Biochemical Pharmacology, Vol. 36, No. 17, pp. 2741–2748, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

ENZYMATIC TRANSFORMATION OF MERCAPTURIC ACIDS DERIVED FROM HALOGENATED ALKENES TO REACTIVE AND MUTAGENIC INTERMEDIATES

SPYRIDON VAMVAKAS, WOLFGANG DEKANT*, KLEMENS BERTHOLD, SABINE SCHMIDT, DIETER WILD and DIETRICH HENSCHLER†

Institute of Toxicology, University of Würzburg, Versbacherstr. 9, D-8700 Würzburg, Federal Republic of Germany

(*Received* 18 *November* 1986; *accepted* 28 *January* 1987)

Abstract—The metabolism of the mercapturic acids *S*-pentachlorobutadienyl-*N*-acetylcysteine (N-Ac-PCBC), *S*-trichlorovinyl-*N*-acetylcysteine (*N*-Ac-TCVC) and *S*-dichlorovinyl-*N*-acetylcysteine (N-Ac-DCVC) by subcellular fractions from male rat liver and kidney homogenates was studied. As a model compound, N-Ac-PCBC, ^{14}C labelled, was synthesised. It was intensively metabolised by cytosolic but not by microsomal enzymes from rat liver and kidney. The major metabolite identified by GC/MS was pentachlorobutadienylcysteine, the amount produced being highest in kidney cytosol. Metabolic conversion of ^{14}C-N-Ac-PCBC by kidney and liver cytosol resulted in covalent binding of radioactivity to protein, binding was strongly inhibited by the β-lyase inhibitor aminooxyacetic acid (AOAA). N-Ac-TCVC and N-Ac-DCVC were also transformed by cytosolic enzymes to the corresponding cysteine conjugates (trichlorovinylcysteine and dichlorovinylcysteine). The three mercapturic acids tested were strong mutagens in the Ames-test after addition of rat kidney cytosol. In the absence of cytosol, N-Ac-TCVC and N-Ac-DCVC were weakly but definitely mutagenic, whereas N-Ac-PCBC was not. In contrast to N-Ac-PCBC, the "direct" mutagens N-Ac-TCVC and N-Ac-DCVC were both transformed to pyruvate by bacterial (*S. typhimurium* TA100) homogenate 100,000 *g* supernatants.

It is concluded that mercapturic acids are deacetylated to the corresponding cysteine conjugates by cytosolic (N-Ac-PCBC, N-Ac-TCVC and N-Ac-DCVC) and bacterial enzymes (N-Ac-TCVC and N-Ac-DCVC) and further cleaved to reactive and mutagenic intermediates by mammalian and/or bacterial β-lyase. The observed activation mechanisms for the mercapturic acids, whose formation from hexachlorobutadiene, tetrachloroethylene and trichloroethylene has been proven, might contribute to the nephrotoxicity and nephrocarcinogenicity of the parent alkenes.

Glutathione conjugation of xenobiotics usually represents a deactivation reaction resulting in the formation of stable mercapturic acids, which are rapidly excreted by the kidney [1]. During recent years, however, two different mechanisms involving glutathione conjugation have been identified which lead to activation of halogenated hydrocarbons to toxic and mutagenic intermediates.

1,2-dichloroethane and 1,2-dibromoethane are conjugated with glutathione to form 2-chloro- or 2-bromoethylglutathione, respectively [2, 3]. From these sulfur half-mustards, highly reactive episulfonium ions may be formed [4]. These strong electrophiles may play an important role in the mutagenicity of 1,2-dihaloalkanes and in the formation of DNA-adducts [5]. *S*-2-haloethyl-*N*-acetylcysteine(s), which are presumed to be formed by renal processing of haloethylglutathione(s), were also found to be strong and direct-acting mutagens [6].

The second multistep activation pathway involving glutathione conjugation has been shown to occur during metabolism of several nephrotoxic and/or nephrocarcinogenic halogenated alkenes. To date, hexachlorobutadiene [7], tetrafluoroethylene [8], chlorotrifluoroethylene [9] and to a small extent trichloroethylene [10] and tetrachloroethylene [11] have all been shown to be conjugated with glutathione under various conditions to form the corresponding *S*-haloalk(en)yl-derivatives. The GSH-conjugates are processed quickly to cysteine conjugates which are finally acetylated to form mercapturic acids [12]. However, thioethers of cysteine with electronegative and/or unsaturated substituents are also substrates for the enzyme β-lyase which cleaves them to reactive, sulfur-containing fragments (presumably thiols), pyruvate and ammonia. Cysteine conjugate β-lyase is present in high concentrations in the proximal tubular cells of rat kidney [13].

Recently, the mutagenicity of *S*-pentachlorobutadienyl-*N*-acetylcysteine (N-Ac-PCBC) was reported [14] and it was postulated that β-lyase contributes to the formation of mutagenic intermediates from this compound. β-Lyase [15], however, does not cleave mercapturic acids, the free amino group of cysteine being a prerequisite for enzymic activity of this pyridoxal phosphate dependent enzyme [16]. Enzymes catalysing a deacetylation of mercapturic acids have been described in rat liver [17] and in rat kidney [16]; the specificities for different mercapturic acids are not yet known.

* Present address: Department of Pharmacology, University of Rochester, 601 Elmwood Avenue, Rochester, NY 14642, U.S.A.

† Correspondence to: Dietrich Henschler, Versbacherstr. 9, D-8700 Würzburg, F.R.G.

In the present study we report on an investigation of the metabolism and the mutagenicity of the mercapturic acids *N*-acetyl-pentachlorobutadienyl-cysteine (N-Ac-PCBC), *N*-acetyl-trichlorovinyl-cysteine (N-Ac-TCVC) and *N*-acetyl-dichlorovinyl-cysteine (N-Ac-DCVC). The analytical identification of the products formed by the reaction of the mercapturic acids with mammalian enzymes and the influence of enzyme inhibition on metabolism, macromolecular binding and mutagenicity were used to clarify the role of β-lyase and deacetylase in the biotransformation and mutagenicity of these compounds.

MATERIALS AND METHODS

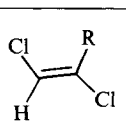
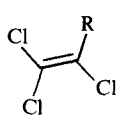
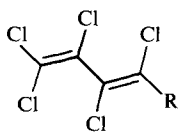
Syntheses. The syntheses of the mercapturic acids of trichloroethylene (TRI), tetrachloroethylene (TETRA) and hexachlorobutadiene (HCBD) were performed as follows: 1.63 g (10 mmol) of *N*-acetyl-L-cysteine were dissolved in 30 ml of dimethylsulfoxide (DMSO), and 2.48 g (20 mmol) of 1,5-diazabicyclo(4,3,0)non-5-ene (DBN) were added. After stirring for 5 min a solution of 10 mmol of the alkene in 20 ml DMSO was added dropwise and stirring was continued for 30 min–1 hr at room temperature. Then the solution was acidified with 25% HCl to pH 1–2 and diluted with 60 ml of water followed by ether extraction. After washing the ether phase with 20 ml of 1N HCl, the ether was evaporated under reduced pressure and the remaining oils were recrystallised from methanol/ether. For *N*-acetyl-*S*-pentachlorobutadienyl-L-cysteine, no crystalline products could be obtained. Radiolabelled ^{14}C-N-Ac-PCBC was synthesised from 1,1,2,3,4,4-(^{14}C)-hexachlorobutadiene (5.3×10^5 dpm/nmol, Radiochemische Abteilung, Farbwerke Hoechst, Frankfurt, FRG; radiochemical purity 99.5%) as described above. The analytical data of the synthetic mercapturic acids are shown in Table 1.

Incubation of ^{14}C-N-Ac-PCBC with mammalian subcellular fractions and 100,000 g supernatants from Salmonella typhimurium *homogenates.* Hepatic and renal cytosolic and microsomal fractions were prepared from male Wistar rats according to Wolf *et al.* [18]. Bacterial homogenate 100,000 *g* supernatants were prepared as described previously [19].

Incubations were carried out in 0.1 M phosphate buffer (pH 8.8) containing hepatic cytosol (10 mg protein/ml) or microsomes (10 mg protein/ml), renal cytosol (5 mg protein/ml) or microsomes (5 mg protein/ml) or bacterial protein (5 mg/ml) together with ^{14}C-N-Ac-PCBC diluted with unlabelled mercapturic acid (0.1 mM, 3.3×10^2 dpm/nmol). For some experiments, the homogenate supernatants were preincubated with aminooxyacetic acid (AOAA, 1 mM) for 10 min. The final incubation volume was 10 ml. Incubations were carried out at 37° in a shaker-water bath for 2 hr. The reaction was stopped by addition of two volumes of methanol. After storage at 4° for 24 hr the incubation mixtures were centrifuged for 10 min. The protein pellet was then washed twice with 5 ml of methanol, the supernatants were combined and concentrated at reduced pressure. The final volume after concentration was 10 ml.

Analytical separation of metabolites from ^{14}C-N-Ac-PCBC. After filtration, aliquots (50 μl) of the supernatants were injected directly into a Waters (Milford, U.S.A.) HPLC System, consisting of two M 6000 A pumps, a U6K injector and a model 660 solvent programmer. For analytical separations, a Waters RCM-module with a Nova-Pak C-18 cartridge (5 mm i.d.) was used. For isolation and purification of metabolites, steel columns (25 × 0.8 cm) filled with Partisil ODS III (Whatman, Maidstone, England) were used. Separation of metabolites was performed by gradient elution: initial conditions—5% aqueous methanol containing 0.1% trifluoroacetic acid (TFA), final conditions—100% methanol;

Table 1. Analytical data of the synthetic mercapturic acids *N*-acetyl-S-dichlorovinyl-L-cysteine (NAcDCVC), *N*-acetyl-*S*-trichlorovinyl-L-cysteine (NAcTCVC) and *N*-acetyl-*S*-pentachlorobutadienyl-L-cysteine (NAcPCBC)

Compound†		mp °C	Mass spectrum m/e (intensity %)	^{1}H-NMR-spectrum* δ-ppm (intensity)	Purity‡ (HPLC)
NAcDCVC		93–96	Methylester: 45 (35), 59 (16), 60 (12), 84 (13), 88 (100), 102 (10), 126 (18), 144 (26), 170 (13), 212 (85 2Cl), 271 (21 2Cl, M^+)	2.0 (3H), 3.3 (2H), 4.6 (1H), 6.6 (1H), 7.0 (1H), 8.4 (1H)	>99%
NAcTCVC		159–160	Propylester: 74 (91), 79 (23), 84 (36), 103 (15), 116 (86), 130 (27), 144 (10), 172 (100), 197 (53 2Cl), 204 (46 3Cl), 232 (12 3Cl), 239 (38 3Cl), 274 (39 3Cl), 333 (6 M^+)	D_2O/Na_2CO_3 2.03 (3H), 2.9–3·6 (2H) 4.3–4.5 (1H), 4.75 (2H)	>99%
NAcPCBC		oil	Methylester: 45 (23), 59 (47), 71 (18), 79 (25), 84 (39), 88 (100), 102 (22), 115 (57 1Cl), 130 (26), 144 (74), 150 (13 2Cl), 185 (18 3Cl), 220 (31 4Cl), 298 (18 5Cl), 304 (24 4Cl), 340 (17 5Cl)	2.0 (3H), 3.6 (2H) 4.7 (1H), 5.4 (2H)	>98%

* 60 MHz, solvent: CD_3CN, standard: tetramethylsilane.
† R = N-acetyl-cysteinyl-.
‡ U.V.-detection (λ = 230 nm), method, see ref. 10.

linear gradient 40 min, flow rate 3 ml/min for steel columns and 20 min, 1.5 ml/min for analytical columns.

To check the separation efficiency, the eluate was also monitored by UV-detection at 280 nm. 0.5 min fractions were collected. Aliquots of the fractions were dissolved in liquid scintillation cocktail (Quickszint 212, Zinsser, Frankfurt, FRG) and counted in a liquid scintillation counter (Tricarb 4000, Packard Instruments, Downers Grove, U.S.A.).

Identification of metabolites. The dry residues (after evaporation of the solvents at reduced pressure) of the fractions containing radioactive metabolites from N-Ac-PCBC were treated with *N*-(bistrifluoroacetyl)-methylamine and BCl_3/methanol. GC/MS was performed on a Finnigan (San Jose, U.S.A.) 4510 GC/MS system as described previously [10, 11].

Determination of macromolecular binding of radioactivity from ^{14}C-N-Ac-PCBC. The protein pellets were resuspended in 4 ml of 6M HCl and heated at 100° in sealed tubes until complete solution was achieved. Aliquots (100 μl) were transferred into Quickszint and counted in a liquid scintillation counter.

Mutagenicity assay. The mutagenicity of the mercapturic acids was tested in the *Salmonella typhimurium* strains TA100 and TA2638 using the preincubation test as described by Maron and Ames [20]. Kidney 100,000 *g* supernatants from male Wistar rats were added to part of the preincubation mixtures. Preincubations were performed at 37° for 120 min. The characteristics of these strains were checked regularly by testing the UV and crystal violet sensitivity, ampicillin resistance and mutability by UV light.

Cleavage of synthetic mercapturic acids by 100,000 g supernatant from Salmonella typhimurium *TA100.* Incubations were carried out in 0.1 M phosphate buffer (pH 7.4) containing mercapturic acid (2 mM), bacterial homogenate 100,000 *g* supernatant (5 mg/ml) and mercaptoacetic acid (5 mM). To some incubations aminooxyacetic acid (1 mM) was added. The final incubation volume was 5 ml. The reactions were carried out at 37° in a shaker-water bath and samples (1 ml) were removed at 0, 10 and 20 min. The reaction was stopped by the addition of ice-cold trichloroacetic acid (0.3 ml). The probes were then centrifuged for 4 min at 12,000 rpm in a Microrapid/k centrifuge (HETTICH, Tuttlingen, F.R.G.) to precipitate protein. Pyruvate formation was quantified colorimetrically in the protein-free solution using a commercial pyruvic acid determination kit (Sigma Chemical Company, St. Louis, MO).

RESULTS

Metabolism of mercapturic acids by mammalian subcellular fractions

^{14}C-labelled *S*-pentachlorobutadienyl-*N*-acetylcysteine (N-Ac-PCBC) was synthesised as a model compound to quantify the metabolism and macromolecular binding of a vinylic substituted mercapturic acid. The synthesis of radiolabelled derivatives of the other mercapturic acids studied was not successful due to the high volatility of the starting materials and the low yield of mercapturic acids obtained; their metabolic transformation was studied by identifying the products of the enzymatic reaction by GC/MS.

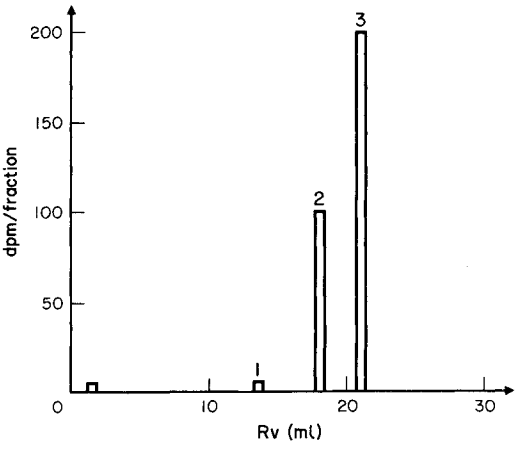


Fig. 1. HPLC-elution profile (Rv = elution volume) of radioactivity after incubation of N-Ac-PCBC with rat kidney 100,000 *g* supernatant. Peak 3 = N-Ac-PCBC, peak 2 = PCBC. Background of 5 dpm subtracted.

^{14}C-N-Ac-PCBC was incubated for 2 hr with cytosol and microsomes from rat liver and kidney. After protein precipitation, the supernatant was subjected to HPLC fractionation. Figure 1 shows the HPLC-elution profiles of ^{14}C-activity obtained from the incubation with kidney cytosol. The formation of metabolites (peaks 1, 2) was dependent on the presence of cytosolic enzymes; peak 3 represents unchanged N-Ac-PCBC. The mass-spectrum of the radioactive compound in peak 2 recorded after trifluoroacetylation and esterification showed several fragments indicating the presence of five chlorine atoms in the molecule (see Fig. 2) and a fragment typical (m/e = 198) for the mass-spectra of thioethers of *N*-trifluoroacetyl-cysteine methyl esters. By comparison with the spectrum of a synthetic reference compound it was identified as pentachlorobutadienylcysteine (see Fig. 2). The mass-spectrum of the minor metabolite (peak 1, Fig. 1) recorded after esterification, contained several fragments (m/e = 218, 159; ^{35}Cl) indicating the presence of at least 3 chlorine atoms in the molecule and the absence of the cysteinyl-residue (spectrum not shown); an unequivocal structure of this metabolite could not be established.

Elution profiles indicating the presence of the above described metabolites of N-Ac-PCBC were also obtained from incubations with liver cytosol; however, the extent of metabolite formation in liver cytosol was only about 50% of that observed in kidney cytosol (data not shown). The β-lyase inhibitor AOAA did not decrease the extent of formation of PCBC from the mercapturic acid, but inhibited the formation of the metabolite in peak 1. Metabolism of N-Ac-PCBC was not observed with microsomes or bacterial protein.

The unlabelled mercapturic acids *S*-trichlorovinyl-*N*-acetylcysteine (N-Ac-TCVC) and *S*-dichlorovinyl-

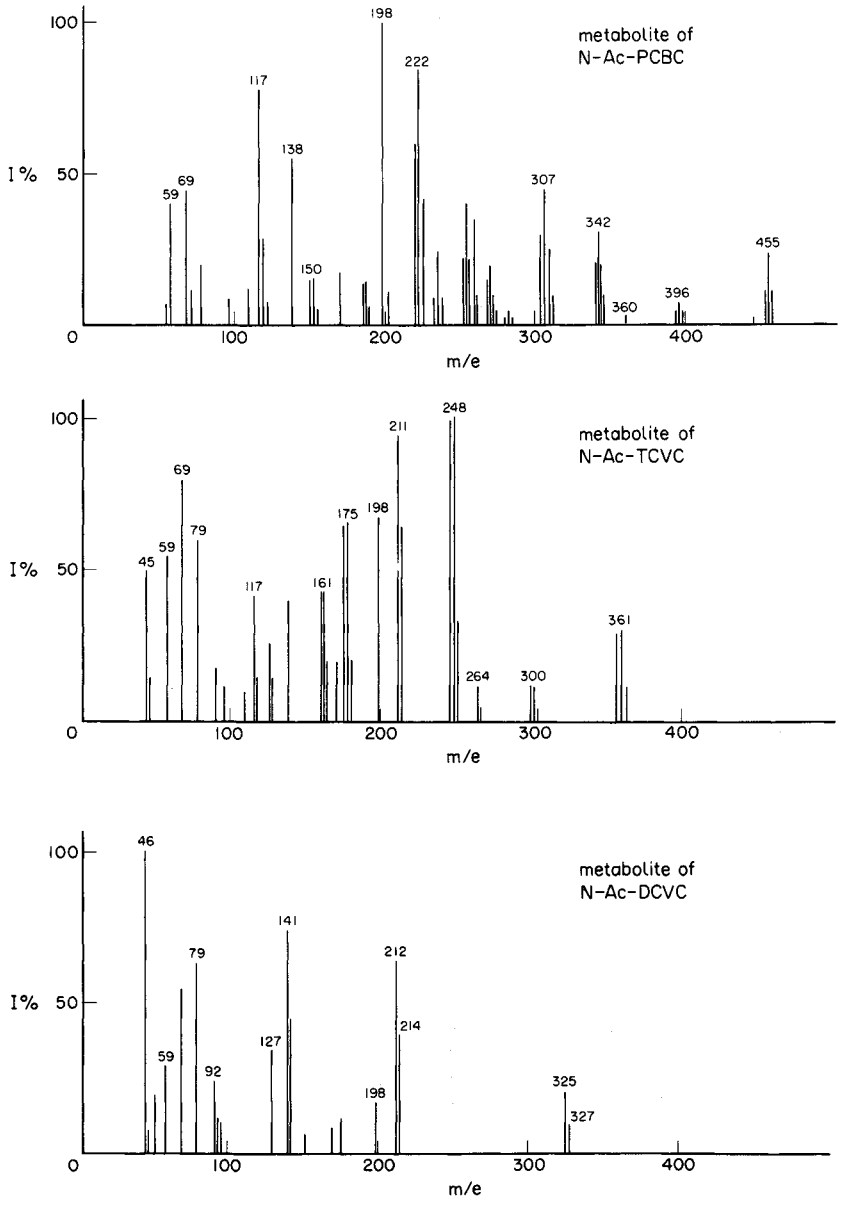


Fig. 2. Mass-spectra of the metabolites (*N*-trifluoroacetylmethylester) formed from N-Ac-PCBC, N-Ac-TCVC and N-Ac-DCVC by cytosolic enzymes from rat liver and kidney.

Table 2. ^{14}C radioactivity covalently bound to protein after incubation of *S*-1,2,3,4,4-pentachlorobutadienyl-*N*-acetylcysteine (0.1 mM, specific activity 3.3×10^2 dpm/nmol) with male rat subcellular fractions in the presence or absence of aminooxyacetic acid (AOAA)

Subcellular fractionation at 100,000 *g*	Covalently bound radioactivity (dpm/mg protein)	
	—	AOAA (1 mM)
Supernatant from rat liver	164 ± 38	52 ± 14
Pellet from rat liver	10 ± 2	10 ± 1
Supernatant from rat kidney	852 ± 86	234 ± 35
Pellet from rat kidney	10 ± 2	10 ± 3
Supernatant from TA100 homogenate	10 ± 2	10 ± 1

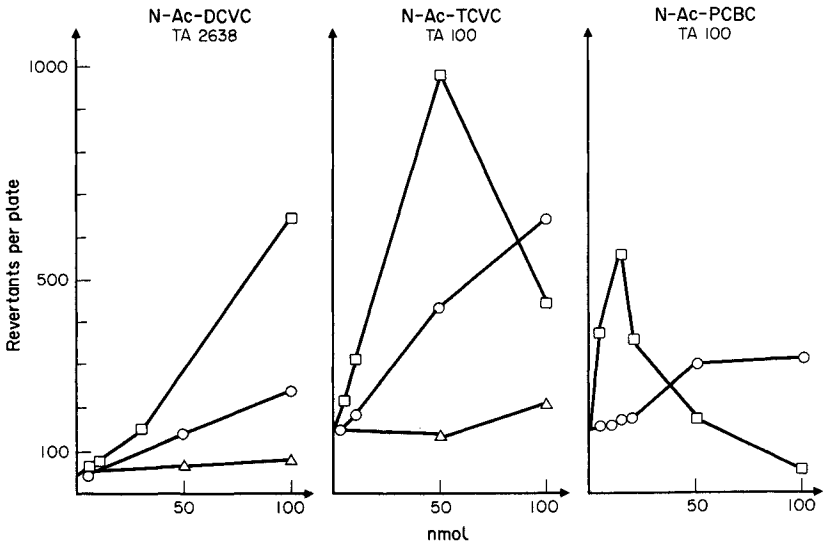


Fig. 3. Mutagenicity of N-Ac-DCVC in *Salmonella typhimurium* TA2638 and of N-Ac-TCVC and N-Ac-PCBC in TA100 (○). Preincubation assay (preincubation time 120 min). To some incubations either (□) male rat kidney cytosol (1 mg protein/plate) or (△) AOAA (1 mM) were added. Experiments were repeated at least three times with consistent results.

N-acetylcysteine (N-Ac-DCVC) were also metabolised by cytosolic enzymes to trichlorovinylcysteine (TCVC) and dichlorovinylcysteine (DCVC) as major metabolites (identified by their mass spectra, see Fig. 2); also traces of chloroacetic acid (formed from N-Ac-DCVC) and dichloroacetic acid (from N-Ac-TCVC) were identified in these incubations by GC/MS of the corresponding methyl esters (spectra not shown).

Macromolecular binding

The amount of radioactivity covalently bound to macromolecules after incubation of ^{14}C-N-Ac-PCBC with liver and kidney subcellular fractions is shown in Table 2. Incubations with 100,000 *g* supernatant from rat liver and kidney resulted in covalent binding of metabolites to macromolecules; AOAA significantly decreased the protein-bound radioactivity. No increase in the levels of ^{14}C activity in the protein pellet above background values was found when N-Ac-PCBC was incubated with 100,000 *g* pellet from male rat liver or kidney or with 100,000 *g* bacterial homogenate supernatants.

Mutagenicity assay

Previous experiments performed in our group showed that the cysteine conjugates *S*-1,2,2-trichlorovinylcysteine (TCVC) and *S*-1,2,3,4,4-pentachlorobutadienylcysteine (PCBC) achieved their highest mutagenic activity in *S. typhimurium* TA100, and *S*-1,2-dichlorovinylcysteine in TA2638 [19]. Deacetylation of mercapturic acids will result in the formation of the corresponding cysteine conjugates; therefore the mutagenic properties of the synthetic mercapturic acids N-Ac-TCVC and N-Ac-PCBC were tested in TA100 and of N-Ac-DCVC in TA2638.

N-Ac-PCBC did not induce a doubling of the spontaneous revertants without the addition of mammalian enzymes, whereas together with rat kidney cytosol it induced a high mutagenic activity (see Fig. 3). Unexpectedly N-Ac-DCVC and N-Ac-TCVC were definitely mutagenic at high concentrations without exogenous activation. Addition of cytosolic protein also resulted in an increase in the mutagenicity of N-Ac-DCVC and N-Ac-TCVC (Fig. 3). The β-lyase inhibitor AOAA diminished the mutagenicity (Fig. 3).

Cleavage of mercapturic acids by bacterial β-lyase

The results of the mutagenicity assays for N-Ac-DCVC and N-Ac-TCVC prompted us to investigate the metabolism of these compounds by bacterial enzymes. Pyruvate is one of the products expected to be formed by deacetylation followed by β-lyase cleavage [15]. Therefore, the metabolism of the mercapturic acids to pyruvate was determined in *S. typhimurium* TA100 homogenates. The results shown in Fig. 4 illustrate the relative rates of pyruvate formation from the mercapturic acids by bacterial enzymes. Incubation of N-Ac-TCVC with bacterial protein resulted in 9.3-fold increase in pyruvate production over control values and of N-Ac-DCVC in a 4.2-fold increase. Incubation of N-Ac-PCBC with bacterial protein did not increase the concentrations of pyruvate over control values. Addition of AOAA (1 mM) to incubations with N-Ac-TCVC and N-Ac-DCVC decreased pyruvate formation by 80%.

DISCUSSION

The results presented indicate that mercapturic acids, which are usually considered to be the end-products of a detoxification pathway *in vivo*, are transformed to mutagenic metabolites by mammalian enzymes, at least in the case of N-Ac-PCBC, N-Ac-TCVC and N-Ac-DCVC. The radioactive labelled model compound N-Ac-PCBC was inten-

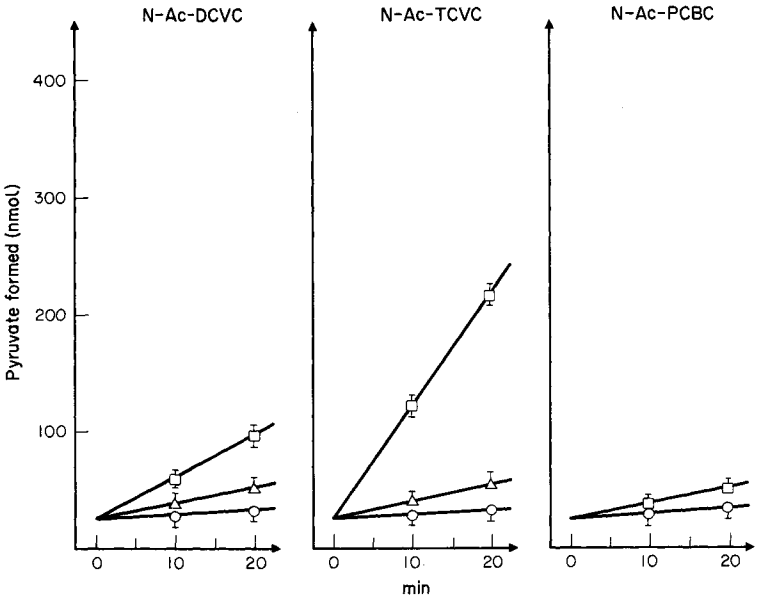


Fig. 4. Cleavage of synthetic mercapturic acids by bacterial 100,000 *g* supernatant. Mercapturic acids were incubated with TA100 100,000 *g* supernatant (5 mg protein/ml) (□). For some experiments (△) AOAA (1 mM) was added to the incubation mixture or the bacterial homogenate was incubated without the addition of substrate (○). Mean of 4 determinations ± SD.

sively metabolised by cytosolic enzymes from rat kidney and liver, resulting in the formation of reactive intermediates binding covalently to protein. Two soluble metabolites were separated by HPLC. The major metabolite was unequivocally identified as pentachlorobutadienylcysteine (PCBC), the formation of the minor metabolite was diminished by the β-lyase inhibitor AOAA [21] whereas PCBC formation was not influenced. The production of these two metabolites suggests that the transformation of the mercapturic acid to reactive intermediates is accomplished by a two-step mechanism. In the first step the mercapturic acid, N-Ac-PCBC, is transformed to pentachlorobutadienylcysteine (PCBC) by a cytosolic deacetylase. Further metabolism of PCBC in a second step results in intermediates which bind to macromolecules. It is known that PCBC is a substrate for cysteine conjugate β-lyase [19, 22]. The involvement of β-lyase—present in both liver and kidney cytosol at approximately equal activities—in the formation of the reactive intermediates is suggested by two findings: (a) both the covalent binding of radioactivity and the formation of the second metabolite observed in HPLC-separations are inhibited by the β-lyase inhibitor AOAA, and (b) the obtained mass spectrum of this second metabolite, whose structure could not be unequivocally elucidated, clearly indicates the absence of a cysteinyl-moiety.

Incubation of N-Ac-DCVC with rat kidney or liver cytosol resulted in the formation of dichlorovinylcysteine (DCVC) and chloroacetic acid and N-Ac-TCVC was transformed to trichlorovinylcysteine (TCVC) and dichloroacetic acid. These data indicate that N-Ac-DCVC and N-Ac-TCVC are also subjected to the sequence of molecular events described above for N-Ac-PCBC. Dichloroacetic acid is presumed to be a product of a β-lyase-mediated cleavage of TCVC [11], chloroacetic acid may be formed by an analogous reaction from DCVC.

The results of our mutagenicity studies also substantiate the suggestion [14] that deacetylation plays an important role in the mutagenicity of N-Ac-PCBC. This compound and the other two mercapturic acids tested in the Ames-test were all transformed to strong mutagens in the presence of cytosolic protein from rat kidney. As we have shown, cytosolic enzymes hydrolyse the mercapturic acids to cysteine conjugates, which are then cleaved by β-lyase present at high activity in cytosolic fractions [16] and in *Salmonella typhimurium* TA100 [19] to mutagenic intermediates presumed to be thioacylchlorides or thioketenes (Fig. 5). Without the addition of mammalian enzymes catalyzing the deacetylation of N-Ac-PCBC, this compound was not unequivocally mutagenic. However, both N-Ac-DCVC and N-Ac-TCVC were weak but definite "direct" mutagens.

To verify the involvement of β-lyase in this effect, the metabolism of the mercapturic acids by bacteria was investigated. In contrast to N-Ac-PCBC which was not cleaved by bacterial homogenate 100,000 *g* supernatants, incubation of the two "directly" mutagenic mercapturic acids N-Ac-DCVC and N-Ac-TCVC with *S. typhimurium* homogenate 100,000 *g* supernatants resulted in pyruvate formation. Pyruvate and the reactive metabolite(s) are formed in equimolar concentrations by the described reaction sequence (Fig. 5). The following three findings are indicative of the key role of β-lyase mediated metabolism of the mercapturic acids as a prerequisite of their mutagenic activity: (1) the pyruvate formation

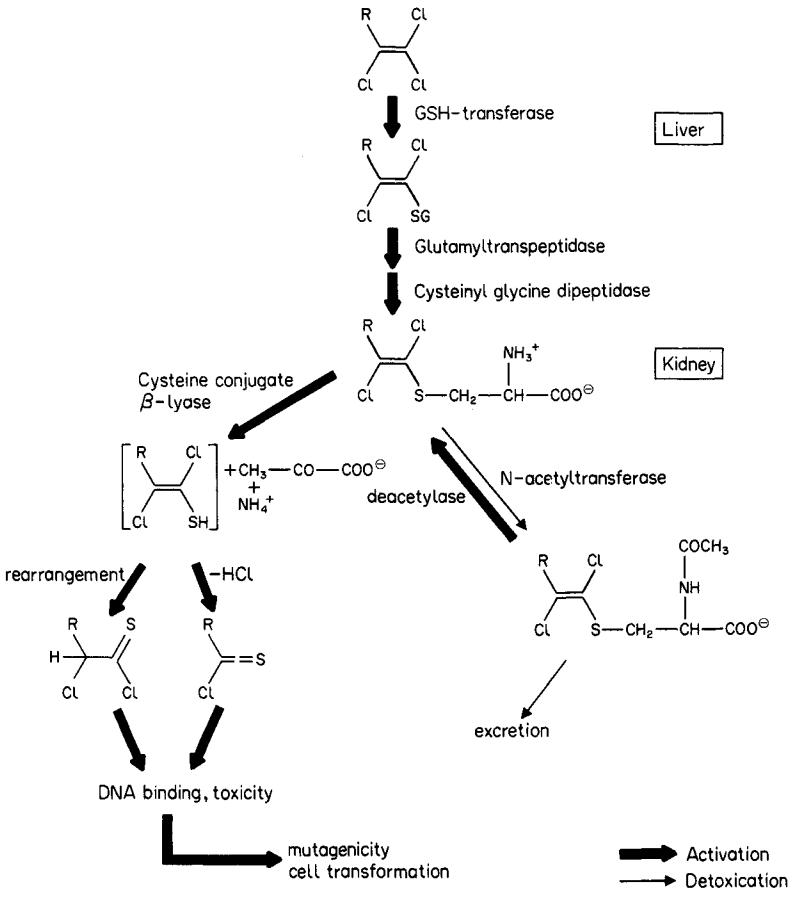


Fig. 5. Schematic of haloethene metabolism through glutathione conjugation. Activation of cysteine adducts by renal β-lyase.

by bacterial homogenate 100,000 *g* supernatants; (2) the direct mutagenic activity of the compounds and (3) the strong inhibition of both (1) and (2) by the β-lyase inhibitor AOAA. We suggest that bacterial enzymes with deacetylating activity (e.g. amidases) transform the mercapturic acids to the corresponding cysteine conjugates which are then cleaved by bacterial β-lyase [20]. The different substrate specificities of these bacterial enzymes towards the three tested mercapturic acids cannot currently be explained.

Our results demonstrate that both renal and hepatic enzymes exhibit a high deacetylating activity for mercapturic acids with unsaturated, electronegative aliphatic substituents. In rat liver and kidney, several different enzymes which might catalyse these deacetylation reactions have been characterised. In rat liver, an enzyme exhibiting a high specific activity for aromatic mercapturic acids [17] is present in cytosol. It does not, however, hydrolyse mercapturic acids with saturated aliphatic substituents. A similar enzyme—acylase III—is also present in rat kidney cytosol and mitochondria [16], catalysing deacetylation of both aromatic and some aliphatic mercapturic acids. An additional enzyme, acylase I, exhibiting high activity towards *S*-alkyl mercapturic acids but markedly lower or no activity towards *S*-aralkyl and *S*-aryl conjugates has been purified from hog kidney and also found in rat liver [16].

The activation of the mercapturic acids by deacetylation is accomplished both in liver and kidney homogenates. In contrast, the *in vivo* toxicity of the cysteine conjugates manifests itself almost exclusively in the kidney [23, 25, 26]. This organ-specific toxicity can be explained by the molecular events causing toxicity *in vivo* (see Fig. 5; for review see Ref. 12 and 15). The initial step in mercapturic acid formation, GSH-conjugation of the parent compound—the halogenated alkene—presumably takes place in the liver [8, 9, 18]; the glutathione conjugates or their metabolic products formed by enzymatic processing [7] are then transported to the kidney, accumulated in the proximal tubular cells by a probenecid-sensitive transport process [27] and cleaved by the enzymes of mercapturic acid formation. It has been shown that both glutamyltranspeptidase [28] and dipeptidases hydrolyzing cysteinylglycine [29] are present at high activities in the brush-border membranes of the proximal tubular cells. The cysteine conjugates formed are substrates for β-lyase, which also exhibits high activity in the renal proximal tubules [13]. The localized distribution of the enzymes involved in this metabolic sequence explains the high organ-specific effects of the halogenated

alkenes. Cysteine conjugates are also substrates for renal cysteine conjugate acetyltransferase [30] which converts them to mercapturic acids, a reaction considered to be a deactivation step resulting in urinary excretion of the products. However, as shown in our study, mercapturic acids can also be deacetylated by renal enzymes; this finding indicates that mercapturic acid biosynthesis may also be regarded as the intermediary step in the toxification of halogenated alkenes which finally contributes to the formation of reactive intermediates. This conclusion is supported by the results of Lock and Ishmael [27] who demonstrated that a single dose of N-Ac-PCBC to female rats resulted in probenecid-sensitive accumulation of the mercapturic acid in the renal cortex and in tubular necrosis in the outer strip of the outer medulla of the kidney accompanied by significant elevations of plasma urea and kidney weight. Therefore, organotropism of N-Ac-PCBC is also likely to be due to the accumulation of the compound in the kidney in addition to the relatively high activity of deacetylase and β-lyase in the renal proximal tubules. However, the relative activities and substrate specificities of renal cysteine conjugate acetyl transferase and deacetylase and their influence on the half-life of excretion of reactive intermediates have not yet been completely elucidated; further studies on the mercapturic acid formation and deacetylation in a system closer to the *in vivo* situation than organ homogenates (e.g. kidney cells in culture or isolated perfused kidney) are needed to quantify the contribution of reactive intermediates formed by the deacetylation reaction to the nephrotoxicity of the parent compounds *in vivo*.

Acknowledgements—This work was supported by the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 172), Bonn and the Doktor-Robert-Pfleger Stiftung, Bamberg. The skilful technical assistance of Ms J. Ahamer, Ms M. Dertinger and Ms S. Seufert is gratefully acknowledged.

REFERENCES

1. L. F. Chasseaud, *Adv. Cancer Res.* **29**, 175 (1979).
2. P. J. van Bladeren, A. van der Gen, D. D. Breimer and G. R. Mohn, *Biochem. Pharmac.* **28**, 2521 (1979).
3. D. L. Hill, T.-W. Shih, T. P. Johnston and R. F. Struck, *Cancer Res.* **38**, 2438 (1978).
4. M. W. Anders, L. H. Lash and A. A. Elfarra, *J. biol. Chem.* (in press).
5. U. Rannug, A. Sundvall and C. Ramel, *Chem.-Biol. Interact.* **20**, 1 (1978).
6. P. B. Inskeep and F. P. Guengerich, *Carcinogenesis* **5**, 805 (1984).
7. J. A. Nash, L. J. King, E. A. Lock and T. Green, *Toxic. appl. Pharmac.* **73**, 124 (1984).
8. J. Odum and T. Green, *Toxic. appl. Pharmac.* **76**, 306 (1984).
9. D. R. Dohn and M. W. Anders, *Biochem. biophys. Res. Commun.* **109**, 1339 (1982).
10. W. Dekant, M. Metzler and D. Henschler, *Biochem. Pharmac.* **35**, 2455 (1986).
11. W. Dekant, M. Metzler and D. Henschler, *J. Biochem. Toxic.* **1**(2), 57 (1986).
12. W. B. Jakoby, J. Stevens, M. W. Duffel and R. A. Weisiger, *Rev. Biochem. Toxic.* **6**, 97 (1984).
13. L. H. Lash, A. A. Elfarra and M. W. Anders, *J. biol. Chem.* **261**, 5930 (1986).
14. D. Wild, S. Schütz and D. Reichert, *Carcinogenesis* **7**, 431 (1986).
15. A. A. Elfarra and M. W. Anders, *Biochem. Pharmac.* **33**, 3729 (1984).
16. M. Tateishi, *Drug Metab. Rev.* **14**, 1207 (1983).
17. S. Suzuki and M. Tateishi, *Drug Metab. Disp.* **9**, 573 (1981).
18. C. R. Wolf, P. N. Berry, J. A. Nash, T. Green and E. A. Lock, *J. Pharmac. exp. Ther.* **228**, 202 (1984).
19. W. Dekant, S. Vamvakas, K. Berthold, S. Schmidt, D. Wild and D. Henschler, *Chem.-Biol. Interact.* **60**, 31 (1986).
20. D. Maron and B. Ames, *Mut. Res.* **113**, 173 (1983).
21. L. H. Lash and M. W. Anders, *J. biol. Chem.* (In press).
22. T. Green and J. Odum, *Chem.-Biol. Interact.* **54**, 15 (1985).
23. A. A. Elfarra, I. Jakobson and M. W. Anders, *Biochem. Pharmac.* **35**, 283 (1986).
24. D. Reichert and S. Schütz, *Biochem. Pharmac.* **35**, 1271 (1986).
25. D. R. Jaffe, C. D. Hassall and A. J. Gandolfi, *J. Toxic. Envir. Health* **11**, 857 (1983).
26. D. R. Dohn, J. R. Leininger, L. H. Lash, A. J. Quebbemann and M. W. Anders, *J. Pharmac. exp. Ther.* **235**, 851 (1985).
27. E. A. Lock and J. Ishmael, *Toxic. appl. Pharmac.* **81**, 32 (1985).
28. B. Tsao and N. P. Curthoys, *J. biol. Chem.* **255**, 7708 (1980).
29. T. McIntyre and N. P. Curthoys, *J. biol. Chem.* **257**, 11921 (1982).
30. M. W. Duffel and W. B. Jakoby, *Molec. Pharmac.* **21**, 444 (1981).