MAMMALIAN DRUG METABOLISM[1]

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ABSTRACT.—Drugs and other chemicals that do not occur normally in mammalian systems are metabolized by a wide variety of enzymes. Reactions catalyzed by these enzymes have been classified into two general phases. Phase I reactions include oxidations, reductions, and hydrolyses, whereas Phase II reactions are broadly defined as conjugation reactions and include glucuronidation, sulfation, acylation, methylation, and conjugation with glutathione. The mechanisms of these biotransformations are outlined to demonstrate how both non-toxic and toxic metabolites are produced. The mammalian metabolism of acetaminophen, a widely used mild analgesic, and R-(+)-pulegone, the major constituent terpene of pennyroyal oil, will be discussed to illustrate specific features of mammalian drug metabolism.

Drugs and other chemicals enter the mammalian system and are metabolized by a variety of enzymes, usually to polar metabolites that are excreted via the kidneys. However, in some cases highly reactive metabolites are formed by a process termed "metabolic activation," and these intermediates can react with tissue macro-molecules to cause a variety of toxic reactions (1). The enzymes involved in mammalian detoxication mechanisms are the same enzymes that catalyze the formation of reactive, toxic metabolites, and the differing structural features of the chemical and its metabolites clearly determine which kind of product is formed.

In 1959, Williams (2) classified the drug metabolic reactions that are catalyzed by mammalian enzymes into two general phases. Phase I reactions include oxidations, reductions, and hydrolyses, whereas Phase II reactions are broadly defined as conjugation reactions and include glucuronidation, sulfation, acylation, methylation, and conjugation with glutathione. The feature that distinguishes mammalian drug metabolism from metabolism in other species is the wide variety of reactions, especially oxidative, that mammals can carry out. Most of the oxidative metabolism is catalyzed by microsomal oxygenases that are located in cellular smooth endoplasmic reticulum. Several isozymes of a peculiar hemo-protein, cytochrome P–450, appear to be especially important. It is noteworthy that some higher plants contain microsomal P–450, which is involved in lignan and flavanoid biosynthesis, terpene oxidation, and alkaloid synthesis.

In this article, proposed mechanisms for the major drug biotransformation pathways will be reviewed followed by a discussion of the mammalian metabolism of the widely used drug, acetaminophen, and the monoterpene, R–(+)–pulegone, by detoxication and toxic pathways.

DISCUSSION

Mammalian Drug Metabolism—Enzymes and Their Reactions

The discussion will be somewhat arbitrarily separated into Phase I and Phase II reactions. For a more detailed description of these reactions and the properties of the enzymes that are involved, see references 3–5.

Phase I reactions include oxidations, reductions, and hydrolyses. Of these, oxidation reactions are the most numerous and are mediated by at least three distinct classes of enzymes, monoamine oxidase, flavin monooxygenase, and cytochrome P–450. Monoamine oxidases are primarily mitochondrial in origin and catalyze the oxidation of primary amines apparently to imines which then hydrolyze to an aldehyde plus ammonia, wherein the oxygen in the aldehyde is

TABLE 1. Phase I Reactions.

1. Oxidations
> Aliphatic Hydroxylations
> Aromatic Hydroxylations
> Oxidation of Heteroatoms (N,S)
> Oxidation of Carbon Alpha to Heteroatoms (N,O,S)
> Oxidation of Catechols
> Epoxidation of Double Bonds

2. Hydrolyses
> Epoxide Hydrolysis
> Ester Hydrolysis
> Amide Hydrolysis

3. Reductions
> Reduction of the Azo Group
> Reduction of the Nitro Group
> Reduction of Quinones
> Reduction of Carbon-Carbon Double Bonds
> Reduction of Aldehydes and Ketones
> Reduction of N-O and S-O Bonds

derived from water. Both flavin monooxygenases and cytochromes P-450 are primarily located in the microsomal fraction of the cell and catalyze oxidation with incorporation of one atom of oxygen from molecular O_2 into substrate and the second atom into water. Reactions of flavin monooxygenase are limited in scope and include oxidation of certain substrates at nitrogen and sulfur. Reactions catalyzed by cytochromes P-450 include oxidation at nitrogen and sulfur as well as several oxidations at carbon.

An abbreviated scheme depicts the biochemical mechanism of cytochrome P-450 (figure 1). With P-450 heme iron in the ferric state, binding of substrate causes a perturbation in liganding of the heme such that it is reduced to the ferrous state by a flavin-containing reductase that utilizes NADPH. The ferrous-hemoprotein complex binds molecular oxygen much as hemoglobin does, except that a second 1-electron reduction rapidly occurs with loss of water and formation of an activated electrophilic oxygenating species that oxidizes the substrate. The oxidized substrate may either be a detoxication product or a toxic metabolite. For example, many benzenoid compounds are oxidized to phenol detoxication products while others form highly toxic epoxides (arene oxides).

FIGURE 1. The cytochrome P-450 oxidation-reduction cycle.
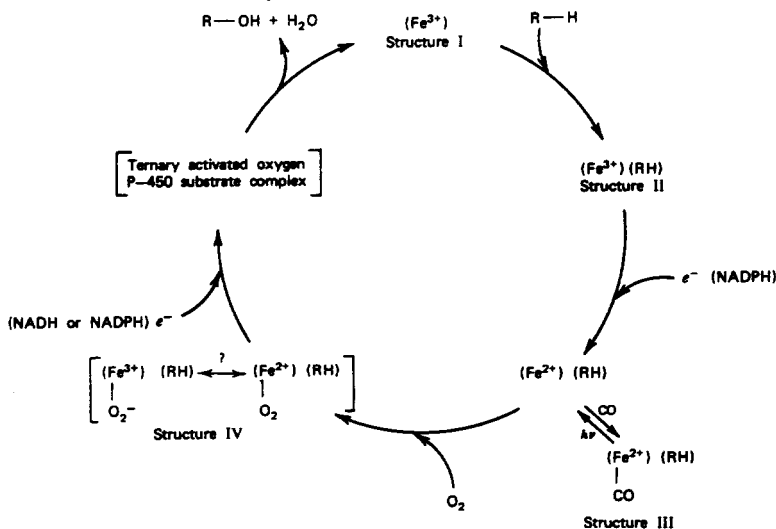
Fortunately, many of the epoxides are further hydrolyzed by another microsomal enzyme, epoxide hydrolase, to diols that, for the most part, are detoxication products. Other important hydrolytic enzymes, esterases and amidases, are also located in the microsomal fraction and hydrolyze a wide variety of esters and amides. Some drugs, such as the widely used local anesthetic and antiarrhythmic agent lidocaine, are ineffectual when administered orally because of rapid hydrolysis by amidases to inactive products. However, several amide and ester prodrugs take advantage of these enzymes to release active drug moieties after absorption of the more lipophilic ester or amide derivative (6). We must note that some esterases are also present in the gut wall and plasma, and a cytoplasmic form of epoxide hydrolase has been identified.

Another Phase I class of metabolic reactions is reduction. Little work has been carried out on reductases, although reduction is an important mechanism that is involved in the generation of detoxification products, therapeutically active metabolites, and toxic products. Aldehyde and ketone reductases are located primarily in the cytosol, whereas azo- and nitro- group and quinone reductases may be microsomal and cytosolic. Of considerable importance is the involvement of the gut flora in the reduction of many drug substances, a topic that will be discussed in other reports.

Phase II reactions include acylations, glucuronidation, sulfation, glutathione conjugation and methylation (table 2). All of these reactions require the forma-

TABLE 2.   Phase II Reactions.

1. Acylations
> Acetylation
> Amino Acid Conjugation

2. Glucuronidation

3. Sulfation
4. Glutathione Conjugation
> Cysteine and Mercapturic Acid
> Synthesis

5. Methylation

tion of reactive donor molecules by the various enzymes involved. For example, acetylation enzymes utilize acetyl-coenzyme A to form an acetylated enzyme intermediate that transfers the acetyl group to an acceptor, usually an amine or hydrazine group (fig. 2). Interestingly, there is pronounced genetic variation in the rates at which various individuals carry out this reaction, and individuals are classified phenotypically as rapid or slow acetylators. Acetylases are located primarily in the cytosol and some in plasma.

Amino acid conjugation requires the formation of an acyl-coenzyme A derivative of a carboxylate moiety on the drug followed by a transferase-mediated reaction with an amino acid amino group to form an amide (figure 3). There is pronounced species variation in the amino acids that act as co-substrates. In man glycine is the most prominent amino acid that conjugates. The enzymes are located mainly in mitochondria although some may be cytosolic.

FIGURE 2.   Formation of acetylated amine metabolites.

Acetyl-coenzyme A

Acetylated N-
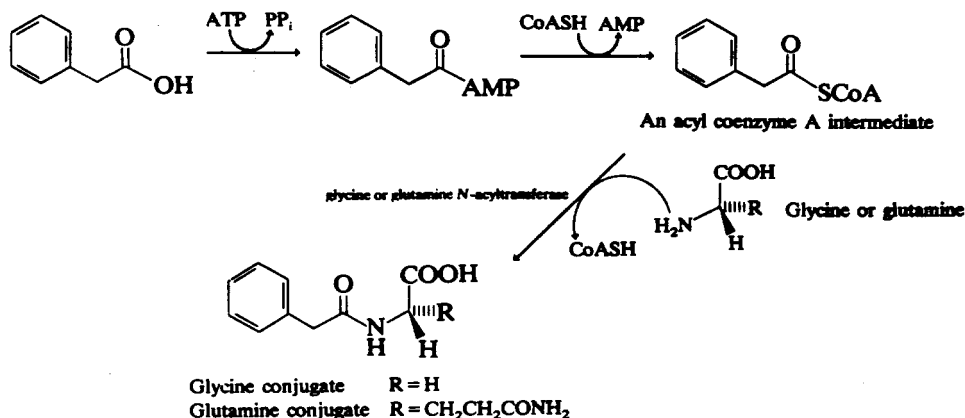acetyltransferase
intermediate
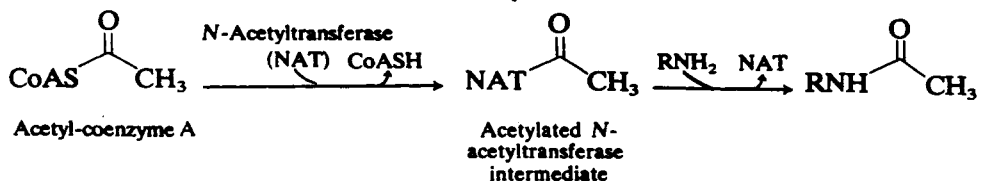
FIGURE 3.   Formation of amino acid conjugates.

Glucuronide formation occurs mainly in the microsomal fraction and is an important route of metabolism for many carboxylic acids, phenols, and alcohols as well as other less acidic groups including amines.   Glucuronyl transferases
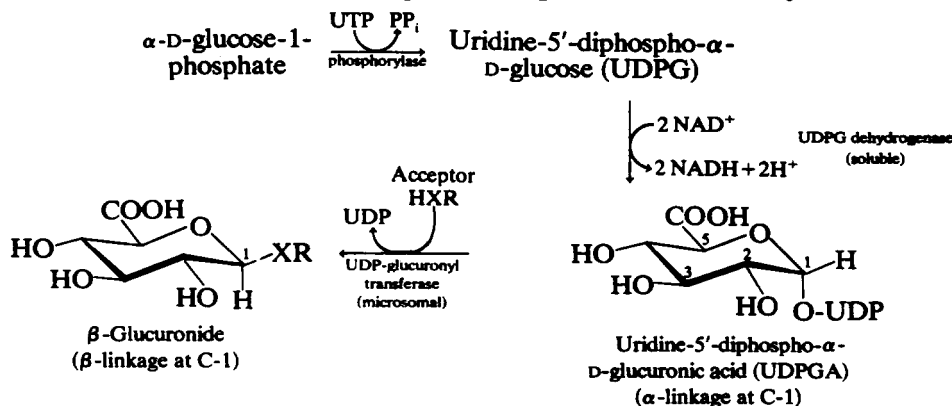

FIGURE 4.   Formation of glucuronide conjugates.

utilize the co-substrate UDPGA to form β-glucuronides, as described in figure 4. The highly polar glucuronide moiety usually leads to rapid excretion of a drug.
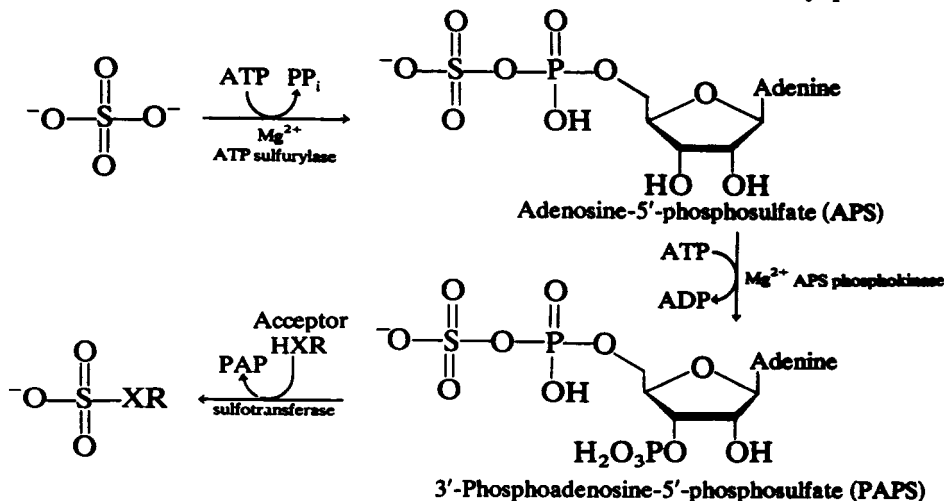Sulfation is another reaction that leads to the formation of very polar con-

FIGURE 5.   Formation of sulfates.

jugates.   The mixed anhydride, PAPS, is the activated co-substrate that is utilized by sulfotransferases (figure 5).   These cytosolic enzymes catalyze sulfation reactions of phenols and other alcohols, as well as some amines.

Glutathione conjugation can occur both enzymatically and non-enzymatically with some highly electrophilic compounds.   Glutathione transferases, which are located primarily in the cytosol, apparently increase the nucleophilicity of the free sulhydryl group of the cysteine residue of the tripeptide glutathione.   Glutathione conjugation is an important mechanism for removal of potentially toxic electrophilic compounds from many body tissues.   Once the conjugates are formed they are degraded by two peptidases to produce cysteine conjugates which are then acetylated to mercapturic acids (figure 6).   Both cysteine conjugates and mercapturic acid conjugates are excreted into the urine.
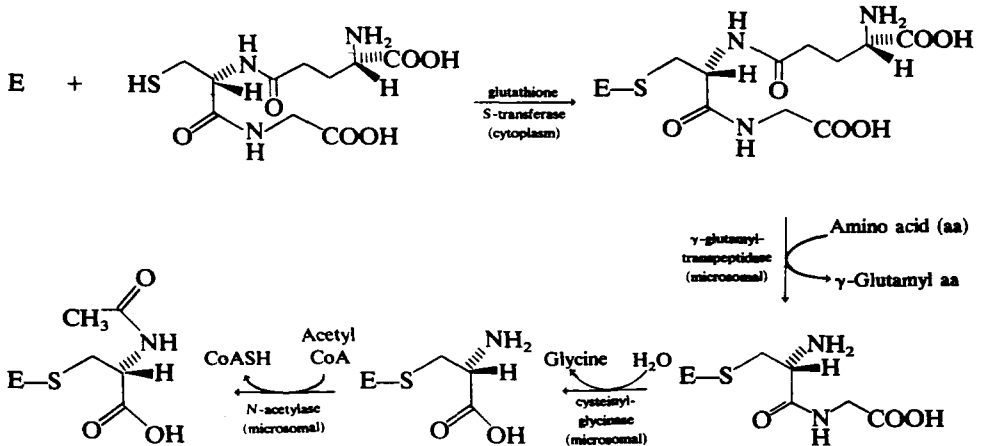

FIGURE 6.   Formation of glutathione conjugates and further degradation to cysteine and mercapturic acid derivates.

Finally, methylation, especially of catechols, is an important biotransformation pathway for both endogenous catecholamines and drugs that either contain or are metabolized to catechol structures.   S-Adenosyl-methionine is the activated co-substrate for methylation (figure 7), and the methyltransferases are primarily cytosolic in origin although some microsomal forms have also been found.
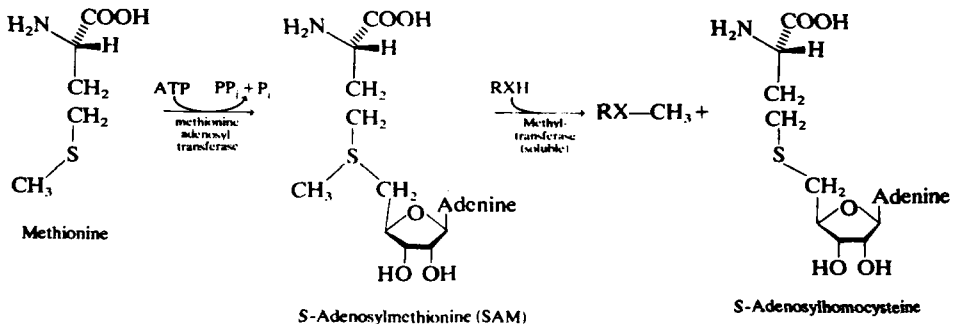

FIGURE 7.   Formation of methylated metabolites.

Acetaminophen—An Example

Acetaminophen is a widely used analgesic drug that can produce serious hepatotoxic and nephrotoxic reactions if ingested in overdose.   The classical studies of Mitchell and coworkers (7) with acetaminophen provided the basis

for a biochemical mechanism of toxicity that has been modified as more has been learned about the drug's metabolism in the mammalian system (8).

This simple 4'-hydroxy-acetanilide undergoes several of the metabolic reactions that we have discussed with the production of both detoxification and toxic products (figure 8). Most of the drug is conjugated as a phenol sulfate and glucuronide. A small fraction of the dose is oxidized to a catechol by cytochrome P-450, and the catechol is methylated by catechol-O-methyl transferase. The drug is also oxidized by cytochrome P-450 to an electrophilic arylating agent thought to be N-acetyl-p-benzoquinone imine. This can be reduced back to acetaminophen, hydrolyzed to benzoquinone and acetanide, or be conjugated with glutathione. If glutathione is depleted, reaction can occur with cellular proteins and death of the cell ensues. Hydrolysis of acetaminophen to p-hydroxy-aniline (not shown) may also lead to toxic reactions, particularly in the kidney (9).

Thus, Phase I reactions of acetaminophen metabolism include amide hydrolysis, aromatic hydroxylation, what is equivalent to N-hydroxylation and dehydration, and reduction. Glucuronidation, sulfation, glutathione conjugation, and methylation occur as Phase II reactions.
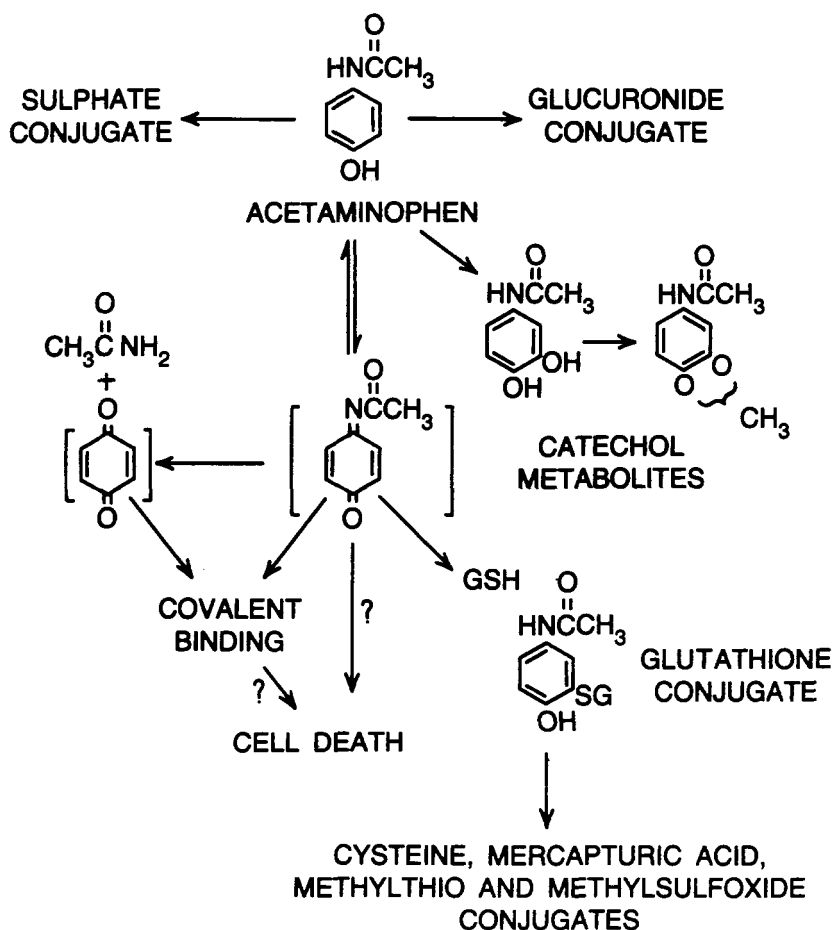

FIGURE 8. Metabolism of Acetaminophen.

Preliminary Studies on the Toxicity and Metabolism of Pennyroyal Oil and Its Constituent Terpenes

Pennyroyal oil is a volatile oil that is obtained by steam distillation of plants of the Labiatae family, in particular *Mentha pulegium* and *Hedeoma pulegioides*.

Because of its distinct mint-like odor, the oil is approved for use in small quantities as a flavoring agent and fragrance component (10).   In addition to its regulated use, pennyroyal oil has been used as a folklore medicine for its reputed abortive properties.   A recent case of massive pennyroyal oil ingestion (estimated 24g) by an 18-year-old girl resulted in massive hepatic necrosis and death (11).   Two other cases have since been reported to the CDC in Atlanta, Georgia, and we have since been personally informed of several other cases of ingestion that have required hospitalization.

We developed an animal model for both the hepatotoxic and lung toxic reactions caused by pennyroyal oil and determined that R–(+)–pulegone (figure 9) is the major terpene responsible for the hepatotoxic reaction (12).   R–(+)–Pulegone is the major terpene component of pennyroyal oil and comprises over 80% of all of the oil that we have examined.   Two other terpenes, isopulegone and menthofuran, are present in much lesser amounts although menthofuran was found to be a potent liver and lung cytotoxin.
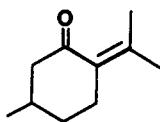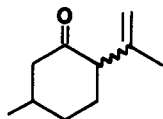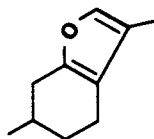

PULEGONE

ISOPULEGONE

MENTHOFURAN

FIGURE 9. Structures of three terpene constituents of pennyroyal oil that cause hepato-toxicity in mice.

In our animal model for toxicity, the BALB/c mouse, we have found that R–(+)–pulegone undergoes extensive oxidative metabolism and some reductive metabolism (unpublished results).   Reduction of the ketone group produces pulegol, and oxidation at virtually every carbon atom has been observed.   Oxidation by cytochrome P–450 seems to be involved in the metabolism of R–(+)–pulegone to its toxic metabolite, and deuterium substitution for hydrogen on the allylic methyl groups has provided evidence for allylic oxidation as a pathway to the toxic metabolite.   Considerable work remains to fully elucidate the mechanism of toxicity.

In conclusion, mammalian drug metabolism involves a wide array of pathways for the formation of detoxication products, therapeutically active metabolites, and toxic products.   Although most, if not all, of the enzymes that catalyze these reactions are found distributed throughout nature, it is the concern we have for our own welfare that provides the impetus for so closely scrutinizing metabolism in mammals.

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American Chemical Society Southeastern Regional Meeting

The 35th ACS Southeastern Regional Meeting will be held from November 9–11, 1983, in Charlotte, North Carolina. The program will include a two-day Symposium on Natural Products, with papers on Structure, Biosynthesis, and Synthesis of Natural Products. Any ASP members interested in presenting a paper at this symposium should contact Dr. David G. I. Kingston, Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061. The deadline for receipt of abstracts is June 15, 1983.