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Review Article

Pharmacology and Biochemistry of Drug Metabolism

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IN RECENT years, much emphasis has been placed on explaining drug action at the subcellular or biochemical level. In order that this be done in a meaningful and precise manner, it is necessary that such parameters as drug absorption, tissue distribution, elimination through excretory pathways, binding to constituents of cells and body fluids, and metabolism be thoroughly explored. Pharmacologists and biochemists appear agreed that the subcellular approach to drug action, whether from the enzymatic or physical biochemical points of attack, will yield a wealth of information concerning sites and mechanisms of drug activity. Metabolism of drugs is the parameter of prime concern in this review. The author will limit himself to a discussion of recent researches undertaken to define general metabolic pathways in which drug substances (and many other organic chemicals) participate. The reader should bear in mind that the related phenomena of drug absorption and elimination play an important role along with drug metabolism in the total spectrum of drug activity. Excellent reviews by Nelson (1), Wagner (2), and Butler (3) cover these topics.

The importance of drug metabolism as a contributing factor to the response noted with any drug *in vivo* is readily apparent upon consideration of the facts that (*a*) the duration of action of a drug can be related to the rate and manner in which it is metabolized, (*b*) the rate of elimination of a drug from the body is markedly dependent upon the physicochemical properties of its metabolic products, (*c*) the ability of a drug to penetrate cellular barriers and reach a site of action may be limited by its rate of biotransformation and the character of its metabolic products, (*d*) the toxicity of a drug can be reversed or, in certain cases, intensified upon conversion to a metabolite, and, (*e*) a metabolite may be formed *in vivo* which itself has a pharmacological effect ("active metabolite").

These points would indicate the importance of knowing the pathways of drug biotransformation as well as the degree of accumulation of metabolic products in tissue. It follows, too, that the toxicity and pharmacological effects of drug metabolites must be understood. From a more practical standpoint, recent legislation covering certain classes of physiologically active material (Food Additives Amendment) has made mandatory the identification and measurement of residual levels of drugs and drug metabolites which conceivably could gain entrance into the human food supply. Reviews on the subject of drug metabolism have appeared by Fishman (4, 135), Brodie, *et al.* (5, 6), and Maynert (7). The book, "Detoxication Mechanisms," by Williams (8) serves as an excellent source of information on the metabolism of drugs and other organic chemicals by providing encyclopedic treatment of the subject.

There is lack of agreement generally on the terms to be used in describing such studies. De-

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toxication is obviously misleading since many drugs can be converted *in vivo* to more toxic compounds as exemplified by certain insecticides containing the —P=S grouping which is converted to the highly toxic —P=O form. This term has, nonetheless, withstood the test of time, and is currently favored by some authors. Others find fault with the use of "drug metabolism" in describing such work, preferring to retain the word metabolism to describe studies on endogenous substrates. The phrase, "drug biotransformation," attributable to Brodie, is perhaps the best terminology in such cases. The present author, however, prefers the use of "drug metabolism" and such will be employed in this review.

Although studies of the metabolic fate of drugs have been carried out since the end of the nineteenth century (9), it is only in the past decade that significant advances have been made concerning the enzymes involved. The excellent work of Axelrod, Brodie, and their co-workers (10–12) has established that the primary site of drug metabolizing activity in the mammal is in the microsomal fraction of the liver, necessary cofactors for the various reactions being found in the soluble fraction. It is of interest that the enzyme systems involved are apparently quite nonspecific in the sense that several reactions can be carried out by the same fraction. Specific enzymes have not been isolated to date. Nearly all of the classical reactions undergone by drugs *in vivo* including hydroxylation, dealkylation from amino nitrogen, ether cleavage, glucosiduronide formation, and reduction of the nitro and azo groupings have been duplicated under *in vitro* conditions using the mammalian liver microsomal system. Further, it has been established that the microsomal enzymes which act upon drugs and other foreign compounds appear unable to carry out the same reactions on endogenous substrates. Isolation and thorough understanding of the activity of the microsomal enzymes which metabolize drugs would seem to be the next step in the chain of events.

Biochemical Reactions Undergone By Drugs.—For purposes of discussion the more important metabolic pathways entered into by drugs can be classified into five groups. These five groups comprise some 15 distinct metabolic reactions, and cover the majority of the biotransformations which drugs and foreign compounds undergo. These are:

1. *Oxidation.*—Reactions considered under this heading are oxidations only in the sense that oxygen (as hydroxyl) takes part or is postulated to take part in one or more of the steps leading to the final metabolite, as proposed by Brodie, *et al.* (5): (*a*) hydroxylation of aromatic rings; (*b*) oxidation of aliphatic side chains to alcohols, ketones, or carboxyl groups; (*c*) dealkylation from oxygen (ether cleavage); (*d*) dealkylation from amino nitrogen; (*e*) deamination; (*f*) sulfoxide formation.

2. *Reduction.*—(*a*) Reduction of the nitro group, (*b*) cleavage and reduction of the azo linkage.

3. *Conjugation Processes.*—(*a*) Glucosiduronide formation, (*b*) formation of aryl sulfates, (*c*) conjugations with glycine, (*d*) acylation.

4. *Hydrolytic Reactions.*—(*a*) Cleavage of esters, (*b*) cleavage of amides.

5. *Exchange Reactions.*—(*a*) Replacement of sulfur by oxygen.

Table I indicates the character of each of these reactions and gives illustrative examples of drug substances known to undergo each of the biotransformations indicated.

Changes in Drug Properties through Metabolism.—Upon metabolism a drug is ordinarily converted to a more polar compound having a lipid solubility much less than that of the parent material. Current knowledge concerning the ability of organic compounds to pass tissue and cellular barriers indicates that it is the molecular form of the compound which is transported to the exclusion of the ionized species (13–15). Thus, the dissociation constant of the drug or metabolite and its inherent lipid solubility appear to be the primary factors governing its passage into cells. This is true whether one considers gut absorption (13), passage through barriers such as the blood-cerebrospinal fluid barrier (16, 17), or kidney elimination (18, 19). Thus it is likely that a drug having been converted to a more polar metabolite will, at physiological pH, be rather rapidly excreted, and will be unlikely to be returned to the tissues. This is indicated by recent studies in these laboratories on the metabolism of C-14 salicylamide and C-14 meprobamate. In neither case was it possible to demonstrate the presence of the relatively more polar metabolites of these drugs (salicylamide → 2,5-dihydroxybenzamide and glucosiduronides; meprobamate → hydroxymeprobamate and glucosiduronides) in body tissues of the rat (20, 21). Through metabolism, then, another factor is brought into play which contributes to termination of drug action, namely that the physical and chemical properties of the metabolite are such that it is unable to pass the lipid-like membranes between plasma and cells,

and it cannot readily be reabsorbed through the renal tubular epithelium.

Binding of drugs to proteins, particularly those of the plasma, can also play a role in determining the length of action of the drug, especially if therapeutic concentrations of free drug can be maintained from the storage depot. Liver, for example, shows a high capacity to bind mepacrine (22); antibiotics, sulfonamides, and certain barbiturates are bound in plasma to a significant degree. Through metabolism, one would expect this type of storage mechanism to be greatly altered. Storage of lipid-soluble drugs in body fat is frequently discussed as a contributing factor to the duration of drug action. This factor has been carefully studied in relation to certain of the thiobarbiturates (19, 23). A drug, upon being converted to a more polar metabolite, would be less lipid-soluble and less likely to be stored in fat depots.

Liver Enzymes Which Metabolize Drugs.— The report by Axelrod (10) who, in 1955, demonstrated that microsomes from rabbit liver could metabolize amphetamine to phenylacetone and ammonia was the first to demonstrate the localization of drug metabolizing activity. His studies of the cofactors involved indicated that the system required both reduced triphosphopyridine nucleotide (TPNH) and oxygen. Upon fractionation of whole liver into nuclei, mitochondria, microsomes, and soluble fraction, the microsomes alone could metabolize amphetamine, but at a very slow rate. Addition of the soluble fraction greatly increased the ability of microsomes to metabolize amphetamine. The soluble fraction containing glucose-6-phosphate and glucose-6-phosphate dehydrogenase serves as a TPNH generating system. Washed rabbit liver microsomes could metabolize amphetamine equally well with added TPNH as with the soluble fraction. Axelrod also showed that the addition of unheated rat liver microsomes to the rabbit microsomes markedly decreased activity, whereas addition of heated rat microsomes did not, suggesting the presence of a heat labile inhibitor in the rat microsomes.

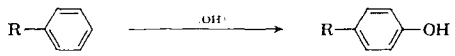
In vitro studies on drug metabolism are ordinarily carried out using microsomes isolated from liver homogenate by differential centrifugation, all necessary cofactors being added in the buffer medium. Some prefer the use of a fraction containing both the microsomes and the soluble fraction, prepared by centrifugation of the whole homogenate at 9,000 × g. Homogenates may be prepared either in isotonic potassium chloride or in 0.25 *M* sucrose, both yielding highly active preparations. General methods of homogenate preparation and fractionation are applicable (47–49). Table II shows the distribution of drug metabolizing activity in various subcellular fractions of rabbit liver. In general, only the liver yields active drug metabolizing preparations; of the reactions listed in Table I, only nitro reduction and glucosiduronide formation occur at other sites in the body. Nitro reductase activity is associated with kidney, heart, lung, and brain, as well as liver. This reaction, however, requires anaerobic conditions and thus differs from the general TPNH-O_2 requirement (35). Coliform bacteria in the intestine can also reduce nitro compounds (50).

Glucosiduronide formation can take place in the kidney cortex and the gastrointestinal tract of several species (51).

The fact that the liver microsomal system appears to act only on exogenous substrates is difficult to explain. The phylogenetic distribution of drug metabolizing activity shows this function to be restricted, in the main, to the mammal. Aquatic animals are unable to carry out the oxidative reactions enumerated in Table I. An explanation for this may lie in the fact that aquatic species have excretory mechanisms which allow rapid removal of unchanged drug from the body, so that metabolic defense mechanisms are unnecessary (52). Brodie (52) suggests the evolution of liver microsomal enzymes in terrestrial animals. In explanation of the fact that endogenous substrates are not attacked by these enzymes, Gaudette and Brodie (30) have studied the dealkylation of a series of foreign and endogenous alkylamines. They show that only those compounds exhibiting a high chloroform/water partition ratio at physiological pH are dealkylated by the microsomal system. It is of interest that the endogenous substrates studied exhibit extremely low partition and are not metabolized. This suggests that the microsomes are protected by a lipid barrier which is impermeable to polar compounds. Such a hypothesis deserves further exploration. Their interesting data are partially reproduced in Table III. McMahon (67) has, in the main, confirmed the observations of Gaudette and Brodie (30) cited above.

Cofactor requirements for the microsomal enzymes carrying out oxidative reactions are quite similar for hydroxylation, ether cleavage, dealkylation, and deamination. pH optima are also similar varying only slightly from about pH 7.0 to 8.5, depending on the particular substrate. Reduced TPN is a general requirement for all of these reactions, as is molecular oxygen. DPNH

TABLE I.—SOME COMMON METABOLIC REACTIONS UNDERGONE BY DRUGS

Pathway or Reaction	Schematically[b]	Drugs which Undergo This Reaction (Reference)
1. Oxidation[a]		
(*a*) Hydroxylation		Acetanilide (24, 11), Salicylic acid (25), Salicylamide 50
(*b*) Side chain oxidation	$R—CH_2CH_3 \xrightarrow{(OH)} R—CHOHCH_3$	Pentobarbital (26, 27)
(*c*) Ether cleavage	$R—O—CH_3 \xrightarrow{(OH)} R—OH + HCHO$	Morphine (28) Phenacetin (29)
(*d*) Dealkylation from amino nitrogen	$R—NH—CH_3 \xrightarrow{(OH)} R—NH_2 + HCHO$	Morphine, Meperidine (12), Monomethyl-4-aminoantipyrine (30), Methamphetamine (31)
(*e*) Deamination	$R—CH_2CHNH_2CH_3 \xrightarrow{(OH)} R—CH_2COCH_3 + NH_3$	Amphetamine, Ephedrine (10)
(*f*) Sulfoxide formation	$R—S—CH_3 \xrightarrow{OH)} R—SO—CH_3$	Chlorpromazine (32, 33, 34)
2. Reduction		
(*a*) Nitro reduction	$R—NO_2 \longrightarrow R—NH_2$	Chloramphenicol (35)
(*b*) Azo reduction	$R—N{=}N—R \longrightarrow 2\ R—NH_2$	Prontosil (36)
3. Conjugation		
(*a*) Glucosiduronide formation	$R—OH + UDPGA \xrightarrow{\text{transferase}} RO\text{-glucosiduronide}$	Salicylates (20, 37), Morphine (38), Meprobamate (21), Codeine (39)
(*b*) Aryl sulfate formation	$R—OH + PAPS \xrightarrow{\text{transferase}} RO—SO_2OH$	Salicylamide (40)
(*c*) Conjugation with glycine	$R—COOH + H_2NCH_2COOH \longrightarrow R—CONHCH_2COOH$	Benzoic acid (41)
(*d*) Acylation	$R—NH_2 + \text{Acetyl-CoA} \longrightarrow R—NHCOCH_3$	Sulfonamides (42)
4. Hydrolytic reactions		
(*a*) Cleavage of esters	$R—COOR' \xrightarrow{\text{esterase}} R—COOH + ROH$	Procaine (43)
(*b*) Cleavage of amides	$R—CONH_2 \xrightarrow{\text{amidase}} R—COOH + NH_3$	Hydroxybenzamide (44)
5. Exchange reactions		
(*a*) Replacement of S by O	$R—CS—R \longrightarrow R—CO—R$	Thiopental (45) Parathion (46)

[a] As proposed by Brodie, *et al.* (5); detailed mechanisms and cofactors for the reactions to be discussed later. [b] Abbreviations used in review are as follows: AMP, ADP, ATP for adenosine mono-, di-, and triphosphates; UDP for uridine diphosphate; UDP-G for uridine diphosphoglucose; UDPGA for uridine dihosphoglucuronic acid; TPN and TPNH for the oxidized and reduced forms of triphosphopyridine nucleotide; DPN and DPNH for the oxidized and reduced forms of diphosphopyridine nucleotide; G-6-PO_4 for glucose-6-phosphate; APS and PAPS for adenosine-5′-phosphosulfate and 3′-phosphoadenosine-5′-phosphosulfate; acetyl-CoA for acetyl coenzyme A; PP for pyrophosphate.

TABLE II.—INTRACELLULAR DISTRIBUTION OF ACETANILIDE HYDROXYLATING ACTIVITY[a]

Cellular Fractions	*p*-Acetylaminophenol Formed, μm.	% Relative Activity
Whole homogenate	1.14	100
Supernatant (9,000 × g)	1.14	100
100,000 × g	...	...
Microsomes	0.01	0.9
Soluble fraction	0.01	0.9
Soluble + microsomes	1.14	100

[a] From Mitoma, *et al.* (24). Fractions equivalent to 250 mg. of liver incubated with 10 μm. of acetanilide. Activity of whole homogenate taken as 100%. The 9,000 × g centrifugation removes unbroken cells, nuclei, and mitochondria, showing that these fractions possess no metabolizing activity.

TABLE III.—LIPID SOLUBILITY AND DEALKYLATION OF A NUMBER OF ALKYLAMINES BY LIVER MICROSOMES[a]

% Compound Extracted into Chloroform	Compound	Relative Activity
Foreign Materials		
>95	Monomethyl-4-aminoantipyrine	100
>95	Ephedrine	110
>95	Demerol	95
>95	Parpanit	80
>95	Methadone	50
>95	Caffeine	20
12	Theophylline	0
Endogenous Compounds		
<3	Adrenaline	0
<3	Sarcosine	0
<3	Choline	0
<3	Creatine	0

[a] Data from Gaudette and Brodie (30). Distribution of each compound between chloroform and pH 7.4 phosphate buffer determined. Microsomes from 1 Gm. rabbit liver incubated with 5 μm. of substrate. Activity of 100 equivalent to the dealkylation of 1 μm. of substrate per hour.

cannot replace TPNH in the oxidative reactions. Nicotinamide is frequently added to homogenates to protect TPN, and Mg^{++} is a required ion. Glucose-6-phosphate and glucose-6-phosphate dehydrogenase are required to maintain TPN in the reduced state. That the drug metabolizing enzymes are not related to the cytochrome chain is indicated by the fact that cyanide does not influence the reactions. Gillette, *et al.* (53), have studied the role of TPNH and oxygen in the oxidative reaction. They show that microsomes contain a TPNH oxidizing system which gives rise to hydrogen peroxide. It is attractive to speculate that the hydrogen peroxide so produced serves as the source of "active hydroxyl" in the oxidative reactions. They further showed that only those tissues possessing significant TPNH oxidase activity could catalyze the dealkylation of monomethyl-4-aminoantipyrine, as shown in Table IV. Further work is required to explain fully this striking parallel.

Brodie (52) shows the requirement for TPNH more directly in a study of the oxidation of hexobarbital, as indicated in Table V.

TABLE IV.—TPNH OXIDATION AND MONOMETHYL-4-AMINOPYRINE DEALKYLATION BY MICROSOMES OF VARIOUS RABBIT TISSUES[a]

Tissue	TPNH Oxidation O.D. 340/5	4-Aminoantipyrine Formed, μm.
Liver	0.189	0.44
Lung	0.065	0.04
Kidney	0.047	0.02
Heart	0.028	0.03
Muscle	0.012	0.01
Brain	0.012	0.00

[a] Data from Gillette, *et al.* (53). TPNH oxidase activity measured spectrophotometrically. Microsomes from 1 Gm. of tissue incubated with 5 μm. of monomethyl-4-aminoantipyrine.

TABLE V.—REQUIREMENT FOR TPNH IN OXIDATION OF HEXOBARBITAL BY LIVER MICROSOMES[a]

Components	Hexobarbital Oxidized, μm.
Microsomes + 1.0 μm. TPN	0
Microsomes + 1.0 μm. TPN + 4 μm. G-6-PO_4	0
Microsomes + 1.0 μm. TPN + 4 μm. G-6-PO_4 + G-6-PO_4 dehydrogenase	0.30
Microsomes + TPNH	0.61
Microsomes + 0.5 ml. soluble fraction + 1 μm. TPN	0.57

[a] Data from Brodie (52).

Species, Sex, and Age Differences in Drug Metabolism.—It was mentioned that aquatic animals do not possess the enzymes necessary to metabolize drugs, this activity being restricted to the mammal. Within the mammalian group, wide quantitative differences are seen in relation to metabolizing activity, as well as some qualitative differences. Thus, amphetamine in rats and dogs is metabolized by hydroxylation of the aromatic ring, whereas in rabbits the compound is deaminated (10). Cats are not able to synthesize glucosiduronides (51).

There are well-known species differences in quantitative response to certain drugs (54, 55). In certain cases these differences are explainable on the basis of relative drug metabolizing activity, there being an inverse relationship between drug metabolizing ability and duration of pharmacological response. Thus, Quinn, *et al.* (55), have shown that the duration of action of hexobarbital is in inverse relationship to hexobarbital metabolism in several species. Their data are shown in Table VI.

Similar results were found in studies of the metabolism of antipyrine, amidopyrine, and aniline, and provide a sound basis for explaining differences in drug duration of action, sensitivity to various drugs, and toxicity.

TABLE VI.—SPECIES DIFFERENCE IN DURATION OF ACTION AND IN METABOLISM OF HEXOBARBITAL[a]

Species	Duration of Action, min.	Biological Half-Life, min.	Relative Enzyme Activity
Mouse	12 ± 8	19 ± 7	598 ± 184
Rabbit	49 ± 12	60 ± 11	196 ± 28
Rat	90 ± 15	140 ± 54	134 ± 51
Man	...	360	...

[a] Data from Quinn, *et al.* (55). Duration of action measured by sleeping time. Biological half-life determined by plotting plasma level *vs.* time at several intervals. Enzyme activity assessed by measuring mcg. of hexobarbital metabolized per gram of liver per hour. 100 mg./Kg. dose of hexobarbital in mouse, rabbit, and rat; 50 mg./Kg. in the dog.

Sex differences in drug metabolism appear to be restricted to the rat. Quinn, *et al.* (55), demonstrated that following a 100 mg./Kg. dose of hexobarbital, female rats sleep about four times as long as males, whereas their enzyme activity is about one-quarter that of the male. It is interesting that hexobarbital metabolizing activity can be significantly altered in rats by administration of testosterone to females and estradiol to males. Control male rats exhibited a hexobarbital metabolizing capacity of 682 mcg. of drug/Gm. of tissue/hr., whereas estradiol-treated males metabolize only 177 mcg./Gm./hr. Female controls metabolized 134 mcg./Gm./hr., whereas testosterone-treated females metabolized 543 mcg./Gm./hr. Sleeping times in these animals were altered to the same degree. Inscoe and Axelrod have recently demonstrated that glucosiduronide formation in rats is also markedly sex-dependent, male animals showing approximately four times the activity of females (56).

Fouts and Adamson (57) report that the newborn rabbit is essentially unable to carry out side chain oxidation, dealkylation, hydroxylation, and deamination. Enzyme activity appears at the second week of life, increasing to adult values at about four weeks. In an experiment to test the possibility of enzyme inhibitors being present in the livers of young animals, the addition of baby rabbit supernatant (9,000 × g) to adult supernatant caused an 82% reduction in the ability of the adult preparation to metabolize amphetamine. Jondorf, *et al.* (58), in a study of drug metabolism in newborn mice and guinea pigs, demonstrated that the newborn mouse is unable to carry out dealkylation, side chain oxidation, and ether cleavage. Enzyme activity appeared at 7 days and reached heightened values at 21 days. It is also of interest that sensitivity of mice to hexobarbital decreased as the animal's ability to metabolize the drug increased. Similar results were obtained in guinea pigs, and these young animals were also unable to synthesize glucosiduronides. These data are significant in that they provide a rational basis for explaining the heightened sensitivity of young animals to certain drugs, mainly central nervous system depressants, apart from differences in blood-brain barrier.

Physiological and Biochemical Control of Drug Metabolizing Enzymes.—Little is known about the extra-hepatic factors which control the activity of the microsomal enzymes. The observation (55, 56) that sex hormones can alter microsomal activity is apparently confined to the rat and does not seem to be of general significance. Remmer (59) has shown that adrenalectomy reduces the capacity of liver microsomes to oxidize barbiturates. Zauder (60) has reported that adrenalectomy in rats destroys the ability of liver slices to conjugate morphine. It is interesting to speculate on the role of the adrenal cortex (and of the pituitary gland) in regulating liver microsomal activity. It is well known, for example, that administration of corticoids increases protein synthesis (61, 62). It might therefore be expected that the adrenal cortical secretions regulate synthesis of microsomal enzymes, at least to some degree. Cochin and Sokoloff (63) have investigated the effects of L-thyroxin on the demethylation of morphine in mice and report that it depresses this function. Since the experiments were carried out in intact animals rather than those which had been thyroidectomized, it is not possible to make a definitive statement regarding thyroxin effects on drug metabolism. Conney and Garren (64) report that thyroxin stimulates the metabolism of zoxazolamine and decreases the metabolism of hexobarbital. In their work with zoxazolamine it is of interest that during the period of greatest zoxazolamine metabolism, the tissue level of G-6-PO_4 and G-6-PO_4 dehydrogenase were also markedly increased.

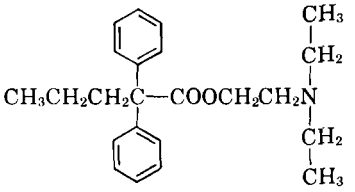
Recent studies by Dixon, *et al.* (65), demonstrate that the alloxan-diabetic rat shows a decreased ability to oxidize hexobarbital and chlorpromazine and to cleave the ether linkage in codeine. Administration of insulin to diabetic rats restores drug metabolizing activity to control values. A relationship is evident between glycogen levels in the liver and drug metabolizing activity, low levels of liver glycogen following alloxan paralleling depressed enzymatic function. McLuen and Fouts (66) show that livers from jaundiced rats possess a markedly decreased ability to carry out oxidation, deamination, ether cleavage, and dealkylation. They note that the effects seen in severely jaundiced animals are quite closely duplicated *in vitro* by the addition

of sodium deoxycholate ($10^{-3}M$) to tissue homogenates, and mention the possibility that effects seen in jaundiced rabbits may be due to increased concentrations of bile acids in the liver.

In 1954, Udenfriend, *et al.* (68), reported that a synthetic system consisting of ascorbic acid, ferrous sulfate, and EDTA in phosphate buffer and an oxygen atmosphere, was able to hydroxylate drugs and other foreign compounds to yield products identical to the hydroxylated metabolites of these drugs obtained *in vivo*. Thus, the hydroxylation of salicylic acid, acetanilide, aniline, and antipyrine was demonstrated (69). It has long been recognized that the administration of certain drugs can cause an increased excretion of ascorbic and glucuronic acids (70), which has been shown to be due to increased synthesis (71). Participation of ascorbic acid in certain drug metabolic reactions is thus suggested. Manthei (72) has shown that ascorbate-treated mice exhibit heightened tissue levels of isoniazid after dosing with this drug, and that a metabolite of isoniazid is recoverable in mice having the same chromatographic characteristics as a compound obtained by chemical reaction of isoniazid and dehydroascorbic acid. In an interesting study of the role of ascorbic acid in aromatic hydroxylation, Axelrod, *et al.* (73), studied the biological half-lives of acetanilide, aniline, and antipyrine in normal and scorbutic guinea pigs. They demonstrated that the biological half-lives of these drugs are significantly increased in the scorbutic animal. Ascorbic acid is known to participate, nonspecifically, in the biological oxidation of tyrosine (74). Before assigning a role to ascorbic acid in drug metabolism, the questions of specificity and detailed mechanism must be answered. For example, barbital, a drug which is not extensively metabolized, has a pronounced effect on stimulating ascorbic acid and glucuronic acid biosynthesis (75). A host of compounds, having different pharmacological effects and being metabolized along different pathways, also induce ascorbic acid synthesis (76). It is interesting to note that liver ascorbic acid is mainly concentrated in the microsomes and supernatant fractions, very little being found in the mitochondria and none in the nuclei (77).

The excellent work of Burns and his colleagues in providing us with details of the mechanism of ascorbic acid biosynthesis and its possible role in drug metabolism is deserving of mention (71, 75, 76, 78, 79).

Drug Metabolism Inhibitors.—Macko, *et al.* (80), reported in 1953 that SKF 525-A (β-diethylaminoethyl diphenylpropylacetate, I) has the ability to potentiate the action of certain spinal cord depressants and central nervous system stimulants. Cook, *et al.* (81), in a detailed study, have shown that the duration of action of hexobarbital is significantly increased under the influence of SKF-525A. Other compounds shown to be potentiated by this agent include secobarbital, amobarbital, phenobarbital, and chloral hydrate (82); *d*-tubocurarine (83); and certain antiepileptic drugs (84). Other compounds known to exert similar effects on drug metabolism include Lilly 18,947 (2,4-dichloro-6-phenylphenoxyethyl diethylamine, II), iproniazid, III, and JB-516 (1-phenylisopropylhydrazine, IV).



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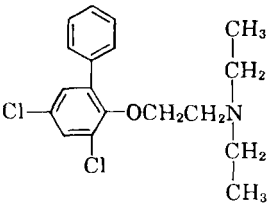

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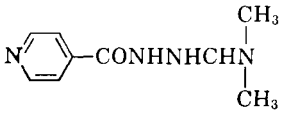

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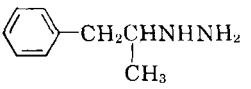

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SKF 525-A has been used extensively as a tool in drug metabolism studies. The mechanism of its action was explained first by Axelrod, *et al.* (85), who showed that it decreased the ability of the liver microsomes to carry out such reactions as side chain oxidation and demethylation and thus increased the duration of action of drugs being metabolized through these pathways. Axelrod, *et al.* (86), further demonstrated in a study of the effects of this compound on hexobarbital action that the increased duration of action was closely paralleled by an increase in the biological half-life of the drug. Of significance in this study was

the fact that upon return of the righting reflex in rats receiving 100 mg./Kg. hexobarbital, plasma levels of hexobarbital were no higher in the SKF 525-A-treated animals than in the control group. This would indicate that the increased sleeping time noted was not due to higher brain concentrations of the barbiturate. It has been shown (81) that administration of SKF 525-A after return of the righting reflex following hexobarbital treatment will not reinduce hypnosis.

In a study of the mechanism of SKF 525-A action, Cooper, *et al.* (87), demonstrated that the compound added to liver homogenates at concentrations of 10^{-4} *M* or greater can prevent oxidation of the barbiturates, hexobarbital, pentobarbital, and secobarbital; the demethylation of aminopyrine and meperidine; the deamination of amphetamine; ether cleavage of codeine, and the formation of morphine glucosiduronide. In order to test the possibility that the inhibition of drug metabolism seen with SKF 525-A might be due to its hydrolytic products, diethylaminoethanol and diphenylpropylacetic acid, *in vitro* experiments were run in which hexobarbital metabolism was studied under the effects of SKF 525-A and the hydrolytic products. Both SKF 525-A and diphenylpropylacetic acid were equally effective in preventing oxidation of the barbiturate, whereas diethylaminoethanol was without effect. SKF 525-A is considerably more active *in vivo* than is diphenylpropylacetic acid, presumably due to the fact that diphenylpropylacetic acid would be rapidly excreted. These authors examined the possibility that SKF 525-A might act by interfering with hydrogen transport from TPNH. They were able to show no effect of diphenylpropylacetic acid on the systems TPN-cytochrome c reductase, glucose-6-phosphate dehydrogenase, and isocitric dehydrogenase.

While the mechanism of action of this compound remains obscure, it has provided a useful tool in studies of drug metabolism in that certain inferences can be drawn about enzymes involved in various of the drug metabolic pathways by noting the quantitative effect of SKF 525-A on the pathway of interest. The possibility of several contributing factors to the mechanism of action of SKF 525-A must not be overlooked. For example, the effects of this compound on drug tissue distribution has not been extensively studied to date. Its ability to reduce the rate of excretion of S-35 pentothal has been reported by Achor and Geiling (88) and should be further investigated in relation to other compounds, quantitative studies being required of excretion rate *vs.* time-course of pharmacological response. The possibility of an interference by SKF 525-A in the function of a normal physiological or biochemical factor controlling the synthesis or function of the liver microsomal enzymes responsible for drug metabolism must not be overlooked.

Fouts and Brodie (89) have reported on the ability of compound Lilly 18,947 to block drug metabolic reactions. They show that this compound is about equally active with SKF 525-A in depressing demethylation, ether cleavage, deamination, and hydroxylation.

JB-516 has been shown by Pearce, *et al.* (90), to decrease the metabolism of hexobarbital in mice, presumably acting by a mechanism similar to that of SKF 525-A and Lilly 18,947. Welch, *et al.* (91), have demonstrated that chlorothion increases hexobarbital sleeping time in mice and, when added to liver slices, decreases the oxidation of the barbiturate. It is pointed out that both control and chlorothion-treated animals awaken when brain levels of hexobarbital are about 25 mcg./Gm. of tissue, again suggesting that receptor sensitization is not involved in the increased duration of pharmacological response.

Compounds Which Stimulate Drug Metabolism.—It was reported by Conney, *et al.* (92, 97), that pretreatment of rats with phenobarbital, barbital, 3-methylcholanthrene, or 3,4-benzpyrene markedly increases the activity of the liver enzymes azo N-demethylase, 3,4-benzpyrene hydroxylase, and zoxazolamine hydroxylase. The data can be confirmed by *in vivo* studies such as the fact that the duration of action of a paralytic dose of zoxazolamine can be shortened by pretreatment of the animals with 3-methylcholanthrene or 3,4-benzpyrene. It is of further interest that chronic administration of aminopyrine or phenylbutazone can increase activity of the liver microsomal enzymes responsible for their own metabolism. The possible implication of these findings in drug tolerance studies is apparent.

Conney, *et al.* (93), provide evidence that the increased activity of the liver microsomal system brought about by pretreatment of rats with phenobarbital is due to increased enzyme synthesis. They show that administration of ethionine, an amino acid antagonist, prevents the phenobarbital-induced increase in microsomal activity. Further, they point out that addition of phenobarbital to *in vitro* incubation mixtures is without effect in increasing the activity of azo N-demethylase activity, whereas administration

of the barbiturate to the intact animal yields homogenates with a substantially greater metabolic capacity. Such pretreated animals also exhibit increased total liver protein and increased liver microsomal protein. This evidence is consistent with a hypothesis of increased enzyme synthesis.

Cramer, *et al.* (94), have shown that the ability of liver homogenates or microsomes to hydroxylate the carcinogen 2-acetylaminofluorene is increased five to ten-fold in rats upon pretreatment with 3-methylcholanthrene. The effects seen with 3-methylcholanthrene were blocked when the animals were pretreated with *d,l*-ethionine. Von der Decken and Hultin (95) have confirmed the enzyme inductive effects of 3-methylcholanthrene in studies of the demethylation and reductive cleavage of *p*-monomethylaminoazobenzene, and the binding of 2-aminonaphthalene to soluble liver proteins. They show also that microsomes from 3-methylcholanthrene-treated rats have an increased capacity to incorporate leucine into protein and a higher RNA content, further substantiating the hypothesis of Conney, *et al.* (93), that increased drug metabolizing activity is due to increased enzyme synthesis.

It is interesting to note that some compounds which can elicit an increase in the activity of the liver microsomal enzymes metabolizing drugs, also markedly increase the biosynthesis and excretion of ascorbic acid (71, 76, 96).

Mechanisms of Specific Metabolic Reactions.—Of the metabolic reactions undergone by drugs listed in Table I, those which are of more general significance biochemically have received a great deal of study in recent years. The mechanisms of such general (and truly "detoxifying") reactions as glucosiduronide formation and aryl sulfate formation have been thoroughly elucidated.

Glucosiduronide Formation.—This reaction occurs with hydroxy compounds primarily, as well as with carboxylic acids and, in certain cases, with amines (N-glucosiduronides), and has been recognized and described for over 60 years (8). The occurrence of this reaction *in vivo* is now known to take place primarily in the liver, although kidney cortex and the gastrointestinal mucosa are also active (51, 98). Regardless of whether an ether type glucosiduronide (from a hydroxy compound) or an ester type glucosiduronide (from a carboxyl group) is formed, carbon atom 1 of glucuronic acid participates in the linkage. Williams (8) covers the mechanism of glucosiduronide formation in detail as well as presenting a historical account of this metabolic pathway. Dutton (99) has outlined the pathway of this reaction as follows

$$\text{glycogen} \xrightarrow{H_3PO_4} \alpha\text{-glucose-1-PO}_4 \quad \text{(Step 1)}$$

$$\alpha\text{-glucose-1-PO}_4 + \text{UTP} \xrightarrow[\text{transferase}]{\text{uridyl}} \text{UDP-glucose} + \text{PP} \quad \text{(Step 2)}$$

$$\text{UDP-glucose} \xrightarrow[\text{UDPG dehydrogenase}]{DPN,O_2} \text{UDP-glucuronic acid} \quad \text{(Step 3)}$$

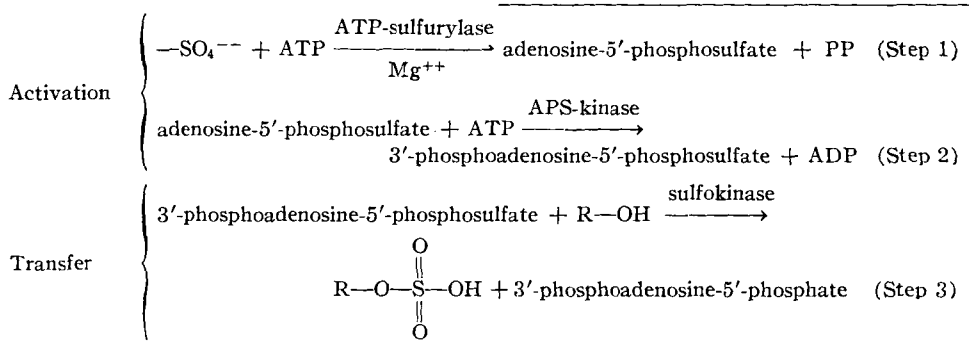
$$\text{UDP-glucuronic acid} + \text{R—OH} \xrightarrow[\text{glucuronyl transferase}]{} \text{R—O-glucuronic acid} + \text{UDP} \quad \text{(Step 4)}$$

That this pathway of metabolism is an important route leading to the elimination of drugs and other foreign compounds from the animal body is obvious; however, its role in normal biochemistry has not been well established. It is known, for example, that endogenous steroids are present in the blood as glucosiduronides (100) as well as being excreted in this form (101). Bilirubin is known to form such a conjugate (102). It has been postulated that steroids are "carried" in the blood as inactive conjugates being released by the enzyme β-glucuronidase which is widely distributed in mammalian tissues (103). The experimental and clinical significance of this is not clear.

Glucosiduronides, whether formed from foreign compounds or endogenous substrates, are rapidly hydrolyzed *in vitro* by the enzyme β-glucuronidase. This technique has proved useful in drug metabolism studies in providing a tool to confirm the suspicion that a conjugate of this type has been formed. The role which β-glucuronidase plays *in vivo* in nuclear. DeDuve (104) described the properties of a class of cytoplasmic particles found in liver, brain, and kidney, known as lysosomes, which appear to contain several enzymes including β-glucuronidase. It is stated that the particles are covered with a lipoprotein layer which is impermeable to the enzyme substrates. Enzyme activity can be released upon treatment of the particles with lecithinase, proteolytic enzymes, various detergents, or carbon tetrachloride. One questions the role which this "latent" β-glucuronidase might play in normal biochemistry.

Sulfate Conjugation.—This reaction represents another metabolic sequence of more general significance which is involved with the metabolism of a number of endogenous sub-

strates, as well as with a host of foreign compounds, including drugs. The so-called "ester sulfates" have been known since the work of Baumann in the last century (105), although the mechanism underlying the formation of such compounds has only recently come to light. Bernstein and McGilvery (106), and DeMeio, *et al.* (107), demonstrated that sulfate conjugation can occur in liver supernatants in the presence of adenylic acid, magnesium ion, and ATP. Lipmann (108) has reviewed developments on this problem, as have Bostrom and Vestermark (109). The generally accepted view for the mechanism of the synthesis is that it involves a two-step process, first, the activation of sulfate, and secondly, the transfer of sulfate to an acceptor molecule. The steps seem to be as follows

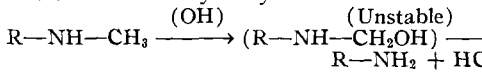


It is of interest that there appear to be at least three sulfate transferring enzymes (sulfokinases) which can take part in the last step of the sequence (108). One of these is called 3′-β-hydroxyl sulfokinase since it catalyzes sulfate transfer to steroids such as dihydroandrosterone, isoandrosterone, and progesterone which have a 3′-β-hydroxyl group. Another kinase appears to react with estrone forming estrone sulfate. The third is apparently quite nonspecific in that it can catalyze the transfer of active sulfate to a wide variety of phenolic acceptor substances. This is the enzyme of interest in drug metabolism studies. Of note in such studies is the suggestion by Anderton, *et al.* (110), that the pKa of the hydroxyl group should lie between 7 and 10 if the compound to which it is attached is to be conjugated with sulfate.

Little information is at hand relative to the role of sulfate conjugation in the normal animal. Certain end products of protein and steroid metabolism are excreted in the urine as sulfates, but the significance of this is not apparent. The lysosomes of DeDuve are stated to contain aryl sulfatase, an enzyme which can hydrolyze the ester sulfate linkage. Some properties of this enzyme have been reported by Webb and Morrow (111) and Roy (112). It appears to be activated by chloride ion (113).

Boyland (114) has reviewed other biochemical conjugations and points out the significance of such reactions as phosphate ester formation and mercapturic acid synthesis through glutathione.

Dealkylation.—The general scheme proposed for the removal of N-alkyl groups is that of Gaudette and Brodie (30) who postulate that "active hydroxyl" plays a role in the mechanism. This is consistent with the TPNH requirement and the formation of hydrogen peroxide during TPNH oxidation (53), which could serve as the source of "active hydroxyl"



Gaudette and Brodie (30) present data indicating that more than one enzyme system is active in the microsomes in the dealkylation reaction. Their data indicate that the removal of alkyl groups from 4-aminoantipyrine is dependent upon the nature of the alkyl group when varied from methyl to dibutyl, whereas dealkylation of the same groups from aniline is not so affected. Additionally, they show that SKF 525-A effectively inhibits the dealkylation of mono- and dimethyl-4-aminoantipyrine, ephedrine, and meperidine, but is without effect on the dealkylation of methyl-, ethyl-, and butylaniline, and the ethyl and butyl 4-aminoantipyrines. Further evidence for the presence of at least two enzymes responsible for dealkylation is indicated by the work of Conney, *et al.* (97), who show that the administration of 3,4-benzpyrene to rats causes an increase in microsomal enzyme activities toward 3-methyl-4-monomethylaminoazobenzene, N-methylaniline, and monomethyl-4-aminoantipyrine, whereas the dealkylation of meperidine and diphenhydramine is decreased. They point out that the mitochondrial system which can dealkylate endogenous substrates such as sarcosine differs from the microsomal system in that

it does not require TPNH and it is blocked by cyanide. The authors postulate that the system dealkylating sarcosine is a cytochrome linked dehydrogenase, the mechanism of the reaction being as follows

$$R{-}NH{-}CH_3 \xrightarrow{-2\,H} R{-}N{=}CH_2 \xrightarrow{HOH}$$
$$(R{-}NH{-}CH_2OH) \longrightarrow R{-}NH_2 + HCHO$$

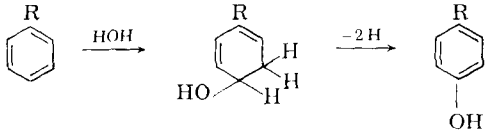
Other oxidative reactions such as sulfoxide formation, ether cleavage, and deamination are postulated to require "active hydroxyl" in sequences similar to that outlined for foreign N-alkylamines.

Aromatic Hydroxylation.—Several studies have confirmed that mammalian liver microsomes are the source of an enzyme(s) able to hydroxylate aromatic compounds (24, 25). TPNH and oxygen are requirements of the system as is the case with other oxidative reactions in the microsomes through which drugs are metabolized. The products of these hydroxylations may be phenols or, less commonly, dihydro diols in the case of condensed ring systems such as naphthalene.

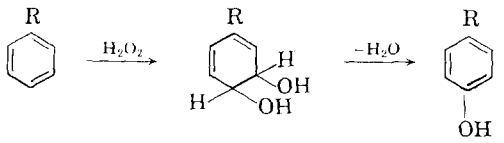
Posner, *et al.* (115), present evidence that more than one enzyme is involved in the hydroxylation mechanism. They cite experiments run with inhibitors showing that the hydroxylation of acetanilide to its 2- and 4-hydroxy derivatives is nearly completely inhibited by 2,2′-dipyridyl whereas the hydroxylation of quinoline is only slightly affected. Microsomes incubated with Tween 80 rapidly lose their ability to hydroxylate acetanilide and quinoline, while the hydroxylation of naphthalene to 1,2-dihydronaphthalene-1,2-diol is little affected.

Endogenous substrates such as steroids (116) and phenylalanine (117) are also hydroxylated. Phenylalanine hydroxylase, while present in a 25,000 × g supernatant of liver, appears to differ from the hydroxylases involved in the metabolism of foreign compounds in that DPNH serves much better than TPNH as a cofactor.

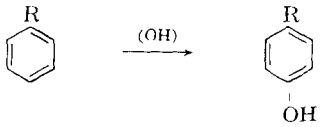
Three possible routes by which an aromatic ring may be hydroxylated have been proposed by Udenfriend, *et al.* (68), in discussion of their Fe^{++}-ascorbic acid system. (*a*) Addition of water to a double bond followed by dehydrogenation



(*b*) Reaction with hydrogen peroxide to form a dihydro-diol intermediate followed by loss of water



(*c*) Direct substitution of hydroxyl for hydrogen



Posner, *et al.* (118), have recently studied the mechanism of aromatic hydroxylation using rabbit liver microsomes incubated in media containing HTO, H_2O^{18}, and in the presence of O_2^{18} in order to determine distribution of hydrogen and oxygen in the metabolite. The conversion of acetanilide to 4-hydroxyacetanilide contains no O^{18} arising from H_2O^{18} which indicates that addition of water plays no role in the mechanism. Phenolic oxygen in the metabolite is derived solely from O_2^{18}. No incorporation of tritium from HTO was found in the 4-hydroxyacetanilide formed. These data do not rule out the possibility that hydrogen peroxide arising during the oxidation of TPNH might be involved in the mechanism as a source of molecular oxygen or "active hydroxyl."

Imai and Sato (119, 120), in an attempt to localize hydroxylases from liver microsomes, report that an acetone powder prepared from microsomes is almost completely unable to carry out hydroxylations, whereas addition of the lipid-rich acetone-soluble fraction restores this function.

Formation of Active Metabolites.—There is now much data in the pharmacological literature suggesting that metabolites of drugs can contribute significantly to total drug response. This is to be expected and in no way conflicts with the general rule that drug metabolites are more rapidly excreted than the parent compound. This possibility should not be overlooked in studies of the metabolic products of any new drug, and is undoubtedly of significance with many older compounds in therapy which have not been fully investigated in the light of recent advances in methodology and understanding of drug metabolism in general.

Thus, the conversion of the barbiturate thiamylal, 5-allyl-5-(1-methylbutyl)-2-thiobarbituric acid, to the active metabolite, secobarbital, was demonstrated *in vivo* by Spector and

Shideman (121), as well as the conversion of the thiobarbiturate, thiopental, to the active species, pentobarbital through exchange of sulfur and oxygen (122). An excellent paper by Butler and Mahaffee (123) shows that the activity of the antiepileptic drug, trimethadione, is in fact due to its demethylated metabolite. Fishman (4) cites several other examples in the barbiturate and anticonvulsants groups in which active metabolites are formed.

It has long been recognized that acetylsalicylic and salicylic acids are converted *in vivo* to the hydroxylated metabolite, gentisic acid (124, 125, 126). This compound also exhibits analgetic properties and reduces fever (127, 128). Conversion of the salicylamide molecule to gentisic acid amide gives rise to a compound exhibiting antihistaminic properties (129), whereas salicylamide itself is devoid of this effect.

Levi and Snow (130) present data showing the *in vivo* conversion of bis-*p*-aminophenylsulfoxide to the corresponding sulfone which has antileprosy properties. Conversion of acetophenetidin to the active analgetic N-acetyl-*p*-aminophenol was demonstrated by Brodie and Axelrod (29). Burns, *et al.* (131), report that a hydroxylated metabolite of butazolidin has a pronounced anti-inflammatory activity as well as being more toxic than the parent drug. A sulfoxide metabolite of the 4-phenylthioethyl derivative of butazolidin has a uricosuric effect much greater than the unchanged drug (132).

Axelrod (133) comments on the formation of active metabolites from endogenous substrates as well as from foreign compounds.

Of related interest is the development of medicinal compounds containing structural groups known to undergo enzymatic attack with release of the active species (drug latentiation) as discussed by Harper (134). A fuller understanding of the metabolism, storage, and transport of drugs and drug metabolites will make this area of medicinal chemistry a fruitful field.

SUMMARY

Studies cited in this review indicate a rapid development of knowledge concerning the biological fate of drugs and other foreign organic chemicals since 1954, coincident with the discovery of systems present in mammalian liver microsomes which catalyze a wide variety of drug metabolic pathways. It is now possible, using this *in vitro* system, to carry out more refined and precise studies of drug metabolism than was the case with organ perfusion techniques and isolation of metabolites from excreta. This subcellular system duplicates exactly the metabolites formed and excreted by the intact animal, so that no question of specificity is involved. The contributions of Brodie, Axelrod, Udenfriend, and their co-workers of the National Institutes of Health are highly significant in the development of current knowledge on drug metabolism.

While recognizing that drugs can be converted *in vivo* to metabolic products exhibiting pharmacological activity, many questions remain to be answered relative to the quantitative importance of such conversions. Modification of drug response through administration of compounds known to stimulate or inhibit the liver microsomal system is an interesting area of study, and probably of future clinical significance. A thorough understanding of the factors operative in the normal animal which control the synthesis and function of the microsomal enzymes must be established in order to open another avenue of effort in modifying drug response. Isolation and characterization of the enzymes involved in drug metabolism is of paramount importance. Further data on the apparent discrimination of the liver microsomal enzymes against endogenous substrates are required to establish this point firmly. Such information will be forthcoming only if studies are carried out with highly purified enzymes, which has not been the case to date.

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