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THE ROLE OF GLUTATHIONE AND GLUTATHIONE S-TRANSFERASES IN MERCAPTURIC ACID BIOSYNTHESIS

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CONTENTS

I. Introduction

Many pathological conditions such as cancer are caused by exposure to toxic agents, and it is important that the toxic effects and metabolism of compounds to which living beings are exposed be understood. Most foreign compounds undergo chemical transformations in the body, including oxidations, reductions, hydrolyses, and syntheses (conjugations), and compounds may undergo one or more of these processes (Williams,

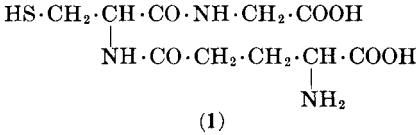
1959). In most cases transformations of foreign compounds result in less active or inert products. The duration of action and toxicity of an exogenous compound are often linked with its metabolism in the body. The transformations are generally catalyzed by enzymes, many of which occur in the liver. The type of chemical change which occurs depends on the structure of the foreign compound but factors such as species, diet, pretreatment with drugs, hormones, or other compounds, and route of administration are also important. The resultant metabolites and unchanged material are usually excreted through the kidney or bile duct, but other channels such as expired air may be involved.

Mercapturic acid biosynthesis is one transformation route that occurs to a greater or lesser extent in many species. Although mercapturic acids were first isolated in 1879, it was only recently established that the initial step in their biosynthesis was the formation of a conjugate between the foreign compound and glutathione. Earlier work on mercapturic acid formation has been reviewed (Williams, 1959; Barnes et al., 1959; Boyland, 1962).

II. Glutathione

A. PROPERTIES

Glutathione occurs in bacteria, plants, and animal tissues as a characteristic component of nearly all living cells. First isolated in a crude form by De Rey-Pailhade (1888), glutathione was isolated in a crystalline form from yeast by Hopkins (1921). Although his earlier work led Hopkins to believe that glutathione was a dipeptide, subsequent studies (Hopkins, 1929) showed that the molecule was a tripeptide containing glutamic acid, cysteine, and glycine. The correct formula (1) was deduced by Pirie and Pinhey (1929) and confirmed by synthesis (Harington and Mead, 1935).



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γ-L-Glutamyl-L-cysteinylglycine (glutathione) is a colorless crystalline compound. Four pK_a values, 2.12, 3.53, 8.66, and 9.12 (Cohn and Edsall, 1943), have been assigned to the two carboxyl, one sulfhydryl, and one amino groups, respectively (Isherwood, 1959). The isoelectric point is at pH 2.8.

The thiol group in glutathione is reactive, undergoing oxidation, forming mercaptides with metals, reacting with various compounds to form *S*-alkyl and *S*-acyl derivatives, and forming semimercaptals with carbonyl compounds (Wieland, 1954; Isherwood, 1959; Waley, 1966). The molecule can be hydrolyzed to the component amino acids. In strong acid, ring closure occurs (thiol group to γ-carbonyl group of glutamyl moiety) to form a thiazoline ring (Calvin, 1954; Goodman and Salce, 1965).

In nature, glutathione occurs in concentrations of 10–200 mg per 100 g tissue and is found mainly in the reduced form (GSH) which is readily interconvertible with the oxidized form (GSSG) (eq. 1):

$$2GSH \rightarrow GSSG + 2e + 2H^+ \quad (1)$$

Glutathione is relatively abundant in yeast and liver cells (Table I) but the amounts present in a given tissue are subject to variation with growth, nutritional state, and hormonal balance of the organism (Knox, 1960). In rat liver, breakdown and synthesis of glutathione are rapid with a half-life of about 4 hr (Waelsch and Rittenberg, 1942). Synthesis of the tripeptide from component amino acids by liver slices has been demonstrated (Braunstein et al., 1948; Bloch, 1949). Catabolism occurs in two stages (Binkley, Davenport, and Eastall, 1959): the glutamyl moiety is first removed by transpeptidation (Hanes, Hird, and Isherwood, 1950) catalyzed by γ-glutamyltransferase (glutathionase) (Kinoshita and Ball, 1953; Binkley, 1961); second, the remaining cysteinylglycine molecule is hydrolyzed to component amino acids by cysteinylglycinase (Binkley, 1952; Semenza, 1957).

TABLE I

Distribution of Glutathione (GSH) in Some Tissues (Glyoxalase Method)

Source	GSH (mg per 100 g tissue)	Ref.
Rat		
Liver	172	Woodward (1935)
Adrenal	109	Woodward (1935)
Brain	102	McIlwain, Martin, and Tresize (1957)
Spleen	92	Woodward (1935)
Kidney	77	Woodward (1935)
Heart	65	Hermann and Moses (1945)
Pancreas	56	Bhattacharya, Robson, and Stewart (1956)
Blood	39	Patterson and Lazarow (1954)
House fly	63	Lipke and Chalkley (1962)
Mosquito	53	Lipke and Chalkley (1962)
Yeast	128	Schroeder and Woodward (1939)
Pea seeds	54	Lawrence (1950)
Potato	11	Schroeder and Woodward (1939)

B. FUNCTIONS

The biological functions of glutathione are not fully understood, but it may be essential for the protection of thiol groups in proteins. It may protect cells against the toxic action of foreign substances including metals and hydrogen peroxide through the action of glutathione peroxidase (Mills, 1960). The utilization of glutathione for the biosynthesis of glutathione derivatives of foreign compounds is perhaps the first definite biochemical process in which glutathione is of obvious benefit to the organism. Glutathione is also involved in several enzymic reactions

as a substrate or coenzyme (Tables II and III). Enzymic reactions involving endogenous substrates (Table II) have been reviewed (Vennesland and Conn, 1954; Jocelyn, 1959; Mapson, 1959; Knox, 1960; Waley, 1966). Reactions outlined in Table III involve exogenous second substrates. Glutathione *S*-transferases (Table

TABLE II

Enzymes Requiring Glutathione (GSH) as a Substrate or Coenzyme

Enzyme system[a]	Source	Reaction catalyzed
GSSG reductase (1)	Yeast, liver	Conversion of GSSG to GSH
GSH peroxidase (2)	Erythrocytes	Oxidation of GSH by hydrogen peroxide
γ-Glutamyltransferase (3)	Kidney, pancreas	Removal of γ-glutamyl moiety from GSH by transpeptidation
γ-Glutamyl lactamase (4)	Pig liver	Removal of γ-glutamyl moiety from GSH as pyrrolidone carboxylic acid
GSH transhydrogenases (5–7)		Reduction of disulfides by GSH, e.g.:
	Rat liver	Insulin
	Ox liver	Homocystine
	Ox kidney	Coenzyme A-GSH disulfide (CoASSG)
Dehydroascorbic acid reductase (8)	Plants	Reduction of dehydroascorbic acid to ascorbic acid by GSH
Glyoxalase (9)	Animal tissues	Hydration of methyl glyoxal to lactic acid; GSH is required as a coenzyme
Maleylacetoacetate isomerase (10)	Liver	Isomerization of maleylacetoacetate to fumarylacetoacetate; GSH is required as a coenzyme
Formaldehyde dehydrogenase (11,12)	Liver, yeast	Oxidation of formaldehyde by NAD; GSH is required as a coenzyme
Indolylpyruvic acid keto–enol tautomerase (13)	Rat liver	Interconversion of tautomers of 3-indolylpyruvic acid; GSH is required as a coenzyme
Sulfur-oxidizing enzyme of *thiobacilli* (14)	*Thiobacillus thioparus*	Oxidation of sulfur to sulfite; GSH is required as a coenzyme

[a] References: (1) Rall and Lehninger (1952); (2) Mills (1957); (3) Binkley and Nakamura (1948); (4) Connell and Hanes (1956); (5) Narahara and Williams (1959); (6) Racker (1955); (7) Chang and Wilken (1966); (8) Crook and Hopkins (1938); (9) Racker (1951); (10) Edwards and Knox (1956); (11) Strittmatter and Ball (1955); (12) Rose and Racker (1962); (13) Spencer and Knox (1962); (14) Suzuki and Silver (1966).

III) are detoxicating enzymes since by catalyzing conjugation with glutathione and rendering nonpolar foreign molecules more hydrophilic, they initiate the first stage of the mercapturic acid pathway for the elimination of foreign compounds from the body. In no case has a glutathione adduct or the mercapturic acid derivative been found to be more toxic than the precursor.

TABLE III

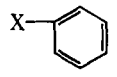
Enzymes Catalyzing the Detoxication of Foreign Compounds by Glutathione (GSH)

Enzyme[a]	Source	Reaction catalyzed
GSH *S*-aryltransferase (1–3)	Liver of many species	Replacement of labile aryl halogen and nitro groups by GSH
GSH *S*-epoxidetransferase (4)	Rat liver; rat kidney	Conjugation of some alkyl and aryl epoxides with GSH
GSH *S*-alkyltransferase (5)	Liver and kidney of several species	Replacement of some alkyl halogen and possibly nitro groups by GSH
GSH *S*-aralkyltransferase (6)	Rat liver; rat kidney	Replacement of labile aralkyl halogen and possibly ester groups by GSH
GSH *S*-alkenetransferases (7–9)	Liver of several species; rat kidney	Conjugation of some $\alpha\beta$-unsaturated compounds with GSH: six different GSH *S*-alkenetransferases catalyzing conjugations of GSH with (i) *cis*-esters, (ii) *trans* esters, (iii) cyclic ketones, (iv) vinyl ketones, (v) *trans* open-chain ketones, and (vi) *trans* aldehydes have been detected
Phosphoric acid triester: glutathione alkyl transferase (10,11)	Rat liver	*O*-Dealkylation of phosphate triesters in the presence of GSH
Nitroglycerine reductase (12,13)	Liver of some species	Denitration of glyceryl trinitrate in the presence of GSH
DDT dehydrochlorinase (14)	DDT-resistant house flies	Removal of "HCl" from DDT in the presence of GSH
Enzyme synthesizing dimethyl selenide from sodium selenite (15)	Mouse liver	Synthesis of dimethyl selenide from sodium selenite in the presence of GSH

[a] References: (1) Booth, Boyland and Sims (1961); (2) Combes and Stakelum (1961); (3) Al-Kassab, Boyland and Williams (1963); (4) Boyland and Williams (1965); (5) Johnson (1966a); (6) Suga, Ohata, Kumaoka, and Akagi (1967); (7) Boyland and Chasseaud (1967); (8) Boyland and Chasseaud (1968); (9) Chasseaud (1967); (10) Fukami and Shishido (1966); (11) Hutson, Pickering, and Donninger (1968); (12) Heppel and Hilmoe (1950); (13) Needleman and Hunter (1965); (14) Lipke and Kearns (1959); (15) Ganther (1966).

III. Mercapturic Acid Biosynthesis

Baumann and Preusse (1879), investigating the metabolism of bromobenzene (**2**, X = Br), and Jaffé (1879), that of chlorobenzene (**2**, X = Cl), showed that these monohalogenobenzenes were excreted as unstable



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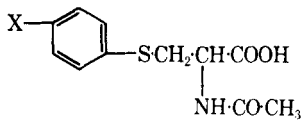
complexes containing acetylcysteine residues, which, on mild treatment with acid, gave arylmercapturic acids (**3**, X = Br or Cl). The nature of the complexes was not ascertained at the time, but compounds con-



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taining *N*-acetyl-L-cysteine residues are called mercapturic acids. Many mercapturic acids have been found in the urine of various animal species [see Stekol (1941) for references to earlier work] to which foreign compounds had been administered (Table IV).

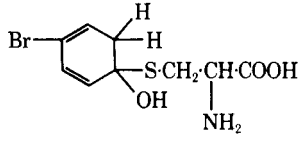
A. SOURCES OF THE CYSTEINE MOIETY

Possible sources of the cysteine moiety in mercapturic acid formation that have been considered include dietary protein, cysteine residues of tissue proteins, and glutathione which is particularly abundant in liver cells.

Stekol (1937a–e, 1939a) found that addition of glutathione or sulfur-containing amino acids to the food of animals given balanced diets did not increase the amount of mercapturic acid formed from bromobenzene or naphthalene. Barnes and James (1957) obtained similar results after administration of cysteine, cystine, or methionine to rabbits followed by the mercapturic acid precursors, chlorobenzene, 1,2,3,5-tetrachloro-6-nitrobenzene, 1,2,3-trichloro-5-nitrobenzene, or benzyl chloride. Experiments by Gutman and Wood (1950) and Marsden and Young (1958), using [^{35}S]-cystine administered to rats simultaneously with bromobenzene and naphthalene, showed that only a small proportion ($<10\%$) of labeled material was excreted as a mercapturic acid. Binkley (1949) demonstrated that the administration of bromobenzene to cystinuric dogs did not decrease excretion of cystine by the animals.

Stekol (1939b) and Mills and Wood (1956) had concluded that the initial stage in mercapturic acid formation involved conjugation of the foreign compound with the cysteine of tissue proteins. Following oral administration of [^{131}I]-iodobenzene to the rat, labeled tissue proteins were isolated (Mills and Wood, 1956), but the radioactivity could have been due to adsorbed molecules not removed by standard washing techniques (Mills and Wood, 1956). A possible mechanism for the formation of mercapturic acids from tissue protein was proposed by Smith, Spencer, and Williams (1950). However, assessment of the relative rates of liver protein synthesis and of mercapturic acid formation led Barnes et al. (1959) to conclude that tissue proteins were unable to provide sufficient cysteine for mercapturic acid production.

Other mechanisms for mercapturic acid biosynthesis have been suggested. Sherwin (1922) proposed that bromobenzene was hydroxylated to *p*-bromophenol, the hydroxyl group was replaced with cysteine, and the product acetylated to give the mercapturic acid. However, halogenophenols are not metabolized to mercapturic acids (Spencer and Williams, 1950) so the mechanism proposed by Sherwin (1922) cannot be considered likely. Rhode (1923) suggested that an intermediate partially reduced ring structure (**4**) was formed *in vivo* from bromobenzene, containing both hydroxyl and cysteine groups.



(**4**)

A perthiol oxidation mechanism was suggested by Teague (1954), whereby the mercapturic acid precursor forms a dihydrodithiol intermediate which conjugates with serine, loses hydrogen sulfide, and is acetylated to the mercapturic acid.

B. GLUTATHIONE AS THE SOURCE OF CYSTEINE

It is remarkable that all three amino acids present in glutathione are used in the metabolism of foreign compounds. Man and some primates excrete phenylacetic acid as the glutamine conjugate, benzoic acid as the glycine conjugate (hippuric acid), and bromobenzene as *p*-bromophenylmercapturic acid. Waelsch (1930) and others (Brand and Harris, 1933) had suggested that glutathione could provide the three constituent amino acids for conjugation with foreign molecules, and although later

TABLE IV

Mercapturic Acid Precursors

Precursor	Group reacting with thiol	Reference to mercapturic acid excretion
Aromatic hydrocarbon[a]	Epoxide intermediate	
Benzene		Zbarsky and Young (1943b)
Naphthalene		Bourne and Young (1934)
Anthracene		Boyland and Levi (1936)
Phenanthrene		Boyland and Sims (1962)
Benz[*a*]anthracene		Boyland and Sims (1964a)
Styrene		James and White (1967)
Arylamine	Hydroxylamine intermediate	
Aniline		Boyland, Manson, and Nery (1963)
2-Naphthylamine		Boyland, Manson, and Nery (1963)
Acetophenetidine[b]		Jagenburg and Toczko (1964)
Arylhalide[a]	Epoxide intermediate	
Bromobenzene		Baumann and Preusse (1879)
Chlorobenzene		Jaffé (1879)
Iodobenzene		Mills and Wood (1953)
1,2-Dichlorobenzene		Parke and Williams (1955)
1-Chloronaphthalene		Cornish and Block (1958)
Halogenonitrobenzene		
1,2-Dichloro-4-nitrobenzene	Cl	Bray, James, and Thorpe (1957a)
1-Bromo-3-chloro-6-nitrobenzene	Br	Bray, James, and Thorpe (1957a)
Pentachloronitrobenzene	NO_2	Betts, James, and Thorpe (1955)
Aralkyl halide		
Benzyl chloride	Cl	Stekol (1938)
Phenethyl bromide	Br	James and White (1967)
1-Menaphthyl chloride	Cl	Hyde and Young (1965)
Aralkyl ester		
Benzyl acetate	$O \cdot CO \cdot CH_3$	Clapp (1967)
1-Menaphthyl acetate	$O \cdot CO \cdot CH_3$	Hyde and Young (1968)
Alkyl phenol		
3,5-Di-*tert*-butyl-4-hydroxytoluene	H of ring methyl	Daniel, Gage, and Jones (1968)
Alkyl halide		
Iodomethane	I	Barnsley and Young (1965)
Bromoethane	Br	Thomson, Maw, and Young (1958)
Allyl chloride	Cl	Clapp (1967)
Nitroalkane		
1-Nitropropane	NO_2	Bray and James (1958)

TABLE IV (*Continued*)

Precursor	Group reacting with thiol	Reference to mercapturic acid excretion
Cycloalkene[a]		
Cyclopentene	Epoxide intermediate	James, Waring, and White (1967)
Halogenocycloalkane		
Bromocyclohexane	Br	James and Jeffery (1964)
Carboxylic acid[b]		
Maleic acid (or fumaric acid)	$\alpha\beta$ Double bond	Kuwaki and Mizuhara (1966)
Isovaleric acid	H on α-carbon	Ohmori and Mizuhara (1962)
Ester		
Ethyl methanesulfonate	$O_3S \cdot CH_3$	Roberts and Warwick (1958a)
Urethane	$O \cdot CO \cdot NH_2$	Boyland and Nery (1965)
Sulfonamide		
Benzothiazole-2-sulfonamide	$SO_2 \cdot NH_2$	Colucci and Buyske (1965)
Sulfur mustard		
Bis-β-chloroethyl sulfide[c]	Cl	Davison, Rozman, and Smith (1961)
$\alpha\beta$-Unsaturated compound		
Ethacrynic acid[b]	$\alpha\beta$ Double bond	Beyer, Baer, Michaelson, and Russo (1965)
Arecoline	$\alpha\beta$ Double bond	E. Boyland and R. Nery (unpublished work)

[a] These compounds are first converted to an intermediate epoxide

[b] Excreted as a cysteine conjugate.

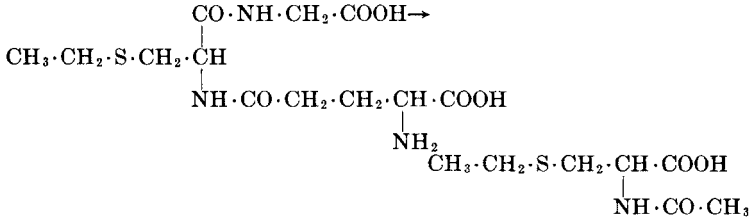
[c] Excreted as glutathione and cysteine conjugates.

experiments (Waelsch and Rittenberg, 1941) failed to show that the glycine moiety of glutathione was used in hippuric acid formation, the possibility that the cysteine moiety was used in mercapturic acid biosynthesis had not been excluded (Waelsch, 1952).

Nakashima (1934) showed that administration of naphthalene to rabbits depleted the glutathione content of the eye lens and liver. A decrease in liver glutathione of the same species dosed with bromobenzene was related to the excretion of the corresponding mercapturic acid (Yamamoto, 1940). A similar parallelism was observed in the rat for a variety of mercapturic acid precursors by Barnes et al. (1959), but glutathione levels in the blood, kidney, and intestine were unchanged. Barnes et al. (1959) also showed that the turnover rate for glutathione in liver was sufficient to sustain mercapturic acid biosynthesis. Other

workers have observed decreases of liver glutathione in animals after administration of mercapturic acid precursors (Binet and Wellers, 1951; Johnson, 1965; Suga, Ohata, and Akagi, 1966).

Stekol (1940, 1941) administered glutathione derivatives of benzyl chloride and *p*-bromobenzyl bromide to rats and isolated the corresponding mercapturic acids from the urine: an analogous result was obtained when the administration of *S*-ethylglutathione resulted in the excretion of ethylmercapturic acid (eq. 2) by rats (Roberts and Warwick, 1958a).



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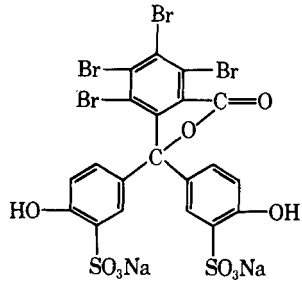
Foxwell and Young (1964) isolated alkylmercapturic acids from the urine of rats dosed with *S*-alkylglutathiones. The corresponding glutathione conjugates have been detected in liver extracts from rabbits and rats after the administration of mercapturic acid precursors, *p*-chlorobenzyl chloride (Bray, Franklin, and James, 1959a) and methyl iodide (Johnson, 1966b). These findings are consistent with the hypothesis that conjugation of glutathione with the foreign compound is a primary step in mercapturic acid formation.

Confirmatory evidence was obtained by Booth et al. (1961), who showed that an enzyme in the supernatant fraction of rat liver homogenates catalyzed the conjugation of glutathione with several foreign compounds, including some that are metabolized to mercapturic acids. This enzyme, which has been named glutathione *S*-aryltransferase (Grover and Sims, 1964), was specific for glutathione but active toward a variety of second substrates. Combes and Stakelum (1961) independently described an enzyme that catalyzed the conjugation of glutathione with sulfobromophthalein (**5**), a dye used as a test for hepatic function (Rosenthal and White, 1925). This enzyme may be glutathione *S*-aryltransferase. A biliary metabolite considered to be the glutathione conjugate has been found in rats (Combes, 1959) and in man (Grodsky, Carbone, and Fanska, 1959) after the administration of sulfobromophthalein (**5**).

The bile of rats dosed with naphthalene contained naphthalene conjugates of glutathione, cysteinylglycine, cysteine, and *N*-acetylcysteine (Boyland, Ramsey, and Sims, 1961), and Boyland (1962) considered the



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changes in mercapturic acid biosynthesis to proceed in the above order since γ-glutamyl-*S*-cysteine derivatives have not been detected. The bile of rats treated with 1,2-dihydronaphthalene and 1,2-epoxy-1,2,3,4-tetrahydronaphthalene contained similar conjugates that can be considered as derivatives of 1,2,3,4-tetrahydronaphthalene (Boyland, Ramsey, and Sims, 1961).

C. BREAKDOWN OF GLUTATHIONE CONJUGATE

Bray and Franklin (1957) demonstrated that liver homogenates from rat, rabbit, and guinea pig liberated *S*-*p*-chlorobenzyl-L-cysteine from *S*-*p*-chlorobenzylglutathione, and the rate of cysteine derivative liberation was parallel to the glutathionase (γ-glutamyltransferase) activity of the preparations. Booth, Boyland, and Sims (1960) have shown that the glutathione conjugate of naphthalene was converted into the cysteine derivative by rat kidney homogenates. This conversion probably proceeds by an initial transpeptidation, involving γ-glutamyltransferase, to give the cysteinylglycine derivative of naphthalene which is then converted to the cysteine derivative. Of 12 rat tissues examined, only the kidney and pancreas were able to transfer glutamyl residues (Revel and Ball, 1959), but in other species, such as the rabbit, all the stages of mercapturic acid formation can occur in the liver. Cysteinylglycine derivatives are hydrolyzed by peptidases (i.e., cysteinylglycinase), present in rat kidney, liver, and pancreas, to cysteine derivatives with removal of glycine (Bray, Franklin and James, 1959a; Booth, Boyland, Sato, and Sims, 1960; Suga, Kumaoka, and Akagi, 1966).

Stekol (1938) isolated *N*-acetyl-*S*-benzylcysteine (benzylmercapturic acid) from the urine of dogs, rats, and rabbits dosed with *S*-benzylcysteine. Zbarsky and Young (1943a) isolated phenylmercapturic acid from the urine of rats following administration of *S*-phenylcysteine in the diet. West and Mathura (1954) showed that 12 arylcysteines were

acetylated to the mercapturic acid in the rat. Acetylation of the cysteine derivative is the final stage in mercapturic acid biosynthesis and has been demonstrated *in vivo* and *in vitro* with liver homogenates or slices (Gutman and Wood, 1951; West, Mathura, and Black, 1951; Bray, Franklin, and James, 1959b; Booth, Boyland, and Sims, 1960; Thomson, Barnsley, and Young, 1963; Barnsley, Grenby, and Young, 1966).

The stages in the formation of a mercapturic acid from 1,2-dichloro-4-nitrobenzene are shown in Figure 1. Initial conjugation is effected by replacement of the labile chlorine atom of the halogenonitrobenzene by glutathione, the reaction being catalyzed by glutathione *S*-aryltransferase (Booth, Boyland, and Sims, 1961). The glutathione conjugate is catabolized in a similar way to glutathione itself, in liver, kidney, and intestine, and the cysteine derivative is acetylated by an acetylase and acetyl-coenzyme A.

IV. Glutathione *S*-Transferases

It has been shown that the initial step in mercapturic acid formation is conjugation of the foreign compound with glutathione, a reaction catalyzed by glutathione *S*-transferases for many substrates. Since glutathione *S*-aryltransferase was described (Booth, Boyland, and Sims, 1961), several other glutathione *S*-transferases have been found: glutathione *S*-epoxidetransferase, catalyzing the reaction of glutathione with many epoxides (Boyland and Williams, 1965); glutathione *S*-alkyltrans-

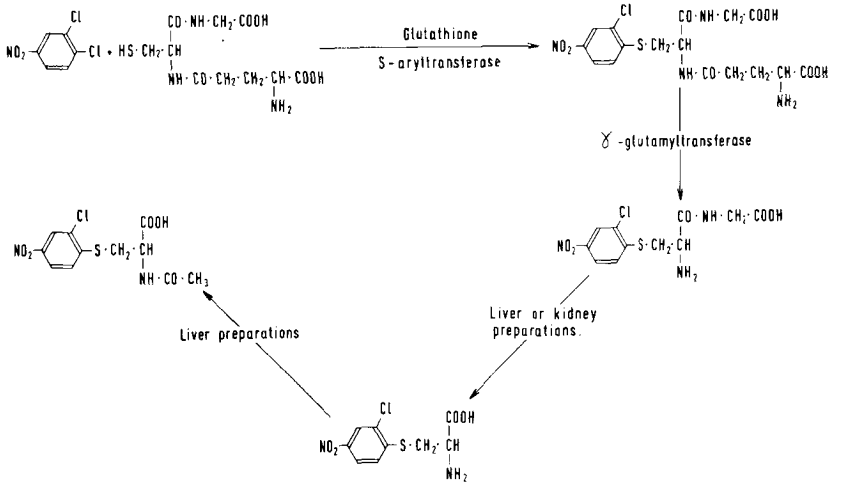


Fig. 1. Mercapturic acid formation from 1,2-dichloro-4-nitrobenzene.

ferase, catalyzing the *S*-alkylation of various alkyl halides (Johnson, 1966a); and several glutathione *S*-alkenetransferases catalyzing the reaction of glutathione with $\alpha\beta$-unsaturated compounds (Boyland and Chasseaud, 1967, 1968).

Reaction of glutathione with some glutathione *S*-transferase substrates is negligible at neutral pH and 37°C or less in the absence of the appropriate enzyme. Other substrates react non-enzymically, although the rate is increased by the addition of enzyme (Booth, Boyland, and Sims, 1961; Johnson, 1966a; Boyland and Chasseaud, 1968). In some cases the reaction of mercapturic acid precursors with glutathione is unaffected by rat liver supernatant, and conjugate formation apparently takes place nonenzymically.

A. GLUTATHIONE *S*-ARYLTRANSFERASE

Using a spectrophotometric assay, glutathione *S*-aryltransferase was studied by Booth, Boyland, and Sims (1961) with 1,2-dichloro-4-nitrobenzene (see Fig. 1) as the second substrate. This enzyme was present in the supernatant fraction of rat liver homogenates and showed a broad pH optimum about pH 8.0. Glutathione *S*-aryltransferase occurs in the livers of many vertebrates (Grover and Sims, 1964; Wit and Leeuwaugh, 1968), but in the rat only small amounts of the enzyme are present in organs other than the liver.

1,2-Dichloro-4-nitrobenzene is particularly suitable for the estimation of glutathione *S*-aryltransferase since, when this compound is conjugated with glutathione, the absorption spectrum changes to a longer wavelength (Fig. 2) (Booth et al., 1961). Measurement at this new wavelength gives a direct reading of the enzymic reaction occurring. Other glutathione *S*-transferases are generally estimated by measuring substrate loss (Boyland and Williams, 1965; Johnson, 1966a; Boyland and Chasseaud, 1967), release of labile groups (Al-Kassab et al., 1963; Boyland and Williams, 1965; Johnson, 1966a), or the reaction products are detected (Booth et al., 1961) or estimated (Suga, Ohata, Kumaoka, and Akagi, 1967) by chromatography.

Glutathione *S*-aryltransferase catalyzes reactions of glutathione with aromatic and other cyclic compounds containing labile halogen or nitro groups. Compounds of this type, which are metabolized to mercapturic acids in rabbits (Table V) include mono-, di-, and trihalogenonitrobenzenes (Bray, James, and Thorpe, 1955a,b, 1956, 1957a, 1958a). This same enzyme probably conjugates glutathione with certain tetrahalogenonitrobenzenes that are metabolized to mercapturic acids (Bray, Hybs, James, and Thorpe, 1953; Betts, James, and Thorpe, 1955) by replace-

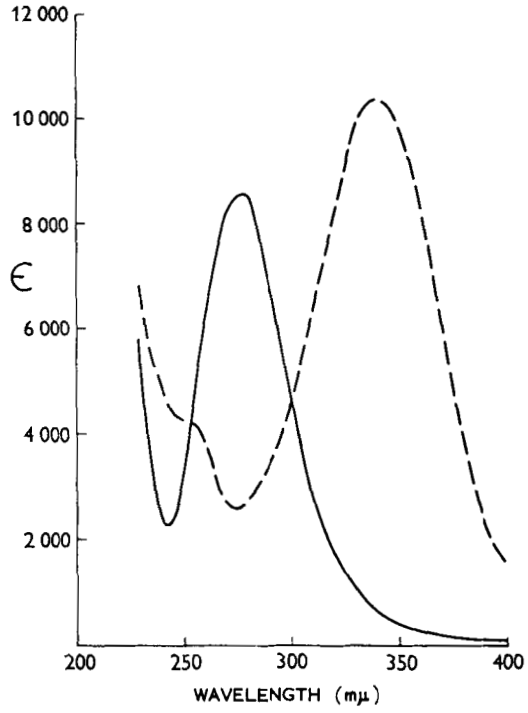
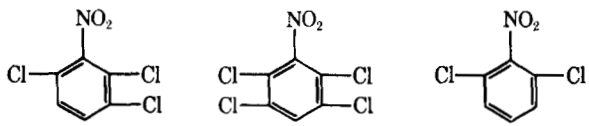


Fig. 2. Absorption spectra in 0.1 M pyrophosphate buffer, pH 8.0: (———) 1,2-dichloro-4-nitrobenzene; (– – –) *S*-(2-chloro-4-nitrophenyl)glutathione.

ment of nitro groups (Al-Kassab et al., 1963). The nitro group is also lost during mercapturic acid biosynthesis from certain trihalogenonitrobenzenes and from pentahalogenonitrobenzene (Betts, James, and Thorpe, 1955; Betts, Bray, James, and Thorpe, 1957), and it appears that the nitro group is lost where it is flanked by halogen atoms and there is another halogen atom in the ring, viz., **6** and **7**. With 1,3-dichloro-2-nitrobenzene (**8**), however, although the nitro group has two neighboring halogen atoms, an additional halogen atom is not present in the ring and no mercapturic acid is formed (Bray, James, and Thorpe, 1957b). In other cases, the halogen atom *ortho* to the nitro group is



(6) (7) (8)

TABLE V

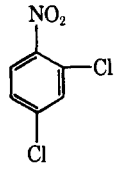
Chloromononitrobenzenes Excreted as Mercapturic Acids after Administration to Rabbits (Adapted from Bray, James, and Thorpe, 1957b)

Position of chlorine atoms[a]	Group replaced by acetylcysteine, and position	Mercapturic acid excreted in urine (% of dose)
2:3:4:5:6[b]	NO_2	37
2:3:4:5[b]	Cl, 2	6
2:3:4:6	NO_2	37
2:3:5:6[b]	NO_2	36
2:3:4	Cl, 2	41
2:3:5	Cl, 2	21
2:3:6	NO_2	10
2:4:5	Cl, 2	30
2:4:6	NO_2	<1
3:4:5	Cl, 4	24
2:3	None	0
2:4	Cl, 2	53
2:5	Cl, 2	27
2:6	None	0
3:4	Cl, 4	45
3:5	None	0
2 (*ortho*)	Cl, 2	7
3 (*meta*)	None	0
4 (*para*)	Cl, 4	7

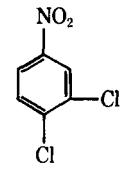
[a] The nitro group is in position 1.

[b] From 33 to 62% of these compounds were unabsorbed.

replaced as in **9**, but *para* replacement occurs where the *ortho* group is absent as in 10. Loss of halogen from a position *meta* to the nitro group has not been observed. The reactivity of a particular center on the aromatic ring is governed by electronic and steric effects exerted by neighboring groups. Thus, certain dihalogenonitrobenzenes form more mercapturic acid than monohalogenonitrobenzenes (Bray et al., 1957a):



(9)

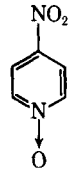


(10)

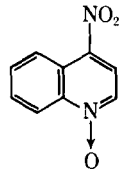
however, no mercapturic acid formation has been observed from dihalogenonitrobenzenes containing groups adjacent or symmetrical in position (Bray et al., 1957b), and the major metabolites are dichloroanilines and glucuronic and sulfuric acid conjugates of phenols.

The stability of the nitro group of some polyhalogenonitrobenzenes has been compared with the extent of mercapturic acid formation; increased lability of the respective halogen or nitro group during alkaline hydrolysis *in vitro* was related to increased mercapturic acid formation *in vivo* (Betts, James, and Thorpe, 1955; Bray et al., 1957a). Examination of the tumor initiatory activity of pentachloronitrobenzene and three tetrachloronitrobenzenes on mouse skin (Searle, 1966) showed that the compound with a stable nitro group, 1,2,3,4-tetrachloro-6-nitrobenzene, was the most potent. This compound only forms very small amounts of mercapturic acid *in vivo* by replacement of a chlorine atom.

The reactions of glutathione with 4-nitropyridine *N*-oxide (**11**) and the carcinogenic 4-nitroquinoline *N*-oxide (**12**) are probably catalyzed by glutathione *S*-aryltransferase (Al-Kassab et al., 1963): the latter reacts rapidly with thiols even in the absence of the enzyme (Nakahara and Fukuoka, 1959).



(**11**)



(**12**)

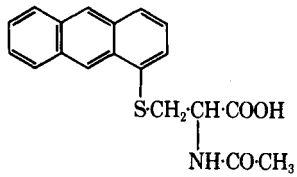
Conjugation of sulfobromophthalein (**5**) with glutathione (Combes and Stakelum, 1961) is possibly catalyzed by glutathione *S*-aryltransferase. Conjugation appears to affect the rate of transport of **5** from liver cells to bile, since the conjugate is transported more rapidly than the free dye (Combes, 1965), but Boyland and Grover (1967) suggested that conjugation was not the rate-limiting step in hepatic dye clearance. In a study of species variation in metabolism, storage, and excretion of the dye, Klaassen and Plaa (1967) showed that biliary excretion was probably the limiting factor in the elimination of (**5**) from the plasma.

Glutathione *S*-aryltransferase activity is present in several species of insect (Cohen and Smith, 1964; Cohen, Smith, and Turbert, 1964); the insect enzyme is inhibited by phthaleins, but the mammalian enzyme is not. Clark, Darby, and Smith (1967) found differences in the glutathione-binding sites of glutathione *S*-aryltransferase from sheep liver and grass grub and showed that the affinity of the enzyme for each sub-

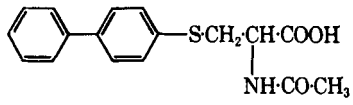
strate (glutathione or aryl compound) was not greatly affected by its combination with the other. House fly supernatant preparations can carry out the mercapturic acid biosynthesis (Sims and Grover, 1965).

B. GLUTATHIONE *S*-EPOXIDETRANSFERASE

Mono-, di-, and trihalogenobenzenes are metabolized to arylmercapturic acids (Smith et al., 1950; Azouz, Parke, and Williams, 1955; Jondorf, Parke, and Williams, 1955; Parke and Williams, 1955), the proportion of which decreases from 25% to 1% of the administered dose with increased halogen substitution in the ring. A free *para* position is required (Azouz et al., 1955) since the hydrogen atom *para* to the halogen is generally replaced by the acetylcysteine residue. Arylmercapturic acids have been isolated from the acidified urine of animals dosed with aromatic hydrocarbons, e.g., 1-anthrylmercapturic acid (**13**) (Boyland and Levi, 1936) and *p*-biphenylmercapturic acid (**14**) (West, Lawson, Miller, and Mathura, 1956) from anthracene and biphenyl, respectively.

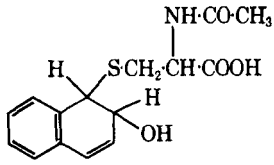


(13)



(14)

In the original work on monohalogenobenzene metabolism (Baumann and Preusse, 1879; Jaffé, 1879), it was recognized that the isolated mercapturic acids were probably excreted in the urine as acid-labile precursors. These precursors have been called "premercapturic acids" and are excreted from animals after administration of benzene, naphthalene, anthracene, and monohalogenobenzenes (Knight and Young, 1958), but they are mercapturic acids and the term premercapturic acid seems unnecessary and misleading. The ammonium salt of the acid-labile mercapturic acid formed from naphthalene by rabbits has been isolated and shown to be *N*-acetyl-*S*(1,2-dihydro-2-hydroxy-1-naphthyl)-L-cysteine (**15**) (Boyland and Sims, 1958). Treatment of this compound (**15**) with cold mineral acid yielded 1-naphthylmercapturic acid together with naphthalene, 1-naphthol, and 2-naphthol. The acid-labile mercapturic acid (**16**) produced by rats and rabbits dosed with bromobenzene has been isolated as the dicyclohexylamine salt (Gillham and Young, 1967). Acid-labile mercapturic acids are probably formed



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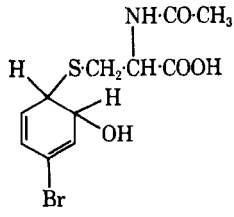
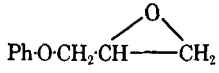


(16)

through an intermediate epoxide (Boyland and Sims, 1958, 1960) that is then conjugated with glutathione. Further evidence for the existence of the epoxide intermediate suggested by Boyland (1950) and Boyland and Sims (1958, 1960) was obtained when Booth, Boyland, and Sims, (1961) showed that *in vitro* conjugations of glutathione with iodobenzene, naphthalene, and phenanthrene would only occur in the presence of rat liver supernatant if rat liver microsomes and NADPH were added and oxygen was present. Although the intermediates were not isolated, a perhydroxylating system was required for the prior formation of the epoxide, since the conjugation of glutathione with 1,2-epoxy-1,2,3,4-tetrahydronaphthalene was catalyzed by rat liver supernatant alone.

Boyland and Williams (1965) showed that the enzyme in rat liver supernatant catalyzing conjugations of glutathione with epoxides was different from glutathione *S*-aryltransferase. 2,3-Epoxypropyl-phenyl ether (**17**) was employed as the second substrate in studies with this



(17)

enzyme because it reacted only slowly with glutathione in the absence of the enzyme. This enzyme, named glutathione *S*-epoxidetransferase, had a pH optimum at pH 6.5 and occurred also in rat kidney and in the livers of other vertebrates (Boyland and Williams, 1965; Wit and Snel, 1968). It is probably responsible for *in vivo* conjugations of glutathione with epoxy derivatives of halogenobenzenes, aromatic hydrocarbons, and other compounds. Some epoxides react with glutathione in the

TABLE VI

Reaction of Epoxides with Glutathione at pH 6.5 and 25° for 1 hr

Which do not react either chemically or enzymically	Which react chemically and at an increased rate enzymically	Which react enzymically but not chemically
1,2-Epoxyethane-1,2-dicarboxylic acid	1,2-Epoxypropane	2,3-Epoxypropyl phenyl ether
9,10-Epoxystearic acid	DL-1,2:3,4-Diepoxybutane	2,3-Epoxypropylbutyrate
1,4-2,3-Diepoxy-1,2,3,4-tetrahydronaphthalene	1,2:5,6-Diepoxyhexane	Styrene oxide
Scopolamine	Triethylene glycol bis-2,3-epoxypropyl ether (Epidyl)	1,2-Epoxy-1,2,3,4-tetrahydronaphthalene
Dieldrin	2,3-Epoxypropyl methacrylate	Resorcinol bis-2,3-epoxypropyl ether

absence of enzyme, others do not react even with the enzyme, and some only if the enzyme is present (Table VI).

The probable pathway for the formation of the acid-labile mercapturic acid from naphthalene is outlined in Figure 3. Although the intermediate epoxide from naphthalene has been prepared synthetically (Vogel and Klärner, 1968), it is very unstable and its metabolism has not yet been studied. Evidence to support the existence of this com-

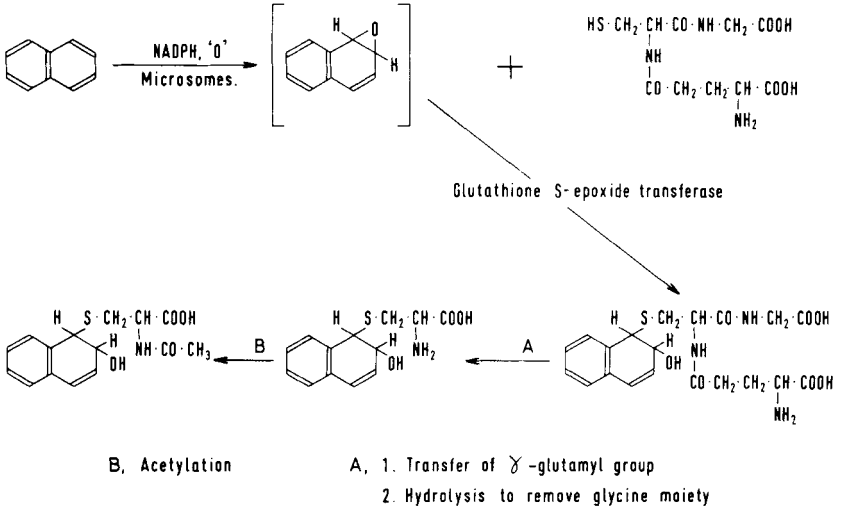
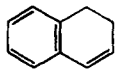
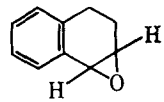


Fig. 3. Formation of acid-labile mercapturic acid from naphthalene.

pound as an intermediate was provided from work on the metabolism of 1,2-dihydronaphthalene (**18**) and its epoxide, 1,2-epoxy-1,2,3,4-tetrahydronaphthalene (**19**). Similar types of metabolites were isolated after both compounds were administered to rabbits (Boyland and Sims, 1960).



(18)



(19)

Acid-labile mercapturic acid derivatives have also been obtained as metabolites of the polycyclic hydrocarbons phenanthrene (Boyland and Sims, 1962) and benz[*a*]anthracene (Boyland and Sims, 1964a). It was shown that formation of acid-labile mercapturic acids from these hydrocarbons proceeds through intermediate epoxides that have been synthesized (Boyland and Sims, 1965a). In these molecules there are a number of reactive double bonds which can undergo epoxidation, but the nature and amounts of the detected metabolites indicated that mercapturic acid formation occurred mainly on the double bond in the *K* region, which is the position in the molecule of the greatest electron density: a measure of this property is given by the bond order of the bond concerned. The amounts of the metabolites of phenanthrene and benz[*a*]anthracene (Fig. 4) produced by mice, rats, and rabbits, has been related to bond order (Boyland, 1964), and it was shown that the relative amounts of metabolites derived from a particular bond increased with increasing bond order (Table VII).

Most known carcinogenic polycyclic hydrocarbons have a high electron density in the *K* region but the role that this bond plays in carcinogenesis remains unknown. The *K* region differs from the other bonds in the molecule in that the metabolism of these compounds yields preferentially mercapturic acids in which the *K* region is involved.

Early work on the metabolism of carcinogenic hydrocarbons indicated that metabolic processes took place in other regions of the molecule, e.g., the metabolic or *M* region (see Fig. 4 and Pullman and Pullman, 1955).

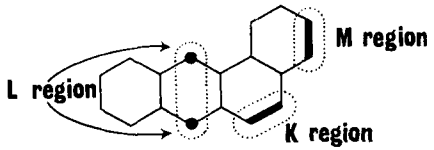
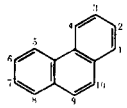
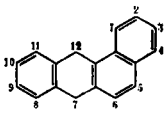


Fig. 4. Benz[*a*]anthracene.

TABLE VII

Relative Amounts of Metabolites of Phenanthrene, and of Benz[*a*]anthracene, Produced by Mice, Rabbits, or Rats in Relation to Bond Orders (From Boyland, 1964)

Bond	Bond order	Relative amounts of metabolites	
	Phenanthrene		
		In urine	Formed by microsomes
2,3 ≡ 6,7	0.623	—	—
3,4 ≡ 5,6	0.702	+	++
1,2 ≡ 7,8	0.705	+++	+++
9,10	0.775	++++	++++
	Benz[*a*]anthracene		
9,10	0.593		—
2,3	0.628		—
1,2	0.695		+
3,4 (*M* region)	0.700		++
10,11	0.731		+
8,9	0.732		+++
5,6 (*K* region)	0.783		++++

It was thought that this was because the *K* region was protected by combination with some enzyme center or other tissue constituent. The reason for the failure of earlier workers to detect or isolate the metabolic products in which the *K* region was involved was due to the instability of these products in acid. When *N*-acetyl-*S*(1,2-dihydro-2-hydroxy-1-naphthyl)-L-cysteine (**15**), which is a metabolite of naphthalene, is treated with cold dilute acid, it loses water to yield mainly the 1-naphthylmercapturic acid. The corresponding mercapturic acid derivatives of phenanthrene and benz[*a*]anthracene decomposed in acid mainly to yield the parent hydrocarbons and diacetylcystine. In early work on hydrocarbon metabolism, the urine was often acidified so that these mercapturic acid derivatives in which the *K* region had reacted were decomposed and not detected.

The *K* region therefore reacts in a different way from the other double bonds of the molecule. The reaction between some epoxides and glutathione, which is an essential stage in mercapturic acid biosynthesis, is catalyzed by glutathione *S*-epoxidetransferase (Boyland and Williams, 1965). 9,10-Epoxy-9,10-dihydrophenanthrene and 5,6-epoxy-5,6-dihydrobenzanthracene may be better substrates than the other isomeric epoxides for the enzyme, which would explain the observed differences in the type of products.

The biochemical changes which phenanthrene and benz[*a*]anthracene undergo are different from the addition reactions which they undergo in the absence of enzymes. The reactions of phenanthrene and benz[*a*]-anthracene with ozone, osmium tetroxide, diazoacetic acid, or perbenzoic acid are almost entirely with the double bond with the highest chemical reactivity, which is the *K* region. On the other hand, the biological oxidation also gives products in which the double bonds with less chemical reactivity are involved. The biological oxidations are in closer agreement with theoretical predictions of bond order than are the non-enzymic chemical reactions.

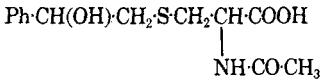
Oxidation of the same hydrocarbons with rat liver microsomes and reduced pyridine nucleotides gave analogous products to those found in urine and bile of animals (Boyland, Kimura, and Sims, 1964).

The bile of rats treated with phenanthrene, benz[*a*]anthracene, and pyrene contained the acid-labile mercapturic acid, cysteine, cysteinylglycine, and glutathione derivatives of the corresponding hydrocarbon epoxides (Boyland and Sims, 1962, 1964a, 1964b), thus providing further evidence as to the nature of the intermediates involved in mercapturic acid biosynthesis.

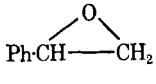
The polycyclic hydrocarbons, benz[*a*]anthracene, dibenz[*a*,*h*]-anthracene (Boyland and Sims, 1965b), 3-methylcholanthrene (Sims, 1966), and 7- and 12-methylbenz[*a*]anthracene (Sims, 1967) formed glutathione conjugates in the presence of rat liver homogenates and are probably partly metabolized as mercapturic acids *in vivo*.

The mercapturic acids detected *in vivo* after administration of lower chlorinated naphthalenes (Cornish and Block, 1958) and the herbicide, 2,6-dichlorobenzonitrile (Wit and van Genderen, 1966), to animals were probably formed through intermediate epoxides. Styrene was probably metabolized as hydroxyphenethylmercapturic acid (**20**) *in vivo* by rabbits and rats (James and White, 1967) through the intermediate styrene epoxide (**21**), as the reaction of this epoxide (**21**) with glutathione is catalyzed by rat liver supernatant (Boyland and Williams, 1965). Bray and Carpanini (1968) showed that about 40% of an administered dose

of thiophene (**22**) was excreted as thienyl 2- and 3-mercapturic acids by rabbits: these metabolites were possibly formed through the intermediate 2,3-epoxy-2,3-dihydrothiophene, which conjugates with glutathione.



(**20**)



(**21**)

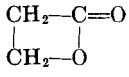
(**22**)

C. GLUTATHIONE *S*-ALKYLTRANSFERASE

Booth et al. (1961) showed that conjugation of 1-bromopropane (**23**) with glutathione was catalyzed by rat liver supernatant. Johnson (1963) described an enzyme that catalyzed the *S*-alkylation of glutathione *in vitro* by iodomethane (**24**), other alkyl halides, and the carcinogenic β-propiolactone (**25**).

(**23**)

(**24**)



(**25**)

This enzyme, named glutathione *S*-alkyltransferase, differed from glutathione *S*-epoxidetransferase (Boyland and Williams, 1965) and from glutathione *S*-aryltransferase (Johnson, 1966a) (Table VIII). With iodomethane (**24**) as the second substrate, the enzyme had a pH optimum at about pH 8.5 and occurred in rat liver and rat kidney in similar amounts.

Several alkyl halides (Bray and James, 1958; Thomson et al., 1958; Grenby and Young, 1960; Barnsley and Young, 1965) and some nitroalkanes (Bray and James, 1958) are excreted as mercapturic acids *in vivo*. In these compounds the halogen or nitro group is replaced by an acetylcysteine residue. Bray, Caygill, James, and Wood (1964) found that, in general, the amount of any given halogeno- or nitroalkane converted into the mercapturic acid *in vivo* decreased with increasing alkyl chain length. Similarly, the *in vitro* studies of Johnson (1966a) showed that the enzymic reaction rates of glutathione with alkyl halides decreased with increasing alkyl chain length.

James (1961) and Bray, Caygill, James, and Wood (1964) found that in addition to the alkylmercapturic acid (**26**), an additional divalent-sulfur-containing metabolite was produced by rabbits dosed with a range of alkyl bromides. Divalent-sulfur-containing metabolites that are identical with those formed from alkyl bromides have also been found in the urine of animals dosed with a corresponding series of 1,2-

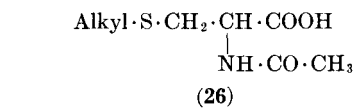
TABLE VIII

Separation of Glutathione *S*-Alkyltransferase, *S*-Aryltransferase, and *S*-Epoxidetransferase from Rat Liver Supernatant (Adapted from Boyland and Williams, 1965)

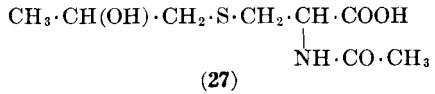
Treatment of rat liver supernatant	Percentage activity remaining toward[a]		
	Alkyl	Aryl	Epoxide
Undialyzed rat liver supernatant	100	100	100
Prolonged dialysis against distilled water	6–13	90–95	70–80
Supernatant obtained after lowering to pH 5.0 and then raising to pH 6.5	7–10	95–100	0–3
Supernatant left after dialysis followed by adsorption with an equal vol. of 20% (w/v) calcium phosphate gel	5–11	46–52	100
Relative activity of partially purified glutathione *S*-aryltransferase and rat liver supernatant; activity of glutathione *S*-aryltransferase taken as 100% in each case	9–17	100	21–24

[a] Substrates used to estimate the activities of these enzymes were methyl iodide, 1,2-dichloro-4-nitrobenzene, and 2,3-epoxypropyl phenyl ether, respectively.

alkenes (James and Jeffery, 1964). Barnsley (1964a,b) showed that the second divalent-sulfur-containing metabolite produced *in vivo* from 1-bromopropane (**23**) was *N*-acetyl-*S*-(2-hydroxy-1-propyl)-L-cysteine (**27**), which is a hydroxyalkylmercapturic acid. This compound (**27**) has been detected in the urine of rats dosed with 1-chloropropane, 1-iodopropane, 1,2-epoxypropane, and 1-chloropropan-2-ol (Barnsley, 1966). Hydroxyalkylmercapturic acids were also obtained after *in vivo* metabolism of corresponding bromocycloalkanes,1,2-epoxy-cycloalkanes, and 1,2-cycloalkenes by rabbits (James et al., 1967). The bromocycloalkanes were also metabolized to the corresponding alkylmercapturic acid and a further unidentified sulfur-containing metabolite, and the latter



(**26**)



(**27**)

was also a metabolite of the epoxy and unsaturated compounds (James et al., 1967).

Sims and Grover (1965) reported that chlorocyclohexenes, α-3,4,5,6-tetrachlorocyclohex-1-ene and γ-2,3,4,5,6-pentachlorocyclohex-1-ene, are conjugated with glutathione by a rat liver enzyme that is probably glutathione *S*-aryltransferase. As these compounds are not planar, it is possible that these and other cycloalkanes are conjugated with glutathione by an enzyme different from glutathione *S*-aryltransferase which is concerned with planar aromatic molecules, or glutathione *S*-alkyltransferase, which is concerned with relatively simple aliphatic molecules.

James and Jeffery (1964) have suggested that mercapturic acids are formed from alkyl halides by two mechanisms: the first would involve a replacement of the halogen atom by glutathione catalyzed by glutathione *S*-alkyltransferase (eq. 3), and the second would proceed by an intermediate stage involving dehydrodebromination, epoxidation, and conjugation of the epoxide with glutathione catalyzed by glutathione *S*-epoxidetransferase (eq. 4). The epoxide may also arise by hydroxylation of the alkyl halide and elimination of HBr (eq. 5). Hydroxyalkylmercapturic acids could also be formed by hydroxylation of the corresponding alkylmercapturic acid or precursor before excretion, as rat liver slices convert *S*-butylcysteine and butylmercapturic acid to hydroxyalkylmercapturic acids (James, Jeffery, Waring and Wood, 1968).

$$\text{Alkyl}\cdot CH_2\cdot CH_2\cdot Br + GSH \rightarrow \text{Alkyl}\cdot CH_2\cdot CH_2\cdot SG + H^+ + Br^- \qquad (3)$$

Hydroxyalkylmercapturic acid formation is restricted to alkyl halides containing more than two carbon atoms since these hydroxyalkylmercapturic acids have not been reported as metabolites of iodomethane (Barnsley and Young, 1965) or bromoethane (Thomson et al., 1963). Bromoethane and 1-bromopropane (**23**) are also partly metabolized to the alkylmercapturic acid sulfoxide (Barnsley, Thomson, and Young, 1964; Barnsley, Grenby, and Young, 1966). The significance of this is unknown, but several *S*-alkyl-L-cysteine sulfoxides occur naturally in plants, e.g., alliin (**28**) in garlic (Virtanen, 1962).

Rabbits and rats dosed with 1-bromobutane excrete in the urine, butylmercapturic acid, (2-hydroxybutyl)mercapturic acid, (3-hydroxybutyl)mercapturic acid, and 3-(butylthio)lactic acid. The latter was also formed from *S*-butylcysteine by rabbit liver slices and may arise by reduction of 3-(butylthio)pyruvic acid produced from *S*-butylcysteine by oxidative deamination or transamination (James et al., 1968).

$$\text{Alkyl}\cdot CH_2\cdot CH_2\cdot Br \xrightarrow{-HBr} \text{Alkyl}\cdot CH{=}CH_2 \xrightarrow{+\text{"O"}} \text{Alkyl}\cdot \underset{\diagdown O \diagup}{CH{-}CH_2}$$
$$\xrightarrow{+GSH} \text{Alkyl}\cdot CH(OH)\cdot CH_2\cdot SG \qquad (4)$$

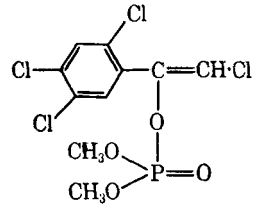
$$\text{Alkyl}\cdot CH_2\cdot CH_2\cdot Br \xrightarrow{+\text{"O"}} \text{Alkyl}\cdot CH(OH)\cdot CH_2\cdot Br \xrightarrow{-HBr} \text{Alkyl}\cdot \underset{\diagdown O \diagup}{CH{-}CH_2} \qquad (5)$$

$$CH_2{=}CH\cdot CH_2\cdot \overset{\uparrow O}{S}\cdot CH_2\cdot CH(NH_2)\cdot COOH$$

(28)

Butylmercapturic acid sulfoxide was detected in rat but not rabbit urine after administration of 1-bromobutane (James et al., 1968).

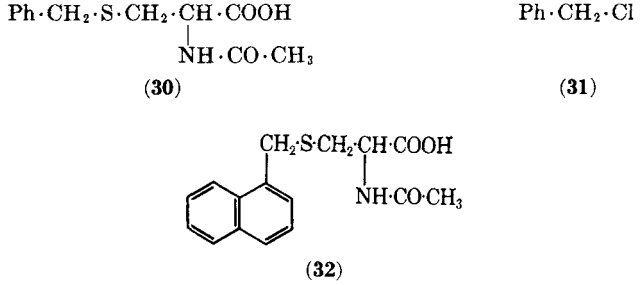
The phosphoric acid triester:glutathione alkyl transferase, described by Hutson, Pickering, and Donninger (1968) appears to utilize glutathione as an acceptor of alkyl groups for the detoxication of 2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate (**29**) and related alkyl homologs by an *O*-dealkylation process (Hutson, Akintonwa, and Hathway, 1967). Products formed on incubation of a rat liver supernatant containing glutathione and (**29**) included *S*-methylglutathione and the *O*-demethylated phosphate diester (Hutson et al., 1968). Fukami and Shishido (1966) showed that the enzyme system that hydrolyzes methyl parathion to desmethyl parathion in rat tissues and insect larvae was glutathione-dependent. The metabolism of *cis* and *trans* isomers of dimethyl 1-carbomethoxy-1-propen-2-yl phosphate (Phosdrin) by mouse liver preparations is different: the *cis* form was *O*-demethylated by a glutathione-dependent enzyme to *S*-methylglutathione and *cis*-monomethyl 1-carbomethoxy-1-propen-2-yl phosphate and the *trans* form was cleaved by a glutathione-independent system to dimethyl phosphate and a ketoester (Morello, Vardanis, and Spencer, 1967). Traces of *S*-ethylcysteine which have been detected after hydrolysis of urine from rats dosed with triethyl phosphate (Roberts and Warwick, 1958b), may have arisen by a similar mechanism. The glutathione-dependent enzymes concerned with these processes may be glutathione *S*-alkyltransferase or separate enzymes.



(29)

D. GLUTATHIONE *S*-ARALKYLTRANSFERASE

Stekol (1938) isolated benzylmercapturic acid (**30**) from the urine of dogs dosed with benzyl chloride (**31**). Mercapturic acids have been isolated after administration of other aralkyl halides to animals (Stekol, 1941; Bray, James, and Thorpe, 1958b; Hyde and Young, 1965, 1968; James and White, 1967). Bray, James, and Thorpe (1958b) found that the proportion of aralkylmercapturic acid excreted decreased with increasing alkyl chain length of the aralkyl halide precursor from 49% for benzyl chloride (**31**) to 4% for 4-bromobutylbenzene. Hyde and Young (1968) have isolated 1-menaphthylmercapturic acid (**32**) from the urine of rats dosed with 1-menaphthyl chloride, bromide, alcohol, acetate, benzoate, and the corresponding glutathione and cysteine conjugates. These findings were confirmed by Clapp (1967), who also detected the corresponding mercapturic acid in the urine of rats dosed with 1-menaphthyl sulfate, phenanthrene 9-carbinol, phenanthrene 9-methyl chloride and acetate, anthracene 9-carbinol, and anthracene 9-methyl chloride and acetate. Benzylmercapturic acid (**30**) was isolated from the urine of rats dosed with benzyl acetate, and 5–9% of the administered dose of 1-menaphthyl alcohol and esters was excreted by rats as 1-menaphthylmercapturic acid (**32**) (Clapp, 1967). Corresponding glutathione, cysteinylglycine, and cysteine conjugates were detected in the bile of rats dosed with 1-menaphthylbutyrate (Clapp, 1967).



The enzyme catalyzing the conjugation of benzyl chloride (**31**) with glutathione has a pH optimum at pH 6.8, and the ratio of activities in rat liver and rat kidney was 100:73 compared with 100:8 for glutathione *S*-aryltransferase estimated with 1,2-dichloro-4-nitrobenzene (see Fig. 1) (Suga et al., 1967). Suga et al. (1967) suggested that this enzyme, which may be named glutathione *S*-aralkyltransferase, was different from glutathione *S*-aryltransferase. This would agree with the findings of Booth et al. (1961) that benzyl chloride (**31**) was a noncompetitive inhibitor of glutathione *S*-aryltransferase. Glutathione *S*-aralkyltransferase has been compared with glutathione *S*-alkyltransferase and *S*-

aryltransferase, and the results suggested that it differed from both these enzymes (Table IX) (E. Boyland and L. F. Chasseaud, unpublished work). Clapp (1967) has shown that rat liver supernatant catalyzes reactions *in vitro* between 1-menaphthyl esters and glutathione, and no reaction was observed in the presence of boiled rat liver supernatant. Rat liver supernatant catalyzes the reaction of glutathione with benzyl formate, but the reaction was slow (E. Boyland and L. F. Chasseaud, unpublished work).

TABLE IX

Activity Ratios Obtained for Glutathione *S*-Alkyltransferase, *S*-Aryltransferase, and *S*-Aralkyltransferase[a]

Rat liver preparations compared	Alkyl	Aryl	Aralkyl
Activity ratio, rat liver supernatant/47% (w/v) ammonium sulfate-fractionated rat liver supernatant	14	0.85	2.7
Activity ratio, dialyzed rat liver supernatant/dialyzed rat kidney supernatant	10	52	4.8

[a] Substrates used to estimate the activities of these enzymes were methyl iodide, 1,2-dichloro-4-nitrobenzene, and benzyl chloride, respectively.

Glutathione *S*-aralkyltransferase possibly catalyzes reactions of glutathione with aralkyl halides, esters, and alcohols *in vivo*. The reaction may be the following, (eq. 6):

$$\text{Aryl}\cdot(\text{CH}_2)_n\cdot\text{X} \rightarrow [\text{Aryl}\cdot(\text{CH}_2)_{n-1}\cdot\text{CH}_2^+] \xrightarrow{+\text{GSH}} \text{Aryl}\cdot(\text{CH}_2)_n\cdot\text{SG} \qquad (6)$$

[X = OH,O·CO·R, halogen]

Phenethylbromide (**33**) is metabolized in rats and rabbits to a hydroxyaralkylmercapturic acid (**34**) as well as phenethylmercapturic acid

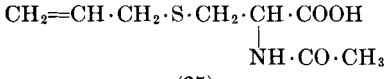
$$\text{Ph}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{Br} \qquad (\mathbf{33})$$

$$\text{Ph}\cdot\text{CHOH}\cdot\text{CH}_2\cdot\text{S}\cdot\text{CH}_2\cdot\text{CH}(\text{NH}\cdot\text{CO}\cdot\text{CH}_3)\cdot\text{COOH} \qquad (\mathbf{34})$$

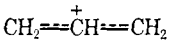
(James and White, 1967), and it is probable that similar hydroxyaralkylmercapturic acids are produced *in vivo* from other related aralkyl precursors, probably by the mechanism outlined for the formation of hydroxyalkylmercapturic acids (James and Jeffery, 1964) (see eqs. 3 and 4). However, James and White (1967) also showed that *S*-phenethylcysteine and phenethylmercapturic acid, which are conjugates of

phenethyl bromide (**33**) were converted *in vivo* and *in vitro* to the hydroxyaralkylmercapturic acid (**34**); this suggests that hydroxylation of the mercapturic acid precursor can occur after conjugation with glutathione and subsequent degradation to the cysteine derivative.

Two mercapturic acids identified in the urine of rats dosed with allyl chloride were allylmercapturic acid (**35**) and 2- or 3-hydroxypropylmercapturic acid, and a metabolite similar to the latter was produced by rats dosed with allyl acetate (Clapp, 1967). The mechanism of formation of these mercapturic acids is unknown but they could occur by initial conjugation of glutathione with an allylic carbonium ion (**36**).



(**35**)



(**36**)

E. GLUTATHIONE *S*-ALKENETRANSFERASES

Reactions of glutathione with $\alpha\beta$-unsaturated compounds RR'C:CR''A, including acetals, aldehydes, esters, ketones, lactones, nitriles, nitrocompounds, and sulfones, are catalyzed by liver preparations from various animal species and by preparations from different rat tissues (Boyland and Chasseaud, 1966, 1967, 1968; Chasseaud, 1967). Reactions with glutathione depend on the relative strength of the electron-attracting group A and on electron repulsion or attraction and probably steric effects exerted by groups R, R', and R''. It appears that at least six different enzymes catalyze reactions of glutathione with various $\alpha\beta$-unsaturated carbonyl compounds [Fig. 5 and Table X (Boyland and Chasseaud, 1968)]. Evidence for this was obtained from heat inactivation experiments (e.g., Fig. 6), ammonium sulfate fractionation studies, enzyme-inhibition studies with *S*-($\alpha\beta$-dicarbethoxyethyl)glutathione, and the distribution of activities in rat liver and rat kidney (Table XI) and in the livers of other animals. These enzymes, named glutathione *S*-alkenetransferases, were different from glutathione *S*-alkyltransferase, *S*-aryltransferase, and *S*-epoxidetransferase and were present in the supernatant fraction of rat liver homogenates (Boyland and Chasseaud, 1968).

The glutathione *S*-alkenetransferase catalyzing the reaction of diethyl maleate (Fig. 5, IA) with glutathione has a pH optimum at pH 7.6 (Boyland and Chasseaud, 1967), is inhibited by the nonenzymic reaction product (Chasseaud, 1967; Boyland and Chasseaud, 1968) and occurs in the livers of several animal species (Boyland and Chasseaud, 1967; Wit and Snel, 1968). The Arrhenius plot for the reaction catalyzed by this enzyme was discontinuous with lower energy of activation below

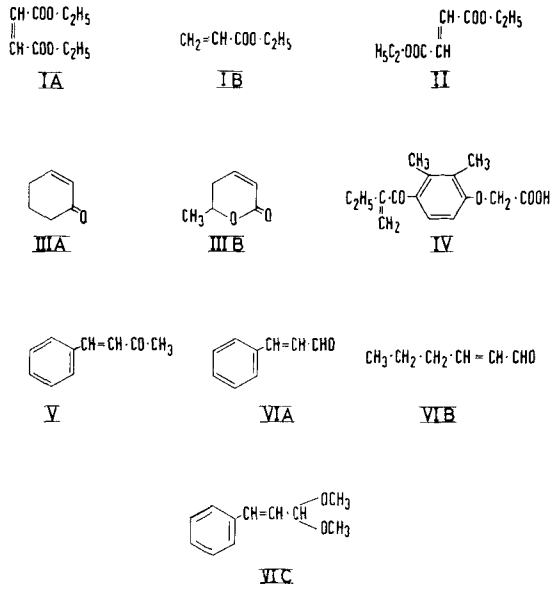


Fig. 5. $\alpha\beta$-Unsaturated carbonyl compounds that are substrates for glutathione *S*-alkenetransferases.

37° (Boyland and Chasseaud, 1968). Within 2 hr of the administration of a dose (0.7 g/kg) of diethyl maleate to the rat, liver glutathione content fell by 90% (Chasseaud, 1967).

Second substrates for glutathione *S*-alkenetransferases (Boyland and Chasseaud, 1967; Chasseaud, 1967) include ethyl acrylate (Fig. 5, IB), vinyl acetate, and acrylonitrile widely used in the plastics industry, a series of alk-2-en-1-als which are present as autoxidation products in fats, oils, and dairy products (Wishner and Keeney, 1965; Day, 1966), β-nitrostyrenes and $\alpha\beta$-unsaturated ketones possessing antifungal and antibacterial properties (Geiger and Conn, 1945; McGowan, Brian, and Hemming, 1948; Schales and Graefe, 1952), and several compounds that are permitted food additives. Second substrates occurring naturally include cinnamaldehyde (Fig. 5, VIA) in cinnamon oils, parasorbic acid (Fig. 5, IIIB) in the ripe berries of the mountain ash (Hauschka, Toennies, and Swain, 1945) and hex-2-en-1-al (Fig. 5, VIB) in a variety of sources (Watanabe, 1958; Forss, Dunstone, Ramshaw, and Stark, 1962; Nursten and Williams, 1967).

Because of their widespread use and occurrence, the toxicities and properties of several of these compounds have been determined (see

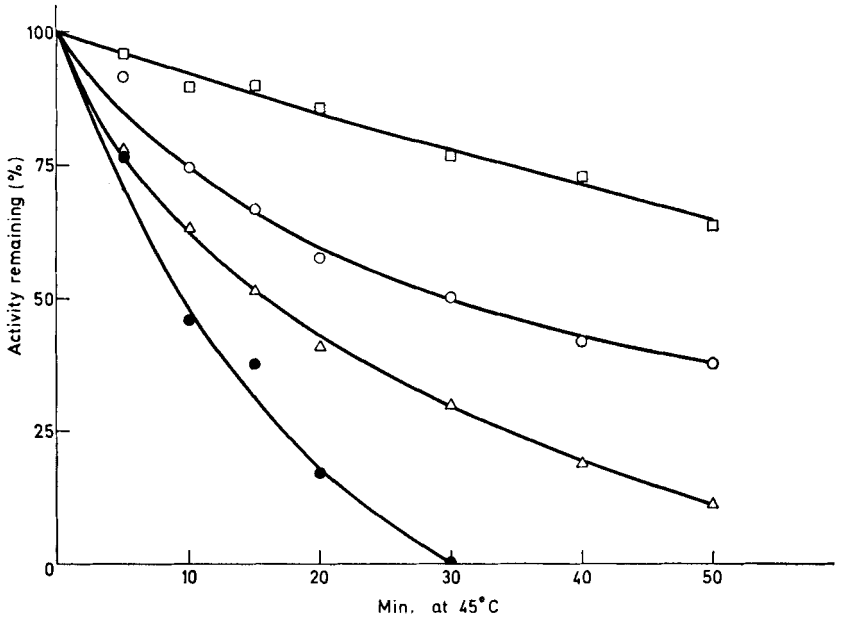


Fig. 6. Reduction in the activities of glutathione *S*-alkenetransferases catalyzing reactions of glutathione with diethyl maleate (IA) (○), cyclohex-2-en-1-one (IIIA) (●), *trans*-benzylideneacetone (V) (□), and *trans*-cinnamaldehyde (VIA) (△) by heating dialyzed rat liver supernatant for 0–50 min at 45° and pH 7.4.

Fassett and Irish, 1963), but studies of their metabolism have been few. The existence of glutathione *S*-alkenetransferases suggests that these compounds are partly metabolized *in vivo* to mercapturic acids, and the probable pathway is outlined in Figure 7. The initial reaction with glutathione probably takes place by addition of the nucleophile (GS^-) to the β-carbon atom of the $\alpha\beta$ double bond strongly polarized by conjugation with electron-withdrawing groups; thiols are known to react with $\alpha\beta$-unsaturated compounds in this manner (Mayo and Walling, 1940; Hurd and Gershbein, 1947; Knuth, Bavley, and Lazier, 1954; Patai and Rappoport, 1964; Friedman, Cavins, and Wall, 1965).

Ethacrynic acid (Fig. 5, IV, Cl for CH_3), a potent new diuretic (Baer and Beyer, 1966), is metabolized in animals to the cysteine conjugate (Porter, 1966).

Glutathione and cysteine conjugates of maleic acid have been found in extracts of calf lens (Calam and Waley, 1963) and the cysteine derivative (**37**) is also present in human urine and animal kidney (Kuwaki and Mizuhara, 1966). The origin of these derivatives is uncertain, and

TABLE X

$\alpha\beta$-Unsaturated Carbonyl Compounds that are Substrates for Glutathione *S*-Alkenetransferases (From Boyland and Chasseaud, 1968)

Enzyme	Type of $\alpha\beta$-unsaturated compound catalyzed	Example	Structure of substrate in Figure 5
1	*cis*-Ester	Diethyl maleate	IA
		Ethyl acrylate	IB
2	*trans*-Ester	Diethyl fumarate	II
3	Cyclic ketone and possibly some lactones	Cyclohex-2-en-1-one	IIIA
		Parasorbic acid[a]	IIIB
4	Vinyl ketone	2,3-Dimethyl-4-(2-methylenebutyryl) phenoxyacetic acid	IV
5	Open-chain *trans* ketone (substituted vinyl ketone)	*trans*-Benzylidene-acetone	V
6	*trans* Aldehyde and possibly acetals	*trans*-Cinnamaldehyde	VIA
		trans-Hex-2-en-1-al	VIB
		Cinnamaldehyde dimethylacetal	VIC

[a] δ-Lactone of 5-hydroxyhex-2-en-1-oic-acid.

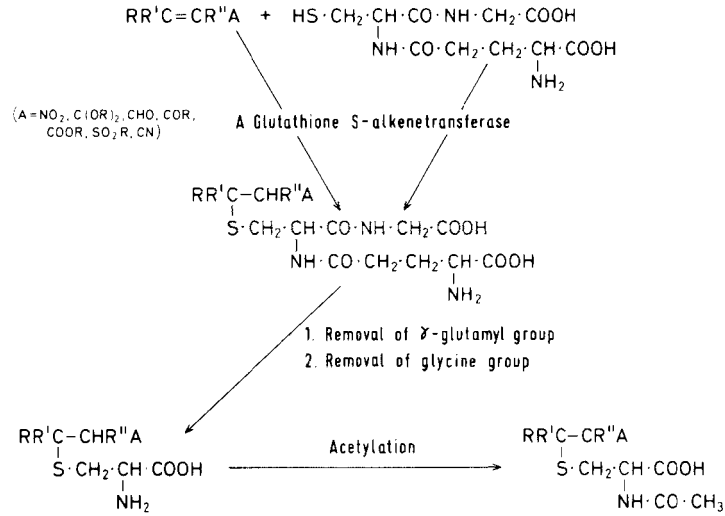


Fig. 7. Mercapturic acid formation from an $\alpha\beta$-unsaturated compound.

TABLE XI

Separation of Glutathione *S*-Alkenetransferases that Catalyze Reactions of Glutathione with $\alpha\beta$-Unsaturated Carbonyl Compounds (Adapted from Boyland and Chasseaud, 1968)

Enzyme preparation and treatment	Relative activity remaining towards: Diethyl maleate (IA)	Diethyl fumarate (II)	Cyclohex-2-en-1-one (IIIA)	2,3-Dimethyl-4(2-methylenebutyryl) phenoxyacetic acid (IV)	*trans*-Benzylideneacetone (V)	*trans*-Cinnamaldehyde (VIA)
Dialyzed rat liver supernatant (control)	100	100	100	100	100	100
Dialyzed rat kidney supernatant	45	33	60	46	17	57
Rat liver homogenate partially purified with 47% (w/v) ammonium sulfate and precipitated with acetic acid	84	150	9	124	150	20
Dialyzed rat liver supernatant heated at 45° and pH 7.4 for 50 min	36	56	4	37	72	15
Dialyzed rat liver supernatant heated at 45° and pH 7.4 for 100 min	23	39	0	0	50	4
Inhibitor constant for competitive inhibition by *S*-($\alpha\beta$-dicarbethoxyethyl)-glutathione	0.5 mM	0.05 mM[a]	—	—	0.44 mM[a]	—

[a] These are apparent inhibitor constants as Dixon plots (Dixon, 1953) were nonlinear at higher inhibitor concentrations.

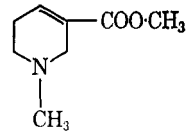
$$HOOC \cdot CH(NH_2) \cdot CH_2 \cdot S \cdot CH(COOH) \cdot CH_2 \cdot COOH$$

(37)

although they could also arise from endogenous fumaric acid, this acid does not react readily with thiols. Boyland and Chasseaud (1967) have found that reaction of glutathione with maleic acid is catalyzed by an enzyme present in rat liver and rat kidney in comparable amounts (Chasseaud, 1967), but a mercapturic acid has not been reported as a metabolite of maleic acid (e.g., Taggart, Angielski, and Morell, 1962). Angielski and Rogulski (1961) studied the reaction of glutathione with maleic acid in the presence of various rat tissue homogenates and suggested that increased losses of glutathione in the presence of kidney homogenates was due to conversion into cysteine which then reacted nonenzymically with maleic acid.

Parasorbic acid (Fig. 5, IIIB) is one of several lactones found to be carcinogenic on subcutaneous injection into rats (Dickens, 1964). However, no carcinogenic effect was observed when this compound was administered orally to rats (Dickens, Jones, and Waynforth, 1966) although the total dose was nearly ten times greater. This result suggests that when administered orally, detoxication of the lactone occurs, partly by conjugation with glutathione.

Arecoline (**38**), an alkaloid present in the seeds of the betel nut palm, is metabolized to a mercapturic acid in the rat (E. Boyland and R. Nery, unpublished work). Nonenzymic reaction of glutathione with this alkaloid occurs readily at pH 7.4, but was not catalyzed by rat liver supernatant (E. Boyland and L. F. Chasseaud, unpublished work).



(38)

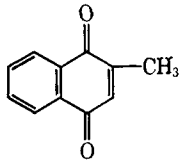
Reactions of glutathione with the $\alpha\beta$, $\gamma\delta$-unsaturated aldehyde, deca-2,4-dien-1-al (**39**) and the acetylenic compound, dimethylacetylenedicarboxylate (**40**) are catalyzed by rat liver preparations (Chasseaud, 1967), but these have not been studied in any detail.

Quinones are important naturally occurring $\alpha\beta$-unsaturated compounds, and although some quinones react with glutathione (Nickerson, Falcone, and Strauss, 1963), and there was evidence that reaction of

$$CH_3 \cdot (CH_2)_4 \cdot CH{=}CH \cdot CH{=}CH \cdot CHO \qquad H_3C \cdot OOC \cdot C{\equiv}C \cdot COO \cdot CH_3$$

(39) (40)

glutathione with menadione (**41**) was catalyzed by rat liver preparations (Chasseaud, 1967), Bray and Garrett (1961) found no mercapturic acids excreted *in vivo* after administration of this (**41**) and related quinones to animals.



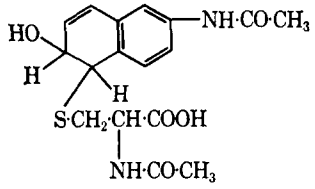
(**41**)

V. Other Mercapturic Acid Precursors

It is not clear whether glutathione *S*-transferases are involved in the metabolism of several compounds that are excreted as mercapturic acids by animals.

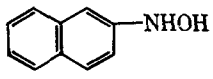
A. ARYLAMINES

Two mercapturic acids have been identified in the urine of animals dosed with 2-naphthylamine, a compound that causes bladder tumors (Boyland, Manson, and Nery, 1963; Boyland and Manson, 1966). One is *N*-acetyl-*S*-(2-acetamido-5,6-dihydro-6-hydroxy-5-naphthyl)-L-cysteine (**42**) and is probably formed through an intermediate epoxide.

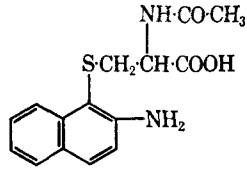


(**42**)

The second mercapturic acid probably arises through the formation of an intermediate, 2-naphthylhydroxylamine (**43**), which, after conjugation with glutathione and rearrangement, yields the mercapturic acid (**44**) (Boyland, Manson, and Nery, 1963).



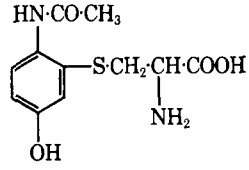
(**43**)



(**44**)

o and *p*-Aminophenyl- and *p*-acetamidophenylmercapturic acids have been detected in the urine of rats and rabbits dosed with aniline and were probably formed through an intermediate hydroxylamine derivative since phenylhydroxylamine, when administered to rats, was converted into *p*-aminophenylmercapturic acid (E. Boyland, D. Manson, and R. Nery, unpublished work).

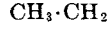
After administration of the analgesic, acetophenetidine, 2% was metabolized in man to the cysteine derivative (**45**) (Jagenburg and Toczko, 1964). This conjugate could have been formed by *N*-hydroxylation and reaction of this derivative with glutathione similar to the metabolism of 2-naphthylamine where **43** → **44**.



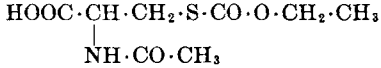
(**45**)

B. URETHANE

In rats, urethane is metabolized and largely excreted in the expired air as carbon dioxide (Boyland and Rhoden, 1949). Metabolites detected in the urine of animals dosed with urethane (**46**) include ethylmercapturic acid (**50**), *N*-acetyl-*S*-carbethoxycysteine (**47**), and ethylmercapturic acid sulfoxide (Boyland and Nery, 1965) and were probably formed through an intermediate *N*-hydroxyurethane which reacts *in vitro* with cysteine producing small amounts of *S*-ethyl and *S*-carbethoxy derivatives.



(**46**)

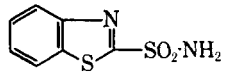


(**47**)

C. BENZOTHIAZOLE-2-SULFONAMIDE

Three urinary metabolites of benzothiazole-2-sulfonamide (**48**) in rats, rabbits, and dogs were identified as benzothiazole-2-mercapturic acid, benzothiazole-2-mercaptan, and benzothiazole-2-thioglucuronic acid (Colucci and Buyske, 1965). All these metabolic products probably arise by initial replacement of the sulfonamide group by glutathione, but this reaction may not be enzyme-catalyzed, as nonenzymic reaction

between benzothiazole-2-sulfonamide (**48**) and glutathione was not significantly increased in the presence of rat liver supernatant (Colucci and Buyske, 1965).



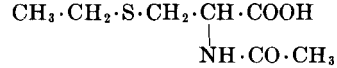
(**48**)

D. ALKYL METHANESULFONATES

Alkylmercapturic acids have been detected *in vivo* after administration of alkyl methanesulfonates to animals (Roberts and Warwick, 1958a; Pillinger, Craig, and Jackson, 1964; Barnsley, 1968).

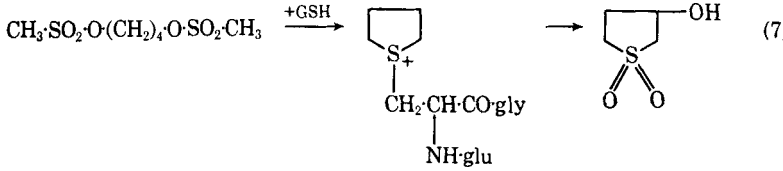
Reaction of ethyl methanesulfonate ("half myleran") (**49**) with glutathione is catalyzed by rat liver supernatant (Booth et al., 1961), presumably with replacement of the methanesulfonate ($SO_3 \cdot CH_3$) moiety by glutathione, as chromatographic analysis of metabolites from the ^{14}C precursor has shown that the mercapturic acid is (**50**) (Roberts and Warwick, 1958a). The glutathione *S*-transferase catalyzing the reaction is not known.

(**49**)



(**50**)

The major urinary metabolite of the antileukemia agent, myleran, in rats was monohydroxytetrahydrothiophene-1,1-dioxide, probably formed by condensation of myleran with glutathione (eq. 7) (Roberts and Warwick, 1959).



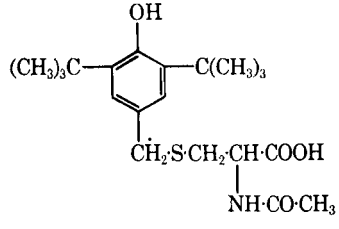
(7)

S-methylglutathione has been detected in the bile of rats dosed with methyl methanesulfonate, and the principal urinary metabolites were *S*-methylcysteine sulfoxide, methylmercapturic acid, and methylmercapturic acid sulfoxide (Pillinger et al., 1964). Barnsley (1968) has shown that metabolites of methyl methanesulfonate also present in rat urine included 2-hydroxy-3-methylsulfinylpropionic acid, methylsulfinylacetic acid, and *N*-(methylthioacetyl)glycine. These are probably formed by oxidation of the cysteine conjugate, *S*-methylcysteine, as similar metabolites have been identified in the urine of rats dosed with

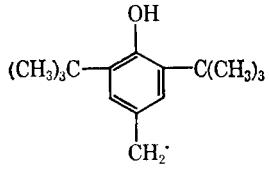
iodomethane (Barnsley and Young, 1965), *S*-methylglutathione (Foxwell and Young, 1964), and *S*-methylcysteine (Barnsley, 1964b). Recently, Sklan and Barnsley (1968) isolated 2-hydroxy-3-methylsulfinylpropionic acid and methylsulfinylacetic acid as dicyclohexylamine salts from the urine of rats dosed with *S*-methyl-L-cysteine. These compounds are also metabolites of methyl methanesulfonate (Barnsley, 1968).

E. 3,5-DI-*TERT*-BUTYL-4-HYDROXYTOLUENE

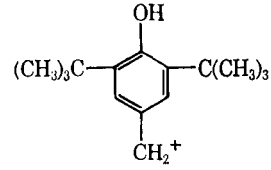
Using spectral and chromatographic techniques, Daniel et al. (1968) showed that a major metabolite of the food antioxidant 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT) in the rat was the mercapturic acid (**51**). Only a trace of this metabolite was present in human urine. Daniel et al. (1968) suggested that the mercapturic acid (**51**) was formed by non-enzymic reaction of the free radical (**52**) with cysteine. Although benzyl alcohol does not give rise to a mercapturic acid *in vivo* (Stekol, 1939b), benzyl chloride does (Stekol, 1938), and it is possible that the mercapturic acid (**51**) is formed from BHT through the intermediate radical (**52**) or carbonium ion (**53**) which conjugates with glutathione.



(**51**)



(**52**)



(**53**)

F. ISOVALTHINE

The amino acid isovalthine (**54**) (*S*-(1-carboxyisobutyl)cysteine) has been found in the urine of hypercholesterolemic patients and of normal cats (Ohmori and Mizuhara, 1962). The biosynthesis of this compound may proceed through a glutathione–isovaleric acid conjugate-precursor which is synthesized from isovaleric acid and glutathione in the presence of ATP and liver homogenates (Kuwaki, Ohmori, and Mizuhara, 1963).

The enzyme catalyzing this reaction does not appear to have been studied. The glutathione–isovaleric acid conjugate is probably catabolized to isovalthine by the same enzymes that catabolize the glutathione conjugates of mercapturic acid precursors (Kuwaki, 1965).

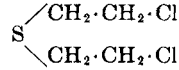
$(CH_3)_2 \cdot CH \cdot CH(COOH) \cdot S \cdot CH_2 \cdot CH(NH_2) \cdot COOH$

(54)

Other cysteine derivatives isolated or detected include *S*-(β-carboxy-*n*-propyl)-L-cysteine (also known as β-isobuteine) and *S*-(β-carboxyethyl)-L-cysteine present in normal child urine (Ohmori, Shimomura, Azumi, and Mizuhara, 1965), and *S*-(carboxymethyl)cysteine present in some animal tissues and in the urine of hypertensive and diabetic patients (Ubuka, Kodama, and Mizuhara, 1967). These derivatives may arise from a mechanism similar to that described above for isovalthine (**54**) or may be enzymically synthesized from the corresponding αβ-unsaturated compound and glutathione by glutathione *S*-alkenetransferases.

G. BIS-β-CHLOROETHYL SULFIDE

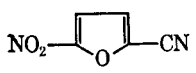
Glutathione conjugates of sulfur mustard (**55**) (bis-β-chloroethyl sulfide) and of bis-β-chloroethyl sulfone were the major urinary metabolites of mustard gas in rodents: some thiodiglycol and other hydrolysis and oxidation products were also formed (Davison et al., 1961). It is not known whether these glutathione conjugates were partially catabolized to cysteine derivatives or mercapturic acids before excretion.



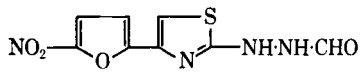
(55)

H. NITROFURANS

Rat liver supernatant catalyzes reactions of glutathione with nitrofurans containing labile nitro groups, e.g., 2-cyano-5-nitrofuran (**56**) (E. Boyland and K. Williams, unpublished work). This indicates that these nitrofurans are partly metabolized to mercapturic acids *in vivo*. The enzyme catalyzing these reactions may be glutathione *S*-aryltransferase or a different enzyme. It appears that carcinogenic nitrofurans (Morris, 1967) such as (**57**) hardly react with glutathione (E. Boyland and K. Williams, unpublished work).



(56)



(57)

VI. Conclusion

Mercapturic acid biosynthesis is becoming increasingly recognized as a pathway for the elimination of foreign compounds from the body. An initial stage in mercapturic acid biosynthesis is the conjugation of glutathione with the foreign compound or a more reactive form of it (e.g., an epoxide). This conjugation is generally catalyzed by glutathione *S*-transferases, although in some cases, nonenzymic reaction, appreciable at physiological pH and temperatures, contributes to conjugation. Although at least ten different glutathione *S*-transferases are known, such a number seems unnecessary since one enzyme would theoretically be sufficient to catalyze the formation of an activated glutathione which could react with foreign compounds. The existence of several glutathione *S*-transferases indicates, however, that both glutathione and the foreign compound are bound to enzymic active sites and that a probable function of glutathione *S*-transferases in the cell is to bring glutathione and the foreign compound together, thereby reducing reaction of the latter with important thiol-containing enzymes and other cellular proteins.

The glutathione conjugates are probably catabolized to cysteine derivatives along the same pathway that operates for glutathione. Acetylation of the cysteine derivative to form a mercapturic acid generally occurs before excretion from the body. Further catabolism of the mercapturic acid may occur, such as oxidation to the sulfoxide and other oxidative reactions, e.g., *S*-methyl-L-cysteine (Barnsley, 1964b; Sklan and Barnsley, 1968). West and Miller (1962) found that administration of *p*-chlorophenylmercapturic acid and [^{35}S]-L-methionine simultaneously to animals resulted in the excretion of [^{35}S]-*p*-chlorophenylmercapturic acid (75% of the dose), suggesting that, as there was appreciable exchange *in vivo*, sulfur plays a part in the metabolism of foreign compounds.

Whereas other conjugative detoxication processes such as glucuronide or sulfate conjugation require the initial formation of a "high energy" or "active" intermediate involving ATP, the reaction of foreign compounds with glutathione does not appear to do so. A possible exception to this may be the reaction of glutathione and isovaleric acid which appears to require ATP (Kuwaki et al., 1963).

The importance of the mercapturic acid biosynthesis in man is unknown. Conjugation with glutathione occurs, but enzyme activities are low compared with some other species (Grover and Sims, 1964; Boyland and Chasseaud, 1967, 1968). Glutathione-conjugating ability is present in the foetus (Grover and Sims, 1964; Boyland and Chasseaud, 1967,

1968) and in the newborn (Vest and Rossier, 1963). Stekol (1946) has shown that acetylation of arylcysteines to mercapturic acids occurs in man, and it is unlikely that the pathway for the catabolism of glutathione conjugates is absent (cf. Revel and Ball, 1959). However, Wainer and Lorincz (1963) were unable to isolate the mercapturic acid after administration of bromobenzene to human subjects. Some animal species may form little or no mercapturic acid due to deficiency in some stage of the mercapturic acid biosynthesis (Bray, Franklin, and James, 1959b).

There are probably many other foreign compounds, at present undescribed, that are substrates for glutathione *S*-transferases. These would be expected to include some compounds capable of providing a sufficiently electrophilic center for reaction with the nucleophilic glutathione. Reaction by a free radical mechanism, although possible, has not been demonstrated in enzyme-catalyzed conjugations with glutathione.

As far as is known, glutathione *S*-transferases do not catalyze reactions of glutathione with endogenous second substrates. The thyroid hormone, thyroxine, and related compounds are not substrates for glutathione *S*-transferases (Grover, 1965). Endogenous compounds that are substrates might include $\alpha\beta$-unsaturated compounds formed by autoxidation of polyenoic acids, alkyl and acyl groups released as carbonium ions in dealkylation or deacylation reactions, or epoxides formed as intermediates in oxidative metabolic reactions. However, the original function of glutathione *S*-transferases is unknown. Boyland (1962) has suggested that nature adapted the existing compound glutathione for a new purpose, that of detoxication.

Acknowledgment

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