METABOLIC FATE OF DRUGS[1]

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This review is based on the literature from 1957 to May, 1960. For earlier work the reader is referred to the systematic treatment of the metabolism of organic compounds by Williams (1). Discussions on metabolic fate have appeared periodically in the *Annual Review of Biochemistry* (2, 3), and in a sense, this review may be regarded as a continuation of that series. However, somewhat different aspects of the field may be expected to be emphasized in a publication designed primarily for pharmacologists. The pharmacologist is more interested in compounds possessing physiological activity and in results obtained in man. He is also more concerned with a complete accounting of the products of metabolism, because he recognizes that therapeutic or toxic actions may not be caused by the drug itself but by a metabolite which represents only a tiny fraction of the drug. At the present time, few, if any, drugs can be completely accounted for after therapeutic doses in man. However, with the continued introduction of more sensitive and specific analytical methods, it would appear that this goal can ultimately be achieved with dividends by providing more secure knowledge upon which a science of structure-activity relationships can be founded.

The papers which were selected for this review are those concerned with the identification and determination of the excretory products of useful drugs. No reference is made to a large number of studies which did not go further than to describe unidentified spots on paper chromatograms. Because they may give results which are grossly misleading for the intact animal, purely *in vitro* investigations of drug metabolism have not been included. For example, cytochrome oxidase causes the disappearance of catechol amines *in vitro* more rapidly than any known enzyme, but *in vivo* this reaction does not appear to occur (4). The enzymatic aspects of drug metabolism are discussed in other reviews (2, 4, 5, 6).

Hypnotics

Barbiturates.—The study of the fate of this series of drugs continues to generate many publications, particularly by German and Japanese investigators, but, except for the work described below, the only new metabolites characterized are some oxidative and hydrolytic products of drugs containing the 1-cyclohexenyl group (7, 8). Other reviews (9 to 12) summarize the evidence that the barbiturates are metabolized by four different routes: oxidation of substituents in the 5-position of the barbituric acid ring,

[1] The following abbreviation has been used: TEPA (triethylenephosphoramide).

hydrolysis (generally a minor reaction), oxidation of alkyl groups attached to a nitrogen atom, and desulfuration of thiobarbiturates. Although it is clear that part of the sulfur of thiobarbiturates is converted to inorganic and organic sulfate (13), evidence for the direct conversion of thiobarbiturates to ordinary barbiturates *in vivo* has not been above objection, inasmuch as the sulfur of thiobarbiturates can be oxidized by the peroxide in ethyl ether employed in extraction. Recently the excretion of barbital after the administration of thiobarbital in man has been demonstrated with a more satisfactory method (14). Reports (15, 16) that N-methylation of a barbiturate (nor-evipal) occurs to some extent in the mouse are unconvincing.

Some new experiments (17) on 2-C^{14}-labeled Phanodorn (5-Δ^1-cyclohexenyl, 5-ethylbarbituric acid) in the rat have indicated that the metabolites can be accounted for as follows: 0 to 1.5 per cent unchanged drug, 12 to 13 per cent 3-hydroxycyclohexenyl derivative, 73 to 74 per cent 3-oxo-

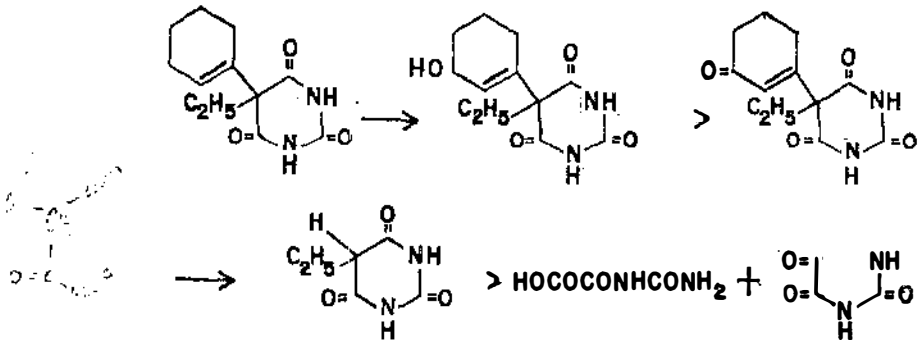


FIG. 1. Metabolism of Phanodorn (5-Δ^1-cyclohexenyl, 5-ethyl barbituic acid).

cyclohexenyl derivative, 9 per cent ethylbarbituric acid, 1 to 2.5 per cent oxaluric acid, 1 to 2 per cent parabanic acid (see Fig. 1). A small amount of ethylbarbituric acid has also been found after the administration of barbital and 5-ethyl, 5-vinylbarbituric acid (18). The removal of substituents in the 5-position probably involves unstable intermediates with a hydroxyl group on the carbon atom immediately adjacent to the barbituric acid ring. The mechanism of the formation of oxaluric and parabanic acid from ethylbarbituric acid is not at all clear. One possible route would be hydrolysis and decarboxylation to yield butyryl urea, which could be converted to oxaluric acid through ω-oxidation followed by β-oxidation.

The formation and subsequent degradation of monoalykl derivates may explain the failure of all other workers to account fully for the products of barbiturate metabolism. This interesting work requires confirmation.

Methyprylon.—This drug (3,3-diethyl-5-methylpiperidine-2,4-dione) appears to be metabolized primarily through a reaction which may be formulated as a dehydrogenation but possibly results from hydroxylation fol-

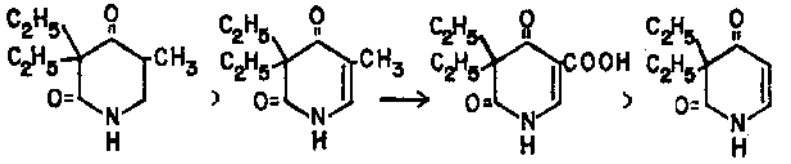


FIG. 2. Metabolism of methyprylon.

lowed by dehydration. As illustrated in Figure 2, the oxidation product (3,3-diethyl-2,4-dioxo-5-methyltetrahydropyridine) has been isolated from the urine of man, the rat, and the dog (19, 20, 21). A similar dehydrogenation was described earlier for 3,3-diethylpiperidine-2,4-dione (22). In the dog the methyl group of the dehydrogenation product of methyprylon has been shown to be oxidized to a hydroxymethyl derivative and then to a carboxylic acid; both of these compounds are excreted in the urine as glucuronides. Part of the carboxylic acid derivative is decarboxylated to 3,3-diethyl-2,4-dioxotetrahydropyridine which also appears in the urine (21).

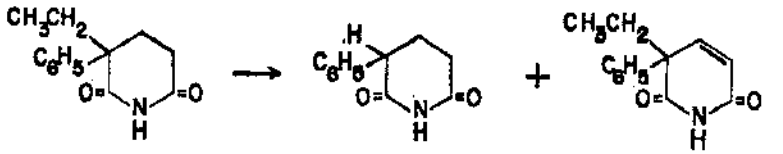


FIG. 3. Unconjugated metabolites of glutethimide.

Glutethimide.—After the administration of this drug to dogs or rats, only traces of unchanged α-ethyl, α-phenylglutarimide appear in the urine (19, 23). In the dog most of the products of the drug are excreted conjugated with glucuronic acid. The unconjugated metabolites, which account for less than 10 per cent of the dose, consist of the dealkylation product, α-phenylglutarimide, and the dehydrogenation product, α-ethyl, α-phenylgluconimide (24 to 27) (Fig. 3). Three glucuronides have been isolated as crystalline derivatives after treatment with diazomethane and acetic anhydride. The aglycones have been shown to have the structures illustrated in Figure 4 (27).

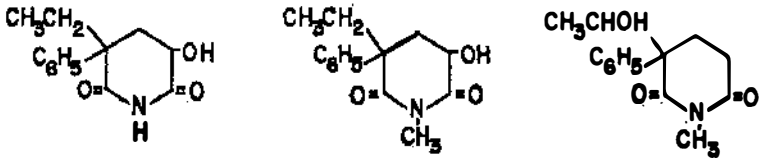


FIG. 4. Aglycones of glucuronides formed from glutethimide.

These compounds prove that hydroxylation occurs in both the ethyl group and the glutarimide ring. The isolation of a 1-hydroxyethyl derivative is noteworthy inasmuch as such compounds have been proposed as inter-

mediates in dealkylations involving rupture of a carbon-carbon bond. The N-methyl group in two of the compounds was probably introduced by the treatment with diazomethane. Although N-methylation has long been recognized as an important biochemical reaction of natural substrates and has been observed in connection with the metabolism of a few foreign amines (e.g., pyridine, quinoline, *p*-methylaminoazobenzene), there is no satisfactory evidence for methylation of an imido group.

Ethinamate and hydroxydione.—The metabolism of ethinamate (1-ethynylcyclohexyl carbamate) (Fig. 5) has been studied in rats. Hydrolytic cleavage of the ester is a minor reaction. Most of the drug is hydroxylated, but the position of the hydroxyl group in the cyclohexyl ring has not been established. About half of the hydroxyethinamate is excreted as such; the remainder is excreted as a glucuronide (28).

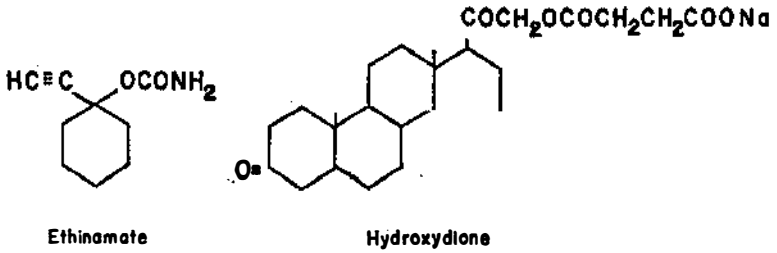


FIG. 5. Structures of ethinamate and hydroxydione.

Hydroxydione (pregnane-21-ol-3,20-dione hemisuccinate sodium (Fig. 5), which has been used for intravenous and basal anesthesia, is metabolized in man by reduction of the 3-keto group to a hydroxyl group which is then conjugated with glucuronic acid. The ester linkage is hydrolyzed. Among the metabolites identified, the principal excretory product is the monoglucuronide of 21,3-α-pregnanediol-20-one; however, the 21-hydroxy group is also partly conjugated. Inasmuch as only 15 to 20 per cent of the drug can be accounted for, oxidation of the side chain to form derivatives of 3-hydroxy- and 3-keto-etiocholanic acid has been suggested (29 to 32).

ANTIEPILEPTICS

Drugs containing an N-alkyl group.—Convincing evidence has been presented that the five drugs shown in Figure 6 (N-methyl derivatives of barbituric acid, hydantoin or oxazolidine 2,4-dione) are almost completely demethylated in man (33). When these drugs are administered chronically in the treatment of epilepsy the concentrations in the body of the products of demethylation far exceed those of unchanged drug. The demethylated derivatives of the five drugs are known to possess antiepileptic activity. Therefore, there is a serious question whether any therapeutic benefits are achieved by administering any of these drugs in preference to the products of demethylation. This is a problem which can be solved only by means of controlled clinical investigation.

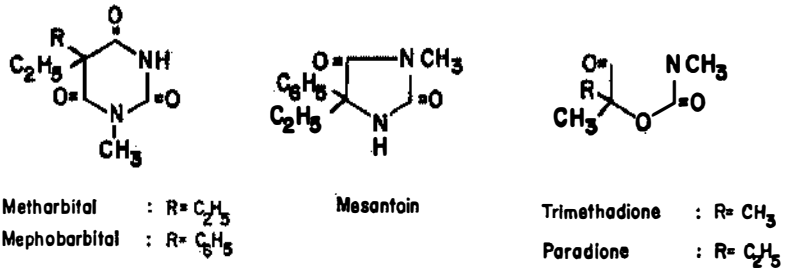


FIG. 6. Structures of antiepileptics.

Barbital and 5,5-dimethyloxazolidine-2,4-dione, the products of demethylation of metharbital and trimethadione, respectively, are eliminated almost completely unchanged, although excretion in man has not been studied as thoroughly as in experimental animals (9, 34). It is not known whether 5-ethyl,5-methyloxazolidine-2,4-dione resulting from the demethylation of paradione completely resists chemical alteration in the body; at least a portion of the compound is excreted unchanged (35). Phenobarbital, which is formed from mephobarbital, is excreted partly unchanged and partly as *p*-hydroxyphenobarbital (36 to 39), which for all practical purposes lacks hypnotic activity (39). Presumably, phenobarbital is also metabolized to other products which are still unknown. In the dog, *p*-hydroxyphenobarbital is excreted entirely conjugated, for the most part with glucuronic acid (39), but in man it is only partly conjugated, and the nature of the conjugation is uncertain (37, 38, 39). Nirvanol (5-ethyl,5-phenylhydantoin) produced by the demethylation of mesantoin is also excreted by man partly unchanged and partly as *p*-hydroxynirvanol (40, 41). The *p*-hydroxynirvanol in dog urine is largely conjugated with glucuronic acid (40); it is not known whether this is also true in man.

Diphenylhydantoin and primidone.—A study with N^{15}-labeled diphenylhydantoin (Fig. 7) has proved that hydrolysis of the hydantoin ring does not occur to an important extent in dogs (42). In man and the dog, a large part of the drug is converted to 5-(*p*-hydroxyphenyl),5-phenylhydantoin, which is subjected to further metabolic alteration (43). Both species excrete about 50 per cent of a dose of diphenylhydantoin as the *p*-hydroxy derivative; 75 per cent of this is conjugated with glucuronic acid, and less than 1 per cent is excreted in the free form (42, 43). Hydroxylation of a phenyl group causes the carbon atom in the 5-position of the hydantoin ring to become asymmetric. The phenolic metabolite in the urine of man consists almost entirely of the levorotatory isomer. The dog excretes both isomers with a slight preponderance of the dextrorotatory form (43).

Primidone (5-ethyl,5-phenyl-4,6-dioxo-hexahydropyrimidine) (Fig. 7) is metabolized in part through oxidation to phenobarbital (44). In man the extent of this conversion is of the order of 15 per cent; in dogs it amounts to only 5 per cent. In rats ethylphenylmalonamide may also be a metabolite (45).

FIG. 7. Structures of diphenylhydantoin and primidone.

ANALGESICS

Morphine and related drugs.—The literature on the narcotic analgesics up to 1957 has been reviewed (46). More recent papers on morphine (47 to 51), levorphanol (52), heroin (53), and codeine (54) are generally in agreement with earlier reports and do not require discussion. A study on the narcotic antagonist, levallorphan (levo-3-hydroxy-N-allylmorphinan), has revealed that all of the common laboratory animals excrete small amounts of the free and conjugated deallylation product, 3-hydroxymorphinan, in the urine (55). Another metabolite which differs from levallorphan in possessing an extra oxygen atom has been isolated from rat urine but not yet identified. Rats excrete only 5 per cent of a large dose of levallorphan as unchanged drug in its free and conjugated forms (55). In contrast, 44 to 60 per cent of a dose of nalorphine can be accounted for as free and conjugated drug in dogs (56). Studies with liver microsomes have shown that nalorphine can be deallylated to normorphine or conjugated with glucuronic acid (48, 57).

6-Dimethylamino-4,4-diphenyl-3-hexanone, which has received clinical use in Japan, has been reported to be metabolized in rats through reduction of the ketone group to a hydroxyl group (58). Whether a similar reduction occurs with methadone, 6-dimethylamino-4,4-diphenyl-3-heptanone, is not known.

Anileridine and propoxyphene.—Anileridine (Fig. 8), which differs from meperidine in having a *p*-aminophenylethyl in place of a methyl group, is metabolized by hydrolysis of the ester and acetylation of the amino group. In man four metabolites have been determined in the urine: unchanged drug (5 per cent), anileridine acid (7 to 14 per cent), acetyl anileridine (0.5 to 2 per cent), and acetyl anileridine acid (1 to 2 per cent). In man, the rat, and the guinea pig an additional 15 to 35 per cent of the dose is excreted as unidentified diazotizable substances; in the rat this fraction consists largely of *p*-acetylaminophenylacetic acid or some closely related compound (59).

The fate of α-dl-propoxyphene (Fig. 8), has been studied in rats and man. Rats given the drug labeled with C^{14} in one of the N-methyl groups excrete 38 per cent of the radioactivity in the expired air, 35 per cent in the feces, and 15 per cent in the urine. The product of mono-demethylation has been isolated from human urine by means of a dinitrophenyl derivative (60).

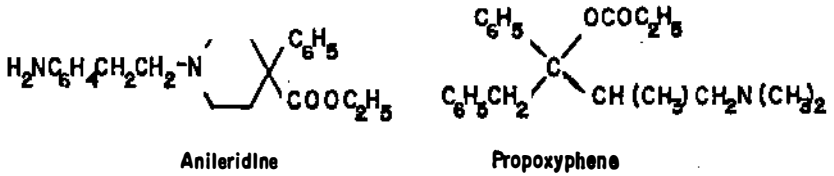


FIG. 8. Structures of anileridine and propoxyphene.

TRANQUILIZING AGENTS

Phenothiazine derivatives.—Promazine and chlorpromazine (Fig. 9) appear to be metabolized by sulfoxidation, hydroxylation, and demethylation. Chlorpromazine sulfoxide has been isolated from the urine of dogs; in man it accounts for about 5 per cent of a dose of the drug (61). The

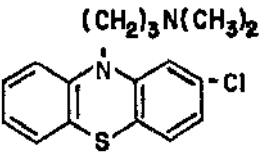


FIG. 9. Structure of chlorpromazine.

pharmacological actions of this metabolite differ qualitatively and quantitatively from chlorpromazine (62); it is a weak sedative in man and is thought to contribute little to the action of the parent compound (63). Sulfoxide metabolites of promazine (64) and mepazine (10[(1-methyl-3-piperidyl)methyl] phenothiazine)(65) have also been reported. A study of the fate in rats of chlorpromazine labeled with C^{14} in one of the methyl groups has proved demethylation to be an important process; as much as 17 per cent of the isotope is exhaled as carbon dioxide in six hours, and considerable amounts are eliminated during the following six hours (66). After the administration of S^{35}-labeled promazine to dogs, the products of monodemethylation of the drug and the corresponding sulfoxide are excreted in the urine, but these two metabolites together account for less than 5 per cent of the dose (64). In man both methyl groups may be removed from the dimethylamino group of chlorpromazine; the mono-methyl and the completely demethylated sulfoxide derivatives have been stated to be the major extractable metabolites, but quantitative data are lacking (67). Removal of the entire dimethylaminopropyl group apparently does not occur, at least in dogs; this is suggested by the fact that radioactive sulfate is a product after S^{35}-labeled phenothiazine but not after labeled promazine (64). However, sulfate has been reported as a metabolite of chlorpromazine in mice (68). Treatment of human urine with β-glucuronidase has been shown to liberate additional metabolites of chlorpromazine that have not yet been definitely identified but may be presumed to contain hydroxyl groups in the

phenothiazine ring (69, 70). No metabolites of promazine or chlorpromazine containing the sulfone group have been detected; chlorpromazine sulfone has been shown to be rapidly metabolized to products of unknown structure by liver microsomes (71).

Reserpine.—In all species examined, only minute amounts of reserpine (Fig. 10) are excreted unchanged in the urine. The earliest study of the fate of this drug revealed that 30 to 60 per cent of an oral or intravenous dose was excreted by mice as 3,4,5-trimethoxybenzoic acid, a compound which is excreted entirely unchanged by this species (72). However, it seems unlikely that hydrolysis to methyl reserpate and trimethoxybenzoic acid is the primary biochemical transformation of reserpine. Rats given reserpine by mouth excrete 22 per cent of the drug in the urine and 45 per cent in the feces as methyl reserpate. However, if the same dose is

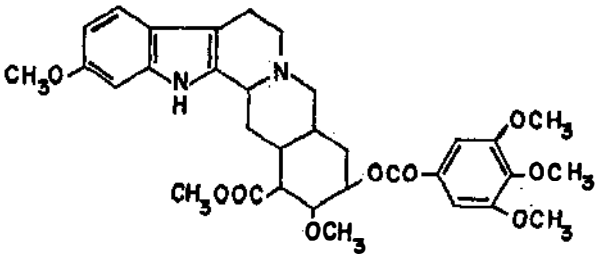


FIG. 10. Structure of reserpine.

given subcutaneously or intraperitoneally, the urinary excretion of methyl reserpate amounts to less than 1 per cent of the dose. It has been demonstrated that the intestine of the rat differs from that of the dog or the monkey in being capable of hydrolyzing reserpine to methyl reserpate. A considerable part of the methyl reserpate formed in the intestine of the rat appears to be absorbed and excreted unchanged in the urine. The very small amount of this compound in the urine after parenterally alministered reserpine indicates that methyl reserpate is not an important intermediary product in the rat when the oral route is avoided. In the dog and the monkey only small amounts of methyl reserpate are found in the tissues and excreta, regardless of the route of administration (73).

Apart from hydrolysis, the only chemical change which has been demonstrated for reserpine is O-demethylation. Rats injected with drug labeled with C^{14} in the 4-methyl group of the trimethoxybenzoyl moiety excrete 24 per cent of the radioactivity in the breath in six hours (74). O-Demethylation products of reserpine and trimethoxybenzoic acid (syringoyl methyl reserpate and syringic acid) have been detected in rat tissues (75), but adequate quantitative data to assess the importance of these metabolites is lacking. No correlation has yet been found between the concentrations of reserpine or its metabolites in the brain and the duration of pharmacological effects (75, 76, 77). It may be that reserpine or one of its metabolites causes

some change in body chemistry which persists long after the drug and its products are completely eliminated, but this can scarcely be accepted as proved until the distribution and fate of all of the metabolites of reserpine are known.

Meprobamate.—The administration of C^{14}-labeled meprobamate to man, dogs, or rats leads to the urinary excretion of four radioactive products in addition to unchanged drug (Fig. 11) (10 to 20 per cent of the dose) (78,

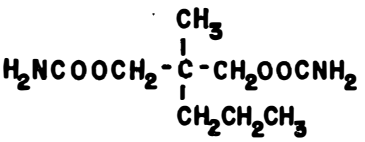


FIG. 11. Structure of meprobamate.

79). Two metabolites appear to be alcoholic derivatives obtained from oxidation of the methyl and propyl groups. One of these has been proved to be 2-hydroxymethyl,2-*n*-propyl-propane-1-3,diol dicarbamate; this compound, which lacks sedative activity, accounts for 60 per cent of the urinary metabolites in dogs (78). The other two products are glucuronic acid conjugates. Acid hydrolysis of one of these yields the hydroxymethyl derivative of meprobamate; the combination with glucuronic acid may involve either the hydroxyl group or the amino groups of the metabolite. Treatment of the other conjugate with acid yields unchanged meprobamate; this substance appears to be an N-glucuronide (80).

N-glucuronides have been considered as metabolites of aromatic amines for over 10 years (81, 82, 83), but much remains to be learned about this class of compounds. They are very rapidly hydrolyzed in acidic solutions but are not attacked by β-glucuronidase (84). Their formation from uridine diphosphate glucuronic acid is catalyzed by the microsomal enzyme system involved in the formation of ether and ester glucuronides (84); however, they are also formed in aqueous solution by the nonenzymatic condensation of amines with glucuronic acid (81, 85, 86). Other drugs reported to form N-glucuronides are 4,4-diaminodiphenylsulfone (86), sulfanilamide (87), and sulfapyridine (88).

CENTRAL NERVOUS SYSTEM STIMULANTS

Iproniazid.—This monoamine oxidase inhibitor, which has been employed clinically as a psychomotor stimulant, is subjected to two different chemical reactions in mice, rats, and rabbits (89) (Fig. 12). One part of

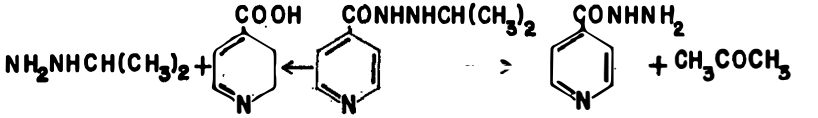
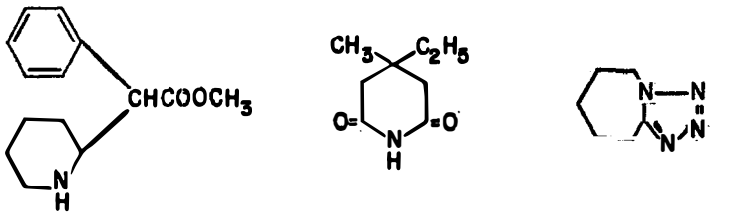


FIG. 12. Metabolism of iproniazid.

the drug is hydrolyzed to isonicotinic acid and isopropylhydrazine; another part is dealkylated to form isoniazid (isonicotinic acid hydrazide) and acetone. After the administration to rats of iproniazid labeled with C^{14} in the isonicotinic acid moiety, 98 per cent of the radioactivity appears in the urine in 24 hours, and almost all of this is in the form of isonicotinic acid and unchanged drug (90). These findings suggest that the principal metabolic pathway for iproniazid involves hydrolysis to isonicotinic acid and isopropylhydrazine, because, if isoniazid were formed to any considerable extent, one would expect to find N-acetyl,N′-isonicotinoylhydrazine and the isonicotinoylhydrazones of pyruvic and α-ketoglutaric acids in the urine (91, 92). However, other investigators have found the acetyl derivative of isoniazid in urine after the administration of iproniazid, although quantitative data are not available (89). When iproniazid containing C^{14} in the isopropyl group is administered to mice, rats, or rabbits, as much as 60 per cent of the isotope is excreted in the breath. Radioactive acetone can be detected in the tissues and excreta; very large doses of iproniazid appear to inhibit the oxidation of acetone to CO_2. The labeled acetone could arise from the dealkylation of either iproniazid or isopropylhydrazine. Two metabolites of iproniazid detected in the urine by paper chromatography have been considered to be isopropylhydrazones of normal tissue constituents, but they have not been definitely identified (89).

Although isopropylhydrazine is known to be quite potent with respect to both toxicity and mono, amine oxidase inhibition, the role of this compound in causing the effects of iproniazide *in vivo* has yet to be clarified. In this connection it is interesting to note that benzohydrazide ($C_6H_5CONHNH_2$) and *p*-chloro- and *p*-methyl-benzohydrazides, which are quite toxic, are extensively hydrolyzed in rabbits to the corresponding carboxylic acids and hydrazine. The less toxic salicylohydrazide and *p*-hydroxybenzohydrazide are less extensively hydrolyzed, and are metabolized primarily through conjugation with glucuronic acid (93, 94, 95).

Other stimulants.—Methylphenidate (Fig. 13) is metabolized primarily through hydrolysis of its ester linkage. After the administration of drug labeled with C^{14} in the carbonyl group to rats, none of the isotope appears in the breath, and only minute amounts are found in the feces. A negligible



Methylphenidate Bemegride Pentylenetetrazole

FIG. 13. Structures of central nervous system stimulants.

quantity of the drug is excreted unchanged. The major urinary product is phenyl(2-piperidyl) acetic acid (62 per cent of the dose). Two other metabolites have been detected but their structures are unknown (96).

The fate of bemegride (Fig. 13) has been studied in a patient who received this drug for the treatment of poisoning with phenobarbital. Unchanged bemegride and a metabolite containing a hydroxyl group were isolated in pure form from the urine. The metabolite gives a positive iodoform test; this reaction proves conclusively that the hydroxyl group is located in the ethyl group on the carbon atom immediately adjacent to the glutarimide ring (97).

Some new work on the metabolism in the rat of pentylenetetrazole (Fig. 13) labeled with C^{14} has yielded interesting results (98). Most of the injected radioactivity appears in the urine in 48 hours. When the urine or extracts of it are subjected to paper chromatography, only one compound can be detected, and this is indistinguishable from unchanged drug. On the other hand, by means of biological methods it has been shown quite clearly that the drug is metabolized to an inactive compound. Furthermore, partial hepatectomy prolongs the sojourn of the drug in the body and nephrectomy is without effect. It would seem that the only explanation for these apparently contradictory observations is that pentylenetetrazole is metabolized in the rat to a very labile conjugate of unknown structure which reverts to the drug in urine. However, it is difficult to imagine what kind of conjugation could be involved.

Cardiac Glycosides

Recent research has revealed that hydroxylation and cleavage of the sugar moieties are important reactions in the metabolism of the cardiac glycosides. In man and the rat digitoxin yields several metabolites, one of which is digoxin (12-hydroxydigitoxin) (99). In the rat injected with digitoxin the digitoxose chain undergoes a stepwise degradation to yield the bis-digitoxosides and the mono-digitoxosides of digitoxigenin and digoxigenin. The principal metabolite of digitoxin in the rat is digoxigenin-bis-digitoxoside (62 per cent of the dose). Unchanged drug accounts for 6 per cent of the dose and digoxin, 20 per cent (100, 101).

The cleavage of the sugars of cardiac glycosides has also been demonstrated in a study of K-strophanthoside and thevetin (102). After oral or subcutaneous administration of strophanthoside, unchanged drug can be detected in the bile but not in the excreta. However, both the urine and the feces contain the product (cymarin) formed from the cleavage of two molecules of glucose. Homogenates of tissues do not cause hydrolysis of strophanthoside but incubation with rat feces leads to a rapid formation of cymarin. Similar findings have been obtained with thevetin. This work raises the question of the relative importance of tissue enzymes and the intestinal flora in the chemical transformations observed with other cardiac

glycosides. The literature on this problem is quite unsatisfactory. For example, some workers have failed to detect any oxidation of digitoxin *in vitro* (103), whereas others find that oxidation and cleavage occur not only in the liver but in blood or plasma (104). One is forced to consider the possibility that the presence or absence of bacteria may play some part in causing the discrepancy in results.

The excretion of the cardiac glycosides into the bile appears to depend upon their polarity or their partition coefficients. After parenteral administration to rats, more than 60 per cent of a dose of a polar drug like ouabain (105) or lanatoside C (106) appears unchanged in the bile. Under the same conditions, less than 10 per cent of a dose of a less polar drug like digitoxin or digoxin enters the bile (106). Secretion into the bile and a limited reabsorption from the gastrointestinal tract provide an explanation for the short duration of action of ouabain and the lanatosides, but further work will be necessary to establish this point.

Sulfonamides

Carbonic anhydrase inhibitors.—A study of the metabolic fate of 2-benzothiazolesulfonamide has provided an explanation for its failure to inhibit

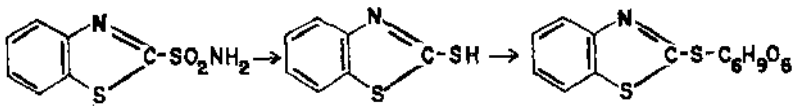
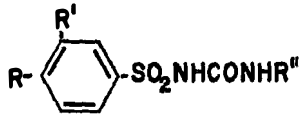


Fig. 14. Metabolism of 2-benzothiazolesulfonamide.

carbonic anhydrase *in vivo* (107). After intravenous administration to dogs, no unchanged drug appears in the urine, but a metabolite accounting for about 25 per cent of the dose can be isolated. This compound has been proved to be the glucuronide of 2-mercaptobenzothiazole (Fig. 14). As such it is the first thioglucuronide (S-glucuronide) to be isolated in pure form. Small amounts of 2-mercaptobenzothiazole and 2-hydroxybenzothiazole have also been isolated from urine. The reduction of the sulfonamide group to a sulfhydryl group is a metabolic reaction which has not been described previously. In the light of earlier observations that thiophenol leads to an increased excretion of ethereal sulfates as well as glucuronides (1), it is possible that mercaptobenzothiazole is also conjugated with sulfuric acid. Acetazoleamide, a carbonic anhydrase inhibitor active *in vivo,* is excreted largely (more than 70 per cent) unchanged (108).

Sulfonylureas.—These drugs, which are useful in the treatment of diabetes mellitus, are metabolized by reactions of substituents in the benzene ring and by modification of the substituted urea group (Fig. 15). In man the only important reaction of tolbutamide is oxidation of the *p*-methyl group to a carboxylic acid group; practically all of the drug is excreted as N-4-carboxybenzenesulfonyl,N′-*n*-butyl urea, which has no hypoglycemic activity (109, 110). Dogs convert tolbutamide to *p*-toluenesulfonyl urea and *p*-toluenesulfonamide, but quantitative data are lacking (111, 112). In the



	R	R'	R"
Tolbutamide :	CH_3	H	n-butyl
Chlorpropamide :	Cl	H	n-propyl
Metahexamide :	CH_3	NH_2	cyclohexyl
Carbutamide :	NH_2	H	n-butyl

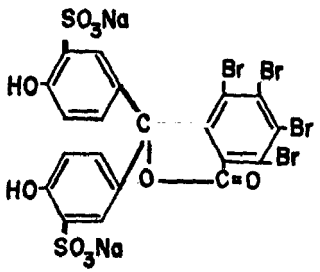
FIG. 15. Structures of the sulfonylureas.

dog chlorpropamide is excreted partly unchanged (27 to 33 per cent), and partly as *p*-chlorobenzenesulfonyl urea (35 to 40 per cent) and the corresponding sulfonamide (16 to 24 per cent) (113). The drug appears to undergo less change in man (114), but *p*-chlorobenzenesulfonamide is also a metabolite in this species (113). Metahexamide has not been studied in man, but the dog excretes some of this drug as 3-amino-4-methylbenzenesulfonamide (115). The only data on the fate of carbutamide were obtained by use of the Bratton-Marshall method for aromatic amines; the results indicated that in man about one third of the excreted amino group was acetylated (116). Now that it is known that aromatic amines may be excreted as N-glucuronides and N-sulfates as well as acetylated derivatives and that some of these conjugates are very labile under conditions required for diazotization, this classical method for dividing excreted amines into free and acetylated fractions does not necessarily yield a meaningful result.

DIAGNOSTIC AGENTS

Bromsulfalein.—Employed clinically to test hepatic function, this drug (Fig. 16) is largely metabolized to derivatives which have the same color. After the administration of S^{35}-labeled bromsulfalein to dogs, four metabolites, none colorless, can be detected in the bile (117). The metabolites appear to be similar in man and all other species studied, although marked quantitative differences occur (118, 119, 120). Attempts to demonstrate conjugation with sulfuric or glucuronic acid have been unsuccessful (121, 122,

FIG. 16. Structure of bromsulfalein.



123), although the drug apparently forms a glucuronide in the presence of liver microsomes (124). A number of studies have shown that the metabolites contain amino acids (120, 122, 123, 125). Although none of the metabolites has been definitely identified, it sems likely that conjugation with glutathione is involved. Hydrolysis of the metabolites yields cysteine, glycine, and glutamic acid (120, 122, 125). Furthermore, in two of the metabolites the sulfur of the cysteine has been demonstrated to be involved in a thioether linkage (125). Compounds similar to the metabolites are formed by the reaction of the drug with glutathione or cysteine *in vitro* (125, 126, 127). The reaction of bromsulfalein with a sulfhydryl group *in vivo* could involve replacement of either a bromine or a hydrogen atom in one of the aromatic rings. Although loss of a bromine atom would seem more likely, an analysis of one of the metabolites isolated from rat bile after the administration of labeled drug indicated that the ratio of Br to S^{35} was the same as in the drug itself (118). It has been shown that after administration to rats one of the metabolites is converted in part to a second metabolite; when the second metabolite is administered, it appears in the bile entirely unchanged (128). If the metabolism of bromsulfalein involves a condensation with glutathione, some of the products detected may arise by stepwise removal of glycine and glutamic acid and the acetylation of the cysteine residue to form a mercapturic acid. The possibility of the introduction of two sulfhydryl-containing moieties into bromsulfalein seems less likely.

Evidence that a condensation with glutathione is the primary step in the formation of mercapturic acids has been strengthened in the past few years. However, larger SH-containing peptides and proteins may sometimes be involved (129). Several foreign compounds leading to mercapturic acids have been shown to cause a decrease in liver glutathione (130). The incubation of rat liver slices with naphthalene leads to the formation of a compound that is almost certainly S-(1,2,dihydro-2-hydroxy-1-naphthyl)-glutathione (131). The ingestion of 1-bromobutane causes the excretion of three metabolites containing sulfur. One of these, after hydrolysis, yields glutamic acid, glycine, and S-butyl-L-cysteine; the second metabolite yields only glycine and S-butyl-L-cysteine. The third compound is *n*-butylmercapturic acid (N-acetyl-S-butyl-L-cysteine) (132).

Anticancer Agents

The availability of a recent review (133) on the fate of drugs employed in the treatment of cancer makes it permissible to confine the present discussion to busulfan and some of the phosphoramides.

Busulfan.—This drug has been studied after separate labeling of the sulfur and the carbon of the tetramethylene chain. After the administration of the S^{35}-labeled drug, the isotope appears in the urine largely in the form of methanesulfonic acid (134, 135). The administration of the C^{14}-labeled drug to man or the rat causes the appearance of a large number of radioactive compounds in the urine (134, 136); this result has been interpreted

as suggesting alkylation of unknown body constituents. In the rat and the rabbit the major metabolite is 3-hydroxytetrahydrothiophene-1,1-dioxide (137, 138) (Fig. 17). The pathway for the formation of this compound may involve the alkylation of a cysteine-containing substance by the drug to form the S-β-alanyltetrahydrothiophenium ion which is then converted to tetrahydrothiophene, which is metabolized by oxidation to the sulfone and hydroxylation.

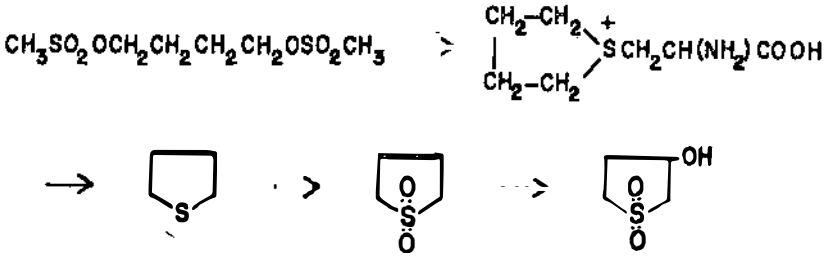


FIG. 17. Metabolism of busulfan.

Phosphoramides.—Inasmuch as alkylating agents have been thought to be highly reactive under biological conditions, it was surprising to discover that considerable quantities of unchanged busulfan appear in the urine of rats (134, 135). Another alkylating agent, N,N′,N″-triethylenephosphoramide (TEPA) is even more stable *in vivo*. In rats this drug is excreted largely unchanged (139). However, in man and the mouse most of it is converted to inorganic phosphate (136, 140). In the rat, the dog, and the rabbit TEPA is a major metabolite of thio-TEPA (Fig. 18) (141, 142); the

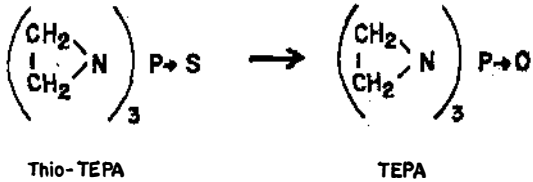


FIG. 18. Metabolism of thio-TEPA.

mouse also degrades this compound almost completely to inorganic phosphate (141). Another thiophosphoramide, OPSPA, which differs from thio-TEPA in having a morpholino group in place of one of the ethylene imino groups, has also been shown to be excreted by man and the rat largely as the oxygen analogue (143). The fact that some of the alkylating agents are excreted unchanged should not be taken to suggest that any therapeutic benefit derived from these substances is not the result of the alkylation of some critical tissue constituent. However, that these drugs do act through alkylation is also unproved.

Miscellaneous Drugs

Nicotine.—Studies in the rat (144) and in the dog (145) with nicotine uniformly labeled with C^{14} have demonstrated that practically all of the isotope is eliminated in the urine. Five metabolites which have been isolated (146 to 149) from dog urine are shown in Figure 19. The position of the hydroxyl group in hydroxycotinine has not been established. All of these compounds with the exception of N-methylnicotine (which has not been sought) have also been detected in the urine of man after smoking or after the ingestion of nicotine (150). The administration to the dog of cotinine, which is less toxic than nicotine, causes the excretion of hydroxycotinine and desmethylcotinine plus some N-methylcotinine (148, 149); presumably,

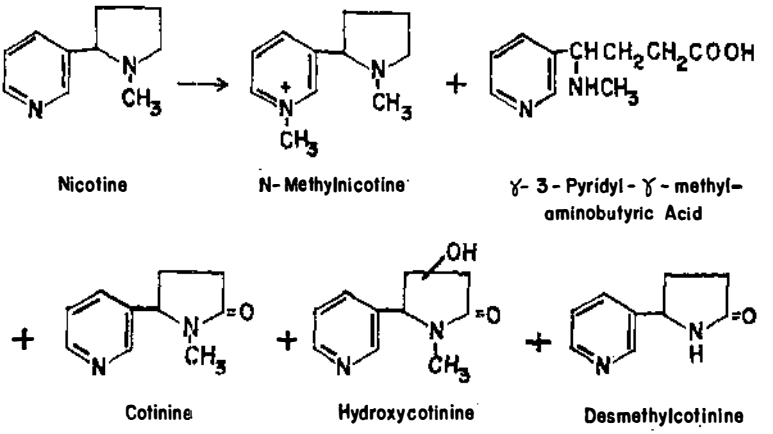


FIG. 19. Metabolism of nicotine.

the latter compound is also a metabolite of nicotine. Earlier work indicated that cotinine was formed primarily by the cyclization of γ-3-pyridyl-γ-methylaminobutyric acid; this reaction proceeds readily *in vitro* under physiological conditions (146, 147). However, the oxidation of nicotine to cotinine by liver microsomes does not appear to involve the amino acid to an important extent; 5′-hydroxynicotine seems a more likely intermediate (151, 152). Also, cotinine could not be detected in the urine of rabbits given γ-3-pyridyl-γ-methylaminobutyric acid (151). 6-Hydroxynicotine is a product of the metabolism of nicotine by bacteria (153, 154); whether this compound is also formed in mammals is not known.

Thiouracil.—The fate of this drug is of interest because it illustrates S-methylation as a biochemical reaction of foreign compounds. After the administration of S^{35}-labeled thiouracil to rats, 60 per cent of the isotope is excreted in the urine as unchanged drug, 6 per cent as 2-methylthiouracil, and 5 per cent as inorganic sulfate (Fig. 20) (155). The desulfuration product, uracil, has also been isolated (156). Other drugs shown to undergo S-methylation are 5-iodo-2-thiouracil (157) and 6-mercaptopurine (158).

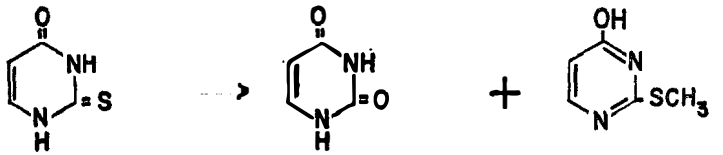


FIG. 20. Metabolism of thiouracil.

Other drugs.—Because of limiations in space it is not possible to discuss recent work on atropine (159), 2-(butylaminomethyl) 1,4-benzodioxane (160), carbromal (161), chlorzoxazone (162), N,N-diethyltryptamine (163), N,N-dimethyltryptamine (164), Dimedion (165), ethyl trichloramate (166), hydralazine (167), isocarboxazid (168), lysergic acid diethylamide (169), mescaline (170), papaverine (171), and zoxazolamine (172).

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