THE METABOLIC FATE AND EXCRETION OF DRUGS[1,2]

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This subject was previously reviewed by Maynert (1) who emphasized the medical or therapeutic aspects. Emphasis here has been given to developments in new types of mechanisms of detoxification which have appeared in the literature since the 1958 review by Brodie, Gillette & La Du (3). The reader should note that many contributions on the metabolic fate and excretion of individual compounds have been omitted and that, for the purpose of this review, compounds which are foreign to the animal body are considered as drugs. A valuable compendium of work in the general field is provided in the monograph by Williams (2).

Hydroxylation

Ring hydroxylation.—The hydroxylation of the aromatic ring of compounds foreign to the body is a common route of metabolism and often causes a change in the pharmacological properties of a drug. For example (see Fig. 1), the muscle relaxant zoxazolamine (2-amino-5-chlorobenzoazole) (I) is converted by man into chlorzoxazone (II) which has the same pharmacological action, but these drugs are also hydroxylated to 6-hydroxyzoxazolamine (III) and 6-hydroxychlorzoxazone (IV), respectively. These compounds are excreted chiefly as glucuronides and are inactive as muscle relaxants (4, 5, 6). Another recent example of drug hydroxylation is given by the conversion of the central nervous depressant butamoxane [2-(*N*-butylaminomethyl)-1,4-benzodioxane-^{14}C] (V) to 6-(or 7-) hydroxybutamoxane (VI) by rats and dogs (7).

Evidence has been obtained to suggest that with some cyclic compounds an epoxide may be an intermediate metabolite in ring hydroxylation. When 1,2-dihydronaphthalene (VII) or 1,2-epoxy-1,2,3,4-tetrahydronaphthalene (VIII) are given to rabbits the same metabolites, *trans*-1,2-dihydroxy-1,2,3,4-tetrahydronaphthalene (IX) and N-acetyl-S-(2-hydroxy-1,2,3,4-tetrahydro-1-naphthyl)-L-cysteine (X) are excreted in the urine (8).

At pH 7.4, the epoxide (VIII) will react with water to form *trans*-1,2-dihydroxy-1,2,3,4-tetrahydronaphthalene (IX) or with GSH to form a derivative which is converted by tissue preparations to the mercapturic acid (X) (see mercapuric acid section). The metabolite (IX) is also formed

[1] The survey of the literature pertaining to this review was concluded in May 1961.

[2] The abbreviations used in this chapter include: GSH (reduced glutathione); NAD (nicotinamide-adenine dinucleotide); $NADPH_2$ (nicotinamide-adenine dinucleotide phosphate, reduced form); UDP (uridine diphosphate).

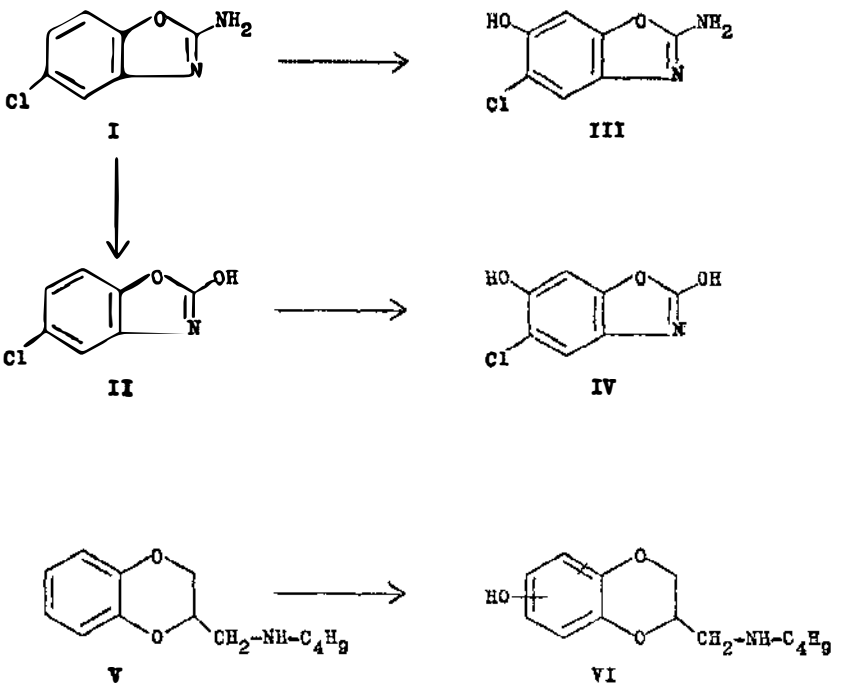

FIG. 1. Recent examples of drug hydroxylations.

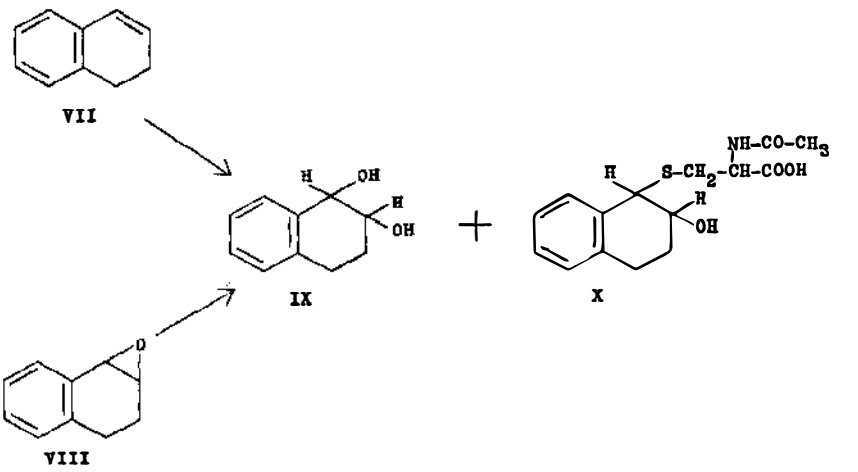

FIG. 2. Urinary metabolites of 1,2-dihydronaphthalene.

by the action of liver microsomes, $NADPH_2$,[3] and oxygen on VII (9), presumably by first converting this compound to the epoxide (VIII). The system consisting of liver microsomes, $NADPH_2$, and oxygen is known to be capable of hydroxylating aromatic rings such as in the formation of 1-naphthol (XI) and 1,2-dihydro-1,2-dihydroxynaphthalene (XII) from naphthalene (XIII) (10), and it now seems likely that the action of this

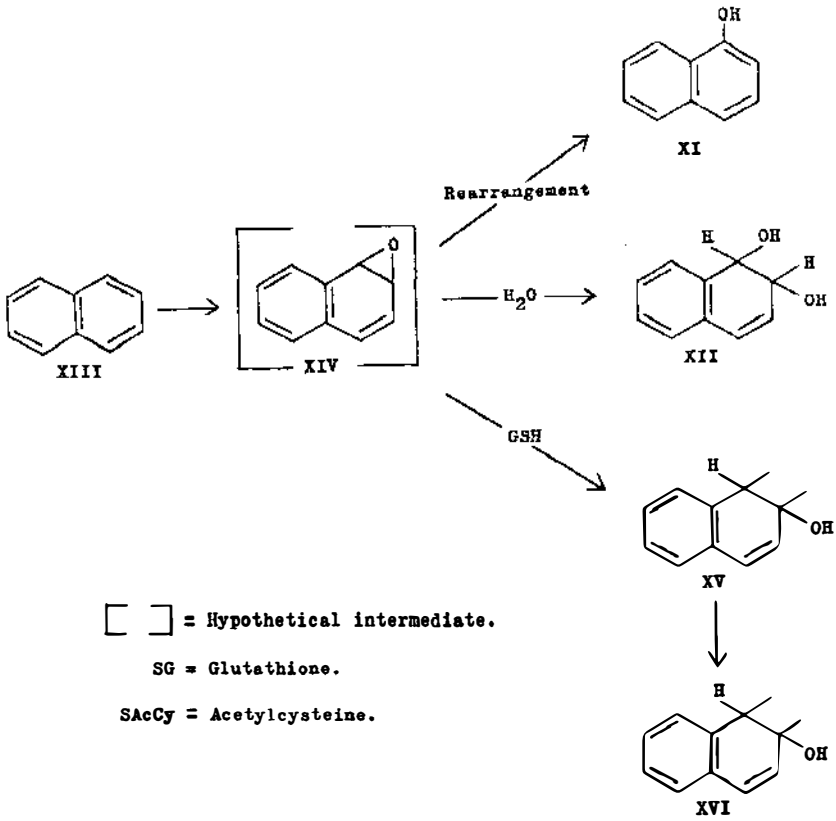

FIG. 3. Possible reactions involved in the hydroxylation of aromatic rings.

system is to form 1,2-dihydro-1,2-epoxynaphthalene (XIV) which could then react either chemically or enzymatically with water to give (XII) or with GSH to give the compound (XV) which is excreted as the mercapturic

[3] In accordance with the newly recommended usage [see *Nature*, **188**, 464–66 (November 5, 1960); *Science*, **132**, 1548–60 (November 25, 1960)], nicotinamide-adenine dinucleotide (NAD) has been substituted for diphosphopyridine nucleotide (DPN); and nicotinamide-adenine dinucleotide phosphate, reduced form ($NADPH_2$), for triphosphopyridine nucleotide, reduced form (TPNH).—EDITOR.

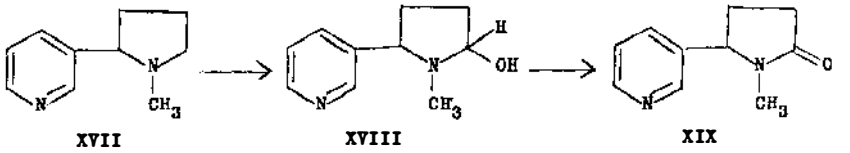

FIG. 4. The metabolism of nicotine by rabbit liver (12).

acid (XVI) (11). The epoxide (XIV) has not yet been synthesized or isolated and so remains hypothetical.

A different system is exemplified by the hydroxylation of nicotine (XVII). Although the metabolism of this alkaloid in dogs and humans has recently been reviewed (1), the demonstration that the first reaction is hydroxylation in the α-position of the pyrrolidine ring is of considerable interest in a consideration of hydroxylation mechanisms. This reaction in which nicotine is converted to hydroxynicotine (XVIII) is carried out by liver microsomes in the presence of $NADPH_2$ and oxygen, and the hydroxynicotine is then oxidized to cotinine (XIX) (12, 13). Hydroxylation of the α-position of the pyrrolidine ring is reminiscent of the enzyme system described by Knox (14) in which quinoline is converted to 2-hydroxyquinoline, and it has been suggested that a similar type of mechanism may be involved in the oxidation of purines by xanthine oxidase (15). A study of the metabolism of the purine antimetabolite, 6-chloropurine, which has been shown to be an effective carcinostatic agent has shown that this compound is oxidized by xanthine oxidase to 2,8-dihydroxy-6-chloropurine (16). The same metabolite

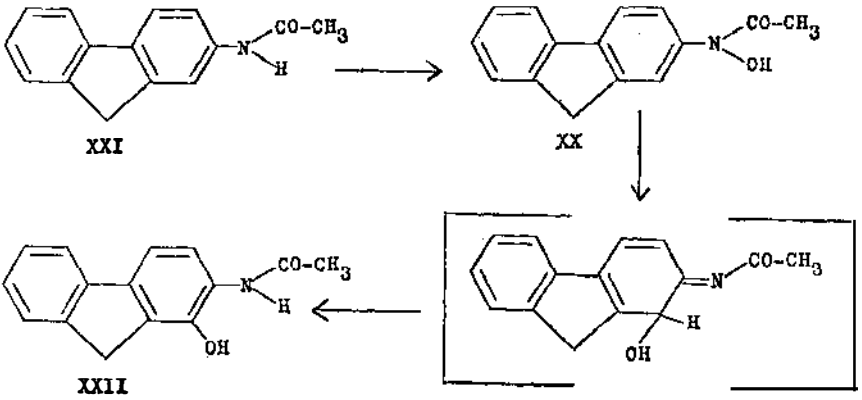

FIG. 5. Possible route of 1-hydroxy-2-acetylaminofluorene formation from 2-acetylaminofluorene (19).

has been identified in the urine of rats which had received 6-chloropurine-8-^{14}C (17).

N-Hydroxylation.—A new type of hydroxylation reaction *in vivo* has been demonstrated by the isolation of the arylhydroxylamine, N-hydroxy-2-acetylaminofluorene (XX) from the urine of rats which had been fed 2-acetylaminofluorene (XXI). This metabolite was present in the urine as a conjugate which was probably an ether-type N-O-glucuronide (18). An investigation of the metabolism of the hydroxylamine (XX) showed that this compound and the parent amine (XXI) produced the same urinary metabolites. The injection of the hydroxylamine (XX) instead of acetylaminofluorene (XXI) caused excretion of smaller amounts of 3-, 5-, and 7-hydroxy derivatives, but relatively more 1-hydroxy-2-acetylaminofluorene (XXII), indicating that the hydroxylamine (XX) may be an intermediate in the formation of the *ortho*-hydroxylation product (19). N-Hydroxyacetylaminofluorene (XX) was shown to be a direct precursor of the acetylaminophenol (XXII) by administering a mixture of 2-acetylaminofluorene-9-^{14}C and unlabelled hydroxylamine (XX) (20). The importance of the hydroxylamine (XX) in the carcinogenic properties of the parent compound is demonstrated by the fact that guinea pigs, which are resistant to tumour by acetylaminofluorene (XXI), did not excrete the hydroxylamine (XX) and that mice are intermediate between rats and guinea pigs both in susceptibility to tumour induction and excretion of the hydroxylamine (XX) (19). N-Hydroxy-2-acetylaminofluorene appears to be a direct carcinogen. On oral administration to Holtzman strain rats it produced tumours in the forestomach, liver, and acoustic duct. By intraperitoneal administration it induced local sarcomas (tumours which are not induced with acetylaminofluorene itself) as well as tumours of the small intestine and acoustic duct (19). The fact that an arylhydroxylamine is converted to an aminophenol *in vivo* indicates that an enzyme system which can carry out this process (probably a real detoxication) occurs in the body. It has previously been suggested that *ortho* and *para* hydroxylation of aromatic amines is attributable to different enzymes because of the different *ortho* to *para* ratio which occurs in different species (21, 22, 23), and it now seems probable that the *ortho* hydroxylation of aromatic amines takes place by rearrangement of N-hydroxy derivatives, probably via the corresponding quinolimide (19, 20). Further examples of oxidation of aromatic amines which have been reported recently are *ortho* and *para* hydroxylation of monochloranilines-^{36}C (24), the formation of 2-amino-4,5-dimethylphenyl sulphate from 3,4-dimethylaniline (25), and 4-amino-3-biphenyl hydrogen sulphate from 4-nitrobiphenyl which is believed to be first reduced to 4-aminobiphenyl (26). Future work may show that the corresponding N-hydroxy derivatives are precursors of these metabolites. The suggestion that N-hydroxylation is a general reaction of aromatic amines (19) is supported by further examples of this type of reaction (27, 28), such as the identification of N-hydroxy-4-acetylaminobiphenyl in the β-glucuronidase treated urine of rats dosed with 4-acetylaminobiphenyl (27).

4-Acetylaminobiphenyl and 2-acetylaminofluorene are structurally related in that the former can be considered as a methylene derivative of 4-acetylaminobiphenyl. The parent amine, 4-aminobiphenyl, and a number of its derivatives are known to induce cancer of the bladder, intestines, liver, and acoustic sebaceous gland (29).

These examples of N-hydroxylation of arylamines occurred with acetyl derivatives but the same change has been found with the primary amine 2-naphthylamine (29, 30). 2-Naphthylamine was the first amine which was shown to induce bladder cancer in dogs (31); it is known to cause bladder cancer in man. The carcinogenic action of 2-naphthylamine on the bladder is almost certainly attributable to some metabolite rather than the amine itself. For this reason the metabolism of 2-naphthylamine has been studied intensively (32) and over 20 metabolites have been identified in urine of animals treated with the amine.

In the United States, a derivative of 2-naphthylhydroxylamine has been detected in the urine of dogs and patients dosed with 2-naphthylamine (28). The *O*-sulfate of 2-naphthylhydroxylamine has been isolated from the urine of dogs which had been given 2-naphthylamine orally (30). This sulfate of the naphthylhydroxylamine is unstable; in acid solution it rearranges to 2-amino-1-naphthyl sulphate which is a well-known metabolite of 2-naphthylamine.

Aryl hydroxylamines are very reactive compounds; in dilute acid they change mainly to *ortho*-aminophenols, through the intermediary formation of a quinolimide ion. The quinolimide ions have some properties similar to those of the alkylating agents. If 2-naphthylhydroxylamine or phenylhydroxylamine are treated with inorganic phosphate in acid solution, they give 2-amino-1-naphthyl phosphate and *o*- and *p*-aminophenyl phosphate respectively. In the presence of the sulphydryl compounds glutathione, cysteine, or acetylcysteine they give S-(aminoaryl) derivatives; the compounds formed in this way from N-acetyl cysteine are mercapturic acids. 2-Amino-1-naphthyl mercapturic acid and *o*- and *p*-aminophenyl mercapturic acids have been identified as metabolites of 2-naphthylamine and aniline. These metabolites are probably formed by the reaction of the aryl hydroxylamine with some sulphydryl compound *in vivo*.

The 2-naphthylhydroxylamine may be the active carcinogen formed from 2-naphthylamine by analogy with the carcinogenic N-acetyl-2-fluorenyl hydroxylamine. As the arylhydroxylamines have similar chemical reactivity to the alkylating agents, such as nitrogen mustard, they may be carcinogenic through some biochemical process analogous to that induced by the alkylating agents. The alkylating agents are usually chemically reactive per se, but the aromatic amines are metabolized into analogous chemically reactive molecules.

The Biosynthesis of Glucuronides

The original finding that glucosiduronic acid derivatives of foreign compounds are synthesized by transfer of glucuronic acid from uridine diphos-

phate glucuronic acid (UDP glucuronic acid) by liver microsomes (33) has been repeatedly confirmed and extended. The enzyme catalyzing the transfer of the glucuronide residue is called UDP transglucuronylase. The UDP glucuronic acid is formed by oxidation of UDP glucose in the presence of oxygen, NAD^3, and the enzyme UDP glucose-dehydrogenase (34). The UDP glucose is formed by the action of pyrophosphorylase on uridine triphosphate and α-glucose-1-phosphate. The formation of uridine triphosphate is dependent on ATP. This system of enzymes and coenzymes is present in the liver of adult mammals except the cat (35) which lacks UDP transglucuronylase. Fetal liver and the livers of newborn animals are often unable to synthesize glucuronides; this is associated with the jaundice of the newborn which is caused by unconjugated bilirubin. The deficient glucuronide synthesis is due to low levels of UDP glucuronic acid and of UDP transglucuronylase (36).

The glucuronide synthesizing system is present in kidney (36) and skin (37) as well as in liver. The work of Hartiala (38) has shown that the mucous membrane of the gastrointestinal tract of the dog, rabbit, and rat, but not the cat, can conjugate *o*-aminophenol with glucuronide. The activity is present in the stomach, duodenum, ileum, and colon. The Finnish authors, Hartiala & Hakkinen (39), suggest that glucuronide synthesis in the gastric mucosa is in some way associated with the occurrence of gastric ulcers. The administration of boiled saccharic acid (an inhibitor of β-glucuronidase) delayed the induction of gastric ulcers in chickens treated with cincophen.

Uridine diphosphate forms derivatives with several carbohydrates including glucose and *N*-acetyl glucosamine. These UDP derivatives might be expected to compete with UDP glucuronic acid and reduce glucuronide synthesis in the presence of UDP glucuronic acid and microsomes. UDP *N*-acetyl glucosamine, however, specifically activates glucuronidation particularly in the presence of ATP (40). The effect is, in part, caused by inhibition of the conversion of UDP glucuronic acid to glucuronic acid 1-phosphate, glucuronic acid, and uridine. The UDP transglucuronylase has been obtained in solution by treatment of the rat liver microsomes with digitonin (40). Hens excrete some phenols, borneol and benzoic acid, as glucuronides, and they are able to conjugate *m*-aminophenol with sulfate (41).

The Metabolism of Sulfur Compounds

Mercapturic acid formation.—Many foreign compounds are excreted in the urine and bile as mercapturic acids. Some of the more recent examples of this route of elimination are shown in Table I.

The first example of mercapturic acid formation from an aliphatic compound was the replacement of the methanesulfonate group by acetylcysteine to give N-acetyl-S-ethylcysteine (ethylmercapturic acid) which was identified in the urine of rats dosed with ^{14}C-ethyl methanesulfonate (42). Ethylmercapturic acid was also identified in the urine of rats dosed with bromoethane (43), and further examples of acetylcysteinyl debrominations were demonstrated by the identification of mercapturic acids on paper

TABLE I

THE FORMATION OF MERCAPTURIC ACIDS

Compound	Species	Mercapturic acid identified	Ref.
^{14}C-Ethyl methanesulfonate	Rats	Ethylmercapturic acid	(42)
Bromoethane	Rats	Ethylmercapturic acid	(43)
1-Bromobutane, -pentane, -hexane, -heptane, or -octane	Rabbits	Corresponding alkylmercapturic acid	(44)
1-Bromopropane or *l*-iodopropane	Rats, rabbits, guinea pigs, mice	*n*-Propylmercapturic acid	(45)
1-Chloropropane	Rats, rabbits	*n*-Propylmercapturic acid	(45)
Naphthalene	Rats, rabbits, mice, hamsters, guinea pigs, man	N-acetyl-S-(1,2-dihydro-2-hydroxy-1-naphthyl)-L-cysteine	(46)
^{14}C-Chlorobenzene	Locusts	*m* and *p*-chlorophenylmercapturic acid	(48)
3,4-Dichloronitrobenzene	Rabbits	N-acetyl-S-(2-chloro-4-nitrophenyl)-L-cysteine	(52)
1,2-Dihydronaphthalene or 1,2-epoxy-1,2,3,4-tetrahydronaphthalene	Rabbits	N-acetyl-S-(2-hydroxy-1,2,3,4-tetrahydro-1-naphthyl)-L-cysteine	(8)

chromatograms of the urine of rabbits treated with 1-bromobutane, -pentane, -hexane, -heptane, and -octane (44). The suggestion that this may be a general metabolic reaction of alkyl halides was supported by the formation of *n*-propylmercapturic acid from 1-bromo-, 1-iodo, and 1-chloropropane in a variety of species (45).

Examples of mercapturic acids with the acetylcysteine residue attached to the aromatic nucleus have been known for many years. Evidence has now been obtained to show that in the case of some aromatic hydrocarbons the mercapturic acids are excreted in the form of acid labile compounds analogous to the naphthalene metabolite, N-acetyl-S-(1,2-dihydro-2-hydroxy-1-naphthyl)-L-cysteine, which is converted by mineral acid at room temperature into 1-naphthylmercapturic acid (46). Benzene, anthracene and the halogenobenzenes form similar acid labile compounds (47), and ^{14}C-chlorobenzene is metabolized to *m*- and *p*-chlorophenylmercapturic acids which are also excreted as acid labile precursors by locusts (48).

Evidence that the initial stage in the biosynthesis of mercapturic acids was the reaction of the foreign compound with GSH was provided by Bray, Franklin & James (49) who identified, by the use of paper chromatography, S-(*p*-chlorobenzyl) glutathione in the liver of a rabbit which had been dosed with *p*-chlorobenzyl chloride. The GSH derivative could be converted to S-(*p*-chlorobenzyl)-L-cysteine by several tissue preparations and the N-acetylation of S-substituted cysteines to the corresponding mercapturic acids was

demonstrated *in vivo* and *in vitro* (50). A similar series of reactions takes place in the metabolism of 3,4-dichloronitrobenzene (XXIII) which is converted to S-(2-chloro-4-nitrophenyl)glutathione (XXIV) by rat liver homogenate. The glycine and glutamic acid of the GSH moiety can be removed by kidney homogenate to form S-(2-chloro-4-nitrophenyl)-L-cysteine (XXV) and this is then acetylated by liver slices to N-acetyl-S-(2-chloro-4-nitrophenyl)-L-cysteine (XXVI) (51), which is the mercapturic acid that is excreted in the urine after dosing rabbits with 3,4-dichloronitrobenzene (52).

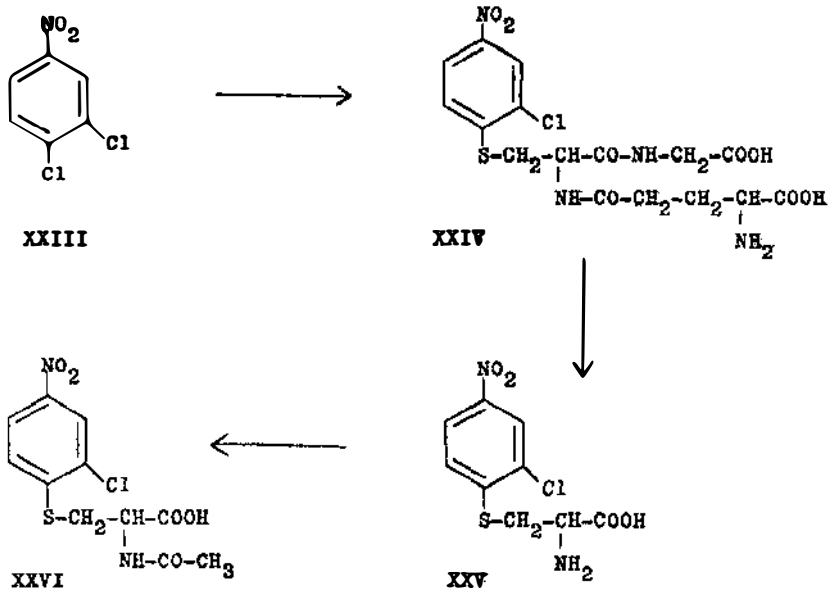

FIG. 6. The formation of *N*-acetyl-*S*-(2-chloro-4-nitrophenyl)-L-cysteine from 3,4-dichloronitrobenzene.

The formation of N-acetyl-S-(1,2-dihydro-2-hydroxy-1-naphthyl)-L-cysteine, which is the mercapturic acid that is excreted in the urine of naphthalene dosed animals, also takes places by analogous stages (11). The enzyme responsible for GSH conjugation has been located in the soluble liver fraction, and other compounds which are excreted as mercapturic acids and form GSH conjugates in the presence of soluble liver fraction are 2,4-dichloronitrobenzene, 2,3,5,6-tetrachloronitrobenzene, bromoethane, 1-bromopropane, ethyl methanesulphonate, bromosulphalein, and benzyl chloride (51). This enzyme, which appears to be a glutathiokinase, is specific for the -SH substrate; it does not activate cysteine or acetylcysteine. Its activation can be conveniently measured by following the reaction

between glutathione and 3,4-dichloronitrobenzene as the substrate and products in the reaction differ in their ultraviolet absorption.

Various foreign compounds have been found to react with peptides. When ^{131}I-iodobenzene was given to rats, the liver and intestine yielded protein conjugates with iodophenyl residues. Homogenates contained iodophenylcysteine and smaller amounts of iodophenylcysteine peptides which were considered to represent intermediates in mercapturic acid formation (53). The urine of rabbits dosed with 1-bromobutane contained metabolites which formed s-butyl-L-cysteine, glycine and glutamic acid on acid hydrolysis (54). The bile of rats treated with naphthalene, 1,2-dihydronaphthalene or 1,2-epoxy-1,2,3,4-tetrahydronaphthalene contained conjugates of GSH, cysteinylglycine, cysteine, and N-acetylcysteine (55). Bromosulphalein, which is used to test hepatic function because it is removed from the blood by the liver, also forms similar metabolites. After administration of bromosulphalein, the bile of several species contains metabolites which yield amino acids after acid hydrolysis. Glycine, glutamic acid, and alanine were identified in man, glycine and glutamic acid in the rat, and glycine in the dog (56, 57). Evidence has been obtained to show that bromosulphalein appears as a conjugate of GSH in the bile of the dog (58) and the rat (59).

Sulfoxide formation.—A number of thioethers are used as therapeutic agents, and in several cases oxidation to the corresponding sulfoxide has been shown to be a metabolic pathway. The antitubercular action of compounds related to ethanethiol was found to be very high *in vivo* but not *in vitro*, suggesting that the action was probably attributable to a metabolite. The metabolism of some of these compounds was investigated by Lowe (60) who found, in agreement with Snow (61), that both ^{14}C-diethyl sulfide and ^{14}C-ethyl thiolbenzoate were converted to ethyl sulfone. It was also demonstrated that the urine of guinea pigs dosed with ^{14}C-ethyl thiolbenzoate contained ethyl methyl sulfoxide.

The first occurrence of a mercapturic acid sulfoxide being formed in the animal body has been demonstrated by Thomson & Young (62). When rats were dosed with bromoethane the urine contained two principal metabolites, ethylmercapturic acid and its sulfoxide N-acetyl-s-ethyl-L-cysteine-s-oxide.

Further examples of the oxidation of thioethers to sulfoxides are demonstrated by metabolic studies on promazine, and the various routes of metabolism have been reviewed (1). One of the main metabolic pathways was sulfoxidation since ^{35}S-promazine was oxidized to promazine-5-oxide or demethylated to 10-(γ-methylaminopropyl) phenothiazine which could also be oxidized to the corresponding sulfoxide, 10-(γ-methylaminopropyl) phenothiazine-5-oxide, all three metabolites having been found in the urine (63). Chlorpromazine is also oxidized *in vivo* by the dog to chlorpromazine-5-oxide (64), and it has now been shown that guinea pig liver contains a

microsomal enzyme system, which requires $NADPH_2$ and oxygen, and catalyzes the oxidation of chlorpromazine and 4-4′-diaminodiphenyl sulfide to chlorpromazine sulfoxide and 4-4′-diaminodiphenyl sulfoxide (65).

Sulfate conjugation.—The mechanism of the conjugation of hydroxyl compounds with sulfate has been clarified during the past decade. A number of distinct sulfokinases have been identified which can transfer sulfate from adenosine-3-phosphate-5′-phosphosulphate to different types of natural phenols and alcohols (66) such as estrogens and steroids and also foreign phenols and alcohols. Simple aliphatic alcohols have been known to be conjugated with sulfuric acid since the work of Neubauer carried out at the beginning of the present century. Swedish workers using ^{35}S-labelled sulfate have now shown that methanol, ethanol, propanol, butanol, and amyl alcohol are all excreted as their sulfuric esters (67) by mice, rats, guinea pigs, and rabbits. This discovery of the conjugations of simple aliphatic alcohols with sulfate needs further investigation from the point of view of alcohol addiction and detoxication and also from the nutritional standpoint since the sulfate conjugation may use sulfur from the dietary proteins.

Aryl sulfamates.—Following the finding that aromatic amines such as aniline and 2-naphthylamine are excreted as aryl sulfamates (68), the carcinogenic 4-amino-3-methylbiphenyl and 4-amino-2′,3-dimethylbiphenyl have also been shown to be excreted as these conjugates (69). The system present in mammalian liver which synthesizes these metabolites has been studied (70). The biosynthesis involves the transfer of sulfate from adenosine-3′-phosphate-5′-sulfatophosphate catalyzed by arylamine sulfakinase. The system is sensitive to oxosteroids; 3-β-methoxyandrost-5-en-17-one in 1 μM concentration inhibits the guinea pig arylamine sulfokinase but activates the rat enzyme (71).

Phosphoric Esters as Metabolites of Foreign Compounds

Since the body tissues contain much more inorganic phosphate than sulfate, it is perhaps surprising that phosphoric esters are not more common than sulfate esters as metabolites of foreign compounds. 2-Amino-1-naphthyl phosphate is readily prepared by oxidation of 2-naphthylamine by perphosphoric acid in the presence of acetone or other ketones (72). This ester, however, does not appear to be a metabolite of 2-naphthylamine in animals. Investigation of the metabolism of 2-naphthylamine in dogs showed that a substance with the properties of *bis*(2-amino-1-naphthyl) phosphate was excreted (73). This ester has been synthesized and its presence in the urine of dogs dosed with 2-naphthylamine confirmed (74). This secondary phosphoric ester may be the precursor of the direct carcinogen in cancer of the bladder induced by 2-naphthylamine, but there are other compounds which appear more likely to be the cause of these tumours. The compound is, however, an interesting metabolite and other phosphate esters will probably be found as metabolites.

The Effect of Carcinogens on Microsomal Enzymes

Many of the metabolic processes involving foreign compounds (e.g., hydroxylation and demethylation) are carried out by microsomes in the presence of $NADPH_2$. The enzyme systems in liver which perform these reactions are greatly increased if the animals are treated with carcinogenic hydrocarbons (75). A similar increase in the rate of metabolism of foreign compounds is induced by some narcotics (76, 77). Earlier work had shown that (*a*) treatment of animals with narcotics increased ascorbic acid excretion (78) and (*b*) carcinogenic hydrocarbons increase the ascorbic acid concentration of the liver in mice (79). More recent work has shown that carcinogenic compounds, including polycyclic hydrocarbons and other types, as well as some narcotics which are not yet known to be carcinogenic, increase the excretion of ascorbic acid in rats (80, 81, 82). The effect on the microsomal enzymes and on ascorbic acid synthesis and excretion are related; the same agent increases the synthesis of enzymes concerned with metabolism of foreign compounds and the enzyme system which synthesizes ascorbic acid.

Since ethionine which blocks protein synthesis inhibits the effect, the increase in the activity of these enzymes in the liver appears to be a true increase of these enzymes by synthesis. The increase in these enzymes by treatment with carcinogens and other substances may be caused by destruction of a suppressor, which normally controls the production of the enzymes, by the treatment. French workers (83, 84) have shown that the increase in production of phage and of enzymes, such as galactosidase, which occurs on treatment of bacteria with radiation or nitrogen mustard is best explained by the destruction of a suppressor which normally controls production of enzymes and phage. The carcinogenic process may be associated with the increase in liver enzymes which occurs in rats treated with carcinogens. The carcinogenic hydrocarbons which produce these effects most markedly in the liver usually produce cancer in other sites. The increase in microsomal enzymes caused by carcinogens may be a stage in carcinogenesis but the relation between the increase in enzyme activity and carcinogenesis is still obscure. The problem, however, seems to be worth investigation both from the aspect of carcinogenesis and of drug metabolism.

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