The Role of Glutathione and Glutathione Transferases in Chemical Carcinogenesis

Brian Coles and Brian Ketterer

Referee: Jack A. Hinson
Department of Biochemical Toxicology
National Center for Toxicological Research
Jefferson, Arkansas

I. INTRODUCTION

The importance of the covalent binding of electrophilic metabolites of carcinogens to macromolecules, in particular DNA, as a critical event in chemical carcinogenesis was established in the 1960s. This was largely through the work of James and Elizabeth Miller and colleagues.[1] Since then, the metabolism of many chemical carcinogens has been investigated and the details of their activation to electrophilic metabolites have been established.[1-3]

That glutathione (GSH) can act as an alternative (competing) nucleophilic site to the nucleophilic portions of DNA, and hence afford protection against genotoxic electrophiles, was first recognized by Boyland and Chasseaud[4] and Chasseaud.[5] It has now been established that GSH reacts with a number of electrophiles derived from carcinogens and that these reactions form an important detoxification mechanism.[5,6] Boyland's laboratory was also the first to show that many of the reactions of GSH with electrophiles are enzyme catalysed.[7] This catalysis was referred to as glutathione *S*-transferase (GSH transferase) activity, and was shown to reside in a number of isoenzymes with broad substrate specificity by Jakoby and colleagues.[8] It is now known that these isoenzymes (E.C.2.5.1.18) are products of a gene superfamily.[9-12]

In this review the role of GSH conjugation in the overall metabolism of carcinogens is discussed both in general and in relation to specific carcinogens.

II. CARCINOGEN METABOLISM

Many lipophilic carcinogens are metabolized to polar conjugates in order to be detoxified and excreted. Metabolism of xenobiotics is usually referred to as phase I and phase II, where phase II metabolism involves the conjugation of phase I products. Oxidative phase I metabolism, generally by cytochrome P450, but occasionally by flavin mixed function oxidation, is of particular relevance to carcinogen metabolism, but carcinogens and their oxidation products may be susceptible to further phase I metabolism (e.g., nitroreduction or the action of epoxide hydrolase). Phase II metabolism includes the formation of glucuronide, sulfate, and glutathione conjugates and usually results in detoxication, as the polar conjugates are readily excreted. Thus, during the metabolism of carcinogens, alternative pathways of phase I and phase II metabolism operate simultaneously, yielding a complex mixture of products.[13]

In general, the metabolism of carcinogens to electrophiles involves at least one oxidative (phase I) step. This may be sufficient to produce a toxic (and in particular genotoxic) species (e.g., polycyclic aromatic hydrocarbon [PAH] diol epoxides), but phase II metabolism is often involved as a critical step in the formation of genotoxic electrophiles. Sulfation in particular is known to be an activation step for weakly electrophilic arylhydroxylamines and benzylic alcohols (see below), but acylation, glucuronidation, and occasionally GSH conjugation can all be activation steps.

The production of electrophiles is a source of danger to the cell, and the detoxication of these electrophiles is therefore essential if toxicity or carcinogenesis is to be prevented. For example, a tissue may be susceptible to chemical carcinogenesis because it is exposed to an electrophile that has the capacity to react with DNA (a genotoxic electrophile), but is poorly detoxified in that tissue and also produces DNA damage that is not repaired correctly.

There are a number of points at which detoxication may occur. It may involve direct reaction with electrophiles, several of which may arise in the metabolism of a particular carcinogen (see Section VII.A, below) or to strong competition from alternative metabolic pathways that do not yield electrophiles. The relative effects of competing pathways on overall metabolism may be changed by the action of selective enzyme inducers or the availability of co-substrate (e.g., reduced nicotinamide dinucleotide phosphate [NADPH], uridine disphosphate [UDP]-glucuronic acid, GSH) utilized in one pathway but not another.

III. THE SPONTANEOUS REACTION OF GLUTATHIONE WITH ELECTROPHILES ARISING DURING CARCINOGEN METABOLISM

A. Tissue Distribution of GSH

The reaction with GSH may be spontaneous or enzyme

B. Coles received B.A. and M.A. degrees from Cambridge University in Cambridge, England, and M.Sc. and D. Phil. degrees from Sussex University in Falmer, Sussex, England. Dr. Coles is currently a Research Associate in the Cancer Research Campaign Molecular Toxicology Research Group in the Biochemistry Department at Middlesex Hospital Medical School at the University of London in London, England. **B. Ketterer** received B.Sc. Hons. and M.Sc. degrees from the University of Western Australia in Nedlands, Western Australia, and a Ph.D. degree from Cambridge University in Cambridge, England. Dr. Ketterer is currently a Professor of Biochemistry in the Cancer Research Campaign Molecular Toxicology Research Group in the Biochemistry Department at Middlesex Hospital Medical School at the University of London in London, England. Dr. Ketterer is also a Fellow of Institute of Biology at the Institute of Biology in London, England.

assisted. If the spontaneous reaction is the only means whereby a particular electrophile is detoxified by GSH, the susceptibility of *in vivo* systems to that electrophile will depend on the concentration of GSH present. Levels are as high as 15 m*M* in the lens, 5 to 10 m*M* in the liver, and 1 to 2 m*M* in many other tissues.[14] Although a tissue may contain GSH, it is not necessarily in all the cells that compose it. For example, the overall concentration of GSH in the brain is 1.5 to 2 m*M*,[15] but histochemical studies show that, while it is abundant in the astroglial cells and the ependymal cells, it is absent from the neuronal stroma. In the lung it is found in the interalveolar, but not the alveolar cells. In the kidney it is rich in the proximal convoluted tubules, but not so apparent elsewhere. In the lens it is highly concentrated in the periphery, but less so in the interior, and so on.[16] In certain cells, most notably the hepatocyte, GSH concentrations vary according to circumstance, being depleted during starvation and by a heavy electrophile load, or having levels higher than normal as the result of a rebound effect following a period of depletion.[17,18]

B. Chemical Reactivity of GSH with Genotoxic Electrophiles

It has been recognized that if an electrophile is to be genotoxic it must have a "high chemical reactivity" in order to react with the weakly nucleophilic positions of DNA bases (e.g., $-O^6$ of guanine).[1,19,20] In general, genotoxic electrophiles are species that have a high degree of localized charge, for example, carbonium ions derived from nitrosoureas, nitrenium and arylcarbonium ions from aromatic amine carcinogens, and benzylic carbonium ions from polycyclic aromatic hydrocarbons. All these species are of high energy compared with, for example, alkyl and aryl halides and aliphatic epoxides. GSH, on the other hand (or more accurately the thiolate anion, since in all cases studied it is the anion that is the reactive species), is a highly polarizable nucleophile. As a consequence of this, the spontaneous reaction of GSH with genotoxic electrophiles is not very rapid. Conversely, the reaction of GSH with polarizable electrophiles, e.g., by Michael addition to conjugated double bonds, is rapid. (For a discussion, see Reference 21.)

Quantitative data for the noncatalytic reaction of electrophiles derived from xenobiotics with GSH are extremely limited, but two examples show the range of rates and illustrate the point made above. The second-order rate constant for the reaction of the mutagen 1-nitropyrene-4,5-oxide with GSH is 1.7×10^{-1} $M^{-1}s^{-1}$,[22] while that for the highly polarizable and nongenotoxic *N*-acetyl-*p*-benzoquinone imine, the cytotoxic metabolite of the drug acetaminophen, is approximately 3×10^4 $M^{-1}s^{-1}$,[23] an order of 10^5 times more reactive. Other reactions of GSH with model electrophiles have been reviewed.[24]

There is a range of nucleophilic species *in vivo*. At one end are protein thiols, which are similar in their susceptibility to electrophiles as GSH itself, while at the other are the nucleophilic sites on DNA, which are toward the polarized end of the nucleophilicity scale, that are susceptible to carcinogenic electrophiles.[21]

IV. GLUTATHIONE TRANSFERASES

A. Importance of Enzymic Catalysis for the Reactions of GSH with Genotoxic Electrophiles

Because GSH detoxifies genotoxic electrophiles poorly by the spontaneous reaction, catalysis of GSH conjugation by the GSH transferases is particularly important and can lead to efficient detoxication.

The rates of noncatalytic reactions of electrophiles with GSH will depend on the thiol concentration and pH. However, the enzyme-catalyzed reaction is less dependent on GSH concentration, since the Km for GSH is of the order of 0.1 m*M*,[25] and reaction rates will fall only when GSH levels approach this value. In addition, there is evidence that, in the case of "Bay region" diol epoxides of benzo(a)pyrene, benz(a)anthracene, and chrysene as substrates, the Km for GSH falls at low GSH concentrations, perhaps due to some conformational change in the enzyme.[26,27] The same argument applies to the electrophilic substrate where Kms are generally in the μ*M* range.

B. Glutathione Transferase Isoenzymes in the Rat

Most of the exploratory work on GSH transferases has been done in the rat and, therefore, in terms of structure, function, and tissue distribution, they provide the standards with which others are usually compared.

Soluble GSH transferases, regardless of their species, are dimeric enzymes and, in the rat, at least 11 subunits have been characterized, although more are known to exist.

Several nomenclatures are used, the two most common being shown. In the nomenclature used in this paper, each subunit is given a number based on the chronological order of its characterization. The basis of the other nomenclature is the mobility of subunits on sodium dodecyl sulfate-polyacrylamide gel electrophoresis.[28]

1. Multigene Families: Primary Structure and Gene Structure

Complete primary structures, deduced from cDNA sequences, are known for subunits 1,[29-31] 2,[32] 3,[33] 4,[34] and 7.[35,36] Based on these sequences, these subunits fall into three categories comprising 1 and 2, 3 and 4, and 7. There is 69% identity in sequence between 1 and 2, 77% identity between 3 and 4, and very little identity among the three categories, showing that these categories represent multigene families. This is supported by studies of gene sequences, now known for subunit 1,[37] subunit 3,[38] and subunit 7,[39] which show that exon-intron structures differ with the family. They have been named alpha (1 and 2), mu (3 and 4), and pi (7), respectively.[9,40] On the basis of present evidence, including complete or partial amino acid sequences, enzymic properties, and immunological cross-reactivity, the genetic relationship among all 11 subunits is believed to be as follows: alpha family, subunits 1, 2, 8, and 10; mu family, subunits 3, 4, 6, 9, and 11; and pi family, subunit 7 only. Subunit 5 has yet to be assigned.[41-45a]

Within a multigene family, subunits may form heterodimers. GSH transferases 1-2, 3-4, 6-9, 3-6, and 4-6 so far having been identified.[44]

The upstream regulatory regions of these genes are clearly of interest with respect to tissue-specific expression and enzyme induction. Those for subunits 1 and 7 have been mapped and show substantial differences.

The subunit 7 gene has sequence motifs associated with "housekeeping" genes, such as a high G and C and CpG content around their promoters, and GC boxes matching the consensus sequence for their binding site of the transcription factor SP1. In addition, it has a phorbol ester responsive element (TRE).[39,46] It has been shown that c-Ha-ras and phorbol esters, both of which can activate the polyoma virus enhancer, act through an enhancer element closely related in sequence to TRE.[47] The 5′ flanking region of subunit 1[37] does not appear to contain either motifs associated with "housekeeping genes" or the phorbol ester responsive element. It contains an element required for maximal basal promoter activity and also a β-naphthoflavone responsive element. As yet, it is not known whether the β-naphthoflavone responsive element is activated directly or indirectly by a trans-acting protein.

2. The Tissue Distribution of GSH Transferases

GSH transferases have been found in almost every rat tissue examined, although not necessarily in every cell type. Their distribution might be expected to parallel that of GSH, but at the moment more information is available about the histochemical localization of GSH transferases than of GSH. The distribution of GSH transferases in any one tissue or cell type may be one of the determinants of its susceptibility to carcinogenesis. GSH-transferase activity using 1-chloro-2,4-dinitrobenzene as substrate (CDNB) varies considerably from high values in the liver and testis to very low values in the nonlactating mammary gland and negligible levels in epididymal sperm.[48] In addition, striking differences in isoenzyme distribution occur from one tissue to another.

The content of GSH transferase subunits in a tissue is readily determined by reverse-phase HPLC analysis of the GSH transferase fraction obtained from tissue homogenates by GSH agarose affinity chromatography.[49] Analyses of the liver, kidney, lung, interstitial cells, and spermatogenic tubules of the testis are shown in Figure 1. In the liver, the organ most active in GSH-dependent detoxication, subunits 1, 2, 3, 4, 6, 8, and 9 are clearly distinguished (both subunits 1 and 8 resolve into two forms).

In some tissues one subunit predominates; for example, in the red blood cell it is subunit 8,[49a] in the small intestine it is subunit 7,[50] while in the lactating mammary gland and the adrenal gland it is subunit 2. In some tissues a subunit usually encountered in small amounts may be abundant. Thus, the testis is rich in subunits 6 and 9 and the brain in subunit 6.[50a]

Immunohistochemistry has revealed interesting differences within tissues; it has shown that in the normal liver, subunit 7 is present in all bile duct cells and in occasional hepatocytes,[51] and that, although subunits 1, 2, 3, and 4 are present in all hepatocytes, they are more abundant in the area around the central vein than in the periportal region.[51,52] In the brain, GSH transferases, like GSH, have been detected only in the astroglial and ependymal cells and not at all in the neuronal stroma.[53]

3. Enzymic Activity

Most of the work done with GSH transferases involves substrates chosen, not because of their biological significance, but because their GSH conjugation results in an optical density change that can be made the basis of a convenient spectrophotometric assay, and much of our current understanding of GSH transferases relates to these substrates (see Table 1). The most commonly used is CDNB, since it is utilized well by most of the rat subunits. Exceptions are subunit 5 and subunit 9.[56,56a] Other substrates are chosen because they are relatively specific for a particular subunit and may be used to detect and quantify it.

Many mutagenic and carcinogenic electrophiles react with GSH to produce conjugates. It is often assumed that they are substrates for GSH transferases, but this has not always been proven, and surprisingly few have been studied with respect to isoenzyme specificity. The spontaneous and enzymic reaction of specific carcinogenic electrophiles with GSH are discussed below.

C. Glutathione Transferase Isoenzymes in Man

1. Multigene Families: Primary Structure and Gene Structure

Studies on GSH transferases of the rat and other laboratory animals have been undertaken with the expectation that they might provide suitable models for the human. As more is known about human GSH transferases, it becomes apparent that information obtained with the rat is indeed relevant to man. The same multigene families are seen in both species, and there is considerable identity in primary structure across the two species.

The first separation of human liver GSH transferases involved a tissue sample from a single individual. Five related "cationic" forms, named α through ε were separated[57] and are now known to be related to the alpha class of rat GSH transferases. Isoenzymes at each extreme of the range of isoelectric points have been shown to be homodimers, while those with intermediate isoelectric points are heterodimers. Homodimers and heterodimers are most readily identified and quantified by reverse-phase HPLC (see Figure 2). The monomeric forms have been named either Y_1 and Y_4,[58] B_1 and B_2,[59] or α_x and α_y,[49] B_1, Y_1, and α_x being the more basic of the two subunits in each nomenclature. Two homogeneous monomers would be expected to give two homodimers and only one heterodimer. The multiplicity of forms that have been observed is yet to be explained.

Two full-length cDNA clones for alpha family subunits, referred to as Ha_1 and Ha_2, have been described[60,61] having 96% identity in the nucleotide sequence of the coding region. Com-

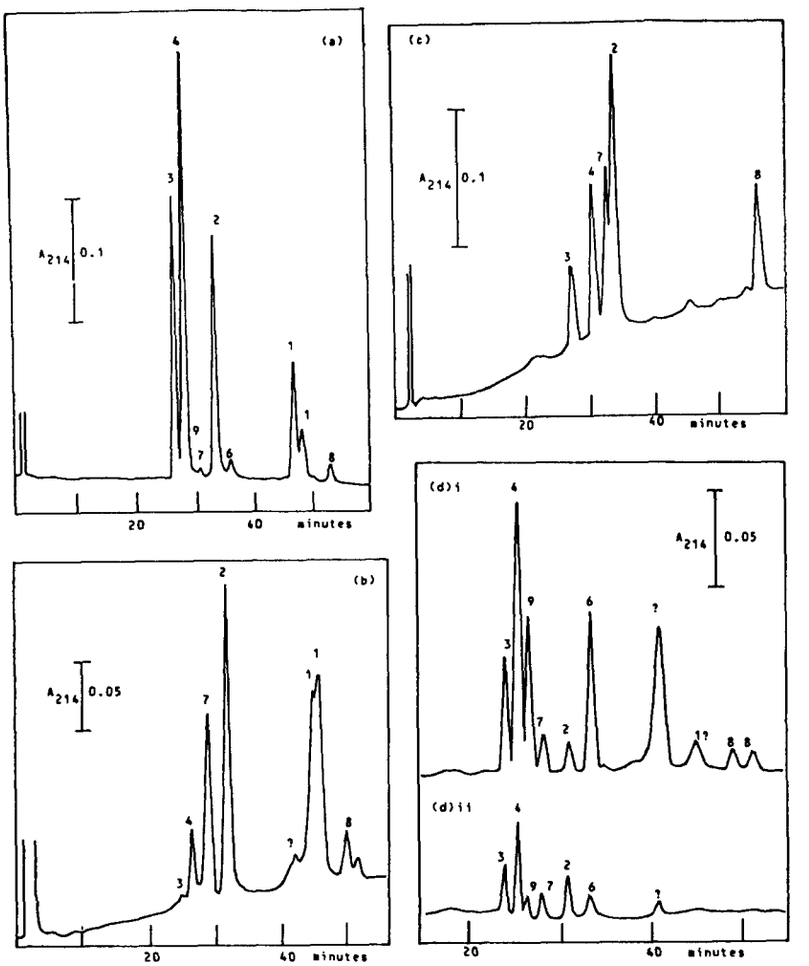

FIGURE 1. GSH transferase composition of the soluble fraction of rat tissues of (a) liver, (b) kidney, (c) lung, (di) spermatogenic tubules, and (dii) interstitial cells of testis as analyzed by HPLC separation of subunits. Data from the Cancer Research Campaign Molecular Toxicology Research Group.

parisons of deduced sequences show that between species, human and rat alpha class GSH transferases are approximately 75% identical in amino acid sequence.

"Near neutral" and "anionic" forms were found in later studies of human liver from various sources and shown to be homologous with the mu and pi families, respectively, in the rat.[40,46,58,60-63] The human π gene has been mapped and shown to have features in its upstream regulatory region similar to those seen in rat subunit 7, for example, sequences associated with "housekeeping" genes, such as a high G and C and CpG content around its promoters, GC boxes matching the common sequence to the binding site of transcription factor SPI, and a phorbol ester/ras responsive element.[46]

Whereas representatives of the alpha family GSH transferases are abundant in all human livers, mu class enzymes are either of low abundance or not present at all. This is illustrated in Figure 2, which shows the analysis of three human livers by HPLC. From genetic studies of 179 liver samples from Chinese, Indian, and Caucasian subjects, it was concluded that the mu locus (GST I) has two expressing genes and a null allele.[64] This conclusion has been supported by other population studies.[65-67] It has been suggested that the null allele either gives no product or an inactive product incapable of forming dimers, with the result that the mu family is either absent or represented by either one or other of two active homodimers or an active heterodimer.[64] Comparison between sequences at present available for human and rat GSH transferases of the mu family once more show considerable homology between the species.[64a]

2. Tissue Distribution

A survey of a number of tissues shows that, in most cases, humans have more CDNB-GSH transferase activity than the

Table 1
Rat GSH Transferases[a]

Substrate	Enzymes by class: Alpha 1-1	Alpha 2-2	Alpha 8-8	Mu 3-3	Mu 4-4	Mu 6-9	Pi 7-7	Unassigned 5-5
1-Chloro-2,4-dinitrobenzene	40.0	38.0	10.0	50.0	20.0	190.0	20	<0.15
1,2-Dichloro-4-nitrobenzene	0.15	0.15	0.12	8.4	0.7	2.4	<0.05	nil
trans-4-Phenyl-3-buten-2-one	0.1	0.1	0.1	0.1	1.2	0.2	0.02	<0.001
1,2-Epoxy-3-(*p*-nitrophenoxy)propane	0.7	0.9	nd[b]	0.2	0.9	<0.5	1.0	25.5
Ethacrynic acid	0.3	2.1	7.0	0.4	1.0	<0.5	4.0	nil
4-Hydroxynon-2-enal[54,55]	2.6	0.7	170.0	2.7	6.9	nd	nd	nd
Cumene hydroperoxide	1.4	3.0	1.1	0.1	0.4	0.04	0.01	12.5
Linoleate hydroperoxide	3.0	1.6	0.2	0.2	0.2	0.06	1.5	5.3
Δ^5-Androstene-3,17-dione	0.23	0.07	nd	0.02	0.002	nd	<0.001	nd

Note: Data are from the Cancer Research Campaign Molecular Toxicology Research Group, unless otherwise stated.

[a] Activities ($\mu mol \cdot min^{-1} \cdot mg^{-1}$) toward model substrates, some of which are used to specify subunits.
[b] nd = not determined.

rat.[68] Many data are now available concerning isoenzyme content of the liver and interindividual variation in that organ. Information in the same detail is rarely available for other tissues, where in most cases the enzymes present are usually identified as "cationic", "near neutral", or "anionic", and, although these broad designations may sometimes equate with alpha, mu, and pi, this equivalence cannot be taken for granted. In an early study, all three forms of GSH transferase, namely, "anionic", "near neutral", and "acidic", were found in the adrenal, testis, and ovary; "anionic" alone occurred in the lung.[69] In certain tissues, such as erythrocyte, platelet,[70] thyroid,[71] placenta, lung, heart, spleen, kidney, pancreas, and liver,[72] the acidic transferase has been identified as GSH transferase π. Subsequently, the kidney has been analyzed, showing that multiple forms of the alpha class together with the pi class are well represented, and small amounts of the mu class are present.[73,74] This pattern is comparable to that found in rat kidney.

Three further mu class enzymes associated with extrahepatic tissues are GST 4, which is muscle specific,[65,74] GST 5, which has so far only been found in brain,[74] and the alpha class GSH transferase 9.9. This last isoenzyme, which has been isolated from skin, differs from hepatic alpha enzymes in its N-terminal sequence, which is identical to that of rat GSH transferase subunit 2 and its higher apparent molecular weight.[75]

Relatively little is known of the occurrence of GSH transferase mu in extrahepatic tissues. However, the availability of the substrate *trans*-stilbene oxide now provides a sensitive means for detecting it and can be used to gather information about it. The considerable variation in isoenzyme content among individuals should result in corresponding variations in their susceptibility to drugs and toxins. An interesting study with mononuclear leukocytes measured activity toward *trans*-stilbene oxide in a survey of smokers who were either control subjects without disease or lung cancer patients. It was found that a greater proportion of control smokers (59%) had *trans*-stilbene oxide-GSH transferase activity than those with cancer (35%), which led to the conclusion that the gene expressing mu may be a host determinant of susceptibility to lung cancer.[76-80]

3. Enzymic Activity

Within the same family, human and rat isoenzymes have similar enzymic activities. Subunits α_x and α_y are associated with Se-independent GSH peroxidase activity, subunit α_x being more active than α_y. GSH transferase μ, like rat subunit 4, has activity toward *trans*-4-phenyl-3-butene-2-one, and GSH transferase π, like rat subunit 7, is a good enzyme for ethacrynic acid and a better Se-independent GSH peroxidase with linoleate hydroperoxide than with cumene hydroperoxide (see Table 2).

Although information is fragmentary, biologically significant substrates are utilized by human transferases, as would be expected from the known behavior of their rat equivalent. For example, this has been demonstrated with the catalysis of the reaction of *N*-acetyl-*p*-benzoquinone imine with GSH. Here, the pi family enzymes 7-7 and π are good catalysts for conjugation, and the alpha class enzymes of rat and man are also good catalysts, but for both conjugation and reduction to acetaminophen.[23] Other examples from PAH chemistry are discussed in Polyaromatic Hydrocarbon Epoxides (see below).

D. Glutathione Transferase Isoenzymes in the Mouse

1. Structure and Nomenclature

The alpha, mu, and pi multigene families have also been recognized in the mouse. These designations have been made largely on the basis of substrate specificity, immunological

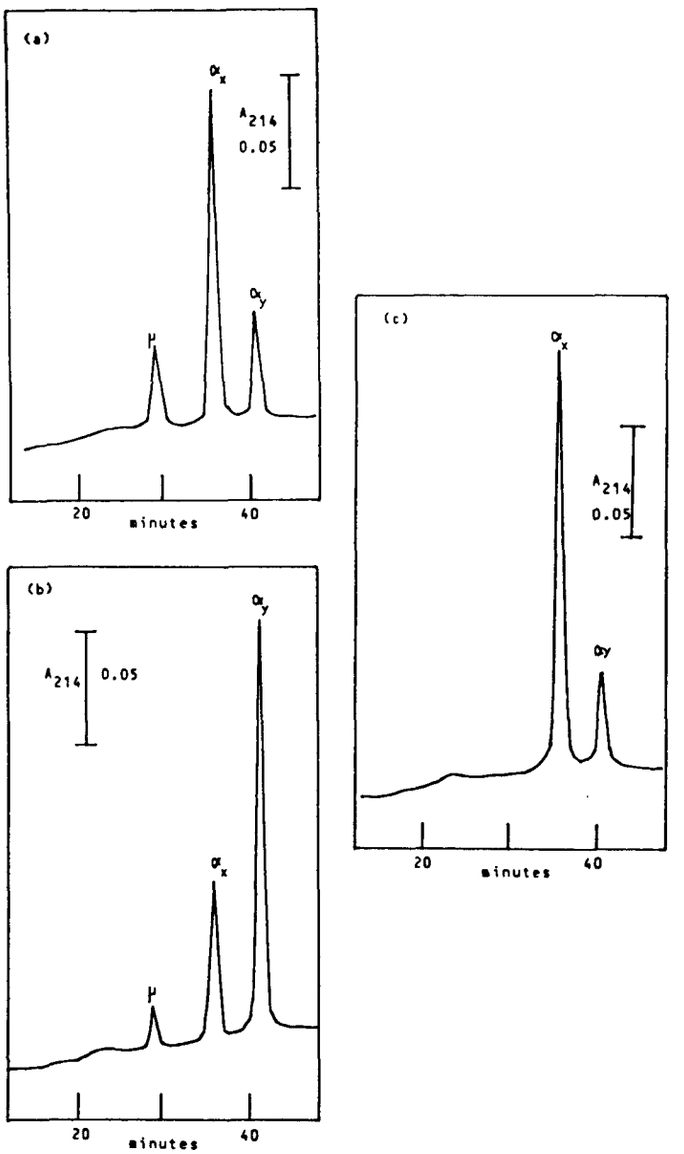

FIGURE 2. Separation of GSH transferase subunits from three human livers by HPLC, showing interindividual variation in enzyme composition. (From Ostlund Farrants, A.-K., Meyer, D. J., Coles, B., Sonthan, C., Aitken, A., Johnson, P. J., and Ketterer, B., *Biochem. J.*, 245, 423, 1987. With permission.) Data from the Cancer Research Campaign Molecular Toxicology Research Group.

cross-reactivity with antisera against rat GSH transferases, and electrophoretic mobility on sodium dodecyl sulfate-polyacrylamide gels.[40,81] Chromatography on hydroxy-apatite has shown that pi type enzymes can be resolved into three forms,[82] but whether these are posttranslational modifications or the products of more than one gene is not known. So far, only one expressing gene has been detected in rat and man. Certainly, the pi family is expressed differently in the mouse compared with rat and man. It is a prominent component in the liver and particularly

Table 2
Human GSH Transferases[a]

	Enzyme by class			
	Alpha		Mu	Pi
Substrate	$\alpha_x\alpha_x$	$\alpha_y\alpha_y$	μ	π
1-Chloro-2,4-dinitrobenzene	55	60	90	72
1,2-Dichloro-4-nitrobenzene	0.3	0.8	0.03	0.6
trans-4-Phenyl-3-buten-2-one	nil	nil	0.36	0.01
1,2-Epoxy-3-(*p*-nitrophenoxy)propane	nil	nil	0.11	1.8
Ethacrynic acid	0.11	0.14	0.081	1.9
Cumene hydroperoxide	6.2	2.1	0.63	0.04
Linoleate hydroperoxide	4.7	1.6	nd	0.44

Note: Data are from the Cancer Research Campaign Molecular Toxicology Research Group.

[a] Activities ($\mu mol \cdot min^{-1} \cdot mg^{-1}$) toward substrates used to specify subunits

abundant in the female liver,[81-83] whereas in many extrahepatic tissues it is said to be absent.[81-83]

2. GSH Transferases Across the Lifespan

A study of GSH-CDNB transferase activity in the female mouse liver, lung, and intestine between the ages of 2 weeks and 18 months show that in the 9 months following birth the activity increases from 40 nmol mg protein^{-1} min^{-1} to 160 nmol mg protein^{-1} min^{-1} and then falls away steeply to postnatal levels by 15 months. Similar changes are seen in lung and intestine and may also occur in other species.[84] Such reduced GSH transferase levels may be associated with the increased susceptibility to disease and drugs that occurs with advanced age.[85]

V. GLUTATHIONE TRANSFERASES IN TUMORS

In tumors, GSH transferases are of interest for several reasons. They may confer an advantage on preneoplastic and neoplastic cells over their normal neighbors, they may confer resistance to chemotherapeutic drugs, and also they may have diagnostic value as tumor markers.

A. Hepatocarcinogenesis in the Rat

An early effect of feeding hepatocarcinogens, such as 3′-methyl-*N*,*N*-dimethyl-4-aminoazobenzene or aflatoxin B_1, is an overall induction in subunits 1 and 3.[86] Subsequently, foci of hepatocytes rich in subunit 7 appear,[87,88] many developing into nodules where, in addition to GSH transferases, epoxide hydrolase, NAD(P)H-quinone oxido-reductase, and UDP-glucuronyl transferase are also seen to be increased.[89] Most foci and nodules redifferentiate into apparently normal liver tissue, but some nodules persist and become neoplastic.[90] The primary hepatomas that result have a similar isoenzyme content to that in the

nodules.[42] During the progression of hepatoma to greater malignancy, all but subunit 7 may be repressed.[42a] Subunit 7 is therefore a very useful marker for both hepatocellular preneoplasia and hepatocarcinoma. It also appears to be ras responsive, since it can be induced *de novo* in rat liver epithelial cells by transformation with a N-*ras* oncogene.[88]

B. Human Tumors

Present evidence suggests that, in human tumors, the expression of alpha enzymes indicates a hepatocellular carcinoma, and GSH transferase π either a cholangiocarcinoma or a metastasis from the colon. Many other human tumors show a common pattern, namely, enhanced expression of GSH transferase π. This has been demonstrated immunohistochemically in cancer of the colon,[91] stomach, pancreas, and uterine cervix[91,92] and by immunoblotting techniques in adenocarcinoma of the breast and lung, nodular small-cell lymphoma and mesothelioma,[93] and metastatic melanoma.[94] It has also been demonstrated by isoelectric focusing in renal cortex tumors.[95] In the case of liver tumors, GSH transferase π, unlike subunit 7 in rat hepatoma, is not a generally recognized marker for human hepatocellular carcinoma.[58,91,96]

Tumor cell lines may also have elevated GSH transferase π, for example, small-cell[97] and nonsmall-cell lung carcinoma cell lines derived from ovarian carcinoma and EJ6 bladder carcinoma.[98] There are human tumor cell lines with very low levels of GSH transferases, for example, a MCF7 mammary carcinoma cell line. Induction of resistance to adriamycin in this MCF7 cell lines, however, has resulted in a 45-fold increase in an acid isoenzyme believed to be GSH transferase π.[99] Neither adriamycin nor its known metabolites are known to be substrates for GSH transferase π, which it induces in MCF7 cells.

The possibility that ras acts through TRE elements of cellular genes is highly relevant to tumor-specific induction of GSH transferase π expression, as amplified or activated ras genes are frequently found in human tumors.

VI. MEMBRANE-BOUND GLUTATHIONE TRANSFERASES

Although soluble GSH transferases have been the most intensively studied, membrane-bound forms also occur. They have been observed in hepatic microsomes and mitochondria and membrane fractions from other cells,[100] for example, the leukotriene C_4 synthase activity of the particulate fraction of rat basophil leukemia cells.[101] When isolated from rat liver, microsomes contain both strongly adsorbed soluble GSH transferases and at least one intrinsic enzyme.[100]

The major intrinsic enzyme is referred to as microsomal GSH transferase. It has a molecular weight of 17,200 and a primary structure that has no apparent homology with the amino acid sequences of known soluble GSH transferase subunits.[100] This enzyme is activated by reagents that react with thiols, including *N*-ethylmaleimide, disulfides, and perhaps also quinones and products of carbon tetrachloride metabolism.[100] This enzyme has appreciable activity toward 1-chloro-2,4-dinitrobenzene and some other model substrates, and low activity toward two carcinogenic electrophiles, namely, 1-nitropyridine-*N*-oxide (10 nmol/min/mg) and benzo(a)pyrene-4,5-oxide (2.4 nmol/min/mg).[100]

In the rat, microsomal GSH transferase is found principally in the liver, although it has been detected in extra-hepatic tissues, and a similar enzyme is found in the liver of other mammals.[100]

Its importance in carcinogenesis is not clear. A microsomal enzyme has been implicated in the metabolism of the renal carcinogen, hexachlorobutadiene.[102] Immobilized microsomal fractions have also been used to catalyze GSH-dependent detoxication of the genotoxic nitrogen mustard, melphalan.[103]

VII. EXAMPLES OF THE DETOXICATION OF CARCINOGENS BY GLUTATHIONE CONJUGATION

A. Polycyclic Aromatic Hydrocarbons

A number of PAH carcinogens have been shown to participate in the GSH conjugation pathway. These include benz(a)anthracene (BA),[104] 7-methyl-benzanthracene, 7,12-dimethyl-benzanthracene,[105] benzo(a)pyrene (BP),[106] and 3-methylcholanthrene.[107] The literature has been reviewed.[108-110] The electrophiles involved are predominantly the k-region epoxides; for example, GSH conjugates form about 70% of the biliary metabolites of BP-4,5-oxide in the rat.[111] The K-region epoxides are positive in bacterial and other mutagenicity tests, and therefore are genotoxic. However, DNA adducts have not been detected in experimental animals and they have not been implicated in carcinogenesis. It is assumed that they are effectively detoxified by GSH transferases and epoxide hydrolase. Table 3 shows that BP-4,5-oxide is utilized by a number of GSH transferase isoenzymes.

The metabolites responsible for the carcinogenic effect of PAH are "Bay region" diol epoxides, which bear a highly reactive benzylic epoxide carbon atom[114] and are poor substrates for epoxide hydrolases. They are, however, substrates for GSH transferase isoenzymes (see Table 3). The importance of GSH conjugation as a protective mechanism for DNA binding of the "Bay region" epoxide BP-7,8-diol-9,10-oxide (BPDE) has been shown in a number of systems, including the rat hepatocyte, in which activation and detoxication occur together, or in *in vitro* systems involving BP epoxides and nuclear DNA.[115,116] BP and other PAHs are not carcinogenic for rat liver, but are carcinogenic for mouse skin and rat mammary gland.[117,118] This may be due to the very high levels of GSH transferase activity found in the rat liver and the much lower levels found in the mouse skin and rat mammary gland.

Epoxidation of PAH is not the only reaction that gives rise to genotoxic electrophiles. In the case of 7,12-dimethylbenz(a)anthracene, activation to a DNA-binding species occurs by methyl oxidation to 7,12-dihydroxymethylbenz(a)anthracene,

Table 3
Activities of Rat GSH Transferases toward Some Electrophiles Derived from Carcinogens and Some Model Electrophiles

Substrate	GSH transferase isoenzyme						Ref.
	1-1	2-2	3-3	4-4	7-7	5-5	
N-Acetyl-*p*-benzoquinone imine	240	48.0	6.0	3.0	60.0	nd	23
Aflatoxin B_1-8,9-oxide	0.0001	0.001	nil	nil	nil	nd	113
Benzo(a)pyrene-4,5-oxide	0.011	0.004	0.087	nd	nd	0.069	112
±*anti* Benzo(a)pyrene-7,8-diol-9,10-oxide	0.1	0.08	0.03	0.33	0.33	nd	26, 27
±*anti* Benz(a)anthracene-3,4-diol-1,2-oxide	nd	nd	nd	2.1	nd	nd	27
±*anti* Chrysene-1,2-diol-3,4-oxide	nd	nd	nd	1.5	nd	nd	27
1-Nitropyrene-4,5-oxide	0.01	0.03	0.30	0.3	0.02	nd	27
1-Nitropyrene-9,10-oxide	0.06	0.01	0.40	0.20	0.08	nd	27
(R)-α-Bromoisovalerylurea	0.005	0.05	0.13	0.14	nil	nd	206
(S)-α-Bromoisovalerylurea	0.016	0.14	0.055	0.015	nil	nd	206

Note: Activities expressed in $\mu mol \cdot min^{-1} \cdot mg^{-1}$. nd = not determined.

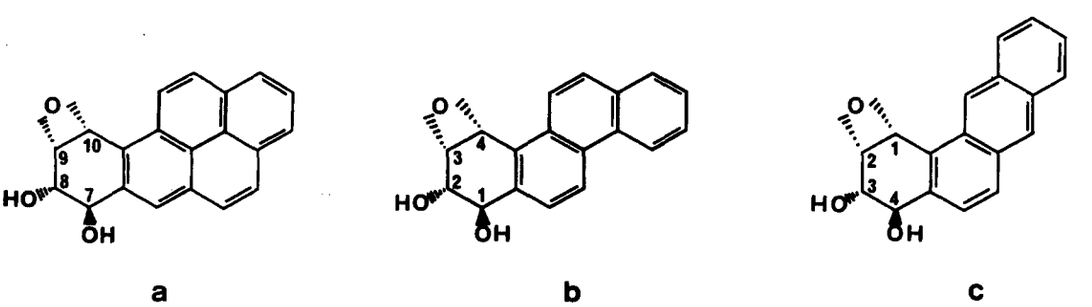

FIGURE 3. Related structures of Bay region diol-epoxides of (a) benzo(a)pyrene, (b) chrysene, and (c) benz(a)anthracene.

followed by sulfation. Dihydroxymethylbenz(a)anthracene-7-sulfate has been shown to be a good substrate for GSH transferases, forming *S*-(12-hydroxymethylbenz(a)-anthracene-7-yl)methyl glutathione. Inhibition of DNA binding by GSH transferase catalyzed conjugation appears to be complete, since binding of the 7-sulfate to calf thymus DNA *in vitro* and the mutagenicity of the sulfate can be abolished by GSH and the GSH transferases of rat liver cytosol.[119] The isoenzyme specificity for this substrate has yet to be determined.

1. Stereoselectivity and Regioselectivity in Glutathione Conjugation of PAH and Related Epoxides

There is now considerable evidence that catalysis of conjugation of "Bay region" diol epoxides and K-region epoxides with GSH occurs stereoselectively. A high degree of stereoselective attack is observed toward the isomers of BPDE and the diol epoxides of benz(a)anthracene and chrysene (*trans*-3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydroxybenz(a)anthracene and *trans*-1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydroxychrysene, respectively).[27] Conjugation catalyzed by GSH transferase 4-4 is specific for the (+)-enantiomer in each case. The three oxides are structurally closely related (Figure 3), and all have the benzylic carbon, at which attack occurs, in the R configuration. GSH transferase 7-7 has also been found to be specific for attack at the R-configured carbon of (+)-*anti*-BPDE.[120]

The conjugation of (±)-*anti*-BPDE has also been examined for the human isoenzymes α through ε (as a mixture), μ, and π, which have been shown to be 59, 60, and 90% selective for the (+) enantiomer, respectively.[121] It is of interest that human GSH transferase μ, although resembling rat GSH transferase 4-4 in that it utilizes *trans*-phenyl-4-buten-2-one as a diagnostic model substrate, is not as selective as GSH transferases 4-4 with respect to the (+)-enantiomer.

Stereoselectivity also occurs for the R-configured carbon of K-region epoxides during catalysis of GSH conjugation by mu class isoenzymes. Some of the most detailed studies are those of Boehlert and Armstrong[122] and Cobb et al.,[123] who used purified optical isomers of azaarene and arene oxides and purified rat isoenzymes 3-3, 3-4, and 4-4. These workers have shown that stereoselectivity is most pronounced with isoenzyme 4-4. For both the R,S- and S,R-epoxide enantiomers, the C-O bond of the

carbon that has the R absolute configuration (R-carbon) is preferentially attacked. The absolute configuration of the carbon atoms of the epoxide ring is more important than the electronic differences between the two carbon atoms. Further studies of phenanthrene-9,10-oxide and GSH transferase 4-4 showed that specificity of attack at the R-carbon was lost by replacing GSH with glycylcysteinyl glutamate ("retro-inverso GSH"),[124] but the reaction rate is only 1/1000 the rate of the reaction catalyzed by GSH.

BP-4,5-Oxide reacts with GSH catalytically at both the 4- and 5- positions. However, when the purified optical isomers are used it is found that (–)-(4R,5S)-BP-oxide is attacked predominantly at the R-configured carbon to form (4S)-glutathionyl-(5S)-hydroxy BP, and (+)-(4S,5R)-BP-oxide is also attacked at the R-carbon to form (5S)-glutathionyl-(4S)-hydroxy-BP.[125-127] The human GSH transferase isoenzyme μ is also specific for the R-carbon of (–)(4R,5S)-BP-4,5-oxide. Conversely, cytosolic GSH transferases from rat kidney and spleen catalyze attack of BP-4,5-oxide, pyrene-4,5-oxide, and benz(a)anthracene-5,6-oxide at the S-carbons.[126] The major isoenzymes of these organs are in the alpha family. Preference for attack at the S-carbon of the K-region epoxides has also been found with cytosolic fractions of rabbit tissues, but the enzymes involved in these studies have not been characterized.[127]

The stereospecificity for attack at the R-carbon of K-region PAH epoxides has been rationalized by a simple model in which the structurally related aromatic rings and epoxide group are arranged in the catalytic site of the enzyme in the same way.[123,126] The PAH diol epoxides mentioned above do not fit into this scheme as readily.

2. Chemical Reactivity vs. Stereoselectivity of Attack in PAH and Related Epoxides

An interesting situation arises when attack is preferred chemically at a more reactive benzylic position of an epoxide, but there is stereochemical restraint of attack because the configuration at the benzylic carbon is unfavorable for GSH transferase-catalyzed reaction. For example, racemic styrene oxide reacts with GSH in the enzyme (rat liver cytosol)-catalyzed reaction preferentially at the R-carbon, i.e., (R)-(+)-styrene oxide at the benzylic carbon and in (S)-(-)-styrene oxide at the nonbenzylic carbon, despite the benzylic carbon being the more reactive.[128]

In the case of *anti*-BP diol epoxide isomers, the (–) *anti*-isomer, which has a S-configuration at the benzylic carbon, is not a substrate for GSH transferases 4-4 and, in fact, appears to be an inhibitor of the enzymic activity toward the (+)-*anti* isomer.[26] In this case, the behavior is presumably due to the complexity of the molecule that leads to restricted access of GSH to the R-configured, nonbenzylic carbon, and to the intrinsic lower reactivity of the nonbenzylic carbon compared with the benzylic (S)-carbon.

B. Aflatoxin B_1 (AFB_1)

A GSH conjugate has been found as a major biliary metabolite of this potent mycotoxin and hepatocarcinogen.[129,130] In the rat, it can account for up to one third of the biliary metabolites of AFB_1 (about 10% of a 0.5-mg/kg dose).[113,131]

Activation of AFB_1 involves cytochrome P-450 oxidation of the 8,9-double bond. The unstable epoxide binds to guanine residues in DNA at the N-7 position.[132-135] The structure of the GSH conjugate has been determined by proton nuclear magnetic resonance (NMR) and mass spectroscopy,[136] and is in agreement with its formation by stereospecific attack of GSH at the 8-position of the 8,9-oxide (Figure 4).

Conjugation of the oxide is an important detoxication route for the epoxide that otherwise hydrolyzes to the diol.[137,138] The diol itself must be regarded as a toxic product since it reacts readily with protein by Schiff base formation,[138] and there is some evidence that the diol can bind to DNA.[139] Thus, GSH conjugation prevents the toxicity of both the epoxide and its hydrolysis product. The possibility that the GSH conjugate could be formed via the ring-opened form of the diol (which is a hemiacetal) has been investigated, but this reaction does not occur.[113]

Experiments with AFB_1 oxide have required the activation of the toxin by microsomal oxidation or peroxo-acid oxidation (although the oxide has recently been isolated in a relatively stable form[139a]). Using microsomal oxidation to activate AFB_1, it has been shown that GSH conjugation occurs only in the presence of the GSH transferases.[113,131,140,141] The catalysis of conjugation has not been investigated in detail, but of those GSH transferase isoenzymes investigated (rat enzymes 1-1, 2-2, 3-3, and 4-4) only enzymes containing subunits 1 and 2 (alpha class) are responsible for activity.[113]

A number of studies have shown how the species resistance to the acute toxic effects of AFB_1 correlate with the GSH transferase activity of their respective liver cytosols to catalyze AFB_1 oxide conjugation.[142-144] In addition, AFB_1 binding to DNA is inhibited by the GSH transferases. For example, experiments using hepatic microsomal activation of AFB_1 in the presence of liver cytosols have shown that GSH conjugation is responsible for 5 to 60% inhibition of DNA binding in rats,[113,145] 50 to 80% in the hamster,[145] and 22 to 38% in the mouse.[146] Studies *in vivo* have given similar results.[146,147] These experiments establish the importance of GSH conjugation as a mechanism that confers resistance to the acute hepatotoxic effects of AFB_1. It would appear that in the rat liver, the GSH transferases become significantly anticarcinogenic if inducers are fed. For example, the powerful inducer ethoxyquin results in a fourfold to fivefold increase in the biliary excretion of AFB_1-GSH conjugate and a marked decrease in genotoxicity as demonstrated by 90% reduction in DNA adducts and greater than 95% reduction in the percentage of liver occupied by preneoplastic foci.[148]

C. Aromatic Amines

GSH conjugation of aromatic amine carcinogens forms an important detoxication route. Extensive study of the metabolism of aromatic amine carcinogens has shown that a critical step in the

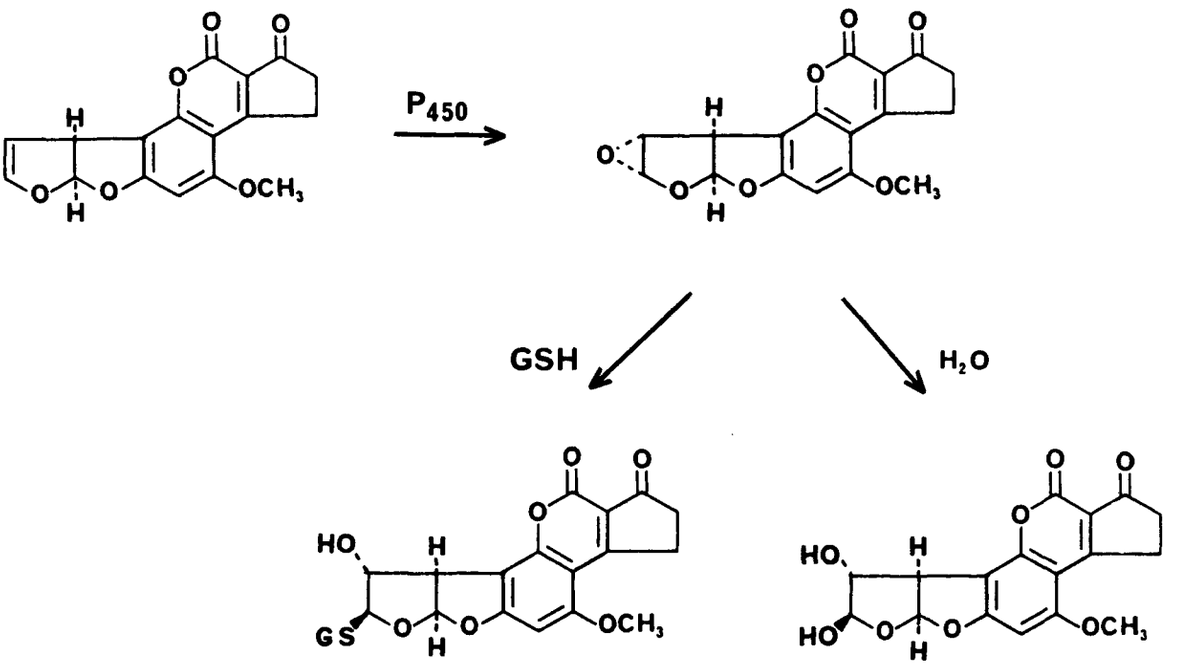

FIGURE 4. Metabolism of aflatoxin B_1 by P450 to the 8,9-oxide and its reaction with GSH and water.

formation of the genotoxic electrophile is oxidation of the amine to the *N*-hydroxy compound.[149] *N*-Hydroxyarylamines have limited electrophilic activity, and although they are known to react directly with DNA (e.g., *N*-hydroxy-2-aminofluorene, see below), a further critical esterification step is usually required to generate the ultimate carcinogen. Several N-O esters are known to be involved, and the route of esterification depends on the carcinogen and the species of animal, for example, sulfation in aminoazodye carcinogens,[150] sulfation and *N*- and *O*-acetylation for aminofluorene and related compounds in the rat,[151-153] and acetylation in the case of benzidine.[154] Other mechanisms are also involved, for example, *N*-hydroxy-2-naphthylamine is activated via *N*-glucuronidation in the dog,[155] and aminoacyl *t*-RNA synthetases have been implicated in the esterification of *N*-hydroxy derivatives of food pyrolysis carcinogens.[156]

These ultimate carcinogens have in common the presence of a good leaving group on the amine nitrogen. *N*-Sulfates spontaneously lose sulfate to form nitrenium ions and their delocalized aromatic carbonium ion tautomers. *N*-Acetoxy compounds react in a similar way, although S_N2 character is more pronounced. In the case of the *N*-glucuronide of 2-napthylamine, the carcinogen is unstable at low pH.[155] In the dog, this results in expression of genotoxicity in the bladder where, during urinary excretion, the glucuronide is exposed to low pH. It should be noted that the parent hydroxylamines also show more pronounced electrophilic character under acid conditions where protonation leads to loss of water and the generation of the electrophilic species.

Although *N*-hydroxylation is a critical step in the generation of genotoxic electrophiles derived from aromatic amine carcinogens, oxidative metabolism also occurs at other sites. In general, these reactions are detoxication steps, since electrophiles are not produced, or those formed do not, apparently, react with DNA. For example, extensive P-450-catalyzed oxidation of aminoazodye carcinogens occurs in the rat to form phenols (excreted as sulfate and glucuronide conjugates).[157]

Nitro PAH are subject to oxidative metabolism that leads to the generation of epoxides and phenols. However, the present evidence is that these are not responsible for the carcinogenic effects of nitro PAH, since the oxides involved are mainly K-region oxides. Activation to genotoxic electrophiles appears to occur by the same route as the aromatic amine carcinogens and therefore involves a reductive or series of reductive steps via the nitroso compound and hydroxylamine to the amine.[158-160]

Aromatic nitroso compounds react rapidly with GSH and thiols in general, and thus may be detoxified by this mechanism. The reaction of nitrosoaromatics with thiols has been studied in detail using nitrosobenzene and thioethyleneglycol[161] (Figure 5). Of particular note in this study is the isolation of the unstable intermediate "semimercaptal", which is the first product of the addition of the thiol to the nitroso compound. The "semimercaptal" may be reduced by excess thiol to the hydroxylamine or the sulfenamide conjugate. Hydrolysis of the sulfenamide forms the parent amine. Alternatively, the "semimercaptal" may rearrange to the sulfinamide.

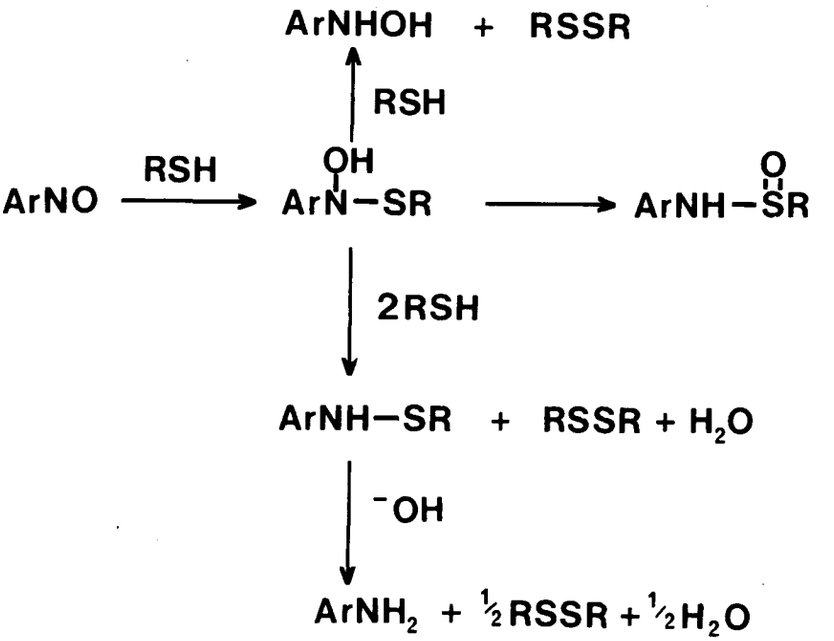

FIGURE 5. Summary of the reaction of nitrosoaromatics (ArNO) with thiols (RSH) via the *unstable "semimercaptal" intermediate.*

The products of the reaction of nitrosoaromatics with GSH will depend on the ratio of reductant (GSH) to nitroso compound and the rate constants for the various reactions in the proposed scheme. A study of the reaction of a series of p-substituted phenylnitroso compounds has shown that the rate of reaction depends on the p-substituent; for example, k (the second-order rate constant for reaction with GSH at pH 7.4 and 37°C) varies from 1.5 for 4-nitroso-*N,N*-dimethylaniline to 80,000 for 4-nitrosoacetophenone.[162] The thiol has less effect. Using nitrosobenzene (k = 5000), the rate constant varies from 1300 with β-mercaptoethanol as the thiol to 12,000 with cysteine or cysteamine.

The ratio of products also depends on the substituent effects. For example, in the case of 4-nitroso-*N,N*-dimethylaniline (electropositive p-substituent), formation of the amine is the preferred pathway, via the unstable sulfenamide conjugate. In contrast, with 4-nitrosoacetophenone (electronegative p-substituent), the hydroxylamine is formed exclusively. Nitrosoaromatics bearing p-substituents of intermediate character (e.g., nitrosobenzene itself) give rise to the sulfinamide conjugate and the hydroxylamine. Thus, substitution with a substituent having a negative Hammet constant appears to form a "hemimercaptal", which is electrophilic and favors nucleophilic attack at the thiol. Substituents with a positive Hammet constant favor attack on the sulfenamide hydroxyl.

These observations may have a bearing on the potential detoxication of aromatic nitroso compounds derived from carcinogens. Those that are effectively electronegatively substituted would react rapidly with GSH to (re)form the hydroxylamine via an electrophilic "semimercaptal" conjugate.

1. N-Methyl-4-Aminoazobenzene (MAB)

GSH conjugates are important constituents of the biliary metabolites of MAB. The major series of GSH conjugates, which form some 70% of the biliary metabolites of a 5-mg/kg dose of MAB in the rat, are derived from aminoazobenzene methimines.[157,163,164] The sequence of reactions involved (Figure 6) is methyl oxidation by cytochrome P-450 to the *N*-hydroxymethyl-4-aminoazobenzene. This dehydrates (presumably spontaneously) to the methimine, which reacts with GSH to give *N*-methylene glutathion-*S*-yl-4-aminoazobenzene. In addition to *N*-methyl oxidation, 4′-hydroxylation, followed by conjugation with either sulfate or glucuronide, occurs. Double conjugates with GSH and either sulfate or glucuronide are the result.[164] *N*-Methylene-glutathione conjugates are relatively unstable giving formaldehyde and 4-aminoazobenzene (or its sulfate or glucuronide conjugate).

There are no reports of the methimine reacting with DNA, and the genotoxic metabolite of MAB results from *N*-oxidation by the flavin mixed-function oxygenase and sulfation of the hydroxylamine to *N*-sulfonyloxy MAB.[150] This dissociates to a nitrenium ion and its delocalized tautomers to give electrophiles that either react with DNA at the C-8 and N^2 positions of guanine residues[165] or with GSH to give 3-, 2′-, and 4′-(glutathion-*S*-yl)- MAB (Figure 4).[166,167] Unlike the *N*-(glutathion-*S*-methylene) conjugates, these are minor metabolites.[168]

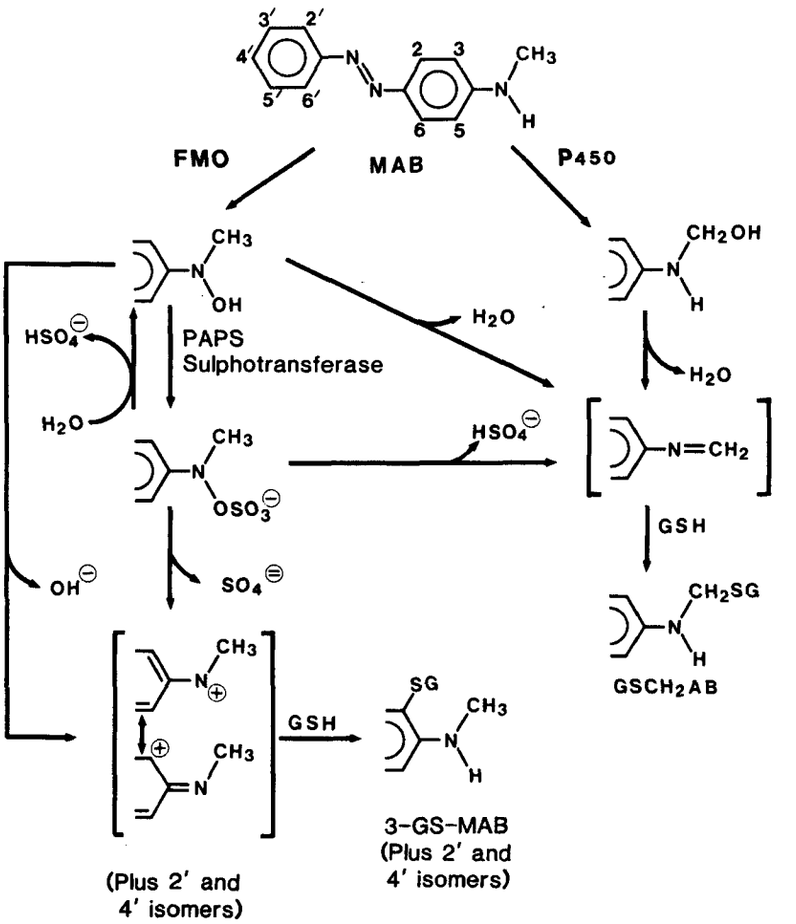

FIGURE 6. Metabolism of *N*-methyl-4-aminoazobenzene: *left*, flavin monooxygenase-catalyzed metabolism to the *N*-hydroxylamine to form the carcinogenic *N*-sulfate and its detoxication by reaction with GSH; *right*, cytochrome P450 oxidation to a methimine and its reaction with GSH.

It is of note that the two sets of detoxication pathways are not as distinct as they may seem. Detailed studies of the reaction of *N*-hydroxy-MAB and *N*-sulfonyloxy-MAB with GSH *in vitro* show that 3-(glutathion-*S*-yl)-*N*-methyl-4-aminoazobenzene and *N*-(glutathion-*S*-methylene)-4-aminoazobenzene are formed in both cases, but that the ratio differs — *N*-hydroxy-MAB reacts mainly via a methimine and *N*-sulfonyloxy-MAB via a nitrenium ion and its delocalized tautomers[169] (Figure 6). The yield of all GSH conjugates is dependent on the GSH concentration and is not affected by liver cytosol or GSH transferases isolated from liver cytosol.[166,168] *In vivo*, the formation of GSH conjugates is profoundly inhibited by GSH depletion with diethyl maleate.[157] Thus, although GSH may play a role in the detoxication of both classes of electrophile, neither appears to be a substrate for rat hepatic GSH transferases, with the result that the genotoxic *N*-sulfate is poorly detoxified.[168]

2. N-Acetyl-2-Aminofluorene (AAF)

GSH conjugates of the hepatocarcinogen AAF have been detected in the bile of rats after administration of the carcinogen and account for some 17% of a 13-mg/kg dose.[170] Activation of AAF occurs in a way similar to that of MAB, i.e., *N*-hydroxylation followed by *N*-sulfation. The *N*-sulfate, *N*-sulfonyloxy-AAF, has been shown to be the electrophile that is responsible for DNA binding of the carcinogen *in vivo*.[151]

The structures of the GSH conjugates have been determined by NMR and mass spectral analyses of the conjugates formed *in vitro*, using the analog of the *N*-sulfate, *N*-acetoxy-AAF. Four conjugates are formed, 1-, 3-, 4-, and 7-(glutathion-*S*-yl)-AAF.[170] The conjugates are formed in a ratio that is in agreement with that predicted from the delocalization of a nitrenium ion to its aromatic carbonium ion tautomers (Figure 7). Subsequent synthesis of the unstable sulfate ester has given direct evidence that the

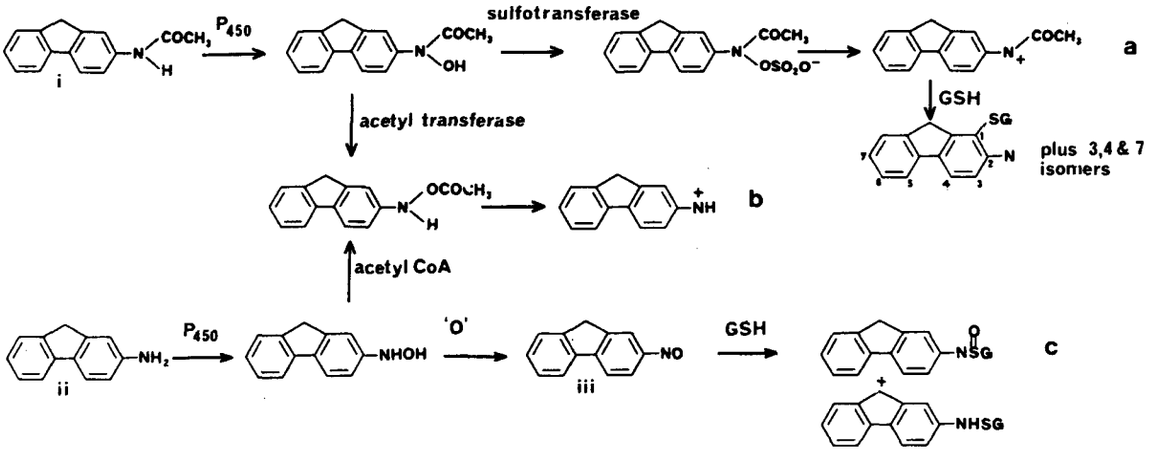

FIGURE 7. Metabolism of *N*-acetylaminofluorene (i) and aminofluorene (ii) (a) cytochrome P450 oxidation to the carcinogenic *N*-sulfate and detoxication with GSH; (c) cytochrome P450 oxidation of aminofluorene to nitrosofluorene (iii) via the hydroxylamine and the reaction with GSH; (b) shows possible routes to a nitrenium ion involving acetyltransferase or acetyl coA-dependent acetylation.

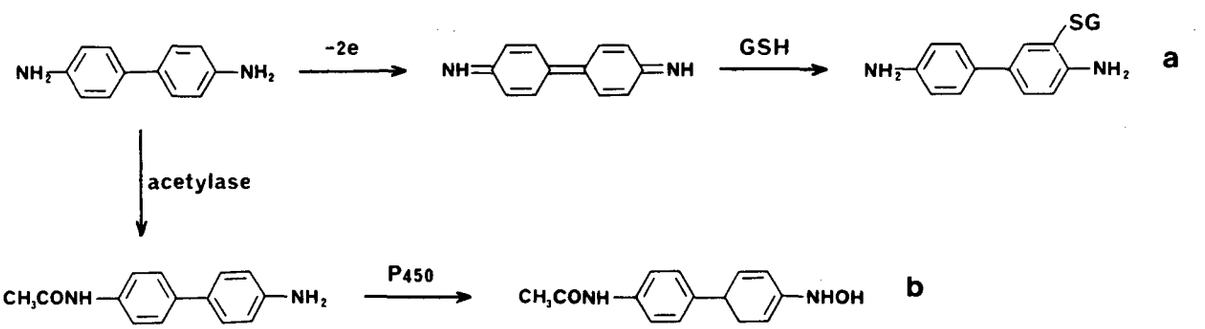

FIGURE 8. Metabolism of benzidine: (a) 2-electron oxidation to the diimine and its detoxication by reaction with GSH; (b) formation of the proximate carcinogen, *N*-acetyl-*N'*-hydroxy benzidine.

same GSH conjugates are formed by reaction of the *N*-sulfate with GSH.[171]

In vivo, only the 1- and 3-isomers are found in bile.[170] The reason for this is not known. *N*-Sulfonyloxy and *N*-acetoxy AAF are not substrates for rat hepatic GSH transferases,[170a] and as with DAB, the excretion of GSH conjugates derived from AAF is directly related to GSH status, a characteristic of nonenzymic GSH conjugation.[172]

In vitro, GSH decreased the binding of *N*-sulfonyloxy-AAF to the DNA of isolated nuclei by 15 to 35%.[172] On the other hand, *in vivo* depletion of GSH, which decreases the biliary excretion of the GSH conjugates, did not appear to affect DNA binding.[172] The interpretation of this apparent paradox may depend on the complex situation that occurs *in vivo,* in that deacetylated adducts of AAF, i.e., 2-aminofluorene (AF), are bound to DNA by an as yet unknown mechanism.[173]

The only electrophilic derivative of AF that has been studied with respect to reactions with GSH is *N*-hydroxyaminofluorene, where the reaction with GSH appears to be slow.[174] In contrast, the reaction of nitrosofluorene, which is an oxidation product of the hydroxylamine, with GSH is rapid. Neither of these reactions were catalyzed by liver cytosol or purified GSH transferases 1-2 or 3-3.[173a] The products of the reaction of GSH with nitroso-AF are a sulfinamide and the reduced analog the sulfenamide (Figure 7).[174] It has also been shown that *N*-OH-AF can be activated by acetyl coenzyme A-dependent acylation. The same product can also be formed from N-OH-AAF by *N-O*-acyltransferase-catalyzed acetyl transfer[152,153] (Figure 7).

3. Benzidine

Benzidine is activated to a DNA-binding electrophile by a similar pathway as other aromatic amines, involving *N*-acetylation and *N'*-hydroxylation, and subsequent *N-O*-acetylation[154] (Figure 8). The GSH conjugates of benzidine have not been studied *in vivo,* but the synthetic *N*-sulfate reacts with GSH to produce a GSH conjugate[154a] (Figure 8).

Benzidine is also metabolized to an electrophile by ram seminal vesicle peroxidase mediated oxidation to benzidine-diimine. The diimine reacts with DNA[175] and GSH,[176] the latter reaction giving a 3-(glutathion-*S*-yl) benzidine conjugate. GSH has been shown to inhibit DNA binding of the diimine by more than 98%,[175] but the significance of this pathway *in vivo* is not known.

4. Heterocyclic Amine Carcinogens from Cooked Food

Heterocyclic aromatic amines, formed in the cooking of food, have been found to be some of the most potent bacterial mutagens known and have been shown to be carcinogenic.[156,177] Several classes of heterocyclics have been isolated.

The most potent bacterial mutagens found in cooked food are those with an imidazoquinoline structure: IQ (2-amino-3-imidazo[4,5-f]-quinoline); MethylIQ (4-methylIQ); MeIQx (2-amino-3,8-dimethylimidazo-[4,5-f]quinoxaline); 7,8-dimethyl-IQx (2-amino-3,7,8-trimethylimidazo-[4,5-f]quinoxaline; 4,8-dimethyl-IQx (2-amino-3,4,8-trimethylimidazo-[4,5-f]quinoxaline). Formation of these carcinogens appears to involve Maillard reaction products and creatinine.[156] IQ, MeIQ, and IQx induce tumors at multiple sites in rodents; for example, IQ has been shown to induce tumors in the liver, stomach, small and large intestines, Zymbals gland, clitoral gland, skin, oral cavity, and mammary gland (for review, see Reference 156). Other products, although formed in greater quantity, are less mutagenic, e.g., 2-amino-1-methyl-6-phenylimidazo-4,5-pyridine (TMIP).[177]

Mutagenic heterocyclics have been isolated from pyrolysates of amino acids or protein: the tryptophan pyrolysis products Trp-P-1 (3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole); Trp-P-2 (3-amino-1-methyl-5H-pyrido[4,3-b]indole); glutamate pyrolysis products GluP1 (2-amino-6-methyldipyrido[1,2-α:3′,2′-*d*]imidazole); GluP2 (2-amino-dipyrido[1,2-α:3′,2′-*d*]imidazole); and two structurally related products AαC (2-amino-9H-pyrido[2,3-b]indole) and 3-methyl-AαC. Other mutagens have been shown to be present in cooked foods, including 1-nitropyrene and dinitropyrenes.[156]

Activation of the heterocyclic arylamine carcinogens, as with other aromatic amines, is thought to require oxidation to the hydroxylamine followed by esterification. *N-O*-Acyl derivatives of Glu-P2 and Trp-P2 have been shown to react with the C-8 carbon of guanine residues in DNA.[178,179] Seryl and propyl t-RNA synthetase may be involved in the activation of *N*-hydroxy-Trp-P2,[180-182] and *N*-hydroxy- Trp-P2 and -Glu-P1 can be activated by an acetyl CoA-dependent enzyme.[183] Sulfation may be involved, in part, in the activation of Glu-P1 and IQ.[184] The hydroxylamines react very poorly with DNA (e.g., *N*-hydroxyamino-Trp-P2[178]).

GSH conjugation has been shown to occur with the hydroxylamines or nitroso compounds, which are their readily formed oxidation products, derived from Trp-P2 and Glu-P1, and this offers a potential detoxication process for carcinogenic aromatic amines.

Nitroso-Trp-P-2 reacts with GSH to produce mainly *N*-hydroxy-Trp-P-2 as well as two GSH conjugates, probably similar in structure to those formed from nitrosofluorene (see above and cf. Figure 5).[185] GSH also reacts slowly with *N*-hydroxy-Trp-P-2. This reaction was subject to catalysis by the GSH transferases present in cytosol, but isoenzyme specificity has not been determined. Three conjugates are formed, two of which appear to be similar to those formed from nitroso-Trp-P-2 and GSH, but the third is a GSH conjugate, which is more mutagenic than the parent hydroxylamine.[185] It is interesting to speculate that this electrophilic GSH conjugate is the "semimercaptal" intermediate referred to above. Whether such "semimercaptal" intermediates are responsible for mutagenic or carcinogenic effects of other arylamine carcinogens is not known.

A mass spectroscopy study of the reaction of nitroso-Glu-P-1 with GSH has also shown GSH conjugates to be formed. The unstable conjugates were analyzed by fast-atom bombardment mass spectroscopy and a scheme for their formation proposed that involves the unstable semimercaptal intermediate (see above).[186] Further studies have shown that a *N*-hydroxyamino sulfonamide conjugate is formed in addition to the sulfinamide conjugate and that the sulfonamide is not a simple oxidation product of the sulfinamide.[187]

IQ is one of the most important of the heterocyclic aromatic amine carcinogens. However, a search for GSH conjugates among the biliary metabolites of IQ in the rat has shown that they are not formed. The major product is a *N*-sulfamate.[188] The metabolism of MeIQ[188a] and MeIQx appears to be similar.[189]

6. Nitropolycyclicaromatic Hydrocarbons

The carcinogenicity of nitropolycyclicaromatic hydrocarbons is associated with reductive metabolism of the nitro group to the *N*-hydroxy compound, which is then thought to be activated by esterification in a similar manner to other aromatic amine carcinogens.[158-160] Since these carcinogens are also PAH, they are susceptible to epoxidation.

The only member of the group in which GSH conjugation has been studied in any detail is 1-nitropyrene, which is the predominant nitrated PAH identified in environmental samples. 1-Nitropyrene is a powerful indirect bacterial mutagen and is tumorigenic in the rat and mouse.[160,190,191] GSH conjugates form approximately one third of the biliary metabolites of 1-nitropyrene in the rat.[22] The conjugates arise from the K-region oxides, mainly from the 9,10-oxide with lesser amounts from the 4,5-oxide (Figure 9). The structures of the GSH conjugates have been determined by mass spectroscopic and NMR studies of the four conjugates isolated from *in vitro* studies of the conjugation of the synthetic 4,5- or 9,10-oxides. Both 4- and 5- or 9- and 10-substitution by GSH occurs, but it was not possible to separate the isomeric conjugates from each other on a preparative scale, and

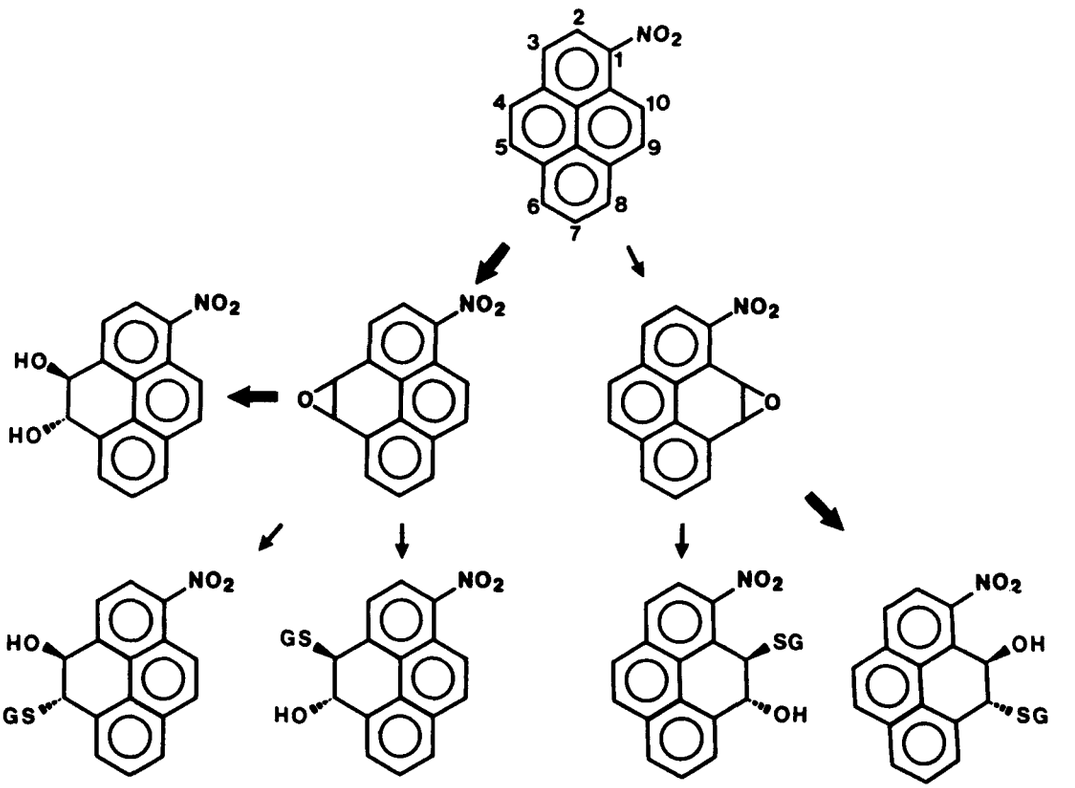

FIGURE 9. Metabolism of 1-nitropyrene in the rat. The major pathways of metabolism are shown by bold arrows. (From Djuric, Z., Coles, B., Fifer, E. K., Ketterer, B., and Beland, F. A., *Carcinogenesis*, 8, 1781, 1987. With permission.)

NMR data were obtained using mixtures. *In vitro*, both K-region oxides have been shown to be substrates for the GSH transferases, they have low K_m (μM range), and low V_{max}. Of the rat isoenzymes tested, those containing the 3 and 4 subunits are the most effective enzymes (Table 4). Isoenzyme 3-3 catalyzed reaction of the 4,5-oxide with GSH gave a 1:1 mixture of the 4- and 5-glutathionyl conjugates, but the 9,10-oxide gave a 2:1 mixture of the 9- and 10-conjugates. This latter observation suggests that there is steric interference caused by the 1-nitro group, which hinders attack at the 10-carbon. The stereochemistry of conjugation has not been examined. It has been suggested that the extensive metabolism of 1-nitropyrene to epoxides rather than by nitro reduction is a major contributing factor to its low carcinogenicity. In contrast, the highly carcinogenic 1,6- and 1,8-dinitropyrenes are able to undergo nitro reduction without extensive competitive metabolism to form epoxides.[22]

1-Nitrosopyrene reacts with GSH *in vitro* to give GSH conjugates, which have yet to be characterized. The reaction is apparently complex, since five GSH conjugates can be separated by HPLC. GSH conjugates are also detected as biliary metabolites after administration of 1-nitrosopyrene to rats. Some of the GSH conjugates are base-labile, which suggests that they are similar in structure to the sulfenamide conjugates formed from other nitrosoaromatics.[22a]

D. Alkylating Agents

The alkylating agent ethylmethane sulfonate was one of the first genotoxic electrophiles shown to be a substrate for rat hepatic GSH transferases *in vitro*.[7] The mercapturic acid *S*-ethyl-*N*-acetylcysteine had been isolated previously from the urine of rats given ethyl methanesulfonate, indicating that GSH conjugation is an *in vivo* pathway of detoxication.[192]

Dimethylnitrosamine is metabolized to a methylating agent, which may also be detoxified by GSH. Thus *N*-acetyl-*S*-methyl cysteine and *S*-methyl cysteine have been detected in rat urine following administration of the carcinogen dimethylnitrosoamine.[193] These presumably arise from the reaction of GSH with dimethyldiazonium hydroxide, which is the supposed electrophilic metabolite of dimethylnitrosamines.[194] An increase in the formation of *N*-7-methylguanine and *O*-6-methylguanine following GSH depletion confirms the suggested role of GSH in dimethylnitrosamine detoxication.[195] It is not known if this reaction is enzymically catalyzed.

Cancer chemotherapeutic agents, such as the bifunctional alkylating agents, also react with GSH. For example, the sulfur mustard *bis*-(2-chloroethyl)-sulfide and its sulfone are metabolized in part to GSH conjugates.[196] The nitrogen mustard melphalan (*bis*-(2-chloroethyl)-phenylalanine) gives three adducts with GSH due to substitution of either one or both of the chlorine

Table 4
kcat, Km, and Catalytic Efficiency of Some Electrophiles Derived from Carcinogens and Some Model Substrates

Substrates	Isoenzyme	kcat(s^{-1})	Km(μM)	kcat/Km($M^{-1}s^{-1}$)	Ref.
N-Acetyl-*p*-benzoquinonimine	2-2	35	0.7	5×10^7	23
1-Nitropyrene-4,5-oxide	4-4	0.3	1.0	3×10^5	22
1-Nitropyrene-9,10-oxide	4-4	0.2	2.0	1×10^5	22
±*anti* Benzo(a)pyrene-7,8-diol-9,10-oxide	4-4	0.23	11	2.1×10^4	27
±*anti* Benz(a)anthracene-3,4-diol-1,2-oxide	4-4	0.044	125	0.7×10^4	27
±*anti* Chrysene-1,2-diol-3,4-oxide	4-4	0.005	105	0.6×10^4	27
(R)-αBromoisovalerylurea	3-3	0.15	2.6×10^3	57	206
(S)-α-Bromoisovalerylurea	2-2	0.26	1.4×10^3	185	206

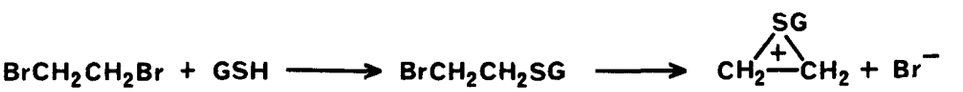

FIGURE 10. Reaction of 1,2-dibromoethane with GSH to form the electrophilic episulfonium ion.

substituents[103] and the formation of *p*-(glutathion-*S*-yl) phenylalanine.[197] The former reaction was catalyzed by microsomal fractions from both rabbit and human liver.[103]

bis-(2-Chloroethyl)nitrosourea is another bifunctional alkylating agent that is enzymically detoxified by GSH. In the rat, it is a substrate for GSH transferase subunits 3 and 4 in a reaction giving nitrite, GSH disulfide, and the urea. In a rat brain tumor cell line, resistance to this drug is associated with induction of GSH transferase subunit 3.[198]

VIII. GLUTATHIONE AND TOXICATION OF ELECTROPHILES

It has been emphasized that the great majority of the reactions of electrophiles with GSH are detoxication processes. However, it has been noted above that the reaction of GSH with *N*-hydroxy-Trp-P-2 produces a genotoxic electrophile as one of the products. Several other cases are known where reaction of alkyl and alkenyl halides with GSH leads to toxic products. This field has been reviewed recently.[199,200]

Ethylene dibromide reacts with GSH to form 1-bromo-2-*S*-glutathionyl ethane. The unstable product is a sulfur mustard, which reacts with nucleophiles such as water, GSH, and the N-7 positions of guanine in DNA via an episulfonium ion.[201-204] Reaction with DNA is thought to be responsible for the genotoxicity of ethylene dibromide. The reaction of the dibromide with GSH requires catalysis by GSH transferase subunits 1, 2, or 3.[204] As can be seen in Figure 10, the episulfonium ion is a charged species with electrophilic properties quite different from those of alkyl halides. From the discussion presented above, it would be expected that the episulfonium ion would be capable of reacting with the bases of DNA, but the alkyl halide would not. This is found to be the case, since the species bound to DNA retain the glutathionyl moiety, indicating that reaction has occurred via the episulfonium ion.

Polyhalogenated alkenes form GSH conjugates, which are genotoxic after further metabolism. For example, hexachlorobutadiene forms the GSH conjugate, *S*-(1,2,3,4,4-pentachlorobutadienyl)-glutathione, which becomes mutagenic after metabolism by the mercapturic acid pathway to cysteinyl derivatives followed by the action of β-lyase to a vinylic mercaptan.[102,200]

IX. OVERVIEW OF GSH TRANSFERASE CATALYSED CONJUGATION OF CARCINOGENS

A. Substrate Specificity

All PAH epoxides so far examined have proven to be substrates for GSH transferases (see above and Table 3), as have other oxides, e.g., AFB_1[113,144] and styrene oxide.[128] Some aromatic amine metabolites may be substrates, e.g., *N*-OH-Trp-P-2[185] and *N*-acetyl-*p*-benzoquinone imine,[23] but a number of the electrophiles derived from aromatic amines (e.g., the esters of *N*-hydroxy AAF and *N*-hydroxy MAB) are not. The basis of substrate specificity is not known. Catalysis may be associated with a multigene family of enzymes, for example, AFB_1-oxide is conjugated catalytically by isoenzymes 1-1, 1-2, and 2-2,[113] i.e., the alpha family. *Anti*-BPDE, on the other hand, is a substrate for all families of the rat GSH transferases, but it is the best substrate for the pi family (rat 7-7 and human π)[121] followed by rat subunit 4 and human GST μ of the mu family.[26]

This has important implications where the anticarcinogenic effects of inducers are concerned. AFB_1 is a poor substrate for GSH transferase subunits 1 and 2 only, with the result that

induction of subunit 1 has a significant effect on the detoxication of AFB_1.[148] BPDE, on the other hand, is a substrate for all subunits in the liver, and a particularly good substrate for subunit 4, which is not subject to induction. Therefore, inducers are expected to have only a small effect in this case.

B. Catalytic Constants, Catalytic Specificity

The ratio of kcat (maximum turnover number, V_{max} expressed in molar units) to Km describes the specificity of an enzyme for its substrate. This ratio, which has the dimensions of a second-order rate constant, cannot be greater than any second-order rate constant on the forward reaction pathway, and therefore sets a lower limit on the rate constant for the association of an enzyme with its substrate. In very efficient enzymes, kcat/Km can approach the value for a diffusion- controlled rate of reaction between enzyme and substrate, for example, kcat/Km for crotonase and fumarase are 2.8×10^8 and 1.6×10^8 $M^{-1}s^{-1}$, respectively.[205] Catalytic specificities for several carcinogen metabolites and some model compounds are presented in Table 4. It can be seen that these vary from the very efficiently catalyzed substrate *N*-acetyl-*p*-benzoquinone imine (NABQI) at 5×10^7 $M^{-1}s^{-1}$ [23] to the very poorly catalyzed substrate (R)-α-bromoisovaleryl urea at 57 $M^{-1}s^{-1}$,[206] with the majority of values around 10^5 $M^{-1}s^{-1}$.

E. Rate Enhancement

The ratio of k_{cat}/K_m to k_2, the second-order rate constant for the spontaneous reaction with GSH, has been mentioned as a possible indicator of rate enhancement[24] brought about by an enzyme. In the case of NABQI and GSH transferase 2-2, this value is 1.7×10^3, but in the case of 1-nitropyrene-4,5-oxide and GSH transferase 4-4 it is 6.5×10^5, which is more than two orders of magnitude greater (Table 5). Thus, although the catalytic specificity of the GSH transferase 2-2 catalyzed reaction of *N*-acetylbenzoquinone imine with GSH is very high, these advantages are offset by the very high second-order rate constant for the spontaneous reaction.

VIII. INDUCTION AND ANTICARCINOGENESIS

A. The Rat

A wide range of chemicals, both naturally occurring and synthetic, induce GSH transferases in the rat, including barbiturates; PAHs;[207] other carcinogens, such as *N,N*-dimethyl-4-aminoazobenzene; certain antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxy-anisole (BHA), and ethoxyquin[87,208,209] the GSH transferase substrate *trans*-stilbene oxide,[210] and natural products such as dithiolthiones isolated from the Brassica family.[211] These inducers appear to be selective for subunits 1 and 3, and the β-naphthoflavone responsive element found in the regulatory region of the subunit 1 gene may be associated with this induction.

The above inducers are anticarcinogenic in those cases where the genotoxic electrophile is a good substrate for GSH transferase subunits 1 and 3. This is illustrated above with respect to the carcinogen aflatoxin B_1-8,9-oxide.

B. The Mouse

The mouse has been used to detect inducing activity in a wide range of compounds. Inducers such as phenobarbitone, BHA, and BHT can increase GSH transferase levels up to tenfold.[208,209] A number of other compounds induce between twofold and fivefold, for example, the coffee bean diterpene esters, kahweol- and cafestrol-palmitates,[212] and also angelica lactone, coumarin, disulfiram, indole-3-carbinol and indole-3-acetonitrile,[213,214] while still others like streptozotocin, diethylnitrosamine,[215] and chronic ethanol induce up to twofold.[216] Induction by extracts of brassicas and tea leaves have also been observed.[212,213] None of the above studies have identifed the isoenzymes affected by the inducers. Many of these inducers are also active in the esophagus, forestomach, and intestine.[212,213,217] Enhanced GSH transferase activity in the forestomach, brought about by some of these inducers, has been shown to reduce benzo(a)pyrene-induced neoplasms in this organ.[218]

Table 5
Approximate Rate Enhancement by the GSH Transferases

Substrate	Isozyme	kcat/k_m M^{-1} s^{-1}	k2*	kcat/Km/k2
N-Acetyl-*p*-benzoquinone imine	2-2	5×10^7	3×10^4	1.7×10^3
1-Nitropyrene-4,5-oxide	4-4	3×10^5	0.17	1.8×10^6

* = The approximate second-order rate constant at pH 7.4

X. CONCLUSIONS

Living organisms encounter a wide range of chemical structures among the xenobiotics to which they are exposed. A characteristic of many enzyme systems that metabolize them is multiplicity (e.g., cytochrome P-450, GSH transferases, UDP glucuronyl transferases, sulfotransferases).[219] However, as far as the GSH transferases are concerned, the multiplicity that exists is not sufficient to enable electrophiles derived from all xenobiotics to be utilized as substrates. The protection afforded by GSH transferases is imperfect, and cytotoxic or genotoxic damage cannot always be avoided. This is well illustrated by AFB-8,9-oxide, which is a poor substrate for the GSH transferases, and the aromatic amines, which are not substrates and which escape the abundant GSH transferase activity of the liver. Similarly, the PAHs which, though effectively dealt with in the liver, may not encounter appropriate isoenzymes in sufficient quantity in mouse skin or rat mammary gland for effective detoxication.

Although these enzymes do not provide a perfect protection, they are valuable to the cell, as is shown by their abundance in

tumor cells that require advantages over the normal neighbors with which they compete and their induction in cells that become resistant to anticancer drugs.

In this review we have concentrated on chemical carcinogenesis. However, GSH transferases also have endogenous substrates, such as lipid and nucleic acid hydroperoxides, and the alkenals, which result from the decomposition of lipid hydroperoxides.[220] The oxygen toxicity that gives rise to these substrates is a constant feature of aerobic metabolism and provides another important reason for the abundance of these isoenzymes in both normal and tumor tissue.

These endogenous substrates may play a role in chemical- and radiation-induced carcinogenesis, but the significance pathway is not as well established as that of the xenobiotic metabolism described above.

REFERENCES

1. **Miller, E. C. and Miller, J. A.,** Mechanisms of chemical carcinogenesis: nature of proximate carcinogens and interaction with macromolecules, *Pharmacol. Rev.*, 18, 805, 1979.
2. **Searle, C. E.,** *Chemical Carcinogens,* ACS Monograph 182, American Chemical Society, Washington, D.C., 1984.
3. **Singer, B. and Grunberger, D.,** *Molecular Biology of Mutagens and Carcinogens,* Plenum Press, New York, 1983.
4. **Boyland, E. and Chasseaud, L. F.,** The role of glutathione and glutathione S-transferases in mercapturic acid biosynthesis, *Adv. Enzymol.*, 32, 173, 1969.
5. **Chasseaud, L. F.,** The role of glutathione and glutathione transferases in the metabolism of chemical carcinogens and other electrophilic reagents, *Adv. Cancer Res.*, 29, 175, 1979.
6. **Ketterer, B., Coles, B., and Meyer, D. J.,** The role of glutathione in detoxication, *Environ. Health Perspect.*, 49, 59, 1983.
7. **Booth, J., Boyland, E., and Sims, P.,** An enzyme from rat liver catalyzing conjugation with glutathione, *Biochem. J.*, 79, 516, 1961.
8. **Habig, W. H., Pabst, M. J., and Jakoby, W. B.,** Glutathione S-transferases, the first enzymic step in mercapturic acid formation, *J. Biol. Chem.*, 249, 7130, 1974.
9. **Mannervik, B.,** The isoenzymes of glutathione transferase, *Adv. Enzymol. Relat. Areas Mol. Biol.*, 57, 357, 1985.
10. **Taylor, J. B., Pemble, S. E., Cowell, I. G., Dixon, K., and Ketterer, B.,** Molecular biology of the glutathione transferases, *Biochem. Soc. Trans.*, 15, 578, 1987.
11. **Pickett, C. B. and Lu, A. Y. H.,** The structure, genetics and regulation of soluble glutathione S-transferases, in *Glutathione Conjugation,* Sies, H. and Ketterer, B., Eds., Academic Press, London, 1988, 137.
12. **Mannervik, B. and Danielson, U. H.,** Glutathione transferases — structure and catalytic activity, *Crit. Rev. Biochem.*, 23, 281, 1988.
13. **Jakoby, W. B. and Habig, W. H.,** Glutathione transferases, in *Enzymatic Basis of Detoxication,* Jakoby, W. B., Ed., Academic Press, New York, 1980, 63.
14. **Kosower, N. S. and Kosower, E. S.,** Glutathione metabolism and function, *Int. Rev. Cytol.*, 54, 109, 1978.
15. **Slivka, A., Myrtilineou, C., and Cohen, G.,** Histochemical evaluation of glutathione in the brain, *Brain Res.*, 409, 275, 1987.
16. **Ashgar, K., Reddy, B. G., and Krishna, G.,** Histochemical localization of glutathione in tumors, *J. Histochem. Cytochem.*, 23, 774, 1975.
17. **Wirth, P. J. and Thorgeirsson, S. S.,** Glutathione synthesis and degradation in fetal and adult liver and Novikoff hepatoma, *Cancer Res.*, 38, 2861, 1978.
18. **Lauterberg, B. H., Yashesh, V., Stillwell, N. G., and Mitchell, J. R.,** The effects of age and glutathione depletion in hepatic glutathione turnover in vivo, *J. Pharmacol. Exp. Ther.*, 213, 54, 1980.
19. **Lawley, P. D.,** Effects of some chemical mutagens and carcinogens on nucleic acids, *Prog. Nucleic Acid Res. Mol. Biol.*, 5, 89, 1966.
20. **Loveless, A.,** Possible relevance of O-6 alkylation of deoxyguanosine to the mutagenicity and carcinogenicity of nitrosamines and nitrosamides, *Nature,* 223, 206, 1969.
21. **Coles, B.,** Effects of modifying structure on electrophilic reactions with biological nucleophiles, *Drug Metab. Rev.*, 15, 1307, 1984/5.
22. **Djuric, Z., Coles, B., Fifer, E. K., Ketterer, B., and Beland, F. A.,** In vivo and in vitro formation of glutathione conjugates from the K-region epoxides of 1-nitropyrene, *Carcinogenesis,* 8, 1781, 1987.

22a. **Coles, B., Howard, P., and Ketterer, B.,** unpublished.

23. **Coles, B., Wilson, I., Wardman, P., Hinson, J. A., Nelson, S. D., and Ketterer, B.,** The spontaneous and enzymic reaction of *N*-acetyl-*p*-benzoquinonimine with glutathione: a stopped-flow kinetic study, *Arch. Biochem. Biophys.*, 264, 253, 1988.
24. **Douglas, K.,** The activity of glutathione in model systems, in *Glutathione Conjugation,* Sies, H. and Ketterer, B., Eds., Academic Press, London, 1988, 1.
25. **Ketterer, B., Meyer, D. J., and Clark, A. G.,** Soluble glutathione isozymes, in *Glutathione Conjugation,* Sies, H. and Ketterer, B., Eds., Academic Press, London, 1988, 73.
26. **Jernström, B., Martinez, M., Meyer, D. J., and Ketterer, B.,** Glutathione conjugation of the carcinogenic and mutagenic electrophile (+/−)-7β,8α-dihydroxy-9α,10α-oxy-7,8,9,10- tetrahydrobenzo(a)pyrene catalysed by purified rat liver glutathione transferases, *Carcinogenesis,* 6, 85, 1985.
27. **Robertson, I. G. C. and Jernström, B.,** The enzymatic conjugation of glutathione with Bay region diol epoxides of benzo(a)pyrene, benz(a)anthracene and chrysene, *Carcinogenesis,* 7, 1633, 1986.
28. **Jakoby, W. B., Ketterer, B., and Mannervik, B.,** Glutathione transferases: nomenclature, *Biochem. Pharmacol.*, 33, 2539, 1984.
29. **Lai, H.-C. J., Li, N.-Q., Weiss, M. J., Reddy, C. C., and Tu, C.-P.-D.,** The nucleotide sequence of a rat liver glutathione S-transferase subunit cDNA clone, *J. Biol. Chem.*, 259, 5536, 1984.
30. **Taylor, J. B., Craig, R. K., Beale, D., and Ketterer, B.,** Construction and characterization of a plasmid containing complementary DNA to mRNA encoding the *N*-terminal amino acid sequence of the rat glutathione transferase Ya subunit, *Biochem. J.*, 219, 223, 1984.
31. **Pickett, C. B., Telakowski-Hopkins, A., Ding, G. J.-F., Argentbright, L., and Lu, A. Y. H.,** Rat liver glutathione S-transferase mRNA and the regulation of the Ya, Yb, and Yc mRNAs by 3-methyl-cholanthrene and phenobarbital, *J. Biol. Chem.*, 259, 5182, 1984.
32. **Telakowski-Hopkins, C. A., Rodkey, J. A., Bennett, C. D., Lu, A. Y., and Pickett, C. B.,** Rat liver glutathione S-transferases. Construction of a cDNA clone complementary to a Yc mRNA and prediction of the complete amino acid sequence of a Yc subunit, *J. Biol. Chem.*, 260, 5820, 1985.
33. **Ding, G. J.-F., Lu, A. Y. H., and Pickett, C. B.,** Rat liver glutathione S-transferase. Nucleotide sequence analysis of a Yb_1 cDNA clone and the prediction of the complete amino acid sequence, *J. Biol. Chem.*, 260, 13268, 1985.
34. **Ding, G. J-F., Ding, V. D-H., Rodkey, J. H., Bennett, C. D., Lu, A. Y. H., and Pickett, C. B.,** DNA sequence analysis of a Yb_2 cDNA clone and regulation of the Yb_1 and Yb_2 mRNAs by phenobarbital, *J. Biol. Chem.*, 261, 7952, 1986.
35. **Suguoka, Y., Kano, T., Okuda, A., Sakai, M., Kitagawa, T., and Muramatsu, M.,** Cloning of the nucleotide sequence of rat glutathione

S-transferase P cDNA, *Nucleic Acids Res.*, 13, 6049, 1985.

36. **Pemble, S. E., Taylor, J. B., and Ketterer, B.,** Tissue distribution of rat glutathione transferase subunit 7, a hepatoma marker, *Biochem. J.*, 240, 885, 1986.
37. **Telakowski-Hopkins, C. A., Rothkopf, G. S., and Pickett, C. B.,** Structural analysis of a rat liver glutathione transferase Ya gene, *Proc. Natl. Acad. Sci. U.S.A.*, 83, 9393, 1985.
38. **Lai, H.-C. J., Qian, B., Grove, G., and Tu, C.-P. D.,** Gene expression of rat glutathione transferases, *J. Biol. Chem.*, 263, 11389, 1988.
39. **Okuda, A., Sakai, M., and Muramatsu, M.,** The structure of the rat GSH transferase P gene and related pseudogenes, *J. Biol. Chem.*, 262, 3858, 1987.
40. **Mannervik, B., Ålin, C., Guthenberg, C., Jensson, H., Tahir, M. K., Warholm, M., and Jornvall, H.,** Identification of three classes of cytosolic glutathione transferases common to several mammalian species: correlation between structural data and enzymic properties, *Proc. Natl. Acad. Sci. U.S.A.*, 82, 7202, 1985.
41. **Meyer, D. J., Christodoulides, L. G., Tan, K. H., and Ketterer, B.,** Isolation, properties and tissue distribution of rat glutathione transferases, *FEBS Lett.*, 173, 327, 1984.
42. **Meyer, D. J., Beale, D., Tan, K. H., Coles, B., and Ketterer, B.,** Glutathione transferases in primary rat hepatomas: the isolation of a form with GSH peroxidase activity, *FEBS Lett.*, 184, 139, 1985.

42a. **Johnson, N., Meyer, D. J., Coles, B., and Ketterer, B.,** unpublished information.

43. **Ketterer, B., Meyer, D. J., Coles, B., and Taylor, J. B.,** Glutathione transferase isoenzymes in the rat: their multiplicity and tissue distribution, in *Microsomes and Drug Oxidations*, Boobis, A. R., Caldwell, J., DeMatteis, F., and Elcombe, C. R., Eds., Taylor & Francis, London, 1985, 166.
44. **Ketterer, B., Meyer, D. J., Coles, B., Taylor, J. B., and Pemble, S. E.,** Glutathione transferases and carcinogenesis, in *Antimutagenesis and Anticarcinogenesis Mechanisms*, Shankel, D. M., Hartman, P., Kada, T., and Hollaender, A., Eds., Plenum Press, New York, 1986, 103.
45. **Scott T. R. and Kirsch, R. E.,** The isolation of a fetal rat liver glutathione S-transferase isoenzyme with high glutathione peroxidase activity, *Biochim. Biophys. Acta*, 926, 264, 1987.

45a. **Coles, B., Meyer, D. J., Lalor, E., Kispert, A., and Ketterer, B.,** unpublished information.

46. **Cowell, I. G., Dixon, K. H., Pemble, S. E., Ketterer, B., and Taylor, J. B.,** The structure of the human glutathione transferase π gene, *Biochem. J.*, 255, 79, 1988.
47. **Imler, J. L., Schatz, C., Wasylyk, C., Chatton, B., and Wasylyk, B.,** A harvey-ras responsive transcription element is also responsive to a tumour promotor and to serum, *Nature*, 332, 275, 1988.
48. **Meyer, D. J., Christodoulides, L. G., Nyan, O., Schuster Bruce, R., and Ketterer, B.,** A comparison of the glutathione transferases (GST) of three extrahepatic organs with different functions — the adrenal, the lactating mammary gland and the male reproductive system, in *Extrahepatic Drug Metabolism and Chemical Carcinogenesis*, Rydstrom, J., Montelius, J., and Bengtsson, M., Eds., Elsevier Science Publishers, Amsterdam, 1983, 189.
49. **Ostlund Farrants, A.-K., Meyer, D. J., Coles, B., Southan, C. Aitken, A., Johnson, P. J., and Ketterer, B.,** The separation of glutathione transferase subunits by using reverse-phase high-pressure liquid chromatography, *Biochem. J.*, 245, 423, 1987.

49a. **Lalor, E., Coles, B., Meyer, D. J., Ålin, P., Mannervik, B., and Ketterer, B.,** unpublished information.

50. **Tahir, M. K., Ozer, N., and Mannervik, B.,** Isoenzymes of glutathione transferase in rat small intestine, *Biochem. J.*, 253, 759, 1988.

50a. **Lalor, E., Coles, B., Meyer, D. J., and Ketterer, B.,** unpublished information.

51. **Tatematsu, M., Mera, Y., Ito, N., Satoh, K., and Sato, K.,** Relative merits of immunohistochemical demonstration of placental, A, B and C forms of glutathione S-transferase and histochemical demonstration of glutamyl transferase as markers of altered foci during liver carcinogenesis in rats, *Carcinogenesis*, 6, 1621, 1985.
52. **Redick, J. A., Jakoby, W. B., and Baron, J.,** Immunohistochemical localization of glutathione S-transferase in livers of untreated rats, *J. Biol. Chem.*, 257, 15200, 1982.
53. **Abromowitz, M., Homma, H., Ishigaki, S., Tansey, F., Cammer, W., and Listowsky, I.,** Characterization and localization of glutathione-S-transferases in rat brain and binding of hormones, neurotransmitters and drugs, *J. Neurochem.*, 50, 50, 1988.
54. **Ålin, P., Danielson, U. H., and Mannervik, B.,** 4-Hydroxyalkenals as substrates for glutathione transferases, *FEBS Lett.*, 179, 267, 1985.
55. **Jensson, H., Guthenberg, C., Ålin, P., and Mannervik, B.,** Rat glutathione transferase 8-8, an enzyme efficiently detoxifying 4-hydroxy-alk-2-enals, *FEBS Lett.*, 203, 207, 1986.
56. **Ishikawa, T., Tsuchida, S., Satoh, K., and Sato, K.,** The subunit structure of a major glutathione S-transferase form, M_T, in rat testis, *Eur. J. Biochem.*, 176, 551, 1988.

56a. **Lalor, E., Meyer, D. J., and Ketterer, B.,** unpublished results.

57. **Kamisaka, K., Habig, W. H., Ketley, J. M., Arias, I. H., and Jakoby, W. B.,** Multiple forms of glutathione S-transferase and their affinity for bilirubin, *Eur. J. Biochem.*, 60, 153, 1975.
58. **Soma, Y., Satoh, K., and Sato, K.,** Purification and subunit structural and immunological characterization of five glutathione S-transferases in human liver, and the acidic form as a hepatic tumor marker, *Biochim. Biophys. Acta*, 869, 247, 1986.
59. **Stockman, P. K. McLellan, L. I., and Hayes, J. D.,** Characterization of the basic glutathione S-transferase B_1 and B_2 subunits from human liver, *Biochem. J.*, 244, 55, 1987.
60. **Tu, C.-P. D. and Qian, B.,** Human liver glutathione S-transferases: complete primary sequence of a Ha subunit cDNA, *Biochem. Biophys. Res. Commun.*, 141, 229, 1986.
61. **Rhoads, D. M., Zarlengo, R. P., and Tu, C.-P. D.,** The basic glutathione S-transferases from human livers are products of separate genes, *Biochem. Biophys. Res. Commun.*, 145, 474, 1987.
62. **Hussey, A. J., Stockman, P. K., Beckett, G. J., and Hayes, J. D.,** Variation in the glutathione transferase subunits expressed in human livers, *Biochim. Biophys. Acta*, 874, 1, 1986.
63. **Kano, T., Sakai, M., and Muramatsu, M.,** Structure and expression of human placental-type glutathione S-transferase (GST-pi) messenger RNA, *Cancer Res.*, 47, 5626, 1987.
64. **Board, P. G.,** Biochemical genetics of glutathione S-transferases in man, *Am. J. Hum. Genet.*, 33, 36, 1981.

64a. **Coles, B., Hayes, J., Meyer, D. J. M., and Ketterer, B.,** unpublished information.

65. **Laisney, V., Van Cong, N., Gross, M. S., and Frezal, J.,** Human genes for glutathione S-transferases, *Hum. Genet.*, 68, 221, 1984.
66. **Strange, R. C., Faulder, C. G., Davis, B. A., Hume, R., Brown, J. A., Cotton, W., and Hopkinson, D. A.,** The glutathione S-transferases: studies on the tissue distribution and genetic variation of the GST 1, GST 2 and GST 3 isoenzymes, *Ann. Hum. Genet.*, 48, 11, 1984.
67. **Harada, S., Abei, M., Tanaka, N., Agarwal, D. P., and Goedde, H. W.,** Liver glutathione S-transferase polymorphism in Japanese and its pharmacogenetic importance, *Hum. Genet.*, 75, 322, 1987.
68. **Baars, A. J., Mukhtar, H., Zoetemelk, C. E. M., Jansen, M., and Breimer, D. D.,** Glutathione S-transferase activity in rat and human tissues and organs, *Comp. Biochem. Physiol.*, 70, 285, 1981.
69. **Sherman, M., Titmuss, S., and Kirsch, R. E.,** Glutathione S-transferase in human organs, *Biochem. Int.*, 6, 109, 1983.
70. **Loscalzo, J. and Freedman, J.,** Purification and characterization of human platelet glutathione-S-transferase, *Blood*, 67, 1595, 1986.
71. **Del Boccio, G., Di Ilio, C., Casalone, E., Pennelli, A., Aceto, A., Sacchetta, P., and Federici, G.,** Purification and characterization of glutathione S-transferases from human thyroid, *Ital. J. Biochem.*, 36, 8,

1987.

72. **Singh, S. V., Kurosky, A., and Awasthi, Y. C.,** Human liver glutathione transferase psi. Chemical characterization and secondary-structure comparison with other mammalian glutathione S-transferases, *Biochem. J.*, 243, 61, 1987.
73. **Tateoka, N., Tsuchida, S., Soma, Y., and Sato, K.,** Purification and characterization of glutathione S-transferases in human kidney, *Clin. Chim. Acta*, 166, 207, 1987.
74. **Suzuki, T., Coggan, M., Shaw, D. C., and Board, P. G.,** Electrophoretic and immunological analysis of human glutathione S-transferase isoenzymes, *Ann. Hum. Genet.*, 51, 95, 1987.
75. **Del Boccio, G., di Ilio, C., Ålin, P., Jörnvall, H., and Mannervik, B.,** Identification of a novel glutathione transferase in human skin homologous with class alpha glutathione transferase 2-2 in the rat, *Biochem. J.*, 244, 21, 1987.
76. **Siedegard, J., Guthenberg, C., Pero, R. W., and Mannervik, B.,** The trans-stilbene oxide active glutathione transferase in human mononuclear leukocytes is identical with the hepatic glutathione transferase mu, *Biochem. J.*, 246, 783, 1987.
77. **Siedegard, J. and Pero, R. W.,** The hereditary transmission of high glutathione transferase activity toward trans stilbene oxide in human mononuclear leucocytes, *Hum. Genet.*, 69, 66, 1985.
78. **Warholm, M., Guthenberg, C., and Mannervik, B.,** Molecular and catalytic properties of glutathione transferase mu from human liver: an enzyme efficiently conjugating epoxides, *Biochemistry*, 22, 3610, 1983.
79. **Siedegard, J., DePierre, J. W., and Pero, R. W.,** Hereditary interindividual differences in the glutathione transferase activity towards trans-stilbene oxide in resting human mononuclear leukocytes are due to a particular isoenzymes, *Carcinogenesis*, 6, 1211, 1985.
80. **Siedegard, J., Pero, R. W., Miller, D. G., and Beattie, E. J.,** A glutathione transferase in human leukocytes as a marker for the susceptibility to lung cancer, *Carcinogenesis*, 7, 751, 1986.
81. **Mannervik, B., Alin, P., Danielson, U. H., Guthenberg, C., Jenssen, H., Ozer, N., Tahir, M., Warholm, M., and Jornvall, H.,** Three classes of mammalian glutathione transferases and their occurrence in the rat and mouse, in *Glutathione S-Transferases and Carcinogenesis*, Mantle, T. J., Pickett, C. B., and Hayes, J. D., Eds., Taylor & Francis, London, 1987, 19.
82. **McLellan, L. I. and Hayes, J. D.,** Sex-specific constitutive expression of the pre-neoplastic marker glutathione transferase, YfYf, in mouse liver, *Biochem. J.*, 245, 399, 1987.
83. **Hatayma, I., Satoh, K., and Sato, K.,** Developmental and hormonal regulation of the major form of hepatic glutathione S-transferase in mice, *Biochem. Biophys. Res. Commun.*, 140, 581, 1986.
84. **Fujita, S., Kitagawa, H., Ishizawa, H., Suzuki, T., and Kitani, K.,** Age-associated alterations in hepatic glutathione S-transferase activities, *Biochem. Pharmacol.*, 34, 3891, 1985.
85. **Stohs, S. J., Al-Turk, W. A., and Angle, C. R.,** Glutathione S-transferase and glutathione reductase activities in hepatic and extra-hepatic tissues of female mice as a function of age, *Biochem. Pharmacol.*, 31, 2113, 1982.
86. **Kitahara, A., Satoh, K., Nishimura, K., Ishikawa, T., Ruike, K., Sato, K., Tsuda, H., and Ito, N.,** Changes in molecular forms of rat glutathione S-transferase during chemical hepatocarcinogenesis, *Cancer Res.*, 44, 2698, 1984.
87. **Sato, K., Kitahara, A., Yin, Z., Waragai, F., Nishimura, K., Hatayama, I., Ebina, T., Yamazaki, T., Tsuda, H., and Ito, N.,** Induction by butylated hydroxyanisole of specific molecular forms of glutathione S-transferase and UDP-glucuronyl transferase and inhibition of development of γ-glutamyl transpeptidase positive foci in rat liver, *Carcinogenesis*, 5, 473, 1984.
88. **Power, C., Sinha, S., Webber, C., Manson, M. M., and Neal, G. E.,** Transformation related expression of glutathione S-transferase P in rat liver cells, *Carcinogenesis*, 8, 797, 1987.
89. **Eriksson, L. C., Sharma, R. N., Roomi, M. W., Ho, R. K., Farber, E., and Murray, R. K.,** A characteristic electrophoretic pattern of cytosolic polypeptides from hepatocyte nodules generated during liver carcinogenesis in several models, *Biochem. Biophys. Res. Commun.*, 117, 740, 1983.
90. **Tatematsu, M., Nagamine, Y., and Farber, E.,** Redifferentiation as a basis for remodelling of carcinogen-induced hepatocyte nodules to normal appearing liver, *Cancer Res.*, 43, 5049, 1983.
91. **Kodate, C., Fukushi, A., Narita, T., Kudo, H., Soma, Y., and Sato, K.,** Human placental form of glutathione S-transferase (GST pi) as a new immunohistochemical marker for human colonic carcinoma, *Jpn. J. Cancer Res.*, 77, 226, 1986.
92. **Sato, K., Satoh, K., Hatayama, I., Tsuchida, S., Soma, Y., Shiratori, Y., Tateoka, N., Inaba, Y., and Kitahara, A.,** Placental glutathione S-transferase as a marker for (pre)neoplastic tissues, in *Glutathione S-Transferases and Carcinogenesis*, Mantle, T. J., Pickett, C. B., and Hayes, J. D., Eds., Taylor & Francis, London, 1987, 127.
93. **Shea, T. C. and Henner, W. D.,** Glutathione transferases in human tumors, in *Glutathione S-Transferases and Carcinogenesis*, Mantle, T. J., Pickett, C. B., and Hayes, J. D., Eds., Taylor & Francis, London, 1987, 227.
94. **Mannervik, B., Castro, V. M., Danielson, U. H., Tahir, M. K., Hansson, J., and Ringborg, U.,** Expression of glutathione transferase in human malignant melanoma, *Carcinogenesis*, 12, 1929, 1987.
95. **di Ilio, C., Del Boccio, G., Aceto, A., and Federici, G.,** Alteration in glutathione transferase isoenzyme concentrations in human renal carcinoma, *Carcinogenesis*, 8, 861, 1987.
96. **Hayes, P. C., Portmann, B., Aldois, P. M., Williams, R., and Hayes, J.,D.,** Glutathione S-transferases and human pathology, in *Glutathione S-Transferases and Carcinogenesis*, Mantle, T. J., Pickett, C. B., and Hayes, J. D., Eds., Taylor & Francis, London, 1987, 175.
97. **Awasthi, Y. C., Singh, S. V., Ahmad, H., Moller, P. C., and Gupta, V.,** Expression of glutathione S-transferase isoenzymes in human small cell lung cancer lines, *Carcinogenesis*, 9, 89, 1988.
98. **Wolf, C. R., Lewis, A. D., Carmichael, J., Ansell, J., Adams, D. J., Hickson, I. J., Harris, A., Balkwill, F. R., Griffen, D. B., and Hayes, J. D.,** Glutathione S-transferase expression in normal and tumour cells resistant to cytotoxic drugs, in *Glutathione S-Transferases and Carcinogenesis*, Mantle, T. J., Pickett, C. B., and Hayes, J. D., Eds., Taylor & Francis, London, 1987, 199.
99. **Batist, G., Tulpule, A., Sinha, B. K., Katki, A. G., Myers, C. E., and Cowan, K. H.,** Overexpression of a novel anionicglutathione transferase in multidrug-resistant human breast cancer cells, *J. Biol. Chem.*, 261, 15544, 1986.
100. **Morgenstern, R. and DePierre, J. W.,** Membrane-bound glutathione transferases, in *Glutathione Conjugation*, Sies, H. and Ketterer, B., Eds., Academic Press, London, 1988, 157.
101. **Bach, M. K., Brashler, J. R., Peck, R. E., and Morton, D. R.,** Leukotriene C-synthetase, a special glutathione S-transferase: properties of the enzyme and inhibitor studies with special reference to the mode of action of V-60,257, a selective inhibitor of leukotriene synthesis, *J. Allergy Clin. Immunol.*, 74, 353, 1986.
102. **Wolf, C. R., Berry, P. N., Nash, J. A., Green, T., and Lock, E. A.,** Role of microsomal and cytosolic glutathione S-transferases in the conjugation of hexachloro-1,3-butadiene and its possible relevance to toxicity, *J. Pharmacol. Exp. Ther.*, 228, 202, 1984.
103. **Dulik, D. M., Fenselau, C., and Hilton, J.,** Characterization of Melphalan-glutathione adducts whose formation is catalyzed by glutathione transferases, *Biochem. Pharmacol.*, 35, 3405, 1986.
104. **Boyland, E. and Sims, P.,** The metabolism of benz(a)anthracene and dibenz(a,h)anthracene and their 5,6-epoxy-5,6-dihydro derivatives by rat liver homogenates, *Biochem. J.*, 97, 7, 1965.
105. **Sims, P.,** The metabolism of 7- and 12-methylbenz(a)anthracenes and their derivatives, *Biochem. J.*, 105, 591, 1967.
106. **Waterfall, J. F. and Sims, P.,** Epoxy derivatives of aromatic polycyclic

hydrocarbons. The properties and metabolism of epoxides related to benzo(a)pyrene and to 7,8- and 9,10-dihydrobenzo(a)pyrene, *Biochem. J.*, 128, 265, 1972.

107. **Sims, P.,** The metabolism of 3-methylcholanthrene and some related compounds by rat liver homogenates, *Biochem. J.*, 98, 215, 1966.
108. **Sims, P. and Grover, P. L.,** Epoxides in polycyclic aromatic hydrocarbon metabolism and carcinogenesis, *Adv. Cancer Res.*, 20, 165, 1974.
109. **Pelkonen, O. and Nebert, D. W.,** Metabolism of polcyclic aromatic hydrocarbons: etiologic role in carcinogenesis, *Pharmacol. Rev.*, 34, 189, 1982.
110. **Gelboin, H. V.,** Benzo(a)pyrene metabolism, activation, and carcinogenesis: role and regulation of mixed function oxidases and related enzymes, *Physiol. Rev.*, 60, 1107, 1980.
111. **Smith, B. R. and Bend, J. R.,** Metabolism and excretion of benzo(a)pyrene-4,5-oxide by the isolated, perfused rat liver, *Toxicol. Appl. Pharmacol.*, 49, 313, 1979.
112. **Nemoto, N., Gelboin, H. V., Pabst, W. H., and Jakoby, W. B.,** K-region benzo(a)pyrene-4,5-oxide is conjugated by homogeneous glutathione S-transferases, *Nature*, 255, 512, 1975.
113. **Coles, B., Meyer, D. J., Ketterer, B., Stanton, C. A., and Garner, R. C.,** Studies on the detoxication of microsomally activated aflatoxin B_1 by glutathione and glutathione transferases *in vitro*, *Carcinogenesis*, 6, 693, 1985.
114. **Jerina, D. M. and Lehr, R. E.,** The Bay region theory: a quantum mechanical approach to aromatic hydrocarbon-induced carcinogenicity, in *Microsomes and Drug Oxidation*, Ullrich, V., Roots, I., Hildebrandt, A. G., Estabrook, R. W., and Conney, A. H., Eds., Pergamon Press, Oxford, 1977, 709.
115. **Hesse, S., Jernström, B, Martinez, M, Moldeus, P., Christodoulides, L., and Ketterer, B.,** Inactivation of DNA binding metabolites of benzo(a)pyrene and benzo(a)pyrene-7,8-dihydrodiol by glutathione and glutathione S-transferases, *Carcinogenesis*, 3, 757, 1982.
116. **Jernström, B., Babson, J. R., Moldeus, P., Holmgren, A., and Reed, D. J.,** Glutathione conjugation and DNA binding of (±)-7β,8α-dihydroxy-9α, 10α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene in isolated rat hepatocytes, *Carcinogenesis*, 3, 861, 1982.
117. International Agency for Research on Cancer, Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, 32, Polynuclear aromatic hydrocarbons, Part 1, 1983.
118. International Agency for Research on Cancer, Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, 33, Polynuclear aromatic hydrocarbons, Part 2, 1984.
119. **Watabe, T., Hiratsuka, A., and Ogura, K.,** Sulfotransferase-mediated covalent binding of the carcinogen 7,12-dihydroxymethylbenz(a)anthracene to calf thymus DNA and its inhibition by glutathione transferases, *Carcinogenesis*, 8, 445, 1987.
120. **Robertson, I. G. C., Jensson, H., Mannervik, B., and Jernström, B.,** Glutathione transferase in rat lung: the presence GSH transferase 7-7, highly efficient in conjugation of glutathione with the carcinogenic (+)-7β,8α-dihydroxy-9α,10α-oxy-7,8,9,10- tetrahydrobenzo(a)pyrene, *Carcinogenesis*, 7, 295, 1986.
121. **Robertson, I. G. C., Guthenberg, C., Mannervik, B., and Jernström, B.,** Differences in stereoselectivity and catalytic efficiencies of three human glutathione transferases in the conjugation of glutathione with 7β,8α-dihydroxy-9α,10α-oxy-7,8,9,10-tetrahydrobenzo(a)pyrene, *Cancer Res.*, 46, 2220, 1986.
122. **Boehlert, C. C. and Armstrong, R. N.,** Investigation of the kinetic and stereochemical recognition of arene and azaarene oxides by isoenzymes A_2 and C_2 of glutathione S-transferase, *Biochem. Biophys. Res. Commun.*, 121, 980, 1984.
123. **Cobb, D., Boehlert, C., Lewis, D., and Armstrong, R. N.,** Stereoselectivity of isoenzyme C of glutathione S-transferase towards arene and azaarene oxides, *Biochemistry*, 22, 805, 1983.
124. **Chen, W.-J. Graminski, G. F., and Armstrong, R. N.,** Dissection of the catalytic mechanism of isoenzyme 4-4 of glutathione S-transferase with alternative substrates, *Biochemistry*, 27, 647, 1988.
125. **Hernandez, O., Walker, M., Cox, R. H., Foureman, G. L., Smith, B. R., and Bend, J. R.,** Regiospecificity and stereospecificity in the enzymatic conjugation of glutathione with (±)-benzo(a)pyrene-4,5-oxide, *Biochem. Biophys. Res. Commun.*, 96, 1494, 1980.
126. **Dostal, L. A., Aitio, A., Harris, C., Bhatia, A. V., Hernandez, O., and Bend, J. R.,** Cytosolic glutathione S-transferases in various rat tissues differ in stereoselectivity with model polycyclic arene and alkene oxide substrates, *Drug Metab. Disp.*, 14, 303, 1986.
127. **Dostal, L. A., Horton, J. K., Harris, C. G., Brier, D. F., and Bend, J. R.,** Stereoselectivity of cytosolic glutathione S-transferases with arene and alkene oxide substrates in various tissues and isolated hepatic and pulmonary cells of the rabbit, *Carcinogenesis*, 8, 1601, 1987.
128. **Watabe, T., Ozawa, N., and Hiratsuka, A.,** Studies on metabolism and toxicity of styrene. VI. Regioselectivity in glutathione S-conjugation and hydrolysis of racemic R- and S-phenyloxiranes in rat liver, *Biochem Pharmacol.*, 32, 777, 1983.
129. **Newberne, P. M. and Butler, W. H.,** Acute and chronic effects of aflatoxin B_1 on the liver of domestic and laboratory animals: a review, *Cancer Res.*, 29, 236, 1969.
130. **Wogan, G. N. and Shank, R. C.,** Toxicity and carcinogenicity of aflatoxins, in *Advances in Environmental Science and Technology*, Petts, J. N. and Metcalf, R. L. Eds., John Wiley & Sons, New York, 1970, 321.
131. **Degan, G. H. and Neumann, H. G.,** The major metabolite of aflatoxin B_1 in the rat is a glutathione conjugate, *Chem.-Biol. Interact.*, 22, 239, 1978.
132. **Lin, J. K., Miller, J. A., and Miller, E. C.,** 2,3-Dihydro-2-(guan-7-yl)-3-hydroxy-aflatoxin B_1, a major acid hydrolysis product of aflatoxin B_1-DNA or -ribosomal RNA adducts formed in hepatic microsome-mediated reactions and in rat liver in vivo, *Cancer Res.*, 37, 4430, 1977.
133. **Croy, R. G., Essigman, J. M., Reinhold, V. N., and Wogan, G. N.,** Identification of the principal aflatoxin B_1-DNA adduct formed *in vivo* in rat liver, *Proc. Natl. Acad. Sci. U.S.A.*, 75, 1745, 1978.
134. **Martin, C. N. and Garner, R. C.,** Aflatoxin B_1-generated by chemical or enzymic oxidation of aflatoxin B_1 causes guanine substitution of nucleic acids, *Nature*, 269, 363, 1977.
135. **Essigman, J. M., Croy, R. G., Nadzan, A. M., Busby, W. F., Reinhold, V. N., and Wogan, G. N.,** Structural identification of the major DNA adduct formed by aflatoxin B_1 in vivo, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 1870, 1977.
136. **Moss, E. J., Judah, D. J., Przblyski, M., and Neal, G. E.,** Some mass-spectral and NMR analytical studies of a glutathione conjugate of aflatoxin B_1, *Biochem. J.*, 210, 227, 1983.
137. **Neal, G. E. and Colley, D. J.,** The formation of 2,3-dihydro-2,3-dihydroxy-aflatoxin B_1 by the metabolism of aflatoxin B_1 in vitro by rat liver microsomes, *FEBS Lett.*, 101, 302, 1979.
138. **Neal, G. E., Judah, D. J., Stirpe, F., and Patterson, D. S. P.,** The formation of 2,3-dihydroxy-2,3-dihydro-aflatoxin B_1 by the metabolism of aflatoxin B_1 by liver microsomes isolated from certain avian and mammalian species and the possible role of the metabolite in the acute toxicity of aflatoxin B_1, *Toxicol. Appl. Pharmacol.*, 58, 431, 1981.
139. **Coles, B. F., Welch, A. M., Hertzog, J., Lindsay Smith, J. R., and Garner, R. C.,** Biological and chemical studies on 8,9-dihydroxy-8,9-dihydro-aflatoxin B_1 and some of its esters, *Carcinogenesis*, 1, 79, 1980.

139a. **Baertschi, S. W., Raney, K. D., Stone, M. P., and Harris, T. M.,** Preparation of the 8,9 epoxide of the mycotoxin aflatoxin B_1: the ultimate carcinogenic species, *J. Am. Chem. Soc.*, 110, 7929, 1988.

140. **Emerole, G. O., Neskovic, N., and Dixon, R. L.,** The detoxication of aflatoxin B_1 with glutathione in the rat, *Xenobiotica*, 9, 737, 1979.
141. **Neal, G. E. and Green, J. A.,** The requirement for glutathione S-transferase in the conjugation of activated aflatoxin B_1 during aflatoxin hepatocarcinogenesis in the rat, *Chem.-Biol. Interact.*, 45, 259, 1983.
142. **Degan, G. H. and Neumann, H. G.,** Differences in aflatoxin B_1 suscep-

tibility of rat and mouse are correlated with the capability in vitro to inactivate aflatoxin B_1 epoxide, *Carcinogenesis*, 2, 299, 1981.

143. **Neal, G. E., Metcalf, S. A., Legg, R. F., Judah, A. J., and Green, J. A.,** Mechanism of the resistance to cytotoxicity which precedes aflatoxin B_1 hepatocarcinogenesis, *Carcinogenesis*, 2, 457, 1981.
144. **O'Brien, K., Moss, E., Judah, D., and Neal, G.,** Metabolic basis of the species difference to aflatoxin B_1-induced hepatotoxicity, *Biochem. Biophys. Res. Commun.*, 114, 813, 1983.
145. **Lotlikar, P. D., Jhee, E. C., Insetta, S. M., and Clearfield, M. S.,** Modulation of microsome-mediated aflatoxin B_1 binding to exogenous and endogenous DNA by cytosolic glutathione S-transferases in rat and hamster livers, *Carcinogenesis*, 5, 269, 1984.
146. **Rahimtula, A. D. and Martin, M.,** Dietary administration of 2(3)-*t*-butyl-4-hydroxyanisole elevates mouse liver microsome-mediated DNA binding and mutagenicity of aflatoxin B_1, *Chem.-Biol. Interact.*, 48, 207, 1984.
147. **Raj, H. J., Clearfield, M. S., and Lotlikar, P. D.,** Comparative kinetic studies on aflatoxin B_1-DNA binding and aflatoxin B_1 glutathione conjugation with rat and hamster livers in vivo, *Carcinogenesis*, 5, 879, 1984.
148. **Kensler, T. W., Egner, P. A., Davidson, N. E., Roebuck, B. D., Pikul, A., and Groopman, J. D.,** Modulation of aflatoxin metabolism, aflatoxin-N^7-guanyl formation and hepatic tumorigenesis in rats fed ethoxyquin: role of induction of glutathione S-transferases, *Cancer Res.*, 46, 3924, 1986.
149. **Miller, E. C., Kadlubar, F. F., Miller, J. A., Pitot, H. C., and Drinkwater, N. R.,** The *N*-hydroxy metabolites of *N*-methyl-4-aminoazobenzene and related dyes as proximate carcinogens in the rat and mouse, *Cancer Res.*, 39, 3411, 1979.
150. **Kadlubar, F. F., Miller, J. A., and Miller, E. C.,** Hepatic metabolism of *N*-hydroxy-*N*-methyl-4-aminoazobenzene and other *N*-hydroxy aryl amines to reactive sulfuric acid esters, *Cancer Res.*, 36, 2350, 1976.
151. **Meerman, J. H. N., Beland, F. A., and Mulder, G. J.,** The role of sulfation in the formation of DNA adducts from *N*-hydroxy-2-acetylaminofluorene in the rat, *Carcinogenesis*, 2, 413, 1981.
152. **Bartsch, H., Dworkin, M., Miller, J. A., and Miller, E. C.,** Electrophilic *N*-acetoxyaminoarenes derived from carcinogenic *N*-hydroxy-*N*-acetylaminoarenes by enzymatic deacetylation and transacetylation in liver, *Biochem. Biophys. Acta*, 286, 272, 1972.
153. **King, C. M.,** Mechanism of reaction, tissue distribution and inhibition of arylhydroxamic acid acyltransferase, *Cancer Res.*, 34, 1503, 1974.
154. **Martin, C. N., Beland, F. A., Roth, R. W., and Kadlubar, F. F.,** Covalent binding of benzidine and *N*-acetyl benzidine to DNA at the C-8 atom of deoxyguanosine in vivo and in vitro, *Cancer Res.*, 42, 2678, 1982.

154a. **Coles, B., Kadlubar, F., and Beland, F.,** unpublished information.

155. **Kadlubar, F. F., Miller, J. A., and Miller, E. C.,** Hepatic microsomal *N*-glucuronidation and nucleic acid binding of *N*-hydroxy arylamines in relation to urinary bladder carcinogenesis, *Cancer Res.*, 37, 805, 1977.
156. **Sugimura, T., Sato, S., Ohgaki, H., Takayama, S., Nagao, M., and Wakabayashi, K.,** Mutagens and carcinogens in cooked food, in *Genetic Toxicology of the Diet*, Alan R. Liss, New York, 1986, 85.
157. **Coles, B., Srai, S. K. S., Waynforth, B., and Ketterer, B.,** The major role of glutathione in the excretion of *N,N*-dimethyl-4-aminoazobenzene in the rat, *Chem.-Biol. Interact.*, 47, 307, 1983.
158. **Beland, F. A., Heflich, R. H., Howard, P. C., and Fu, P. P.,** The in vitro metabolic activation of nitro polycyclic aromatic hydrocarbons, in *Polycyclic Hydrocarbons and Carcinogenesis*, Harvey, R. G., Ed., American Chemical Society, Washington, D.C., 1986, 371.
159. **Rosenkranz, H. S. and Mermelstein, R. J.,** The genotoxicity, metabolism and carcinogenicity of nitrated polycyclic aromatic hydrocarbons, *Environ. Sci. Health*, 23, 221, 1986.
160. **Tokiwa, H. and Ohnishi, Y.,** Mutagenicity and carcinogenicity of nitroarenes and their sources in the environment, *Crit. Rev. Toxicol.*, 17, 23, 1986.
161. **Klehr, H., Eyer, P., and Schafer, W.,** On the mechanism of reaction of nitrosoarenes with thiols, *Biol. Chem.*, Hoppe-Seyler, 366, 755, 1985.
162. **Diepold, C., Eyer, P., Kampffmeyer, H., and Reinhardt, K.,** Reaction of aromatic nitroso compounds with thiols, in *Reactive Intermediates II, Part B, Advances in Experimental Medical Biology*, Snyder, R., Parke, D. V., Kocsis, J. J., Jollow, D. J., Gibson, C. G., and Witmer, C. M., Eds., Plenum Press, London, 136B, 1982, 1173.
163. **Ketterer, B., Srai, S. K. S., Waynforth, B., Tullis, D. L., Evans, F. E., and Kadlubar, F. F.,** Formation of *N*-(glutathion-*S*-methylene)-4-aminoazobenzene following metabolic activation of the *N*-methyl group of the carcinogen, *N*-methyl-4-aminoazobenzene, *Chem.-Biol. Interact.*, 38, 287, 1982.
164. **Coles, B., Srai, S. K. S., Ketterer, B., Waynforth, H. B., and Kadlubar, F. F.,** The identification of 4′-sulphonyloxy-*N*-(glutathion-*S*-methylene)-4-aminoazobenzene. A compound conjugated with both sulfate and glutathione, which is a major biliary metabolite of *N,N*-dimethyl-4-aminoazobenzene, *Chem.-Biol. Interact.*, 43, 123, 1983.
165. **Tullis, D. L., Dooley, K. L., Miller, D. W., Baetcke, K. P., and Kadlubar, F. F.,** Characterization and properties of the DNA adducts formed from *N*-methyl-4-aminoazobenzene in rats during a carcinogenic treatment regime, *Carcinogenesis*, 8, 577, 1987.
166. **Kadlubar, F. F., Ketterer, B., Flammang, T. J., and Christodoulides, L.,** Formation of 3-(glutathion-*S*-yl)-*N*-methyl-4-aminoazobenzene and inhibition of aminoazodye nucleic acid binding in vitro by reaction of glutathione with metabolically generated *N*-methyl-4-aminoazobenzene-*N*-sulphate, *Chem.-Biol. Interact.*, 31, 265, 1980.
167. **Ketterer, B., Kadlubar, F. F., Flammang, T. J., Carne, T., and Enderby, G.,** Glutathione adducts of *N*-methyl-4-aminoazobenzene formed in vivo and by reaction of *N*-benzoyloxy-*N*-methyl-4-aminoazobenzene with glutathione, *Chem.-Biol. Interact.*, 25, 7, 1979.
168. **Srai, S. K. S., Ketterer, B., and Kadlubar, F. F.,** Two biological reactive intermediates in aminoazodye carcinogenesis and their glutathione adducts, in *Reactive Intermediates II, Part B, Advances in Experimental Medical Biology*, Snyder, R., Parke, D. V., Kocsis, J. J., Jollow, D. J., Gibson, C. G., and Witmer, C. M., Eds., Plenum Press, London, 136B, 1982, 1297.
169. **Coles, B., Ketterer, B., Beland, F. A., and Kadlubar, F. F.,** Glutathione conjugate formation in the detoxification of ultimate and proximate carcinogens of *N*-methyl-4-aminoazobenzene, *Carcinogenesis*, 5, 917, 1984.
170. **Meerman, J. H. N., Beland, F. A., Ketterer, B., Srai, S. K. S., Bruins, A. P., and Mulder, G. J.,** Identification of glutathione conjugates formed from *N*-hydroxy-2-acetylaminofluorine in the rat, *Chem.-Biol. Interact.*, 39, 149, 1982.

170a. **Ketterer, B. and Beland, F.,** unpublished information.

171. **Van den Goorbergh, J. A. M., Meerman, J. H. N., de Wit, H., and Mulder, G. J.,** Reaction of 2-acetylaminofluorene-*N*-sulfate with RNA and glutathione: evidence for the generation of two reactive intermediates with different reactivities toward RNA and glutathione, *Carcinogenesis*, 6, 1635, 1985.
172. **Meerman, J. H. N. and Tijdens, R. B.,** Effect of glutathione depletion on the hepatotoxicity and covalent binding to rat liver macromolecules of *N*-hydroxy-2-acetylaminofluorine, *Cancer Res.*, 45, 1132, 1985.
173. **Beland, F. A. and Kadlubar, F. F.,** Formation and persistence of arylamine DNA adducts in vivo, *Environ. Health Perspect.*, 62, 19, 1985.

173a. **Ketterer, B. and Kadlubar, F.,** unpublished information.

174. **Mulder, G. J., Unruh, L. E., Evans, F. E., Ketterer, B., and Kadlubar, F. F.,** Formation and identification of glutathione conjugates from 2-nitrosofluorene and *N*-hydroxy-2-aminofluorene, *Chem.-Biol. Interact.*, 39, 111, 1982.
175. **Yamazoe, Y., Roth, R. W., and Kadlubar, F. F.,** Reactivity of benzidine diimine with DNA to form *N*-(deoxyguanosin-8-yl)-benzidine, *Carcinogenesis*, 7, 179, 1986.
176. **Wise, R. W. Zenser, T. V., and Davis, B.,** Prostaglandin H-synthase

oxidation of benzidine and *o*-dianisidine: reduction and conjugation of activated amines by thiols, *Carcinogenesis*, 6, 579, 1985.

177. **Felton, J. S., Knize, M. G., Shen, N. H., Andresen, B. D., Bjeldanes, L. F., and Hatch, F. F.,** Identification of mutagens in cooked beef, *Environ. Health Perspect.*, 66, 17, 1986.
178. **Hashimoto, Y., Shudo, K., and Okamoto, T.,** Metabolic activation of a mutagen, 2-amino-6-methyldipyrido[1,2-α:3′,2′-d]imidazole. Identification of 2-hydroxyamino-6-methyldipyrido[1,2-α:3′,2′-d] imidazole and its reaction with DNA, *Biochem. Biophys. Res. Commun.*, 92, 971, 1980.
179. **Hashimoto, Y., Shudo, K., and Okamoto, T.,** Activation of a mutagen, 3-amino-1-methyl-5H-pyrido[4,3-b]indole. Identification of 3-hydroxyamino-1-methyl-5H-dipyrido[4,3-b]indole and its reaction with DNA, *Biochem. Biophys. Res. Commun.*, 96, 355, 1980.
180. **Yamazoe, Y., Shimada, M., Kamataki, T., and Kato, R.,** Covalent binding of *N*-hydroxy-Trp-P-2 to DNA by cytosolic proline dependent system, *Biochem. Biophys. Res. Commun.*, 107, 165, 1982.
181. **Yamazoe, Y., Shimada, M., Shinohara, A., Saito, K., Kamataki, T., and Kato, R.,** Catalysis of the covalent binding of 3-hydroxyamino-1-methyl-5H-pyrido[4,3-b]indole to DNA by a L-proline and adenosine triphosphate-dependent enzyme in rat hepatic cytosol, *Cancer Res.*, 45, 2495, 1985.
182. **Yamazoe, Y., Tada, M., Kamataki, T., and Kato, R.,** Enhancement of binding of *N*-hydroxy-Trp-P-2 to DNA seryl tRNA synthetase, *Biochem. Biophys. Res. Commun.*, 102, 432, 1981.
183. **Shinohara, A., Saito, K., Yamazoe, Y., Kamataki, T., and Kato, R.,** DNA binding of *N*-hydroxy-Trp-P-2 and *N*-hydroxy-Glu-P-1 by acetyl coA-dependent enzyme in mammalian liver cytosol, *Carcinogenesis*, 6, 305, 1985.
184. **Nagao, M., Fujita, Y., Wakabayashi, K., and Sugimura, T.,** Ultimate forms of mutagenic and carcinogenic heterocyclic amines produced by pyrolysis, *Biochem. Biophys. Res. Commun.*, 114, 626, 1983.
185. **Saito, K., Yamazoe, Y., Kamataki, T., and Kato, R.,** Activation and detoxication of *N*-hydroxy-Trp-P-2 by glutathione transferases, *Carcinogenesis*, 4, 1551, 1983.
186. **Saito, K. and Kato, R.,** Glutathione conjugation of arylnitroso compounds: detection and monitoring labile intermediates in situ inside a fast atom bombardment mass spectrometer, *Biochem. Biophys. Res. Commun.*, 124, 1, 1984.
187. **Umemoto, A., Grivas, S., Yamaizumi, Z., Sato, S., and Sugimura, T.,** Non-enzymatic glutathione conjugation of 2-nitroso-6-methyldipyrido[1,2-a:3′,2′-d]imidazole (NOGlu-P-1) in vitro; *N*-hydroxysulfonamide, a new binding form of arylnitroso compounds and thiols, *Chem.-Biol. Interact.*, in press.
188. **Turesky, R. J., Skipper, P. L., Tannenbaum, S. R., Coles, B., and Ketterer, B.,** Sulfamate formation is a major route for detoxication of 2-amino-3-methylimidazo[4,5-f]quinoline in the rat, *Carcinogenesis*, 7, 1483, 1986.

188a. **Coles, B. and Ketterer, B.,** unpublished results.

189. **Turesky, R. J., Aeschbacher, H. U., Wurzner, H. P., Skipper, P. L., and Tannenbaum, S. R.,** Major routes of metabolism of the food borne carcinogen 2-amino-3,8-dimethylamidazo[4,5-f]quinoxaline in the rat, *Carcinogenesis*, 9, 1043, 1988.
190. **Hirose, M., Lee, M.-S., Wang, C. Y., and King, C. M.,** Induction of rat mammary gland tumors by 1-nitropyrene, a recently recognised environmental mutagen, *Cancer Res.*, 44, 1158, 1984.
191. **El-Bayoumy, K., Hecht, S. S., Sackl, T., and Stoner, G. D.,** Tumorigenicity and metabolism of 1-nitropyrene in A/J mice, *Carcinogenesis*, 5, 1449, 1984.
192. **Roberts, J. J. and Warwick, G. P.,** Mode of action of alkylating agents in formation of S-ethyl cysteine from ethyl methanesulphonate, *Nature*, 179, 1181, 1958.
193. **Hemminki, K.,** Dimethylnitrosamine adducts excreted in rat urine, *Chem.-Biol. Interact.*, 39, 139, 1982.
194. **Jensen, D. E., Lotlikar, P. D., and Magee, P. N.,** The in vitro methylation of DNA by microsomally activated dimethylnitrosamine and its correlation with formaldehyde production, *Carcinogenesis*, 2, 349, 1981.
195. **Frei, E., Bertram, B., and Wiessler, M.,** Reduced glutathione inhibits the alkylation by *N*-nitrosodimethylamine of liver DNA in vivo and microsomal fraction in vitro, *Chem.-Biol. Interact.*, 55, 123, 1985.
196. **Davidson, E., Rozman, R. S., and Smith, P. K.,** Metabolism of *bis*-β-chloroethyl sulfide (sulfur mustard gas), *Biochem. Pharmacol.*, 7, 65, 1961.
197. **Dulik, D. M. and Fenselau, C.,** Conversion of melphalan to 4-(glutathionyl)phenylalanine. A novel mechanism for conjugation by glutathione S-transferases, *Drug Metab. Disp.*, 15, 195, 1987.
198. **Smith, M. T., Evans, C. G., Doane-Setzer, P., Castro, V. M., Tahir, M. K., and Mannervik, B.,** Denitrozation of 1,3-*bis*(2-chloroethyl)-1-nitrosourea by class mu glutathione transferases and its role in cellular resistance in rat brain tumor cells, *Cancer Res.*, 49, 2621, 1989.
199. **Peterson, L. A. and Guengerich, F. P.,** Comparison of and relationships between glutathione S-transferase and cytochrome P-450 systems, in *Glutathione Conjugation*, Sies, H. and Ketterer, B., Eds., Academic Press, London, 1988, 193.
200. **Dekant, W., Lash, L. H., and Anders, M. W.,** Fate of glutathione conjugates and bioactivation of cysteine S-conjugates by cysteine conjugate β-lyase, in *Glutathione Conjugation*, Sies, H. and Ketterer, B., Eds., Academic Press, London, 1988, 415.
201. **van Bladeren, P. J., Breimer, D. D., Rotteveel-Smijs, G. M. T., DeJong, R. A., Buijs, W., and van der Gen, A.,** The role of glutathione conjugation in the mutagenicity of 1,2-dibromoethane, *Biochem. Pharmacol.*, 29, 2975, 1980.
202. **Ozawa, N. and Guengerich, F. P.,** Evidence for formation of an *S*-[2-(*N*′-guanyl)ethyl]glutathione adduct in glutathione-mediated binding of the carcinogen to DNA, *Proc. Natl. Acad. Sci. U.S.A.*, 80, 5266, 1983.
203. **Schasteen, C. S. and Reed, D. J.,** The hydrolysis and alkylation activities of *S*-(2-haloethyl)-L-cysteine analogues — evidence for extended half lifes, *Toxicol. Appl. Pharmacol.*, 70, 423, 1983.
204. **Inskeep, P. B. and Guengerich, F. P.,** Glutathione mediated binding of dibromoalkanes to DNA: specificity of rat glutathione S-transferases and dibromoalkane structure, *Carcinogenesis*, 5, 805, 1984.
205. **Ferscht, A.,** Enzyme structure and mechanism, W. H. Freeman, San Francisco, 1985.
206. **teKoppele, J. M., Coles, B., Ketterer, B., and Mulder, G. J.,** Stereoselectivity of rat liver glutathione transferase isoenzymes for α-bromoisovaleric acid and α-bromoisovalerylurea enantiomers, *Biochem. J.*, 252, 137, 1988.
207. **Igarashi, T., Irokawa, N., Ono, S., Ohmori, S., Ueno, K., and Kitagawa, H.,** Difference in the effect of phenobarbital and 3-methylcholanthrene treatment on subunit composition of hepatic glutathione S-transferase in male and female rats, *Xenobiotica*, 17, 127, 1987.
208. **Benson, A. M., Batzinger, R. P., Ou, S.-Y. L., Bueding, E., Cha, Y.-N., and Talalay, P.,** Elevation of hepatic glutathione S-transferase activities and protection against mutagenic metabolites of benzo(a)pyrene by dietary antioxidants, *Cancer Res.*, 38, 4487, 1978.
209. **Benson, A. M., Cha, Y.-N., Bueding, E., Heine, H. S., and Talalay, P.,** Elevation of extra-hepatic glutathione S-transferase and epoxide hydratase activities by 2(3)-tert-butyl-4- hydroxyanisole, *Cancer Res.*, 39, 2971, 1979.
210. **Di Simplicio, P., Jensson, H., and Mannervik, B.,** Identification of the isoenzymes of glutathione transferases induced by trans-stilbene oxide, *Acta Chem. Scand.*, *B*37, 255, 1983.
211. **Pearson, W. R., Windle, J. J., Morrow, J. F., Benson, A. M., and Talalay, P.,** Increased synthesis of glutathione S-transferases in response to anticarcinogenic antioxidants, *J. Biol. Chem.*, 258, 2052, 1983.
212. **Lam, L. K. T., Sparnins, V. L., and Wattenberg, L. W.,** Isolation and identification of kahweol palmitate and cafestrol palmitate as active constituents of green coffee beans that enhance glutathione S-transferase activity in the mouse, *Cancer Res.*, 42, 1193, 1982.

213. **Sparnins, V. L., Venegas, P. L., and Wattenberg, L. W.,** Glutathione S-transferase activity: enhancement by compounds inhibiting chemical carcinogenesis and by dietary constituents, *J. Natl. Cancer Inst.*, 68, 493, 1982.
214. **Sparnins, V. L., Chuan, J., and Wattenberg, L. W.,** Enhancement of glutathione S-transferase activity of the esophagus by phenols, lactones and benzyl isothiocyanate, *Cancer Res.*, 42, 1205, 1982.
215. **Agius, C. and Gidari, A. S.,** Effect of streptozotocin on the glutathione S-transferases of mouse liver cytosol, *Biochem. Pharmacol.*, 34, 811, 1985.
216. **David, R. M. and Nerland, D. E.,** Induction of mouse liver glutathione S-transferases by ethanol, *Biochem. Pharmacol.*, 32, 2809, 1983.
217. **Parchment, R. E. and Benson, A. M.,** Glutathione S-transferase isoenzymes of mouse intestine: differential induction by 2(3)-tert-butyl-4-hydroxyanisole, *Biochem. Biophys. Res. Commun.*, 119, 1015, 1984.
218. **Sparnins, V. L. and Wattenberg, L. W.,** Enhancement of glutathione S-transferase activity of the mouse forestomach by inhibition of benzo(a)pyrene-induced neoplasia of the forestomach, *J. Natl. Cancer Inst.*, 66, 769, 1981.
219. **Jakoby, W. B.,** *Enzymatic Basis of Detoxication*, Vols. I and II, Academic Press, New York, 1980.
220. **Ketterer, B., Tan, K. H., Meyer, D. J., and Coles, B.,** Glutathione transferases: a possible role in the detoxication of DNA and lipid hydroperoxides, in *Glutathione S-Transferases and Carcinogenesis*, Mantle, T. J., Pickett, C. B., and Hayes, J. D., Eds., Taylor & Francis, London, 1987, 149.