tion is stimulated by both L-serine and L-proline. This suggests that 4HAQO may be activated by both seryl- and prolyl-tRNA synthetases in rat tissues. Aminoacyl-tRNA synthetases which are capable of 4HAQO-activation, may have an unique conformation enabling them to aminoacylate the N-hydroxyl group of the carcinogen. Carcinogenic aromatic amines and nitro compounds undoubtedly require metabolic activation for their reactivity *in vivo*. The first activation step of these compounds seems to be formation of N-hydroxyderivatives, and it has been suggested that the final activation step is N-hydroxyesterification¹⁴. Seryl-tRNA synthetase and some other species of aminoacyl-tRNA synthetase may participate *in vivo* in the activation of not only 4HAQO but also some other N-hydroxy compounds through their aminoacylating capacity.

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K region benzo(α)pyrene-4,5-oxide is conjugated by homogeneous glutathione S-transferases

We report direct evidence that conjugation with glutathione (GSH) is a significant mechanism for detoxification of the epoxides of polycyclic aromatic hydrocarbons. We believe that the level of the enzymes may be directly relevant to the carcinogenic potential of the hydrocarbons, which are common pollutants¹. They are metabolised by microsomal mixed function oxygenases and associated enzymes, to detoxified as well as carcinogenic forms^{2–4}. One of the metabolic products of benzo(α)pyrene is the 'K' region epoxide, benzo(α)pyrene-4,5-oxide. Such epoxides bind to macromolecules, are mutagenic and may be the carcinogenic forms of the polycyclic aromatic hydrocarbons⁵. Benzo(α)pyrene-4,5-oxide is converted enzymatically either to the dihydrodiol by hydrolysis⁶ or to a thioether by conjugation with GSH⁷. The glutathione S-transferases (EC 2.5.1.18) detoxify many compounds^{8,9} including alkyl epoxides¹⁰ and arene oxides^{11–12}. Now that pure glutathione S-transferases are available (five rat^{10,12,13} and five human, K. Kamisaka, W. H. H., J. N. K., I. M. Arias and W. B. J., unpublished), we have been able to evaluate directly the extent to which these enzymes can produce GSH conjugates of the polycyclic aromatic oxide and, presumably, detoxify it.

Incubation of each of the enzymes with benzo(α)pyrene-4,5-oxide and GSH in standard assay conditions⁷ always resulted

Table 1 Specific activity (nmol min⁻¹ mg⁻¹) of homogeneous rat and human glutathione S-transferases

Enzyme	Benzo(α)pyrene-4,5-oxide*		Alkyl epoxide†	Bromosulphophthalein‡
	Exp. 1	Exp. 2		
Rat transferase A	100	88	100	530
B	14	11	0§	6
C	127	99	0§	18
E	51	70	6,100	0§
AA	8	4	0§	4
Human transferase β	25	—	0§	10
δ	33	29	0§	1

*Incubation was for 2 min at 37 °C in 0.4 ml of 10 mM Tris-chloride, pH 7.5, 50 μM ³H-benzo(α)pyrene 4,5-oxide in 15 μl of methanol and 0.5 mM GSH. After precipitation of protein with 0.5 ml acetone and centrifugation, the solution was subjected to thin-layer silica gel chromatography with *n*-butanol-*n*-propanol-2 M ammonium hydroxide (2:1:1). Radioactivity in the R_f 0.06 component was measured⁷. Two series of experiments were conducted with different amounts of the same enzyme. The values presented have been corrected for the small amount of conjugate, about 1 pmol min⁻¹ mg⁻¹, produced in the absence of enzyme.

†Specific activity with 1,2-epoxy-3-(*p*-nitrophenoxy)propane as described before¹⁰.

‡Determined spectrophotometrically as described before¹².

§A value of zero implies that nanomolar concentrations of enzyme did not produce conjugate in an amount greater than that of a control in the absence of enzyme.

in the formation of the GSH conjugate (Table 1). Two other substrates of the enzymes are included for comparison: an alkyl epoxide, 1,2-epoxy-3-(*p*-nitrophenoxy)propane, and an aryl halide of pharmacological significance, bromosulphophthalein. Both compounds form the appropriate thioethers of GSH. Each of the purified enzymes was active in the conjugation of the polycyclic hydrocarbon epoxide with glutathione although relative activity varied considerably (Table 1). We stress that available information on the rat and human transferases (Kamisaka *et al.*, unpublished) suggests that these enzymes represent a large portion, 10% and 2%, respectively, of the total protein extractable from liver. Furthermore, one of the enzymes, transferase B¹⁴, is identical to ligandin¹⁵, a protein known to form a covalent derivative *in vivo* with 4-dimethylaminoazobenzene¹⁶. Knowledge of the role of the glutathione S-transferases should clarify our understanding of the routes of carcinogen activation and detoxification.

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