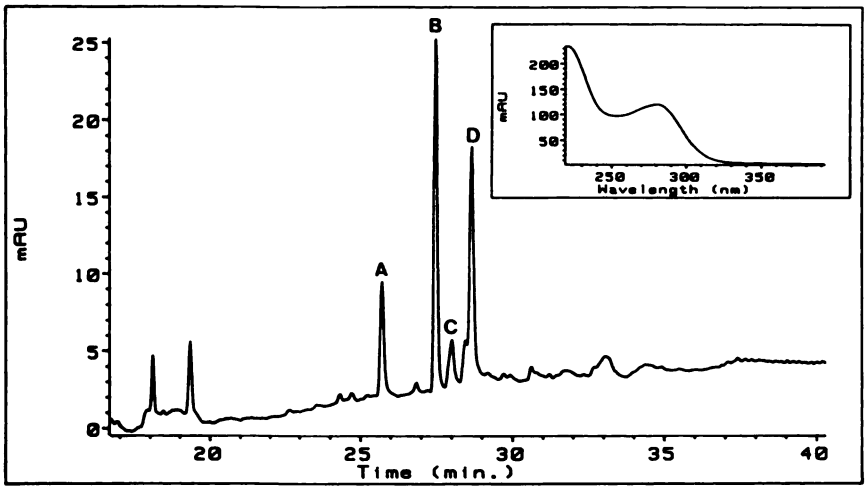

Fig. 4. Reversed phase HPLC separation (UV detection at 280 nm) of urinary metabolites formed in the isolated rat kidney perfused with 100 μM PCBG. Chromatography was performed on a Waters liquid chromatography system (see Materials and Methods for chromatographic conditions). Identities of chromatographic peaks are as follows: *A*, PCBCG; *B*, PCBG; *C*, PCBC; *D*,*N*-Ac-PCBC. *Inset*, UV absorption spectrum of metabolite A from perfusion urine.

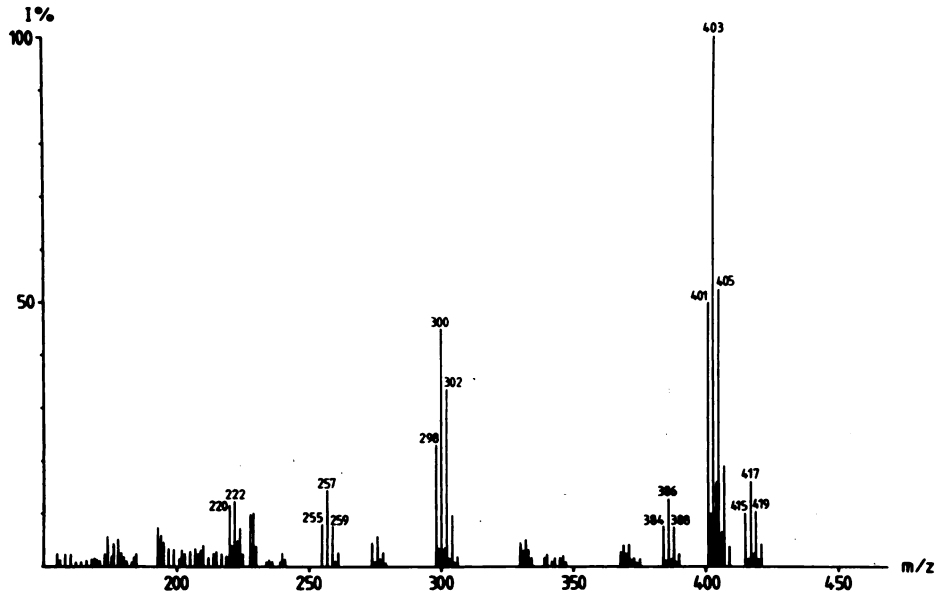

Fig. 5. FAB mass spectrum of metabolite A from urine formed in the isolated rat kidney perfused with 100 μM PCBG. To obtain FAB spectra, the dry residue obtained after metabolite isolation by HPLC was dissolved in glycerol (containing 10% HCl). Spectra were recorded with a Varian MAT CH-1 spectrometer with argon as reagent gas. FAB gun voltage was 7 kV.

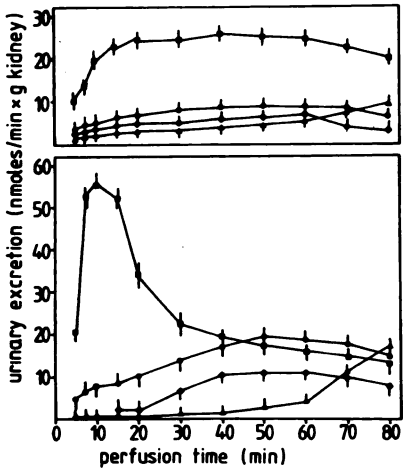

Fig. 6. Urinary excretion of PCBG (*triangles*), PCBCG (*diamonds*), PCBC (*circles*), and *N*-Ac-PCBC (*squares*) after addition of 20 μM (*open symbols*) or 100 μM (*closed symbols*) PCBG to the perfusate. Values are means ± standard deviations from three isolated perfused kidneys.

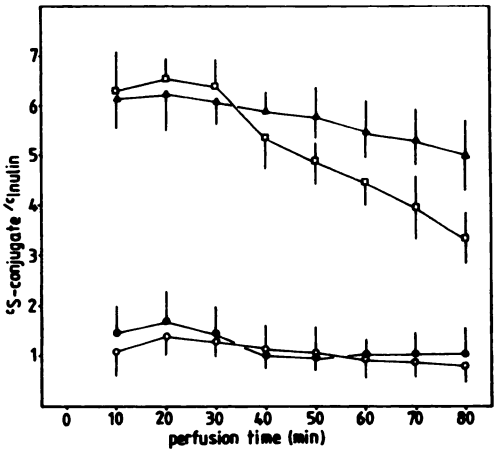

Fig. 7. Fractional clearance in the isolated perfused rat kidney of 20 μM PCBG without (▲) or with 50 μM probenecid (●), and 20 μM PCBC without (□) or with 50 μM probenecid (○). Values are means ± standard deviations from three isolated perfused rat kidneys.

both *S*-conjugates are 6-fold higher than unity, nonfiltering uptake and excretion is most probably the predominant pathway for the renal processing of these compounds. Similar data have been reported by Anderson *et al.* (18) for the *in vivo* renal elimination of glutathione and by Newton *et al.* (19) for the clearance of an acetaminophen glutathione *S*-conjugate by the isolated perfused rat kidney. These authors suggest the presence of an active transport mechanism for glutathione and glutathione *S*-conjugates in the kidney as demonstrated in erythrocyte membranes (20) and hepatic canalicular plasma membranes (21). Recent work (22) suggests the existence of a sodium-dependent transport system for glutathione and the GSH glutathione conjugate *S*-(1,2-dichlorovinyl)glutathione (11) in renal basal-lateral membrane vesicles and isolated rat kidney cells.

After uptake from the basal-lateral pole of the tubular cell, *S*-conjugates are metabolized to excretable mercapturic acids or activated to toxic metabolites. In the case of PCBC, metabolism contributes considerably to its disappearance from perfusion medium. The only metabolite with an intact pentachlorobutadienyl moiety, *N*-Ac-PCBC, is formed in the kidney by renal *N*-acetyl transferases and rapidly excreted into the tubular lumen or transported back into the perfusion medium to be excreted subsequently into urine, presumably by a high affinity tubular secretion mechanism as proposed by Lock *et al.* (14).

The rapid decline of *N*-Ac-PCBC excretion at 100 μM PCBC can be explained by the potent toxicity of PCBC at this concentration, which produces severe cellular damage and disturbance of active transport. At the low concentration of 20 μM, the excretion of both PCBC and *N*-Ac-PCBC decreased continuously, most likely as a result of the continuous decline of the PCBC concentration in the perfusion medium.

PCBG requires processing by GGT and dipeptidases to PCBC for activation by β-lyase or for acetylation of N-acetyltransferase to yield N-Ac-PCBC. No significant differences were observed in the rate of urinary excretion of *N*-Ac-PCBC in experiments with either PCBC or PCBG (Figs. 2 and 6)

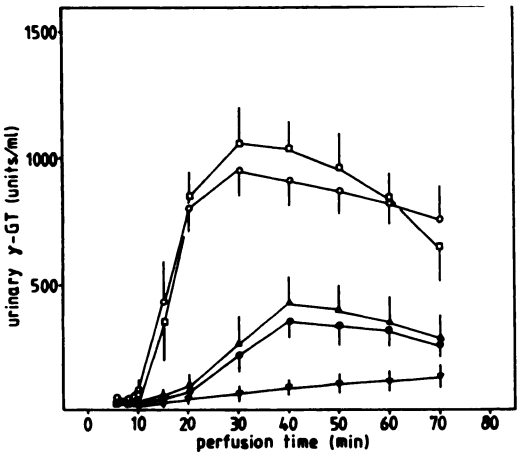

Fig. 8. Effects of 20 μM PCBC without (□) and with 50 μM probenecid (○), and 20 μM PCBG without (▲) and with 50 μM probenecid (●), and of 50 μM probenecid alone (▽) on the urinary GGT activity in the isolated perfused rat kidney. Values are means ± standard deviations from three independent experiments.

This may be due to the high concentration of GGT and dipeptidases in the brush border of the kidney. These enzymes very rapidly transform PCBG to PCBC. At 20 μM PCBG, the major metabolite, *N*-Ac-PCBC, is continuously excreted in urine together with smaller amounts of PCBC and PCBCG. At 100 μM, the initial pronounced mercapturic acid formation rapidly decreased, indicating the potent toxicity of PCBG at this concentration. Cellular transport and/or enzymatic *N*-acetylation as prerequisites for the excretion of *N*-Ac-PCBC are obviously susceptible to the toxic action of PCBG whereas PCBC and PCBCG, most probably formed by brush border GGT and dipeptidase, become major urinary metabolites. Furthermore, their uptake by renal epithelial cells may be inhibited by the toxic effects of PCBG, including both direct damage to transport systems and mitochondrial damage resulting in disturbance of energy-dependent transport.

Both HCBD *S*-conjugates are evidently eliminated predominantly by nonfiltering mechanisms, which may be concluded from their high fractional clearances. Accordingly, addition of probenecid to the perfusion medium reduced fractional clearance values to unity, which indicates that only glomerular filtration is operative under these conditions. Hook *et al.* (23) have previously reported that 30 mg/kg probenecid did not influence the nephrotoxicity of HCBD in rats, whereas higher doses were shown by Lock and Ishmael (13) to provide protection against the nephrotoxicity of HCBD, PCBG, and PCBC. Uptake of PCBG does not appear to be sensitive to probenecid, inasmuch as the toxicity of PCBG in isolated rat kidney cells was only slightly inhibited by 1 mM probenecid (24), whereas tubular transport of *N*-Ac-PCBC was strongly suppressed by 50 μM probenecid (14).

In our experiments, probenecid reduced renal elimination of PCBG and PCBC predominantly by reduction of mercapturate excretion, whereas PCBCG and PCBC, assumed to be formed from PCBG mainly in the tubular lumen (25), became the major urinary metabolites of PCBG. Because 50 μM probenecid did not protect the kidney against the toxicity of either *S*-conjugate, a marked influence on their basal-lateral uptake is unlikely.

The results obtained in our study clearly demonstrate the outstanding capacity of the kidney to process the nephrotoxic HCBD *S*-conjugates PCBC and PCBG. Strong evidence is provided for the existence of efficient nonfiltering pathways for their elimination. Our findings also show not only that the renal metabolism of HCBD *S*-conjugates is responsible for their nephrotoxicity but also that their toxic effects decisively influence the metabolic pathways of these compounds. Further work is necessary to identify the ultimate reactive metabolite formed in the kidney and to elucidate its role in nephrotoxicity and nephrocarcinogenicity.

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References

1. Chasseaud, L. F. Conjugation with glutathione and mercapturic acid excretion, in *Glutathione: Metabolism and Function* (W. B. Jakoby and I. M. Arias, eds.). Raven Press, New York, 77–114 (1976).
2. Fischer, L. J., M. D. Green, and A. W. Harman. Studies on the fate of the glutathione and cysteine conjugates of acetaminophen in mice. *Drug Metab. Dispos.* **13**:121–126 (1985).
3. Dekant, W., M. Metzler, and D. Henschler. Identification of *S*-1,2-dichlorovinyl-*N*-acetyl-cysteine as a urinary metabolite of trichloroethylene: a possible explanation for its nephrocarcinogenicity in male rats. *Biochem. Pharmacol* **35**:2455–2458 (1986).
4. Dekant, W., M. Metzler, and D. Henschler. Identification of *S*-1,2,2-trichlorovinyl-*N*-acetylcysteine as a urinary metabolite of tetrachloroethylene: bioactivation through glutathione conjugation as a possible explanation of its nephrocarcinogenicity. J. Biochem. Toxicol. **1**:57–72 (1986).
5. Reichert, D., and S. Schütz. Mercapturic acid formation is an activation and intermediary step in the metabolism of hexachlorobutadiene. Biochem. Pharmacol. **35**:1271–1275 (1986).
6. Elfarra, A. A., and M. W. Anders. Renal processing of glutathione conjugates; role in nephrotoxicity. *Biochem. Pharmacol.* **33**:3729–3732 (1984).
7. Wolf, C. R., P. N. Berry, J. A. Nash, T. Green, and E. A. Lock. Role of microsomal and cytosolic glutathione *S*-transferase in the conjugation of hexachloro-1:3-butadiene and its possible relevance to toxicity. J. Pharmacol. Exp. Ther. **228**:202–208 (1984).
8. Jones, T. W., R. G. Gerdes, K. Ormstad, and S. Orrenius. The formation of both a mono- and a bis-substituted glutathione conjugate of hexachlorobutadiene by isolated hepatocytes and *in vivo* administration to the rat. Chem.-Biol. Interact. **56**:251–267 (1985).
9. Green, T., and J. Odum. Structure/activity studies of the nephrotoxic and mutagenic action of cysteine conjugates of chloro- and fluoroalkenes. Chem.-Biol. Interact. **54**:15–31 (1985).
10. Dekant, W., D. Schrenk, S. Vamvakas, and D. Henschler. Metabolism of hexachloro-1,3-butadiene in mice: *in vivo* and *in vitro* evidence for activation by glutathione conjugation. *Xenobiotica*, in press.
11. Lash, L. H., and D. P. Jones. Uptake of the glutathione conjugate *S*-(1,2-dichlorovinyl)glutathione by renal basal-lateral membrane vesicles and isolated kidney cells. *Mol. Pharmacol.* **28**:278–282 (1985).
12. Schaeffer, V. H., and J. L. Stevens. The transport of *S*-cysteine conjugates in LLC-PK$_1$ cells and its role in toxicity. Mol. Pharmacol. **31**:506–512 (1986).
13. Lock, E. A., and J. Ishmael. Effect of the organic acid transport inhibitor probenecid on renal cortical uptake and proximal tubular toxicity of hexachloro-1,3-butadiene and its conjugates. Toxicol. Appl. Pharmacol. **81**:32–42 (1985).
14. Lock, E. A., J. Odum, and P. Ormond. Transport of *N*-acetyl-*S*-pentachloro-1,3-butadienylcysteine by rat renal cortex. Arch. Toxicol. **59**:12–15 (1986).
15. Inoue, M., K. Okajima, and Y. Morino. Renal transtubular transport of mercapturic acids *in vivo*. Biochim. Biophys. Acta **641**:122–128 (1981).
16. Dekant, W., S. Vamvakas, K. Berthold, S. Schmidt, D. Wild, and D. Henschler. Bacterial β-lyase mediated cleavage and mutagenicity of cysteine conjugates derived from the nephrocarcinogenic alkenes trichloroethylene, tetrachloroethylene, and hexachlorobutadiene. Chem.-Biol. Interact. **60**:31–45 (1986).
17. Newton, J. F., and J. B. Hook. The isolated perfused kidney. *Methods Enzymol.* **77**:94–105 (1981).
18. Anderson, M. E., R. J. Bridges, and A. Meister. Direct evidence for interorgan transport of glutathione and that the non-filtration renal mechanism for glutathione utilization involves γ-glutamyl transpeptidase. Biochem. Biophys. Res. Commun. **96**:848–853 (1980).
19. Newton, J. F., D. Hoefle, M. W. Gemborys, G. H. Mudge, and J. B. Hook. Metabolism and excretion of a glutathione conjugate of acetaminophen in the isolated perfused rat kidney. J. Pharmacol. Exp. Ther. **237**:519–524 (1986).
20. Kondo, T., M. Murao, and N. Taniguchi. Glutathione *S*-conjugate transport using inside-out vesicles from human erythrocytes. Eur. J. Biochem. **125**:551–554 (1982).
21. Inoue, M., T. P. M. Akerboom, H. Sies, R. Kinne, and I. M. Arias. Biliary

transport of glutathione *S*-conjugates by rat liver canalicular membrane vesicles. J. Biol. Chem. **259**:4998–5002 (1984).
22. Lash, L. H., and D. P. Jones. Renal glutathione transport. J. Biol. Chem. **259**:14508–14514 (1984).
23. Hook, J. B., M. S. Rose, and E. A. Lock. The nephrotoxicity of hexachloro-1:3-butadiene in the rat: studies of organic anion and cation transport in renal slices and the effect of monooxygenase inducers. *Toxicol. Appl. Pharmacol.* **65**:373–382 (1982).
24. Jones, T. W., A. Wallin, H. Thor, R. G. Gerdes, K. Ormstad, and S. Orrenius. The mechanism of pentachlorobutadienyl-glutathione nephrotoxicity studied with isolated rat renal epithelial cells. *Arch. Biochem. Biophys.* **251**: 504–513 (1986).
25. Jones, T. W., H. Thor, and S. Orrenius. *In vitro* studies of mechanisms of cytotoxicity. Food Chem. Toxicol. **24**:769–773 (1986).

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