

# The Role of Glutathione in Detoxication

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Glutathione (GSH) is a strong nucleophile which reacts well with soft electrophiles, but poorly with both weak and strong electrophiles. Weak electrophiles have low reactivity with all nucleophiles while strong electrophiles react well with weak nucleophiles including superabundant  $H_2O$ .

There are enzymes, the GSH transferases, which catalyze GSH conjugation with all the types of electrophiles described above. In order to deal with the wide variety of potential substrates, a multiplicity of GSH transferases exists—each tissue having its own collection and each enzyme having a different substrate specificity. These enzymes are often very abundant, e.g., in the rat liver cytosol, their concentration is 0.2 mM.

The following substrates are considered in some detail: 1-chloro-2,4-dinitrobenzene, the electrophile derived metabolically from paracetamol (*N*-acetylminiquinone?), benzo(a)pyrene-4,5-oxide, cholesterol-5 $\alpha$ ,6 $\alpha$ -oxide, benzo(a)pyrene-7,8-diol-9,10-oxide and the electrophiles derived metabolically from aflatoxin B<sub>1</sub> (the 2,3-oxide?). According to the substrate, optimal enzyme rates vary over seven orders of magnitude from  $10^{-5}$  to  $10^{-12}$  mole/min/mg.

Despite the wide embrace of the GSH transferases, not all metabolically produced electrophiles are substrates. We know of the following examples: *N*-methylol-4-aminoazobenzene and its 4'-hydroxy derivative (these are soft electrophiles and react well with GSH noncatalytically), *N*-sulfonyloxy-*N*-methyl-4-aminoazobenzene, *N*-sulfonyloxy-*N*-acetyl-2-aminofluorene (these are strong electrophiles which do not react selectively with GSH) and *N*-hydroxy-2-aminofluorene which appears to react only slowly with GSH. It is of interest in the present context that all these compounds are derived from either arylamine or arylamide carcinogens.

Whether the reaction be enzymic or nonenzymic, conjugation with GSH is a very important means of detoxication accounting in some cases for up to 60% of the biliary metabolites. As seen in the example of aflatoxin B<sub>1</sub>, very low enzymic rates observed *in vitro* are sufficient to account for apparently high rates of biliary excretion of GSH conjugates.

GSH transferases have evolved other functions apart from the catalysis of GSH conjugation. GSH transferase B participates in the hepatic uptake of bilirubin and the intracellular distribution of the heme prosthetic group. It also has GSH peroxidase activity which suggests that it might participate in the detoxication of by-products of oxygen utilization including those produced by the action of cytochrome P-450. It is shown that GSH transferase B inhibits lipid peroxidation *in vitro*.

## Glutathione

Glutathione (GSH),  $\gamma$ -glutamylcysteinylglycine, is a molecule with the following important properties. Its cysteinyl residue provides a nucleophilic thiol important for the detoxication of electrophilic metabolites and metabolically produced oxidizing agents. Its net negative charge and overall hydrophilicity greatly increases the aqueous solubility of the lipophilic moieties with which it becomes conjugated. Its molecular weight (307) ensures that its adducts are preferentially secreted via the biliary system which selects molecules of molecular weight greater than 300 to 500 according to the species (1). Its unique tripeptide structure, including the *N*-terminal glutamyl residue linked via a  $\gamma$ -glutamyl peptide bond, provides for specificity in GSH-enzyme

interaction. It is apparently universal in nature, often at high intracellular concentrations, e.g., in the rat liver it is 5-10 mM (2), while glucose is 5 mM, ATP 3 mM and ascorbic acid and alanine 2 mM (3).

## Conjugates

In mammals, GSH conjugates are often further metabolized by hydrolysis and *N*-acetylation, either in the gut or in the kidney, to give *N*-acetylcysteinyl conjugates known as mercapturic acids, which are excreted in the urine (4). It might be asked why conjugation with cysteine does not occur in the first place. Presumably GSH provides a means whereby the pool of cysteine for detoxication is kept separate from the pool of cysteine for protein synthesis.

## Factors Affecting GSH Conjugation

**Nucleophilic Character of GSH.** GSH exists as two nucleophilic species at physiological pH, GSH

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and its thiolate anion. Concentrations of the latter, the stronger nucleophile, are approximately 1% of GSH concentration [ $pK_a$  of GSH = 9.2 (5, 6)].

**Nature of Electrophilic Center.** Reactions with which we are mostly concerned are nucleophilic substitutions (including opening of the strained oxirane ring) and nucleophilic additions to a polarized double bond. The mechanism of GSH conjugation may be examined in relation to the two following extremes. (a) The electrophilic center is soft, and the nucleophile initiates reaction by causing polarization of the electrophile: in these circumstances strong nucleophiles such as GSH and GS<sup>-</sup> will react best. (b) The electrophilic center is hard and capable of initiating reaction by generation of polarization in a weak nucleophile and therefore reaction is less dependent on GSH.

## Glutathione Transferases

Numerous xenobiotics have been shown to be excreted as mercapturic acids (4), and it has been suggested that enzymes, the GSH transferases, catalyze the initial conjugation with GSH (7). It will be shown below that enzymic catalysis is not always involved in GSH conjugation; nevertheless, GSH transferases are widespread in both animals and plants (8). They appear to have evolved later than GSH itself, but are found in animals as primitive as the flatworm (9). In a highly evolved animal such as the rat, the organism as a whole has a multiplicity of GSH transferases (10). All tissues appear to possess some activity; most have more than one enzyme species—usually a spectrum of isoenzymes which is characteristic of the tissue (11, 12). The multiplicity of GSH transferases is probably due to the need to deal with a large range of electrophiles. However, it is not clear what the electrophiles encountered by various tissues are, although it is assumed that in the liver, which is so important in detoxication, most of the electrophiles are derived from xenobiotics.

## Detoxication by Glutathione in the Rat Liver

In the present paper we will concentrate on detoxication by GSH and GSH transferases from the rat liver, the area which has been the object of most of our researches. The detoxication of arylamines and arylamides will be compared with that of other compounds.

### Rat Liver Glutathione Transferases

**Occurrence.** The greatest source of GSH transferases is the cytosol, where the concentration may range from 0.2 to 0.5 mM (13), according to whether

or not the transferases have been induced by agents such as phenobarbitone, BHA, TCDD, etc. (14-16). Other GSH transferases have been detected in the membrane of the hepatic microsomes and the mitochondria (17-19).

**Structure and Function.** The soluble isoenzymes number at least seven, each with a similar dimeric structure in which the monomers fall into three categories defined by their apparent molecular weights as  $Y_a$  (MW 22,000),  $Y_b$  (MW 23,500) and  $Y_c$  (MW 25,000) (20). GSH transferases A, C, D (?) and E are all homodimers of  $Y_b$ , but vary in their isoelectric points and substrate specificities, indicating that the monomer referred to as  $Y_b$  represents more than one polypeptide (21). GSH transferase AA is a  $Y_c$  homodimer, while GSH transferase B is separable into two forms, one of which is a  $Y_a$  homodimer  $Y_aY_a$  and the other a heterodimer  $Y_aY_c$  (20, 22) (Figs. 1 and 2). GSH transferase B can be grouped together because of their possession of GSH peroxidase activity (23) and similarities in the structures of  $Y_a$  and  $Y_c$  which have at least two-thirds of their amino acid sequences in common despite the greater molecular weight of the latter monomer (24). There is, however, one important difference between  $Y_a$  and  $Y_c$ .  $Y_a$ , in addition to its enzymic properties, possesses a high affinity binding site for

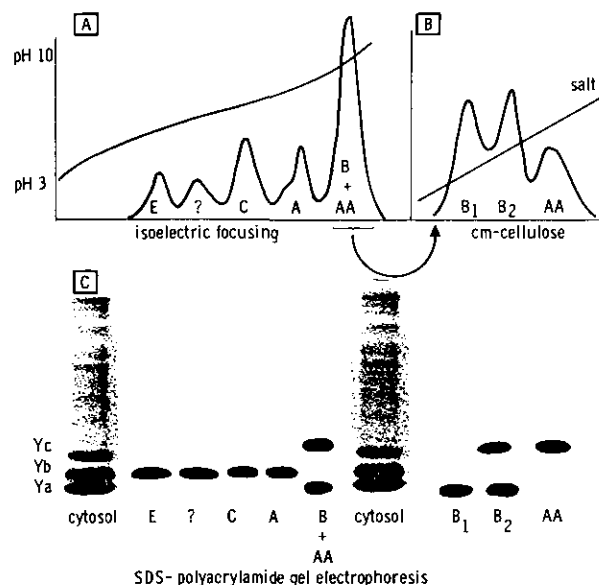
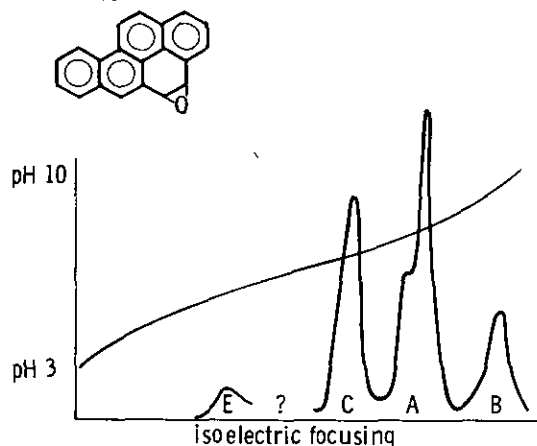


FIGURE 1. Separation and subunit compositions of GSH transferases from rat liver soluble supernatant fraction: (A) the GSH transferase fraction is separated by gel chromatography on Sephadex G100 and submitted to isoelectric focusing; (B) the fraction focusing at pH 9-10 is then separated further on CM cellulose in 10mM phosphate pH 6.7 using a 0-0.2M gradient; (C) the subunit composition of the various GSH transferases is shown by sodium dodecyl sulfate polyacrylamide gel electrophoresis.

benzo (a) pyrene-4,5-oxide



benzo (a) pyrene-7,8-diol-9,10-oxide

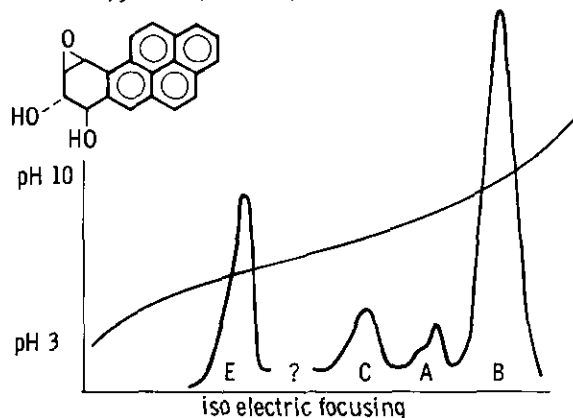


FIGURE 2. Substrate specificity of two BP-oxides towards GSH transferases separated by isoelectric focusing as in Fig. 1. The ordinate indicates both pH gradient and enzyme activity (the scale for BP-4,5-oxide is three orders of magnitude greater than that for BP-7,8-diol-9,10-oxide) (Table 1).

compounds with lipophilic moieties (25, 26); this property defines GSH transferase B, otherwise known as ligandin (27).

To say that there are seven GSH transferases in the rat liver is a conservative estimate. There is heterogeneity in  $Y_a Y_a$  (24) and also GSH transferase A (a  $Y_b$  dimer), both of which give two isoelectric forms. It is probable that all these variations, large and small, in GSH transferases are adaptations which enable interaction with the wide range of substrate structures which it is assumed hepatic GSH transferases are required to detoxify. The evolution of a high affinity binding site which characterizes  $Y_a$  has been shown to be important for a number of nonenzymic functions, e.g., the hepatic uptake of bilirubin (28, 29), the release of mitochondrial heme for utilization in the hepatocyte (30) and also, according to a mathematical model of the hepatocyte, the uptake and intrahepatocellular transport of lipophilic substrates (13).

**Combined Catalytic and Noncatalytic Conjugation with GSH.** A number of electrophiles undergo GSH conjugation by substitution or addition reactions at appreciable noncatalytic rates. Many, but not all, such reactions are also catalyzed by GSH transferases.

Where both catalytic and noncatalytic reactions occur, it is of interest to assess what the relative importance of these two reactions might be *in vivo*, but little of the necessary data is available. Data are available for the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) (an extremely useful substrate used in the laboratory to identify and quantitate GSH transferase activity since unlike other electrophiles it is a good substrate for many GSH transferases; see Table 1) and GSH. In using them it is necessary to assume that kinetic data obtained *in vitro* can be applied to the intrahepatocellular milieu. There the concentration of GSH transferases is known to be high, at least 0.2 mM, and aqueous-

Table 1. GSH transferase activity.

Substrate	GSH transferase activity, $\mu$ mole substrate conjugated/mg enzyme/min <sup>a</sup>					Reference
	B	AA	A	C	E	
1-Chloro-2,4-dinitrobenzene	11	14	62	10	0.1	(7)
1,2-Dichloro-4-nitrobenzene	$3 \times 10^{-3}$	$8 \times 10^{-3}$	4.3	2	0	(7)
Ethacrynic acid	0.26	0.30	0	0.1	0	(7)
trans-4-phenyl-3-buten-2-one	$1 \times 10^{-3}$	— <sup>b</sup>	$20 \times 10^{-3}$	0.4	0	(7)
Benzofluoranthene-4,5-oxide	$11 \times 10^{-3}$	$4 \times 10^{-3}$	$90 \times 10^{-3}$	$100 \times 10^{-3}$	$70 \times 10^{-3}$	(38)
Cholesterol-5 $\alpha$ , 6 $\alpha$ -oxide	$100 \times 10^{-6}$	— <sup>b</sup>	— <sup>b</sup>	— <sup>b</sup>	— <sup>b</sup>	(71)
Aflatoxin B <sub>1</sub> -2,3-oxide <sup>c</sup>	$30 \times 10^{-6}$	— <sup>b</sup>	$3 \times 10^{-6}$	$9 \times 10^{-6}$	— <sup>b</sup>	(50)
Benzofluoranthene-7,8-diol-9,10-oxide	0.7	— <sup>b</sup>	(0.2) <sup>d</sup>	(0.3) <sup>d</sup>	(1.2) <sup>d</sup>	(72)

<sup>a</sup> GSH transferase activity is expressed as  $\mu$ mole substrate conjugated per mg enzyme per minute under varying conditions. They do not necessarily represent  $V_{max}$ .

<sup>b</sup> Not determined.

<sup>c</sup> The reactive metabolite so marked is a postulated rather than a proven structure.

<sup>d</sup> Rough estimates based on competition for the reactive metabolite by the GSH transferase in question and DNA.

free concentrations of lipophiles such as CDNB are assumed to be low, owing to the presence of abundant membrane lipid into which most of the CDNB will partition. Low substrate concentration and high enzyme concentration will ensure that the contribution of the noncatalytic rate will be small. Using data for GSH transferase B only and assuming an intracellular concentration of GSH to be 10 mM, we calculate that the contribution of the noncatalytic rate is only 0.01% of the total rate (31, 32). In tissues in which the lipid fraction is not so abundant and the enzyme activity is low, the contribution of the noncatalytic reaction might be expected to be higher.

However, the principal role of hepatic GSH transferases is to detoxify electrophiles produced metabolically. The intracellular-free concentration of electrophiles generated *in vivo* by enzymes with slow rates such as the mixed function oxygenases is likely to be low and, therefore, by analogy with CDNB the contribution of the noncatalytic reaction to the total will be negligible. Mathematical models set up to describe these systems support this conclusion (33).

## GSH Conjugation of Electrophilic Species Formed In Vivo

### The Reactive Metabolite of Paracetamol

Paracetamol is believed to be oxidized at the endoplasmic reticulum to *N*-acetyliminoquinone, which undergoes nucleophilic addition of GSH followed by rearrangement to give 3[glutathion-S-yl]paracetamol (Fig. 3). (34). This reaction proceeds well both noncatalytically as well as catalytically (35), but on the basis of the above discussion the noncatalytic contribution *in vivo* should be negligible (33). Preliminary results suggest that the reactive metabolite of paracetamol is a better substrate for GSH transferases A and C than for GSH transferase B (36).

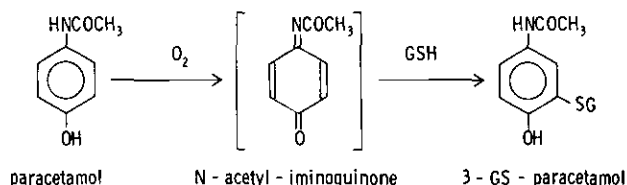


FIGURE 3. Activation and GSH conjugation of paracetamol (34).

### Benzo(a)pyrene-4,5-oxide (BP-4,5-oxide)

It is hard to judge as yet how typical the cases of CDNB and *N*-acetyliminoquinone are. None of the following cases conform with these examples. For example, BP-4,5-oxide (Fig. 2) is relatively stable and

unreactive towards GSH (it is a soft and weak electrophile) yet GSH conjugation is catalyzed quite effectively by GSH transferases (37-39). A comparison of the purified GSH transferases shows that BP-4,5-oxide is a much better substrate for GSH transferases A and C than for B and E (Table 1 and Fig. 2) (38).

### BP-7,8-diol-9,10-oxide

BP-7,8-diol-9,10-oxide (Fig. 2), the ultimate carcinogen of BP (40, 41), is the opposite of BP-4,5-oxide in that it is highly reactive and compared with CDNB is a much harder electrophile capable of reacting rapidly with weak nucleophiles, including water. The amount of noncatalytic reaction with GSH at physiological concentrations is negligible; nevertheless, in the presence of GSH transferases, GSH conjugation occurs (41). In contrast to BP-4,5-oxide, GSH transferases B and E are more effective catalysts than GSH transferases A and C (41) (Table 1 and Fig. 2).

### Cholesterol-5 $\alpha$ ,6 $\alpha$ -oxide

Cholesterol-5 $\alpha$ ,6 $\alpha$ -oxide is a suspected mutagen which may be generated from endogenous cholesterol during lipid peroxidation (42, 43). Conjugation with GSH is catalyzed *in vitro* by transferase B, but not by A or C (Table 1), perhaps due to the hydrophobic site of transferase B, which is known to bind cholesterol (44). Nonenzymic reaction with GSH is negligible.

### Aflatoxin B<sub>1</sub>-2,3-oxide (AFB<sub>1</sub>-oxide)

The ultimate carcinogen of AFB<sub>1</sub> (assumed to be the 2,3-oxide) (45) formed by activation of AFB<sub>1</sub> by a liver microsomal fraction resembles BP-7,8-diol-9,10-oxide in being a highly reactive strong electrophile with a negligible noncatalytic reaction with GSH in the absence of GSH transferases (46-48) (Fig. 4).

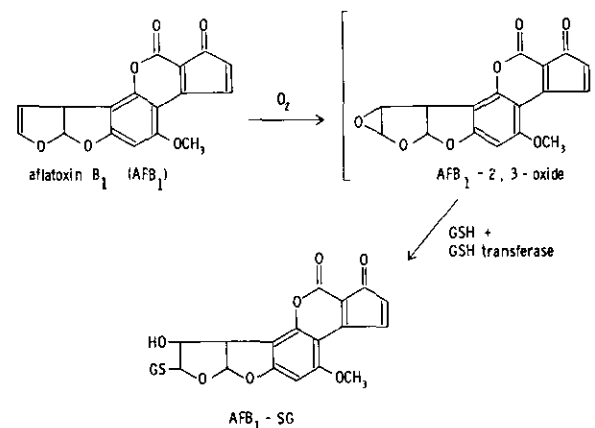


FIGURE 4. Activation and GSH conjugation of aflatoxin B<sub>1</sub> (47).

Data are not yet available for GSH transferase E, but as with the above diol-epoxide GSH transferase B is a much more effective enzyme than GSH transferases A and C (48) (Table 1).

### Reactive Metabolites of *N,N*-Dimethyl-4-aminoazobenzene (DAB)

Metabolism of DAB or *N*-methyl-4-aminoazobenzene (MAB) gives rise to two different types of electrophile for which there is no evidence of an enzyme-catalyzed GSH conjugation.

The *N*-methyl oxidation of MAB or 4'-OH-MAB gives an intermediate which is a soft electrophile reacting readily with GSH and believed to be an *N*-methylol or a methimine (49). GSH conjugation of the intermediate derived from MAB gives *N*-(glutathion-S-methylene)-4-aminoazobenzene (GSCH<sub>2</sub>AB) (49) or, in the case of 4'-OH-MAB, 4'-OH-GSCH<sub>2</sub>AB which is sulfated *in vivo* to 4'-sulfonyloxy-GSCH<sub>2</sub>AB (50) (Fig. 5). In *in vitro* experiments involving the microsomal oxidation of MAB in the presence of GSH, the yield of GSCH<sub>2</sub>AB is directly proportional to the GSH concentration in the incubation (49),

which is characteristic of a nonenzymic reaction. The reaction is not dependent on the microsomal GSH transferases which are present in the incubation nor is it affected by added soluble GSH transferases.

The other electrophile results from *N*-hydroxylation of MAB followed by sulfation. The resulting *N*-sulfonyloxy MAB forms a highly reactive cation which exists as the nitrenium ion and the 3, 2' and 4' carbonium ions (Fig. 6) (51).

*N*-Sulfonyloxy MAB, unlike the other ultimate carcinogens BP-7,8-diol-4,5-oxide and AFB<sub>1</sub>-oxide discussed above, is not a substrate for GSH transferase B (52, 53). This is surprising, since GSH transferase B or ligandin was first isolated as a protein that bound activated aminoazodye carcinogens covalently (54, 55) and was subsequently shown to have a noncovalent binding site for *N,N*-dimethyl-4-aminoazobenzene (56) and 4-sulfonyloxyazobenzene (57), which are structures related to the ultimate carcinogen. Three of the four possible cysteinyl residues per mole of GSH transferase B, Y<sub>a</sub>Y<sub>a</sub> react covalently with a proportion of the *N*-sulfonyloxy-MAB produced *in vivo*: similarly, three cysteinyl residues in Y<sub>a</sub>Y<sub>a</sub> also react with hydrophobic thiol

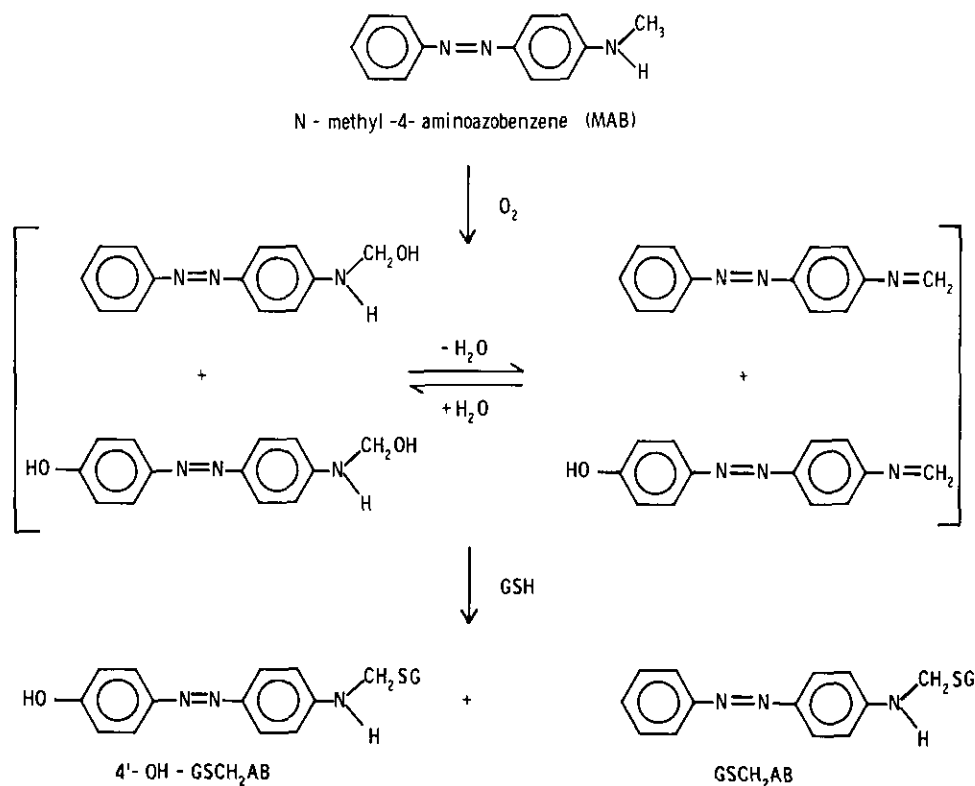


FIGURE 5. *N*-Methyl oxidation of *N*-methyl-4-aminoazobenzene to generate *N*-methylol or methimine intermediates and their reaction with GSH (50, 51).

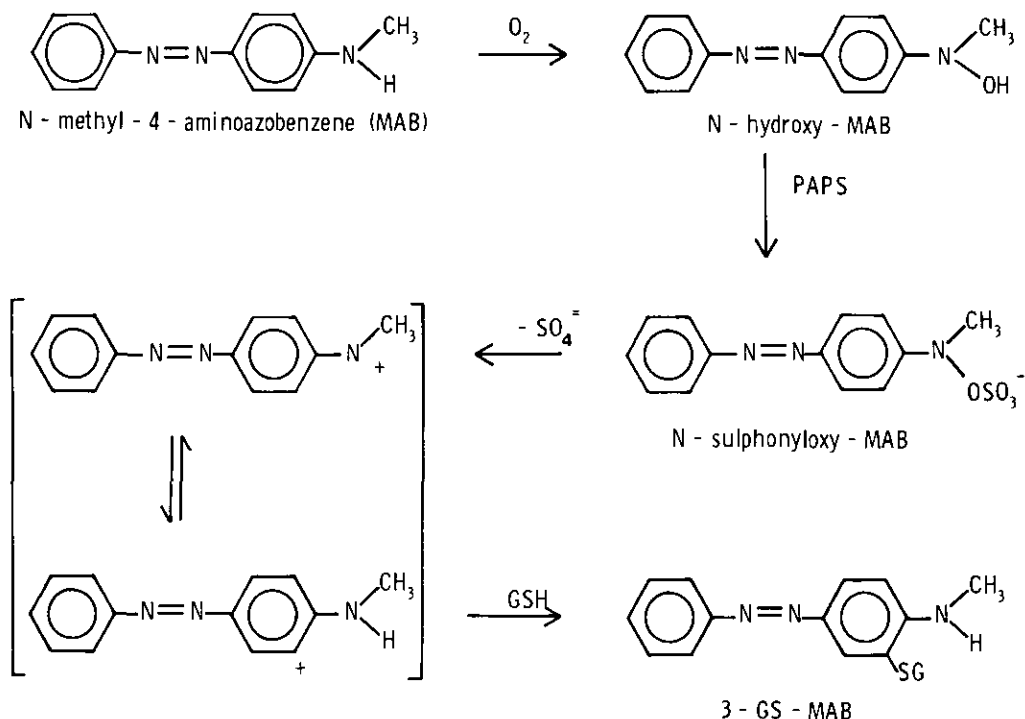


FIGURE 6. *N*-Oxidation of *N*-methyl-4-aminoazobenzene to generate the strongly electrophilic nitrenium and carbonium ions and their reaction with GSH (53).

reagents such as *p*-chloromercuribenzoate and without substantial loss of enzyme activity (22). It is therefore possible that *N*-sulphonyloxy MAB does not orient at the enzyme surface in a manner favorable to GSH conjugation. If unfavorable orientation does occur, it would appear to be a property shared by both the soft and hard electrophilic metabolites of MAB.

Although conjugation of *N*-sulphonyloxy-MAB is noncatalytic *in vivo*, the product distribution of GSH conjugates is more selective *in vivo* than *in vitro*. Thus 3-GS-MAB, but neither 2'-GS-MAB nor 4'-GS-MAB which are formed *in vitro*, is found in bile (52).

### *N*-Acetyl-2-Aminofluorene (AAF)

Unlike DAB, AAF does not give rise to soft electrophilic metabolites which react readily with GSH. Like DAB, AAF gives a highly electrophilic *N*-sulphonyloxy metabolite which is the ultimate carcinogen (58, 59). Studies with the relatively stable *N*-acetoxy-AAF (60) and more recently, *N*-sulphonyloxy-AAF (61), show reaction with GSH to be poor and to give rise to several conjugates, namely 1-, 3-, 4-, and 7-GS-AAF (Fig. 7). This reaction is not affected by GSH transferases. Yet as with DAB the product distribution of GSH conjugates is more selective *in*

*vivo* than *in vitro*. Only 1- and 3-GS AAF are found in bile (60).

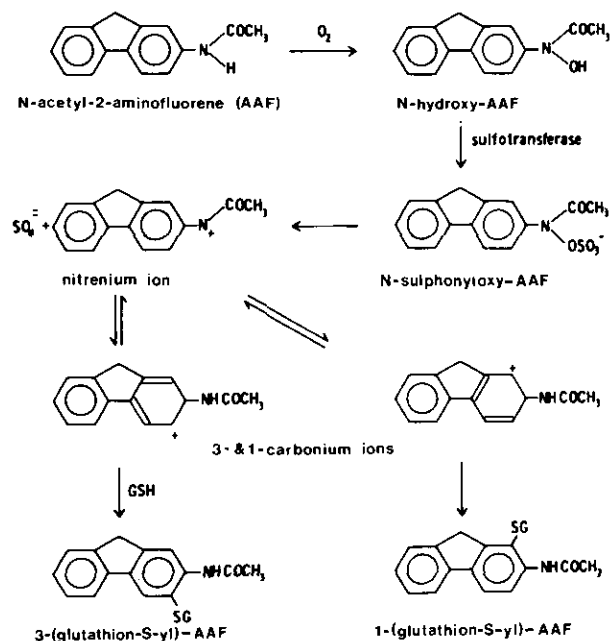


FIGURE 7. *N*-Oxidation of *N*-acetyl-2-aminofluorene to generate the strongly electrophilic nitrenium and carbonium ions and reaction with GSH (60).

## The Question of Aminofluorene (AF) Adducts

AAF gives rise to both AAF and AF adducts to DNA (62, 63). The electrophilic metabolite most likely to bring this about is *N*-acetoxy-AF resulting from enzymic transacetylation of *N*-OH-AAF (64). Another possible electrophilic metabolite is *N*-OH-AF resulting from *N*-deacetylation of *N*-OH-AAF (62-64). *N*-OH-AF has been shown to react with GSH *in vitro* to give *N*-(glutathion-S-yl)AF (see Fig. 8) (66). This reaction is relatively slow and not catalyzed by GSH transeferases (65). A similar product might be expected to be produced more rapidly by *N*-acetoxy-AF; however, no such GSH conjugate is observed in bile (65). If, as expected, electrophilic derivatives of AF are generated in the cytoplasm, their fate remains to be determined.

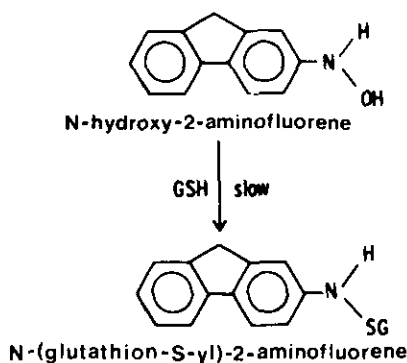


FIGURE 8. Reaction of *N*-hydroxy-2-aminofluorene with GSH (65).

## Quantitative Importance of GSH Conjugation in Detoxication

The importance of GSH conjugation in the detoxication of a xenobiotic depends on the extent to which the xenobiotic is metabolized to electrophiles.

In the case of DAB, soft electrophiles are produced in quantity which react noncatalytically with GSH. Of the biliary metabolites, 60% occur as GSCH<sub>2</sub>AB or 4'-sulfonyloxy GSCH<sub>2</sub>AB (50). Even the ultimate carcinogen, *N*-sulfonyloxy MAB, which is a hard electrophile, reacts noncatalytically with GSH; it gives a conjugate accounting for 0.2% of the total dose, which is two orders of magnitude higher than binding to DNA (67). The competition between GSH and DNA *in vivo* is much more in favor of GSH than expected from *in vitro* experiments. Since these reactions are noncatalytic, the yield is presumably very susceptible to intracellular GSH concentrations.

Since AAF is not metabolized to soft electrophiles, the effect of GSH on detoxication is expected

to be of a low order. No data are available for AAF itself, but even when the proximate carcinogen *N*-OH-AAF is administered, 17% of the total biliary conjugates are AAF-GSH adducts (60).

Paracetamol forms a soft electrophile and, as would be expected, its GSH conjugate, 3-glutathion-S-yl paracetamol, is very important in detoxication and can account for 60% of the biliary metabolites (68).

Benzo(a)pyrene produces a range of electrophiles *in vivo*, including not only the soft and weak electrophile BP-4,5-oxide and the hard electrophile BP-7,8-diol-9,10-oxide discussed above, but also a number of other electrophiles including BP-9,10-diol-7,8-oxide (40) BP-9-phenol-4,5-oxide (?) (70) and other oxides of BP (40), apart from the stable BP-4,5-oxide. As would be expected of a compound which is extensively metabolized to a number of electrophiles, GSH-conjugation is a major excretory pathway accounting for up to 60% of the total biliary metabolites (70).

In summary, the generation of a soft, but not weak electrophile should lead to high levels of GSH conjugation. This can be enzymic or nonenzymic; the former is assumed to be particularly effective and may even result in GSH depletion (35). The conjugation of soft but weak electrophiles requires enzymic intervention to bring about effective GSH conjugation. The conjugation of hard electrophiles also requires enzymic intervention, because these compounds run the risk of attack by water. It might be significant that GSH transferase B which has a hydrophobic binding site is an effective enzyme with those hard electrophiles which are conjugated with GSH. So far, the two examples which have been studied (BP-7,8-diol-9,10-oxide and aflatoxin oxide) have been epoxides. Two examples of hard electrophiles, the GSH conjugation of which is not catalyzed, are *N*-sulfonyloxy derivatives of MAB and AAF and the carbonium/nitrenium ions derived from them.

Reference to Table 1 shows that rates of enzyme-catalyzed GSH conjugation can vary from 62  $\mu$ mole/min/mg enzyme to 4 pmole/min/mg enzyme, i.e., they can vary over seven orders of magnitude. It might be supposed that the lowest rates observed are not significant biologically; however, in the case of aflatoxin B<sub>1</sub>, one can calculate from the *in vitro* rates of oxidation, coupled with enzyme-catalyzed GSH conjugation, that the rate of AFB<sub>1</sub>-SG formation in a 12-g rat liver should be 1.7  $\mu$ mole/min. This is close to the observed biliary output of AFB<sub>1</sub>-SG in rats given an intraperitoneal dose of 2 mg AFB<sub>1</sub>, where the mean rate of excretion over 4 hr is 1.8  $\mu$ mole/min (48). Although the experimental system is not ideal, the evidence is strong that a low

enzyme rate, such as the above, can result in the excretion of 20% (48) of the biliary metabolites as the glutathione conjugate.

## Detoxification of Oxygen

The action of the mixed function oxidation system is associated with the release of small quantities of oxygen radicals capable of initiating lipid peroxidation. Such processes have been monitored both *in vivo* and *in vitro* during xenobiotic metabolism by the release of hydrocarbon gases (73-75). GSH is thought to participate in the inhibition of such lipid peroxidation processes since they are accompanied by a loss of hepatic GSH (76) and the release of GSSG into the bile (77). Moreover, GSH-dependent cytosolic factors are present in rat liver which inhibit lipid peroxidation initiated enzymically *in vitro* by ADP-Fe<sup>3+</sup> plus NADPH (78).

Our studies indicate that GSH by itself is only a partial inhibitor of lipid peroxidation in the *in vitro* system, whereas GSH plus liver cytosol yield almost pure (complete) inhibition (Fig. 9). Extending

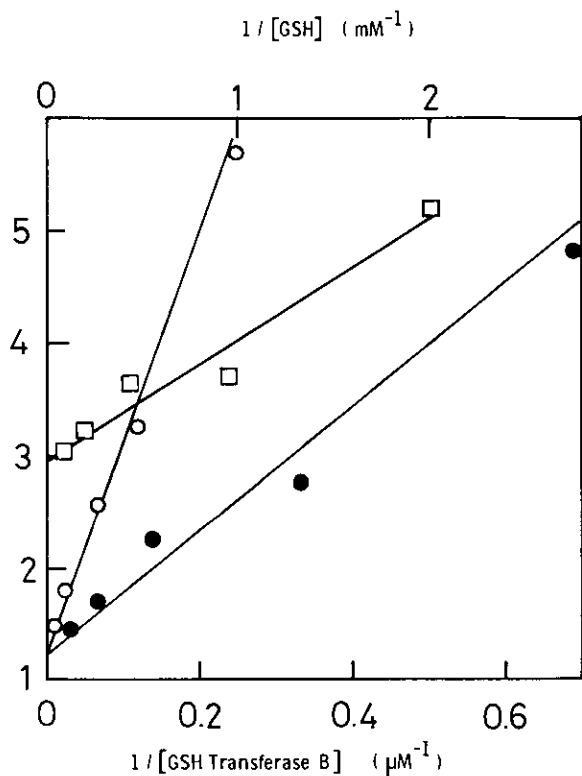


FIGURE 9. Inhibition of lipid peroxidation by GSH and GSH transferase B: (□) GSH alone; (•) GSH (2mM) plus purified GSH transferase B; (○) GSH (2mM) plus dialyzed rat liver cytosol expressed according to its content of GSH transferase B. Lipid peroxidation induced in rat liver microsomes by ADP-Fe<sup>3+</sup> plus NADPH was assayed by malondialdehyde formation.

the studies of Burk et al. (79), we find that purified GSH transferase B, which displays Se-independent GSH-peroxidase activity with lipid hydroperoxides, is also a pure inhibitor (Fig. 9); GSH transferases A and C do not inhibit. Using frontal elution analysis of liver cytosol on Sephadex G-100 (Fig. 10), we have demonstrated that the total GSH-dependent inhibitory activity of rat liver is accounted for (approx. 50% each) by GSH transferase B and the Se-dependent GSH peroxidase. These enzymes presumably inhibit lipid peroxidation through reaction with H<sub>2</sub>O<sub>2</sub> and fatty acid and steroid hydroperoxides; however, the possibility of GSH reaction with other active oxygen species remains to be studied.

## Conclusions

The evidence is very good that GSH and GSH transferases, both abundant in cells, provide a system which is important in the detoxication of electrophiles having the potential for cytotoxic or genotoxic damage.

The GSH transferases are multiple, but appear to be related genetically and to have arisen by divergence. It is assumed that a multiplicity of enzymes is required to deal with a multiplicity of substrates; however, it is shown that not all electrophiles, notably those derived from DAB and AAF, are substrates. The net cast by the GSH transferases is by no means perfect.

In the rat liver, an unusual GSH transferase has evolved that appears to perform two additional tasks associated with detoxication. This is GSH transferase B, which *in vitro* evidence suggests is

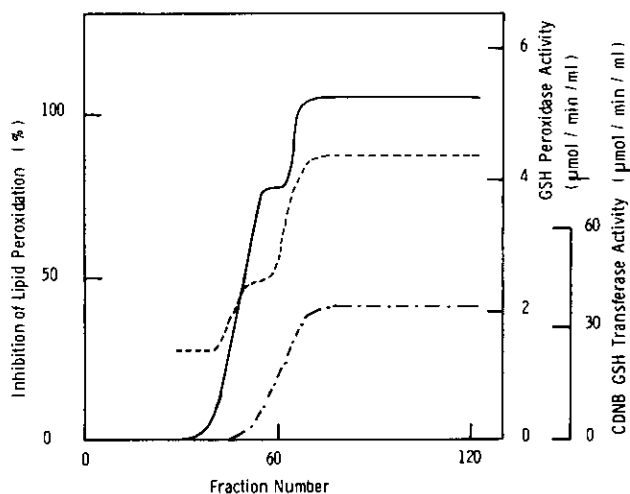


FIGURE 10. Separation by frontal elution chromatography of components of rat liver cytosol which inhibit lipid peroxidation: (---) inhibition of lipid peroxidation; (-•-) CDBN GSH transferase activity; (—) GSH peroxidase activity (with cumene hydroperoxide).



necessary for the release of heme from mitochondria for use by the apoenzymes, notably cytochrome P-450, and which also appears (again from *in vitro* evidence) to participate in the detoxication of by-products of the utilization of O<sub>2</sub> by the P-450 system.

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