THE METABOLIC FATE OF DRUGS[1]

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Material available for a review on the metabolic fate of foreign compounds is very plentiful and the subject is rapidly growing for it covers not only the metabolism of drugs but also that of pesticides, detergents, cosmetics, industrial chemicals, food additives and any other type of chemical compound that man may contact. The results of metabolic studies are potentially of very wide application and, to mention some of the less obvious applications, one could include detection of racehorse doping, human forensic toxicology, resistance of invertebrates to pesticides, and teratogenesis due to various compounds. However, the editor, fortunately for the authors, requires a "fiercely selective" review. Previous articles on this subject in these *Annual Reviews* have emphasized therapeutic aspects and new types of metabolic reactions [Maynert (1); Boyland & Booth (2)]. In this review, we have made an almost random selection of topics, hoping that we have chosen some that are of general interest and which illustrate the general principles of drug metabolism.

Meprobamate and Other Carbamate Esters

Carbamates of various kinds have received much attention as pharmacological agents. Some of the older hypnotics such as 1,3-dichloroisopropyl carbamate (aleudrine[2]) and 1-methylbutyl carbamate (hedonal) belong to this group, as does the well-known anticholinesterase, physostigmine (eseroline methylcarbamate), the toxic constituent of the Calabar bean. In recent years, renewed attention has been directed towards the carbamates particularly those derived from glycols and used as tranquilizers and those derived from N-methyl- and N-dimethylcarbamic acid having anticholinesterase activity and developed as insecticides. Until recently little was known about the metabolic fate of carbamic acid esters, but it is now clear that there are interesting differences in metabolism between the esters of the type, NH_2COOR, and the type $R_1R_2N \cdot COOR$ (where R_1 or R_2 or both are alkyl groups). Broadly speaking one can say that the ester link in the first type is stable in the body, whereas in the second type there is a tendency for the link to undergo hydrolysis, and this leads to considerable differences in the pharmacological properties of these two types of esters.

N-Methyl carbamates.—The metabolic fate of carbamate insecticides which are compounds of the second type has been considered in a review by Casida (3) and therefore will only be outlined briefly here. These compounds have

[1] The survey of the literature pertaining to this review was concluded in May 1963.

[2] Not to be confused with aleudrin, a synonym for isoprenaline or isoproterenol.

the general formula, $R_1R_2N \cdot COOR$, where R is often an aromatic or heterocyclic system, and R_1 is CH_3, and R_2 is H or an alkyl group (e.g., the insecticide, sevin, where R = 1-naphthyl, $R_1 = CH_3$ and $R_2 = H$). The reactions which may occur in the body with these compounds are (*a*) hydrolysis of the ester linkage to yield the N-substituted carbamic acid and an alcohol or phenol, and (*b*) oxidative demethylation by which an N-methyl group is removed as formaldehyde. The extent of these reactions depends upon the nature of the group R, although further investigations are needed *in vivo*. The insecticide, sevin, for example, when fed to rats yields 1-naphthol conjugates in the urine, and, if carbonyl-^{14}C-sevin is administered, radioactive CO_2 is eliminated in the expired air. These observations suggest that the following metabolic changes take place:

$$CH_3NH \cdot {}^{14}CO \cdot O \cdot C_{10}H_7 \rightarrow C_{10}H_7OH + CH_3NH \cdot {}^{14}COOH \rightarrow {}^{14}CO_2 + CH_3NH_2$$

When compounds of this type, for example, *p*-nitrophenyl dimethylcarbamate, are incubated with rat liver microsomes in the presence of reduced triphosphopyridine nucleotide ($NADPH_2$) and oxygen, formaldehyde-yielding products are formed. There is thus evidence for oxidative demethylation, which, for the *p*-nitrophenyl compound just mentioned, proceeds as follows:

$$(CH_3)_2N \cdot CO \cdot O \cdot C_6H_4NO_2 \rightarrow CH_3 \cdot (CH_2OH) \cdot N \cdot CO \cdot O \cdot C_6H_4NO_2$$
$$\rightarrow HCHO + CH_3NH \cdot CO \cdot O \cdot C_6H_4NO_2$$

Neostigmine, the dimethylcarbamate of *m*-hydroxyphenyltrimethylammonium bromide, is a compound whose metabolism depends upon whether it is given orally or by intramuscular injection, although it provides satisfactory relief of the symptoms of myasthenia gravis if given by either route. On injection, 67 per cent of the drug is excreted unchanged but when given orally to patients only 5 per cent is excreted unchanged [Nowell, Scott & Wilson (4)]. If the urine of these patients is examined chromatographically only the unchanged drug can be detected after injection, but on oral administration two metabolites can be detected, one of which is *m*-hydroxyphenyltrimethylammonium [Scott, Nowell & Wilson (5)]. The site of hydrolysis of neostigmine has not yet been determined. However, the phenolic metabolite is pharmacologically active and an intravenous dose of 5 mg has a therapeutic effect equivalent to that produced by 0.4 mg of neostigmine and is much more rapid in onset. The activity of the metabolite thus explains the efficacy of neostigmine orally or by injection despite the difference in metabolic fate.

Meprobamate and its congeners.—Meprobamate and the related carisoprodol and mebutamate, form a group of carbamates which behave metabolically in a different way from the N-methylcarbamates. In these compounds, the carbamate ester group is stable *in vivo*, since only 1–2 per cent of the ^{14}C of carbamate-labeled meprobamate appears in the expired CO_2 of dogs receiving the drug [Walkenstein et al. (6)]. They are metabolized by oxidation of the longer alkyl substituent attached to C_2 of the propan-1,3-diol portion

of the molecule, and by glucuronide formation to form an apparently new type of N-glucuronide [Walkenstein et al. (6); Berger (7)].

In dogs, the major metabolite of meprobamate is a hydroxy derivative which was formulated by Walkenstein et al. (6) as 2-hydroxymethyl-2-propyl-1,3-propanediol dicarbamate (I, $R = CH_2O \cdot CONH_2$), but further work by Ludwig et al. (8) showed this structure to be incorrect with respect to the position of the hydroxy group. They synthesized (I) and 2-methyl-2-(2′-hydroxypropyl)-1,3-propanediol dicarbamate (II, $R = CH_2O \cdot CO \cdot NH_2$) and showed that hydroxymeprobamate from human and dog urine was identical with (II) and not (I).

$CH_3 \cdot CH_2 \cdot CH_2 \cdot CR_2 \cdot CH_2OH$ — I

$CH_3 \cdot CHOH \cdot CH_2 \cdot CR_2 \cdot CH_3$ — II

Hydroxymeprobamate (II) is pharmacologically inactive and nontoxic, for doses as high as 7000 mg/kg did not kill mice, whereas the LD_{50} of meprobamate in mice is 800 mg/kg. There is also another major metabolite of meprobamate in human and dog urine, a glucuronide which on treatment with acid yields meprobamate and glucuronic acid [Ludwig et al. (8)]. This glucuronide is interesting because meprobamate does not contain the usual groupings which conjugate with glucuronic acid. However, it appears that it could be formulated as an N-glucuronide of meprobamate. A similar glucuronide has been claimed for ethinamate (1-ethynylcyclohexyl carbamate) in rabbits [Tsukamoto, Yoshimura & Tatsumi (9)]. Further metabolites of meprobamate have been shown to occur in the urine of dogs and rabbits by Yamamoto, Yoshimura & Tsukamoto (10) who claim six metabolites apart from unchanged meprobamate. Three of these are glucuronides, one of which is the N-glucuronide of Ludwig et al. (8) and another is the O-glucuronide of hydroxymeprobamate. The third glucuronide has not been identified. The three other metabolites are not conjugated, and were identified as hydroxymeprobamate (II), the corresponding ketone of (II) namely 2-methyl-2-(2′-oxopropyl)-1,3-propanediol dicarbamate or ketomeprobamate (III) and the ω-oxidation product, 2-methyl-2-(3′-carboxypropyl)-1,3-propanediol dicarbamate (IV; $R = CH_2O \cdot CO \cdot NH_2$).

$CH_3 \cdot CO \cdot CH_2 \cdot CR_2 \cdot CH_3$ — III

$HOOC \cdot CH_2 \cdot CH_2 \cdot CR_2 \cdot CH_3$ — IV

In fact, all the metabolites of meprobamate except the N-glucuronide are those which could be expected as a result of the ω- and $(\omega-1)$-oxidation of the *n*-propyl chain of the drug.

Carisoprodol is the N-isopropyl derivative of meprobamate, and has muscle relaxant and analgesic properties. Since this drug is an N-substituted carbamic ester it could be expected to undergo dealkylation to meprobamate. This, in fact, does occur but is of little significance from the pharmacological point of view for the amount of meprobamate occurring in the plasma is not

more than 2–3 per cent of the administered dose of carisoprodol. The major metabolite in the dog is hydroxycarisoprodol which has a probable structure analogous to the structure of hydroxymeprobamate (II) [Douglas, Ludwig & Schlosser (11)]. Very little carisoprodol is excreted unchanged, but the urine contains, in addition to hydroxycarisoprodol, small amounts of hydroxymeprobamate and meprobamate.

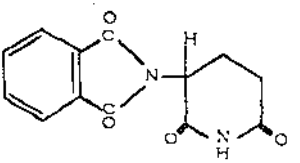
Mebutamate, a centrally acting hypotensive agent, is the *sec*-butyl analogue of meprobamate. Like the latter it is metabolized by oxidation in the dog, and its major metabolite (60 per cent of dose) is hydroxymebutamate which is relatively nontoxic and devoid of the pharmacological properties of the parent drug. Mebutamate also gives rise to considerable amounts of conjugated glucuronic acid (equivalent to 30–40 per cent of the dose) but the nature of this compound is not yet known [Douglas et al. (12)].

The extent of hydroxylation *in vivo* of meprobamate and congeners is proportional to their lipid solubility and this is to be expected if hydroxylation occurs in the liver microsomes. Lipid solubility and extent of hydroxylation are in the sequence carisoprodol > mebutamate > meprobamate and the percentage of the dose of these drugs excreted unchanged in the urine is **<1**, **2** and **10**, respectively [Douglas, Ludwig & Schlosser (11)].

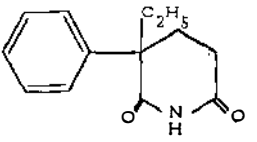
The metabolism of meprobamate is also related apparently to tolerance to the drug. In man and in animals, meprobamate becomes less effective on repeated administration and this may be due partly to accelerated metabolism by hydroxylation to inactive products [Douglas, Ludwig & Smith (13)]. Rats made tolerant to meprobamate excrete only half of the amount of the unchanged drug that nontolerant rats excrete. Furthermore, the tolerant animals excrete the metabolites more rapidly than the nontolerant [Phillips, Miya & Yim (14); Kato (15)]. The metabolism of meprobamate by whole rats, rat liver, or rat liver microsomes is also markedly accelerated by pretreatment of the animals with a number of unrelated drugs such as phenobarbital, glutethimide, nikethamide, etc., but SKF 525A had the opposite effect [Kato & Vassanelli (16); Kato, Chiesara & Frontino (17)]. Similar observations were made with carisoprodol [Kato et al. (18)]. Furthermore, a sex difference in metabolism was observed in rats, for the male metabolised carisoprodol 2.5 times more rapidly than the female [Kato et al. (18)].

Derivatives of Glutarimide

There are two derivatives of glutarimide, namely α-phthalimidoglutarimide or thalidomide (V) and α-ethyl-α-phenylglutarimide or glutethimide (VI), which are of great interest both from a metabolic and pharmacological point of view. V has been described as a mild sedative in man, and VI as a mild hypnotic. Thalidomide, as is now well-known, is teratogenic, whilst glutethimide shows virtually no side-effects. The metabolic fates of these two compounds are completely different, for V is metabolised mainly by hydrolysis and VI by hydroxylation. The amide links in V are unstable at physiological pH values, whereas in VI they are stable. This may be due to the fact that V is a derivative of the unstable α-aminoglutarimide whilst VI is not.



V. Thalidomide



VI. Glutethimide.

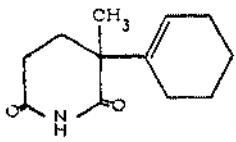
Glutethimide.—It will be convenient to consider glutethimide first, because its metabolism has been more completely elucidated. It is, moreover, an excellent example of a drug occurring in optically active forms, the metabolic fates of which are different [Keberle, Hoffman & Bernard (19); Bütikofer et al. (20); Keberle, Reiss & Hoffman (21)]. The drug itself is a *dl*-form.

In the dog glutethimide (labelled with ^{14}C) is excreted almost entirely in the urine as hydroxylated metabolites conjugated with glucuronic acid. Two conjugated glucuronides, each accounting for about 45 per cent of the dose, are excreted and they are derived from different optical antipodes. The two optical forms of glutethimide have been separated and shown to undergo different routes of metabolism. The (+)-glutethimide is metabolised by hydroxylation of the glutarimide ring to α-ethyl-α-phenyl-α'-hydroxyglutarimide. This metabolite is almost entirely excreted as a glucuronide except for a small proportion (2 per cent of the dose) which is dehydrated and excreted as α-ethyl-α-phenylglutaconimide. The (−) isomer, on the other hand, is metabolized by hydroxylation of the ethyl group to α-(1-hydroxyethyl)-α-phenylglutarimide which again is almost entirely excreted as a glucuronide except for about 4 per cent which loses acetaldehyde to yield α-phenylglutarimide. The glucuronides display no sedative activity, but the glutaconimide has pharmacological properties similar to glutethimide but is less active. The amount formed is so small that it probably plays little, if any, part in the action of glutethimide. The α-phenylglutarimide possesses no sedative properties.

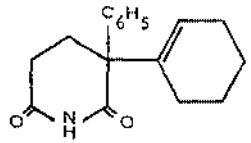
The metabolism of glutethimide in the rat is similar to that in the dog, but, by using rats with biliary fistulae, it was possible to show that enterohepatic circulation of glutethimide metabolites occurred on a considerable scale. About two-thirds of the radioactivity of the administered drug is excreted in the bile largely as glucuronides. These glucuronides were isolated after administration of radioactive glutethimide and then administered to rats. They were less well absorbed than glutethimide itself but once in the blood stream they were easily excreted via the kidneys without spreading to the central nervous system or other lipoid tissues.

The metabolism of N-methyl-α-ethyl-α-phenylglutarimide (N-methylglutethimide) has also been examined in the dog [Keberle et al. (22)]. This compound, like the parent glutethimide, is resolvable into (+) and (−)-forms. Each optical form gives rise to two glucuronides. The (+)-form is partly demethylated to (+)-glutethimide which is then oxidized to the α'-hydroxyglutethimide. The (−)-form is also demethylated to (−)-glute-

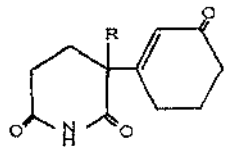
thimide which is then oxidized to the other hydroxyglutethimide. Both hydroxyglutethimides are excreted as glucuronides. However, each optical form is also oxidized at the N-methyl group to the corresponding N-hydroxymethyl-α-ethyl-α-phenylglutarimide which is excreted as a glucuronide of a new type, i.e., that of an N-hydroxymethyl group. Its formation supports the view that N-demethylation proceeds by oxidation of the methyl group to hydroxymethyl which is then removed as formaldehyde. The N-hydroxymethyl compound survives in the body because conjugation with glucuronic acid occurs before it is all converted into glutethimide. Many N-methylated drugs are known to be demethylated in the body, and it is possible that the glucuronides of the intermediate N-hydroxymethyl compounds are formed but have not been found. This suggests that when N-demethylation occurs, the glucuronide of the intermediate should be considered as a possible metabolite.



VII



VIII



IX . a, R = CH_3; b, R = C_6H_5

It is also of interest to mention two glutarimides analogous to glutethimide but containing cyclohexenyl rings. These are α-methyl- and α-phenyl-α-(1-cyclohexenyl)-glutarimides (VII and VIII). These compounds are metabolised in rabbits partly by oxidation of the cyclohexenyl ring to form ketones, (IXa and b) [Tsukamoto & Yoshimura (23)].

Thalidomide.—The metabolic fate of thalidomide (V) is of interest because any of its actions, sedative, neurotoxic or embryotoxic, could be due to a metabolite. Several publications have now appeared on its metabolic fate and a clear picture is now emerging (24 to 30).

Thalidomide is very sparingly soluble in water and its low toxicity was attributed to limited absorption from the gastrointestinal tract [Somers (24)]. However, Beckman (25) and MacKenzie & McGrath (26) using ^{14}C-thalidomide showed that it was readily absorbed from the gastrointestinal tract in rats, peak blood levels of radioactivity being reached in 15 min and

peak brain levels in 0.5 to 1 hr. Radioactivity was also excreted in the urine, but very little of this was due to unchanged thalidomide. Beckman (25) also showed that in rats 21 per cent of the administered radioactivity was eliminated in the bile but again very little of this was unchanged thalidomide. The first metabolite of thalidomide to be isolated in crystalline form from urine was 4-phthalimidoglutaramic acid (X of Table I and Figure 1). This was isolated from rabbit urine by Smith, Williams & Williams (27) who claimed it to be a major metabolite of thalidomide in man, rat, and rabbit. These workers further claimed that the animal urines also contained derivatives of 3-hydroxyphthalic acid thus suggesting that thalidomide underwent aromatic hydroxylation *in vivo*.

TABLE I

LIST OF NAMES OF THALIDOMIDE METABOLITES

V	α-Phthalimidoglutarimide or thalidomide
X	4-Phthalimidoglutaramic acid or phthaloylisoglutamine
XI	2-Phthalimidoglutaramic acid or phthaloylglutamine
XII	α-(*o*-Carboxybenzamido) glutarimide
XIII	2-Phthalimidoglutaric acid or phthaloylglutamic acid
XIV	4-(*o*-Carboxybenzamido) glutaramic acid
XV	2-(*o*-Carboxybenzamido) glutaramic acid
XVI	2-(*o*-Carboxybenzamido) glutaric acid
XVII	Phthalic acid
XVIII	α-Aminoglutarimide
XIX	4-Aminoglutaramic acid or isoglutamine
XX	2-Aminoglutaramic acid or glutamine
XXI	2-Aminoglutaric acid or glutamic acid

Studies by Faigle et al. (28) with ^{14}C-thalidomide showed that after a single oral dose of 100 mg/kg, rats excreted about 80 per cent of the radioactivity in one day (97 per cent in 10 days), just over half of the ^{14}C appearing in the urine and just under half appearing in the faeces. The same workers (28) examined the fate of the labeled drug in dogs which were fed 100 mg/kg daily for 10 days. In these animals 28 per cent of the radioactivity was found in the urine and 64 per cent in the faeces. Most of the faecal radioactivity (97 per cent) was due to unchanged thalidomide. Apart from thalidomide, six other labeled compounds were identified and estimated isotopically in the urine. Similar results were obtained by Beckman (29) in man and the rat. The results of Faigle et al. (28) and Beckman (29) are summarised in Table II. From this table it is obvious that 4-phthalimidoglutaramic acid (X) is a major excretory product in man, and that α-(*o*-carboxybenzamido)glutarimide (XII) is the major excretory product in the rat and the dog. However, these figures may not be of great significance because the studies of Williams and his co-workers (30, 31) have shown that thalidomide is unstable

FIG. 1. The pathways of hydrolysis of thalidomide.

at physiological pH values. If a solution of thalidomide in phosphate buffer pH 7.4 (the solubility of thalidomide is about 40–50 μg/ml) is incubated at 37°, it gradually undergoes spontaneous hydrolysis to yield 12 hydrolysis products (compounds X to XXI of Table I) all of which have been identified by two-dimensional paper chromatography of the thalidomide solution. In one hour about 8 per cent of the thalidomide is hydrolysed and in 24 hr, 80 per cent is hydrolysed. The possible pathways of hydrolysis are shown in Fig. 1. In fact, Williams and co-workers (30, 31) have been able to isolate in crystalline form from the urine of rabbits dosed with thalidomide, all the compounds listed in Table I. The compounds listed in Table II are therefore spontaneous hydrolysis products. Thalidomide undergoes spontaneous hydrolysis

TABLE II

METABOLITES FOUND IN THE URINE OF VARIOUS ANIMALS DOSED WITH ^{14}C-THALIDOMIDE

Compound[a]	Percentage of Dose		
	Man[b]	Rat[b]	Dog[c]
V	1.0	2.2	1.8
X	53.2	7.4–14.1	2.2
XI	5.0	8.3–16.0	13.6
XII	29.5	35.2–54.1	22.9
XIII	3.5	7.5–15.5	9.0
XIV and XV	—	5.3–13.0	—
XVI	2.3	2.4	4.7
XVII	2.7	2.9	6.1

[a] See Table I for names of compounds.
[b] Beckman (29).
[c] Faigle et al. (28).

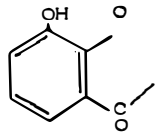
at pH values above 6, and the higher the pH, the more rapid is the hydrolysis. In 0.1 N NaOH, the hydrolysis of thalidomide is, for all practical purposes, almost instantaneous. This means that the speed and extent of spontaneous hydrolysis of thalidomide in the body depends upon the pH it encounters in the gastrointestinal tract and in the body tissues, so that a dose of thalidomide can be equivalent to giving the drug plus the hydrolysis products. Williams and co-workers (30, 31) have also found that whilst thalidomide is the major faecal excretory product in rabbits, the faeces also contain substantial amounts of hydrolysis products, particularly XII. There is therefore evidence that thalidomide is partly hydrolysed before absorption from the gastrointestinal tract. These hydrolysis products and thalidomide are detectable in the blood and brain of rats 1–2 hr after dosing with the drug.

The biological activities of some of these hydrolytic products have been investigated. Compounds X and XI have no teratogenic effect upon white

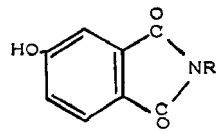
New Zealand rabbits which are known to be affected by thalidomide. Thalidomide itself prolongs the hexobarbital sleeping time in rats, but X, XI, XIII, XIV, XV, and XVII do not, and it appears that the sedative effect of thalidomide may be due to the parent drug rather than metabolites [Smith, Williams & Williams (27); Schumacher et al. (31)]. Since the hydrolytic products of thalidomide are largely derivatives of glutamic acid it is possible that they could interfere with the glutamine and glutamic acid metabolism of the embryo by affecting the enzymes involved. Tests have been made on some of these enzymes *in vitro* and it has been found that 2-(*o*-carboxybenzamido)glutaric acid (XVI) is an inhibitor of rat brain glutamine synthetase, glutamate dehydrogenase and L-glutamate decarboxylase, but the inhibition is not very marked (inhibitory concentration 10^{-2} to 10^{-3} *M*). None of the other metabolites inhibit glutamine synthetase. Compounds XIV and XVII also inhibit glutamate dehydrogenase and XIII is a more powerful inhibitor of glutamate decarboxylase than XVII. The significance of these enzymic inhibitions is not yet known in relation to the effects of thalidomide.

Roath, Elves & Israël (32, 33) have studied the effect of thalidomide and some of its hydrolysis products upon the transformation of human leucocytes in culture. Glutethimide had no effect on these cultures, but thalidomide had an inhibitory effect in so far as there was a decrease in the percentage of cells transformed into the "blast" and "intermediate" types, compared with normal. Morphological abnormalities in the cultures were also observed in the presence of thalidomide. This effect was also shown by XV and XVI, but not by X, XI and XIII.

The hydroxylation of thalidomide *in vivo* was reported by Smith et al. (27) and further work has shown that derivatives of 3- and 4-hydroxythalidomide (XXII and XXIII) (R = glutarimide ring) occur in rabbit urine [Schumacher et al. (31)].



XXII. 3-Hydroxy derivatives.



XXIII. 4-Hydroxy derivatives.

The hydroxy thalidomides have been synthesized and shown to produce abnormalities in chick embryos [Boylen, Horne & Johnson (34)]. Five hydroxy compounds, namely, 3- and 4-hydroxythalidomide, 2-(3′- and 4′-hydroxyphthalimido)glutaramic acid, and 2-(4′-hydroxyphthalimido)glutaric acid were found to produce abnormalities in chick embryos when injected into the yolk sac. The incidence of these abnormalities was reduced to almost the same levels as in controls if L-glutamine were injected simultaneously with the hydroxy compounds. This would suggest that glutamine metabolism is interfered with by thalidomide or its metabolites.

BARBITURATES

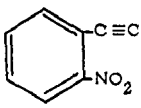
The general pathways of metabolism of this important group of drugs are now well known, but much research is still needed to clarify the nature of the metabolites of specific barbiturates which, moreover, could be of medico-legal significance in cases of suspected barbiturate poisoning where none of the unchanged drug remained in the body or excreta. Paper chromatography techniques have been developed for identifying the metabolites of barbital, phenobarbital, cyclobarbital, propallylonal, pentobarbital, butabarbital, and hexobarbital and it is claimed that a chromatographic pattern specific for each drug can be obtained with urine extracts [Frey, Sudendey & Krause (35)]. The use of gas chromatography for the separation and identification of barbiturates and their metabolites has also been recommended [Vanden-Heuvel, Haahti & Horning (36); Svendsen & Brochmann-Hanssen (37)].

Phenobarbital.—That the main metabolite of phenobarbital in man and the dog is *p*-hydroxyphenobarbital is now well known [Butler (38)]. More recently the metabolism of phenobarbital labelled with ^{14}C in the 2-position of the barbiturate ring has been examined in the rat [Glasson & Benakis (39)]. The rats exhaled no radioactive CO_2 indicating that the barbiturate ring of this drug is not destroyed in this species. On intravenous administration of the drug (90 mg/kg), 45 per cent of the dose was excreted in the urine in 24 hr and seven radioactive metabolites were detected. These consisted of unchanged phenobarbital (27.5 per cent of the urinary ^{14}C), *p*-hydroxyphenobarbital (19 per cent) and its glucuronide (27 per cent) and possibly *o*-hydroxyphenobarbital (2.5 per cent). A minor metabolite (<1 per cent) was another conjugate of hydroxyphenobarbital. The two remaining metabolites accounted for 20 per cent of the urinary radioactivity, one being a conjugate of the other. They were not identified, although they were not parabanic acid, oxaluric acid or urea which could be metabolites if the barbiturate ring were disrupted. The same authors [Glasson & Benakis (40)] showed that in rats pretreated with carbon tetrachloride, the rate of elimination of radioactivity after injection with ^{14}C-phenobarbital, and the hydroxylation and conjugation of the drug, were retarded.

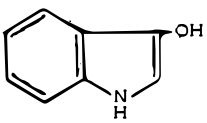
Secobarbital (*Seconal*).—Seconal is a widely used barbiturate of short duration of action containing an allyl and a secondary amyl group. The allyl group in barbiturates is usually stable to biological attack and, in fact, Seconal is metabolized through its *sec*-amyl or 1-methylbutyl, group. Apart from unchanged seconal, two metabolites have been identified in the urine of rabbits, namely hydroxyseconal [5-allyl-5-(3′-hydroxy-1′-methylbutyl)barbituric acid] and seconal carboxylic acid [5-allyl-5-(3′-carboxy-1′-methylbutyl)barbituric acid] [Tsukamoto, Yoshimura & Ide (41)]. Seconal is thus metabolized in part by an ω- and an $(\omega-1)$-oxidation of the 1-methylbutyl side chain. The formation of hydroxyseconal introduces a new asymmetric carbon atom into the molecule and assuming that Seconal itself, which is a *dl*-form, remains unresolved, two diastereoisomeric hydroxyseconals could be

formed. The metabolite isolated has $[\alpha]_D^{19}+19.3°$ and appeared to be the (+)-form of hydroxyseconal. Other metabolites of Seconal were formed but not identified [Tsukamoto et al. (41)] although Waddell (42) had earlier reported that Seconal formed a hydroxyseconal in the dog which was excreted free (15 per cent of dose) and conjugated with glucuronic acid (15 per cent).

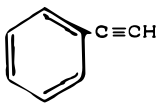
Methohexital and the metabolism of acetylenic links.—Methohexital is a barbiturate containing an acetylenic link in one of the 5-substituents, i.e., 5-allyl-5-(1′-methylpent-2′-ynyl)-1-methylbarbituric acid, which is an ultrashort acting barbiturate in rats, dogs, monkeys, and man. Since the allyl group in barbiturates is usually stable *in vivo*, and the acetylenic link is relatively resistant to biological attack, it is of much interest to know which of the two 5-substituents, the allyl or the 1-methylpentynyl, of methohexital is metabolized *in vivo*. At this point, however, it might be useful to review briefly previous information on the metabolism of some drugs containing the acetylene linkage. From earlier work on *o*-nitrophenylacetylene (XXIV) and phenylacetylene (XXVI) it is known that the acetylenic linkage can be metabolized *in vivo*, for the first compound yields indoxyl (XXV) *in vivo* and the second is slowly converted into phenylacetic acid (XXVII).



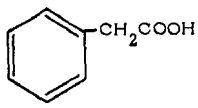
XXIV



XXV



XXVI



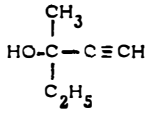
XXVII

XXIV. *o*-Nitrophenylacetylene. XXV. Indoxyl.

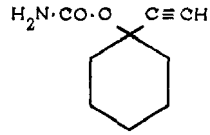
XXVI. Phenylacetylene. XXVII. Phenylacetic acid

It is to be noted, however, that the ethynyl group (-C≡CH) in these compounds is attached directly to an aromatic ring. In most of the drugs containing the ethynyl group, it is attached to an aliphatic system, examples of which are methylparafynol (Dormison) (XXVIII), ethinamate (XXIX), pargyline (XXX), and butynamine (XXXI). In all these drugs, the acetylenic system is stable *in vivo*, and metabolic change, if any, occurs elsewhere in the molecule. Methylparafynol (XXVIII) is metabolized by conjugation at the OH group to give the corresponding glucuronide [Smith & Williams (43)]; ethinamate (valamin) (XXIX) is largely hydroxylated in the cyclohexane ring to hydroxy-ethinamate [McMahon (44)]; pargyline (XXX) is mainly excreted unchanged, although an unidentified metabolite amounting to only 3 per cent of the dose is formed [Taylor & Krause (45)] and butynamine (XXXI) is mainly N-demethylated to 3-methyl-3-(*tert*-butylamino)but-1-yne [McMahon & Easton (46)].

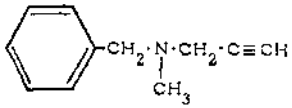
Methohexital contains three groups which could be metabolized, namely, the 1-N-methyl group and the two 5-substituents. In some barbiturates, such



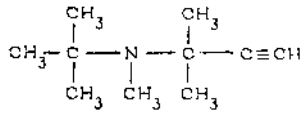
XXVIII



XXIX



XXX



XXXI

XXVIII. Methylparafynol.

XXIX. Ethinamate.

XXX. Pargyline.

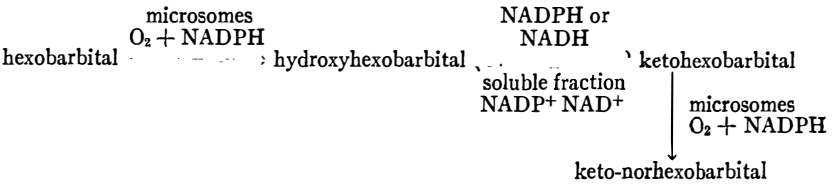
XXXI. Butynamine.

as N-methylphenobarbital (mephobarbital), N-methyl groups are extensively but slowly metabolized to CO_2. However, by using methohexital with ^{14}C in the N-methyl group, it could be shown that very little demethylation (*ca.* 1 per cent) of this drug occurred in the rat or dog [Welles, McMahon & Doran (47)]. The main urinary metabolite of methohexital in the dog and the rat was hydroxymethohexital in which the acetylene and ethylene links were unchanged, hydroxylation having occurred at the $(\omega-1)$-carbon atom of the 1-methylpentynyl group. The short anaesthetic action of methohexital (8–10 min) is believed to be due to a fall in blood level caused by distribution rather than by metabolism and excretion. In rats receiving the ^{14}C-drug by injection, nearly 83 per cent of the radioactivity is excreted in the faeces and about 19 per cent in the urine in 48 hr, whereas the values found in the dog are, faeces, about 30 per cent, and urine, 50–60 per cent. However, there is a very rapid biliary excretion of the injected drug, for in the rat more than 50 per cent of the administered radioactivity is excreted in the bile in one hour and a total of 78 per cent in 24 hr. The major product excreted in the bile is probably the hydroxylated metabolite. In the dog excretion of metabolites was not as rapid as in the rat.

Dihydro barbiturates.—On electrolytic reduction, phenobarbital is reduced in the 2-position to primidone or 2-deoxyphenobarbital. One metabolic reaction of primidone is oxidation back to phenobarbital. Another example of this reaction has now been found with the reduced form of ethylhexobarbital, that is 5-ethyl-5-(1′-cyclohexenyl)-4,6-dioxohexahydropyrimidine. On administration to rabbits this compound yields keto-ethylhexobarbital which is also a metabolite of ethylhexobarbital [Yoshimura & Tsukamoto (48)].

Enzymic aspects of barbiturate metabolism.—There are three main aspects of barbiturate metabolism and these are (*a*) oxidation of a 5-substituent, (*b*)

removal of an N-methyl group, and (*c*) degradation of the barbiturate ring; but the enzymic aspects of these reactions have not yet been considered in detail. The main enzyme studies of barbiturates have been concerned with the stimulation or inhibition of the oxidation process by other drugs and by physiological factors such as sex, strain, age, species and environment and this aspect has been adequately reviewed recently [Conney & Burns (49); Netter (50); and Remmer (51)]. However, details of the enzymic aspects of the various steps in the metabolism of barbiturates have as yet received little attention. A beginning has been made with hexobarbital. This compound gives rise *in vivo* to hydroxyhexobarbital, keto-hexobarbital and keto-norhexobarbital. The conversion of hexobarbital to hydroxyhexobarbital is carried out by an NADPH-dependent enzyme requiring molecular oxygen and occurring in liver microsomes, but the conversion of hydroxyhexobarbital to keto-hexobarbital is carried out by an NAD- or NADP-dependent enzyme present in the soluble fraction of liver homogenates. Furthermore, the second enzyme catalyses a reversible reaction and is inhibited by microsomes [Toki, Toki & Tsukamoto (52, 53)]. Hexobarbital is not demethylated by liver slices, but both its hydroxy- and keto-derivatives are converted into keto-norhexobarbital [Okui & Kuroiwa (54)]. It would appear that oxidation to keto-hexobarbital occurs before demethylation and that the sequence of reactions from hexobarbital to keto-norhexobarbital is as follows:



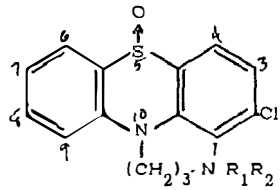
Pretreatment of rats with phenobarbital stimulates all the above reactions, for the *in vitro* conversion of hexobarbital to hydroxyhexobarbital and of keto-hexobarbital to keto-norhexobarbital is increased by 50 per cent whereas the conversion of keto-hexobarbital to hydroxyhexobarbital is increased by 150 per cent. However, the reverse reaction is reduced, possibly due to a stimulation of processes leading to complete oxidation of the cyclohexenyl ring [Okui & Kuroiwa (54)].

In the case of N-methylbarbital and N-methylphenobarbital, the 5-substituents (i.e., ethyl and phenyl), unlike the cyclohexene ring in hexobarbital, are not readily metabolized, but the compounds are demethylated [Kuroiwa (55)]. If rats are pretreated with phenobarbital, the demethylation of methylbarbital is increased tenfold, and the demethylation of methylphenobarbital is increased from negligible to considerable amounts [Okui & Kuroiwa (54)]. The demethylation occurs in the liver but not in the kidney [Kuroiwa (55)].

Chlorpromazine and its Derivatives

The metabolic fate of chlorpromazine is a difficult problem because various reports have suggested that more than 20 metabolites are formed, and

there is the further complication that the bile is as important a channel of excretion as the urine. A consideration of the chlorpromazine molecule (XXXIIa without O at 5), and of previous work on phenothiazine and its simple derivatives such as methylene blue, suggest several possibilities.



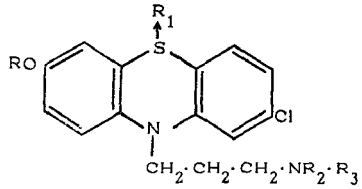
XXXII

(a) $R_1=R_2=CH_3$. (b) $R_1=CH_3$, $R_2=H$. (c) $R_1=R_2=H$.

These are (*a*) oxidation of the heterocyclic sulphur atom to a sulphoxide or a sulphone; (*b*) dealkylation, which could result in the removal of one or both of the terminal methyl groups or even the removal of the whole of the dimethylaminopropyl group; (*c*) the hydroxylation of the ring, which could occur at seven different positions, followed by conjugation with glucuronic or sulphuric acid; (*d*) oxidation of the terminal dimethylamino group to yield an N-oxide; and (*e*) splitting of the central ring to diphenylamine derivatives. Any combination of these reactions could occur, so that the possible number of metabolites is quite large. Numerous papers have been published on the metabolism of chlorpromazine, but relatively few of these will be quoted here. The oxidation of the heterocyclic sulphur was first reported by Salzman & Brodie (56) who found chlorpromazine 5-oxide (XXXIIa), as a urinary metabolite of the drug in man and the dog. Subsequently, demethylation of the terminal nitrogen of the dimethylaminopropyl group to give desmonomethylchlorpromazine and desdimethylchlorpromazine [Ross, Young & Maass (57, 58)] and the aromatic hydroxylation and conjugation of chlorpromazine were reported [Lin et al. (59)]. The formation of free radicals from chlorpromazine and its metabolites *in vivo* has been considered to occur but whether these are of any pharmacological significance is an open question [Forrest & Forrest (60); Lagercrantz (61)].

The use of ^{35}S-chlorpromazine has shown that in rats 40–50 per cent of single doses (12 mg/kg) are excreted in the urine and an equivalent amount in the faeces, the excretion of radioactivity being complete in 72 hr [Emmerson & Miya (62)]. About a half of the urinary radioactivity is made up of four compounds, the main one (12 per cent of the dose) being unchanged chlorpromazine. The other three were the sulphoxides of chlorpromazine (XXXIIa) and its mono-demethylated (XXXIIb) and di-demethylated (XXXIIc) derivatives. The amounts of these compounds excreted in the urine by rats after a single dose of chlorpromazine were XXXII, 12.3; XXXIIa, 5.0; XXXIIb, 5.2; and XXXIIc, 2.3 per cent. Rats pretreated with the drug, by being given daily an oral dose of 25 mg/kg for 14 days, and

then given the ^{35}S drug, excreted the same amounts of XXXII and XXXIIb, but XXXIIa decreased (3.6 per cent) and XXXIIc increased (4.2 per cent), suggesting that demethylation but not the total sulphoxidation was enhanced by pretreatment. The sulphoxide metabolites are also excreted by man and the dog, the total sulphoxides in the dog being similar to that in the rat. In man, however, the excretion of the sulphoxides is of less importance and amounts to only about 6 per cent of the dose whilst the unchanged drug excreted is very small (0.2 per cent) [Goldenberg & Fishman (63)]. It has been claimed that man excretes six sulphoxides after chlorpromazine, and three of these are XXXIIa, b and c [Fishman & Goldenberg (64)].



XXXIII

FIG. 2. Hydroxylated metabolites of chlorpromazine.

	R	R_1	R_2	R_3
a	H	—	CH_3	CH_3
b	$C_6H_9O_6$	—	CH_3	CH_3
c	H	—	CH_3	H
d	$C_6H_9O_6$	—	CH_3	H
e	H	—	H	H
f	$C_6H_9O_6$	—	H	H
g	H	O	CH_3	CH_3
h	$C_6H_9O_6$	O	CH_3	CH_3

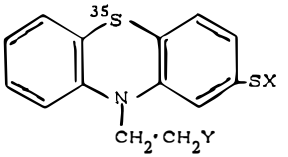
There are several publications which suggest that the major urinary metabolites of chlorpromazine in man are polar and water-soluble. Four of these metabolites have been claimed to be glucuronides of hydroxylated phenothiazines [Lin et al. (59)]. On analogy with phenothiazine itself, hydroxylation could be expected to occur in positions 3 or 7 or both, but hydroxylation in the other free positions, 1, 4, 6, 8 and 9, cannot be excluded. Since hydroxylation could be combined with sulphoxidation and demethylation, a relatively large number of glucuronides are possible. Four of these glucuronides have now been identified in dog and human urine, and since the aglycones of these glucuronides also occur in the free state in the urine, this means that 8 more metabolites of chlorpromazine have been identified [Fishman &

Goldenberg (65)] (see Fig. 2, XXXIII a–h). All these compounds (see formulae above) are derivatives of 7-hydroxychlorpromazine, and include the 5-oxide, the desmonomethyl and desdimethyl 7-hydroxychlorpromazines and their glucuronides. Fishman & Goldenberg (65) have stated that additional hydroxy derivatives occur in dog urine and still more in human urine. In fact, hydroxylation and glucuronide formation is a more important pathway of biotransformation in man than in the dog. Whether or not these hydroxy metabolites have pharmacological activity does not appear to have been reported, but it is interesting to note that Posner et al. (66), working with model metabolites of promazine, have suggested that hydroxylated metabolites may be pharmacologically active. The hydroxypromazines were synthesized and tested for pharmacological activity, i.e., potentiation of hexobarbital sleeping time, and inhibition of stimulated locomotor activity and conditioned responses, in the rat and mouse. The 4-hydroxy derivative was almost as active as promazine, whilst the 2-hydroxy compound was markedly less active than promazine except in the sleeping time test, when it was found on intravenous administration to be about as active as promazine. However, it should be noted that 2- and 4-hydroxypromazine are synthetic compounds and not proved metabolites of promazine. In the case of imipramine (Tofranil), an analogue of promazine, the hydroxy metabolite is pharmacologically active (see XXXVIb).

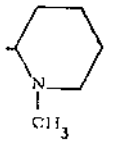
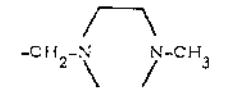
The formation of the N-oxide of chlorpromazine has now been proved and the compound has been isolated from human and dog urine [Fishman, Goldenberg & Heaton (67)]. This substance is excreted by humans in amounts equivalent to 0.7 per cent of the dose. In dogs the excretion of this metabolite is greater than in man, amounting to 2.0 to 3.5 per cent of the dose. On keeping this N-oxide for several weeks, it decomposes to give formaldehyde and desmonomethylchlorpromazine. It is interesting to note that the N-oxide and its decomposition product, the desmonomethyl derivative are nearly as active pharmacologically as the parent drug [Posner et al. (66)].

It was suggested earlier that the splitting of the phenothiazine ring of chlorpromazine to yield diphenylamine derivatives was a possible reaction *in vivo*. If this occurred, then one might expect a release of sulphur which would appear in the urine as sulphate. However, using ^{35}S-chlorpromazine in rats, Emmerson & Miya (62) have reported that the urine contained negligible amounts of radioactive inorganic sulphate, and this suggests that, at least as far as the rat is concerned, the ring system in chlorpromazine is biologically stable.

Other phenothiazines.—At least 50 per cent of chlorpromazine is excreted in the bile and this is also a characteristic of other phenothiazines. The metabolism of three such compounds has been reported recently [Zehnder et al. (68, 69)]. These compounds, thioridazine (XXXIVa), Sandoz E.T. 758 (XXXIVb), and thiethylperazine (XXXIVc), were prepared with ^{35}S in position 9. Whether they were given orally or by injection they were excreted

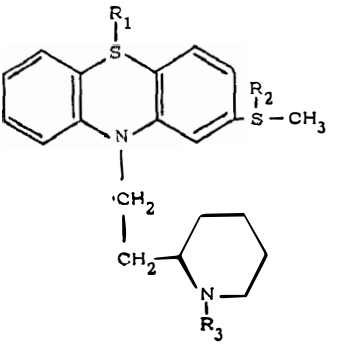


XXXIV

	X	Y
a) Thioridazine	CH_3	
b) Sandoz E.T.758	CH_3	
c) Thiethylperazine	C_2H_5	

largely in the bile as metabolites, less than 10 per cent of the dose appearing in the urine.

Some of the metabolites of XXXIVa have been identified in bile and urine and estimated by reverse isotope dilution. The metabolites found are those expected on analogy with chlorpromazine and those expected from metabolism of the two side chains. However, these metabolites account for only a minor proportion of the radioactivity of the bile and the urine, and it appears that the major transformation products of thioridazine (80 per cent) are glucuronides of unknown constitution derived from XXXIVa and its norderivative. Eight metabolites of thioridazine, which occur in bile and urine, have been identified as the 5-oxide (XXXVa), the side-chain sulphoxide (XXXVb), the disulphoxide (XXXVc) and the disulphone (XXXVd) of thioridazine, and the corresponding sulphur oxidation products of northioridazine (XXXVe–h). Of these compounds, the disulphoxides (XXXVc & g) occur as the major component in both bile and urine. Using the N–$^{14}CH_3$ form ($R_3 = {}^{14}CH_3$ in formula XXXV) of thioridazine, it can be shown that 30 to 40 per cent of thioridazine is demethylated in rats. A relatively unusual type of metabolite are the disulphones, (XXXVd & h), for sulphone formation *in vivo* has not been reported for chlorpromazine or any other phenothia-

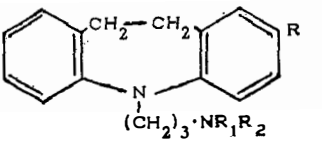


XXXV

Metabolite	R_1	R_2	R_3
a)	O	—	CH_3
b)	—	O	CH_3
c)	O	O	CH_3
d)	O_2	O_2	CH_3
e)	O	—	H
f)	—	O	H
g)	O	O	H
h)	O_2	O_2	H

zine except methylene blue, the sulphone of which was described as far back as 1905 as occurring in the urine of animals injected with the dyestuff [Underhill & Closson (70)].

Imipramine (*Tofranil*).—Imipramine (XXXVIa) is an analogue of promazine in which the heterocyclic sulphur atom has been replaced by an ethylene group. The expected metabolic reactions of this compound, allowing for the fact that it has no sulphur, are hydroxylation and demethylation as in the case of chlorpromazine, and this is what has been found. However, the ethyl-



XXXVI

(*a*) Imipramine R=H, $R_1=R_2=CH_3$

(*b*) 2-Hydroxy-imipramine R=OH, $R_1=R_2=CH_3$

(*c*) Metabolite R=H, $R_1=CH_3$, $R_2=H$

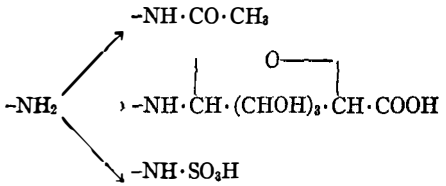
ene bridge between the two benzene rings is also open to metabolic attack, possibly by oxidation and fission.

Three metabolites of imipramine have been identified in the urine of man and the rabbit. The first two metabolites are 2-hydroxyimipramine (XXXVI b) and its glucuronide (XXXVI, $R = O \cdot C_6H_9O_6$) which account for 40 to 50 per cent of the dose in the rabbit but only 6 to 10 per cent in man. The hydroxy compound can also be formed *in vitro* by the action of ascorbic acid and iron on imipramine. The other metabolite is the monodemethylated compound (XXXVIc) which is a relatively minor product. [Hermann & Pulver (71); Schindler (72); Pulver, Exer & Hermann (73)]. The importance of demethylation in determining the nature of the pharmacological action of imipramine, amitriptyline, and promazine has been emphasized by Bickel, Sulser & Brodie (74).

Sulfonamides

The introduction of the sulfonamide drugs for the treatment of acute bacterial infections in the mid-1930's started a new era in chemotherapy, but the later development of antibiotics in the 1940's relegated the sulfonamides to a place of secondary importance. However, the appearance of side-effects and of resistant strains, and the problem of secondary infections have lead to an attitude of restraint in the use of antibiotics and there is consequently a renewed interest in sulfonamide drugs. A number of new sulfonamides with a long duration of action have been made in recent years and it appears that the sulfonamides are staging a come-back [Neipp, Sackmann & Tripod (75)]. The duration of action of a sulfonamide drug is an important consideration, since those with long duration of action can be administered less frequently to provide the necessary therapeutic effect than those of short or medium duration of action. The reason for the long or short duration of action in sulfonamide drugs is not clear, but it may be related to protein-binding or metabolic fate.

From the point of view of metabolic fate, there are two main structural components in a sulfonamide, $H_2N \cdot C_6H \cdot SO_2 \cdot NHR$ (in which the first amino group is referred as N^4 in the following text, and the second as N^1), to be considered, namely, the N^4-amino group and the substituent group R. The N^4-amino group is now known to undergo three reactions *in vivo*. These are acetylation, N^4-glucuronide formation and N^4-sulfate or sulfamate formation:



Acetylation.—This is a well-known reaction of sulfonamide drugs, but the extent to which it occurs varies with the individual, species, and the sulfona-

mide. It is, nevertheless, a reaction of major significance, for the acetylated drug is therapeutically inactive and could be responsible for kidney damage if it is sparingly soluble. Acetylation of the N^4-amino group occurs in most species of animals except the dog and the fox [Leibman & Anaclerio (76); Bridges & Williams (77)], although, in the case of sulfanilamide only, both the dog and the fox can acetylate the N^1-amino group so that sulfacetamide, $NH_2 \cdot C_6H_4 \cdot SO_2 \cdot NH \cdot CO \cdot CH_3$, is a minor metabolite (about 9 per cent) of the drug in these species [Bridges & Williams (77)]. In all other species examined and with all sulfonamide drugs tested, N^4-acetylation has been found to occur. The process, however, is very variable and a few recent examples are quoted to illustrate this. With the drug sulfasomisole (5-*p*-aminobenzenesulfonamido-3-methylisothiazole), the extent of acetylation in three species was rabbit > man > rat [Bridges & Williams (78)] whilst with sulfamethomidine (4-*p*-aminobenzenesulfonamido-6-methoxy-2-methylpyrimidine) the order was rat > rabbit > man [DiCarlo et al. (79, 80)]. Different sulfonamides are acetylated to different extents in the same species; thus for four sulfonamides in man the extent of acetylation of 1-g doses in 24 hr was sulfamethoxypyridazine > sulfisoxazole > sulfamethomidine > sulfadimethoxine as measured by the free and total drug excreted in the urine [DiCarlo et al. (80)]. Of the common laboratory animals, it is probably true to say that the rabbit acetylates sulfonamides to the highest extent. However, the amount of acetyl derivative excreted in the urine probably depends upon the rate of acetylation and of deacetylation, and in the case of the rabbit, deacetylation occurs to only a minor extent. In the hen, deacetylation occurs very actively in the kidney and the hen thus appears to be a poorer acetylator of sulfonamides than the rabbit [Bridges & Williams (77)].

N^4-Glucuronide formation.—This reaction of sulfonamides is a relatively minor one and these glucuronides occur in urine to no more than a few per cent of the dose. Their formation is spontaneous and nonenzymic and depends upon pH and the availability of free glucuronic acid. Their spontaneous synthesis can occur in blood or urine. They are also labile especially at acid pH values, so that any N-glucuronide found in urine is the result of two opposing reactions, synthesis and hydrolysis, both dependent upon the pH of the medium [Bridges & Williams (81)]. One could expect all sulfanomide drugs to form N-glucuronides, and many have been reported as occurring in urine as minor metabolites (e.g., sulfanilamide, sulfacetamide, sulfathiazole, and sulfamethylthiadiazole [Bridges & Williams (81); Uno & Ueda (82); Uno & Okazaki (83)].

N-Sulfates or sulfamates.—The N-sulfates of sulfonamides have been reported to occur as minor urinary products of a number of sulfonamides (e.g., sulfasomizole, sulfisoxazole, sulfathiazole, and sulfamethylthiadiazole [Bridges & Williams (77); Uno (84); Uno & Ueda (82); Uno & Okazaki (83)]. These compounds are not formed spontaneously and are presumably formed *in vivo* by enzymic transfer of sulfate from 3′-phosphoadenosine-5′-phosphosulfate (PAPS) [see Roy (85)].

In order to illustrate the quantitative importance of the N^4-conjugates of sulfonamide drugs, two examples are quoted below:

Drug:	*Free*	*N^4-acetyl*	*N^4-glucuronide*	*N^4-sulfate*
Sulfathiazole	63	29	0.8	0.5
Sulfisoxazole	56	18	3.4	1.0

The above values in percentage of dose were obtained in human urine, 48 hr after dosing with 1 g of sulfathiazole [Uno & Ueda (82)] and 24 hr after a dose of 2 g of sulfisoxazole [Uno (84)].

Other metabolites of sulfonamides.—Apart from the N^4-amino group, the group R in the formula, $H_2N \cdot C_6H_4 \cdot SO_2 \cdot NHR$, could also be metabolized. However, there is a considerable dearth of information on this point pertaining to the newer sulfonamide drugs. The following drugs have been shown to form *in vivo*, products which are derived from the metabolism of the R group, sulfamethomidine [DiCarlo, Malament & Phillips (86)], sulfathiazole [Uno (84)], sulfadimethoxine, sulfamethoxypyridazine [Koechlin, Kern & Engelberg (87)], sulfamethoxypyrazine [Bertazzoli, Chieli & Ciceri (88)], sulfasomizole [Bridges & Williams (78)], sulfisoxazole [Uno (84)], and sulfamethylthiadiazole [Uno & Okazaki (83); Uno (84)].

Most of the sulfonamides listed in Table III are drugs of medium or long duration of action and they all undergo *in vivo* some kind of change in the heterocyclic substituent. What happens in these heterocyclic groups is at present not known with any certainty. In fact, the only sulfonamide drug for which the metabolism of the heterocyclic moiety has been elucidated is that of sulfapyridine, which is known to be oxidized *in vivo* to 5′-hydroxysulfapyridine which is excreted as a glucuronide [Scudi & Childress (89)]. The drugs listed in Table III form glucuronides in which the glucuronic acid is associated in some way with the heterocyclic system. The amounts of these glucuronides formed are quite large in some cases such as sulfadimethoxine, sulfamethomidine, and sulfamethoxypyridazine, but quite small (about 2 per cent of the dose) in the others. In some instances, the glucuronide is probably that of a hydroxy derivative of the drug, e.g., sulfamethomidine, but in others, glucuronic acid is attached to the drug in an unknown manner for the glucuronide on hydrolysis often releases the unchanged parent drug as the aglycone. The elucidation of the nature of these glucuronides is therefore a problem of considerable interest.

Sulfamethomidine.—This drug forms two main metabolites in man, the N^4-acetyl derivative and a glucuronide, which is not an N^4-glucuronide. The glucuronic acid in the second metabolite is attached to the pyrimidine ring in ether linkage [DiCarlo, Malament & Phillips (86)]. Sulfamethomidine glucuronide on diazotization and coupling with N-(1-naphthyl)ethylenediamine forms an azo-glucuronide which on hydrolysis with acid or alkali yields azo-sulfanilic acid or azo-sulfanilamide, thus showing that hydroxylation *in vivo* had not occurred in the benzene nucleus of the drug.

TABLE III

Some Heterocyclic Sulphonamides which Form Glucuronides

Drug	R	Duration of action	Percentage of glucuronide formed	Proposed nature of glucuronide	Reference
Sulfamethomidine		medium	+ (man)	glucuronide of hydroxysulphamethomidine of unknown structure	(86)
Sulfathiazole		short	3-4 (man)		(84)
Sulfadimethoxine		long	> 50 (man)	unknown	(87)

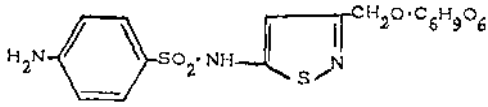
Sulfa-somizole		medium	2 (rabbit, dog)		(78)
Sulf - isoxazole		medium	2 (man)		(84)
			30–35 (man)	--	(92)
Sulfa-methoxy-pyridazine		long	14–19	unknown	(92)
Sulfa-methoxy-pyrazine		long	+	unknown	(88)
Sulfa-methyl-thiadiazole		medium	1	unknown	(84)

Sulfathiazole.—This drug undergoes in man all the known reactions of sulfonamide drugs. In 48 hr, 97 per cent of a 1-g dose is excreted in the urine, 63 per cent being unchanged sulfathiazole, 29 per cent N^4-acetylsulfathiazole, 0.8 per cent sulfathiazole N^4-glucuronide, and 0.5 per cent sulfathiazole N^4-sulfate. In addition to these, 3.8 per cent of the dose is excreted as a glucuronide containing a free aromatic amino group. This glucuronide (m.p. 176–178°) gives equimolecular amounts of sulfathiazole and glucuronic acid on hydrolysis. On the basis of infrared and ultraviolet spectra Uno (84) considers it to be an N^1-glucuronide, which is a new type of N-glucuronide.

Sulfadimethoxine.—This long-acting sulfonamide (Madribon) is particularly interesting because it shows a marked species difference in metabolic fate. In the rabbit and rat, it is extensively acetylated to N^4-acetylsulfadimethoxine and little, if any, glucuronide is formed. In man, however, the drug is converted to a considerable extent to a glucuronide, and acetylation is much less than in the rabbit and the rat [Fust et al. (90); Koechlin, Kern & Engelberg (87)]. Sulfadimethoxine is an extreme example of species difference, for, in the authors' laboratory, it has been found that acetylation in the rabbit can be as high as 90 per cent of the dose with practically no glucuronide formation, whereas in man glucuronide accounts for 60 per cent or more of the dose, and acetylation may be less than 20 per cent. The structure of the glucuronide of sulfadimethoxine which is not an N^4-glucuronide is thus of great interest. On acid hydrolysis, the glucuronide yields sulfanilic acid and this shows that the glucuronic acid is not attached through a hydroxyl group to the benzene nucleus [Koechlin, Kern & Engelberg (87)]. In the authors' laboratory, the glucuronide has been found to release sulfadimethoxine on treatment with snail glucuronidase, so that the glucuronide may not be a derivative of a hydroxylated sulfadimethoxine, but possibly some kind of N-glucuronide.

Sulfasomisole.—This drug is regarded as having a medium duration of action [Adams et al. (91)]. In the rabbit, it is highly acetylated, and in man it forms the expected sulfonamide metabolites which are the N^4-acetyl derivative (about one third of the dose) and small amounts (<1 per cent) of the N^4-glucuronide and N^4-sulfate. In the rabbit and dog, but not in man and the rat, it forms a second glucuronide (about 2 per cent of the dose) which may be that of an oxidation product of sulfasomizole [Bridges & Williams (78)]. The structure XXXVII has been tentatively suggested for this glucuronide.

Sulfisoxazole.—This drug is also a sulfonamide of medium duration of action. It is not highly acetylated in man, rat or rabbit [Bertazzoli, Chieli &



XXXVII. Hydroxysulfasomisole glucuronide.

Ciceri (88); DiCarlo et al. (79)]. In man about 80 per cent of a 2-g dose is excreted in 24 hr, the metabolites being those expected, i.e., unchanged sulfisoxazole (56 per cent), N^4-acetylsulfisoxazole (18 per cent), N^4-glucuronide (3.4 per cent), and N^4-sulfate (1.0 per cent). In addition to these, there is a second glucuronide (2 per cent of the dose) which was isolated from human urine as an ammonium salt [Uno (84)]. This compound yielded equimolecular proportions of sulfisoxazole and glucuronic acid on hydrolysis. The benzene nucleus is not hydroxylated, and the metabolite is not an N^1-glucuronide of sulfisoxazole. Furthermore, the methyl substituents of the drug were not oxidized. From infrared and ultraviolet absorption spectra studies, it was suggested that the compound was an N^2-glucuronide of sulfisoxazole.

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