Microbial Transformations of Bioactive Compounds

Volume II

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PREFACE

The microbial world consists of an incredibly diverse variety of living creatures which thrive by their remarkable abilities to adapt to an ever-changing environment. Part of this ability to adapt is attributable to the formation of enzymes as they are needed, and to the presence of certain constituitive enzymes. In general, these enzymes perform critical catabolic and anabolic functions for the organisms which produce them. Some of these functions are highly specific, and the enzymes involved are capable of interacting only with specific classes of substrates. Other enzymes possess relatively broad substrate specificities and these are commonly associated with biodegradative processes. The field which we know as microbial transformations has been largely concerned with attempting to understand and harness the enzymatic potential of microorganisms and to elucidate pathways and mechanisms operative in microbial transformation processes.

Biotechnological developments have occurred with staggering rapidity during the past decade and these have included advances in the microbial transformation field. Activity in the area of microbial transformations most probably reflects the availability of new analytical, chemical, and biochemical tools as well as our clearer perceptions of the nature of enzymes involved in metabolic interconversions. This is evident, for example, in the now widely-recognized presence of the cytochrome P-450 monooxygenase systems in all classes of microorganisms. Our present day technologies have enabled a more precise determination of metabolic pathways and of the mechanisms of enzymatic transformations of even the most complicated types of organic and inorganic substrates.

Many of the philosophies adapted to microbial transformation research were derived from earlier work with the steroids. Perhaps the greatest burst of research activity in the microbial transformation field began in the 1940s and extended over a period of three decades during which steroid transformations were vigorously and successfully developed. Processes were established on an industrial scale particularly with the steroid hormones whose combined microbiological and chemical syntheses are among the finest scientific endeavors of this century. Certainly the successes realized with the steroids were instrumental in stimulating microbial transformation research activity with other classes of physiologically active compounds like the alkaloids, antibiotics, cannabinoids, and the prostaglandins.

The tools and philosophies of microbial transformations have also been extended to research broadly concerned with problems of pharmacological and toxicological significance. Evidence for this may be seen in the extensive literature on biotransformations of environmentally important compounds like pesticides, aromatic hydrocarbons, and other xenobiotics. The utilization of microbial systems as tools in drug development work has been widely described under the "microbial models for mammalian drug metabolism" concept, and advances have been made in determining how microorganisms in the intestines achieve xenobiotic transformations of possible consequence to the host.

These volumes were assembled with the intent of bringing together contemporary research efforts in the microbial transformation field with an additional focus on physiologically active agents. Primary emphasis has been devoted to work reported during the past 10 years, and chapters have been prepared by individuals actively engaged in microbial transformation work. Notably absent from these volumes is a chapter dealing with the steroids, a subject which has been extensively reviewed elsewhere. The book begins with notes on the historical development of the microbial transformation field, and a chapter dealing with fundamental methodologies is also included. It is

intended to provide a comprehensive and well-referenced look at the microbial transformation field as it exists today and to illustrate some of the current and future directions possible with it. If these volumes provide the stimulation necessary to lure others into this fascinating area of research, we will feel that it has been a success.

John P. Rosazza

THE EDITOR

John P. Rosazza, Ph.D., is Professor and Head of the Division of Medicinal Chemistry and Natural Products of the College of Pharmacy at the University of Iowa. He joined the faculty there as an Assistant Professor in 1969 following a year of postdoctoral study at the University of Wisconsin in the area of Pharmaceutical Biochemistry, and was promoted to the rank of Associate Professor in 1972 and full Professor in 1977. He received the B.Sc. degree in Pharmacy from the University of Connecticut in 1962, the M. Sc. degree in 1966 and the Ph.D. degree in 1968 from the same institution. Graduate degrees were received with research specialties in fermentation chemistry and organic chemistry. The Ph.D. degree was completed under the auspices of a training grant from the NIH in the area of Natural Products.

Dr. Rosazza has given service to several organizations. He serves as a referee for the Journal of Natural Products; the Journal of Pharmaceutical Sciences; Bioorganic Chemistry; Applied and Environmental Microbiology; and Antimicrobial Agents and Chemotherapy. He is a member of the editorial advisory boards for the Journal of Natural Products, Applied and Environmental Microbiology and Antimicrobial Agents and Chemotherapy. Dr. Rosazza has organized symposia for the American Society of Microbiology, the American Chemical Society and the American Society of Pharmacognosy. He is a member of the Executive Committee and is Librarian of the Division of Microbial and Biochemical Technology of the ACS. He is President of the American Society of Pharmacognosy. In addition, he is a consultant for the National Cancer Institute and for several industrial firms, and has served as a member of the Bio-Organic Natural Products study section for review of NIH grants.

Dr. Rosazza has pursued strong interests in microbial chemistry, biochemistry and natural products chemistry through the development of a vigorous program of research in the area of microbial and enzymatic transformations of organic compounds. Major emphases in his work have been on: microbial transformations of naturally occurring antitumor compounds; the applications of microbial systems as models for mammalian xenobiotic metabolism; and the development of microbial and enzymatic systems for use in organic synthetic processes. This work has been strongly supported by grants and contracts from the National Cancer Institute and from industry. Along with his fellow students and coworkers, Rosazza has published his work in numerous journals having published some 60 manuscripts to date.

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Chapter 1

MICROBIAL TRANSFORMATIONS AS A MEANS OF PREPARING MAMMALIAN DRUG METABOLITES

Robert V. Smith and John P. Rosazza

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I. INTRODUCTION

New drug development is a complex process which normally occurs in several definitive phases. Following the initial discovery of interesting physiological activity, further investigations are pursued in order to: determine the scope of action of the new drug in higher animals and ultimately in man; systematically modify the chemical structure of the active agent to devise a more selective drug with improved therapeutic activity; and to prepare suitable dosage forms to insure stability and the most effective means of drug administration to the host. The underlying thrust of all these efforts is the enhancement of desired therapeutic effects while diminishing unwanted side effects.

Very few drugs are able to stimulate single and specific responses in complex living systems. Undesirable side effects which are commonly experienced may in some cases be attributed to the intrinsic nature of a drug, or they may be caused by metabolites formed by the action of single or mixed groups of enzymes as the drug passes through actively metabolizing organs in the body. In such cases, studies relating to absorption, distribution, excretion, and metabolic transformations of new drugs assume major importance. It is through such studies that some of the underlying reasons for drug action and/or toxicity may be gained, as well as a firm base for structure activity relationship studies. For similar reasons, metabolism studies provide clues to the mechanisms of toxicity of other xenobiotics.

A. Microbial Models of Mammalian Metabolism

We first summarized our views on microbial models of mammalian metabolism 7 years ago[1] in a paper concerned with aromatic hydroxylations of selected substrates. Since then, we have expanded on concepts relevant to the microbial models hypothesis in a trilogy of reviews which were concerned with metabolic type-reactions common to both microbial and mammalian metabolic systems;[2] methodology used in the microbial transformation area;[3] and the fundamental biochemical bases which allow for the valid comparison of diverse metabolic systems such as mammals and microorganisms.[4] In this chapter, we bring together concepts presented in our earlier work, and we introduce new ideas and present additional examples of metabolic transformations which clearly underline the applicability of microbial transformations as a means for preparing mammalian drug metabolites.

While the need for conducting metabolic studies of drugs and other xenobiotics is clear, numerous problems hinder their rapid and satisfactory completion. These include the usual deficiencies in animal models where species variation is observed in response to the drug. This phenomenon may often be traced to differences in pathways and degrees of metabolic transformations. Analytical difficulties may occur especially concerning the complete establishment of the structures of metabolites which are often available only in miniscule (i.e., microgram) amounts. Further problems may be posed by the inability to detect nascent/highly reactive intermediates and in the production of sufficient amounts of metabolites for complete biological evaluation.

Solutions to some of the problems encountered in typical drug metabolism studies have included the application of sophisticated GC/mass spectral/computer systems and NMR methods employing Fourier transform computer processes which enable structure elucidation work on very small amounts of some metabolites. Production of sufficient quantities of drug metabolites or potential metabolites for complete biological evaluation remains as a continuing and major problem, especially with complex molecules whose syntheses may be extremely complicated. Attempts to circumvent the problem of metabolite availability have included the use of microsomal preparations or organ perfusion systems. However, these have obvious limitations in providing more than milligrams of metabolites; quantities usually insufficient for comprehensive biological/toxicological evaluation.

Smith and Rosazza[1] suggested that microbial systems might be defined which possess the capability of mimicking the metabolic systems of mammals in the production of drug metabolites. Such systems were labeled, "Microbial Models of Mammalian Metabolism". In principle, microbial systems consisting of one or several microorganisms would be used to mimic the metabolic transformations by mammals. Under ideal conditions, microbial and mammalian xenobiotic metabolism studies would be conducted in parallel and common microbial/mammalian metabolites would be produced by simple fermentation scale-up methods using the microbial systems. Microorganisms might also be employed in predictive fashion where microbial drug metabolites would presumably be similar or identical to those formed by corresponding mammalian systems. The isolated microbial metabolites might then be used to establish analytical systems for use in the identification of mammalian metabolites.

Much recent work underlines the amazing metabolic capabilities of single fungal, yeast, or bacterial cultures which perform useful biotransformation reactions. We first suggested that it might be difficult to find a single microorganism which could achieve many of the transformations achieved by a single mammalian liver.[1] Numerous recent examples, however, may now be cited which display the versatilities of microbial systems. These systems have been found to hydroxylate, *O*- and *N*-dealkylate, and also form conjugative products of xenobiotics or their metabolites.

1. Metabolic Reactions Common to Mammalian and Microbial Systems

Most drugs and other xenobiotics entering higher organisms are chemically altered by the action of enzymes. In mammals, the process of drug metabolism has been subdivided into Phase I and Phase II biotransformation reactions. Phase I transformations are those involving oxidative, reductive, and hydrolytic reactions while Phase II reactions include conjugation processes, also known as synthetic reactions involving the drug or its metabolites and common biochemical intermediates found in the body. A schematic representation of these phenomena is given in Figure 1. Such biotransformation reactions are often associated with inactivation, activation, or termination of biological activity of drugs.

Table 1 lists typical Phase I and Phase II biotransformation reactions. It is noteworthy that many of these mammalian type-reactions are found in microbial systems as well. Summaries of such reactions have been provided by Smith and Rosazza,[2] Beukers et al.,[5] Fonken and Johnson,[6] Skryabin and Golovleva,[7] and Kieslich.[8]

The use of microorganisms as metabolic tools has become even more important with the high degrees of predictability possible with given substrates and specific organisms. It is now possible to select microorganisms for the purpose of achieving specific metabolic type reactions. This is especially true in the cases of aromatic hydroxylations, *O*- and *N*-dealkylations, steroid hydroxylations, and ester or amide hydrolyses. Representative examples of cultures possessing broad capabilities of achieving aromatic hydroxylation, *O*- and *N*-dealkylations are shown in Table 2.

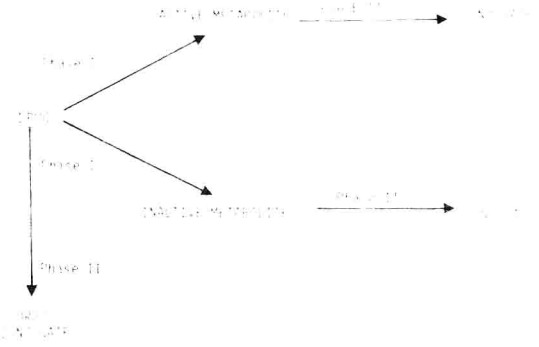

FIGURE 1. Possible metabolic fates of drugs as they pass through the mammalian organism.

Table 1
A LISTING OF COMMON MAMMALIAN DRUG METABOLISM REACTIONS

Reaction Type	Substrates
(Phase I transformations)	
Hydroxylations	Aliphatic, alicyclic and aromatics
N-dealkylation	Secondary, tertiary amines
O-dealkylation	Alkyl-alkyl and alkyl-aryl ethers
S-dealkylation	Alkyl-alkyl and alkyl-aryl sulfides
S-oxidation	Sulfides, sulfoxides
N-oxidation	Primary, secondary and tertiary amines, amides
Deamination	Primary and secondary amines
Dehydrogenations	Primary and secondary alcohols; aldehydes
	Trans-dihydrodiols
(Phase II transformations)	
Glucuronidation	Alcohols, phenols, enols, hydroxylamine, carboxylic acid, primary and secondary amines, thiols
Sulfate conjugation	Alcohols, phenols, enols, amines
Glycine conjugates	Carboxylic acids
Acetylation	Primary amines, hydrazines, hydrazides, sulfonamides
N-methylation	Primary, secondary, tertiary amines
O-methylation	Catechol, phenol

2. General Strategy for Use of Microbial Models of Mammalian Metabolism

Methods employed in microbial transformation experiments have been described in detail in Chapter 2 of this volume and elsewhere.[3,4] It is emphasized that considerable control of fermentation conditions may be required in order to favor the formation of single microbial metabolites of drug substrates. Fermentation parameters are commonly varied in searching for optimum conditions for metabolite production since it is well known that enzymes produced by microbial cells are highly dependent on environmental conditions. Much experimental latitude exists by the choice of culture, medium, and incubation conditions.[2,3]

Cultures for transformation studies may be obtained from rich natural environments like sewage or soil, or from standardized culture collections.[4] Judicious use of the literature will greatly facilitate the selection of cultures for the purposes of conducting specific types of reactions. For example, a listing like Table 2 may be constructed by examination of the current literature for desired reactions. The likelihood of obtaining

Table 2
REPRESENTATIVE EXAMPLES OF CULTURES PERFORMING AROMATIC HYDROXYLATION, O-DEMETHYLATION OR N-DEMETHYLATION REACTIONS

Culture	Aromatic hydroxylation (Ref.)	Dealkylation O-R (Ref.)	Dealkylation N-R (Ref.)
Aspergillus alliaceus NRRL 315	23	17	—
Aspergillus flavus X-158-UI	—	17	—
Aspergillus ochraceus ATCC 1008	1,18,26	—	—
Aspergillus ochraceus 398-UI	—	—	20
Aspergillus niger ATCC 9142	1,18,19	—	—
Aspergillus niger ATCC 11394	27	—	—
Botrytis alii NRRL 2502	—	25	—
Cunninghamella bainieri ATCC 3065	—	15,16,17	—
Cunninghamella bainieri ATCC 9244	1,19,11	17	20
Cunninghamella blakesleeana ATCC 8688a	1,19	20,17	21
Cunninghamella echinulata NRRL 3655	19	15,16,17	20
Cunninghamella echinulata NRRL 1387	19	—	—
Cunninghamella echinulata NRRL 1386	—	25	—
Cunninghamella elegans ATCC 9245	19	16,17,30	—
Curvularia lunata NRRL 2178	1,26	—	—
Gliocladium deliquescens 1086	1,18,26	—	—
Helicostylum piriforme QM 6945	1	16	—
Microsporum gypseum ATCC 11395	—	16	20,24
Mucor mucedo UI-4605	—	16	20
Penicillium brevi-compactum ATCC 10418	—	25	20
Penicillium chrysogenum ATCC 10002	1	—	—
Penicillium duclauxii NRRL 2020	26	16	—
Pseudomonas putida	—	9	—
Pseudomonas fluorescens	—	10	—
Rhizopus stolonifer NRRL 1472	1	—	—
Sepedonium chrysospermum ATCC 13378	—	16,17,22	—
Streptomyces griseus UI 1158	1	15,16,17	20
Streptomyces griseus ATCC 10137	28	28	28
Streptomyces griseus ATCC 13968	—	31	—
Streptomyces lincolnensis ATCC 25466	—	—	20,32
Streptomyces platensis ATCC 13865	—	17	29
Streptomyces punipalus NRRL 3529	—	15	20
Streptomyces rimosus ATCC 23955	1,18,19	16	—
Streptomyces spectabilicus ATCC 27465	—	31	—

Note: ATCC — American Type Culture Collection, Rockville, Maryland
NRRL — Northern Regional Research Laboratories, USDA, Laboratories, Peoria, Illinois
UI — University of Iowa, College of Pharmacy

cultures mentioned in contemporary literature is much higher than from the older literature (i.e., 20 years ago). Most often, pure culture strains of microorganisms are used in microbial transformation work, although mixed culture procedures have been occasionally used. The mixed culture systems, however, may present complications in reproducing experimental conditions.

Methods which we use have been described.[14] Briefly, we have found that stainless steel capped Delong culture flasks are simple to use and afford reproducibility in aer-

ation of shaken-flask cultures. A two-stage fermentation protocol has been routinely employed, and drug substrates are normally added to incubation media 24 hr after the inoculation of Stage II cultures. Samples of drugs are normally added as concentrated solutions in water miscible solvents like dimethylsulfoxide, acetone, alcohol, or dimethylformamide. Alternatively, solutions in methylene chloride or water in the presence of wetting agents like Tween 80 (0.001%) have also been used.[3] Samples are withdrawn from substrate containing incubation mixtures at appropriate time intervals (2 hr to 240 hr) and are assayed by GC, TLC, HPLC or other appropriate methods. All of our screening procedures are conducted in 125 m*l* Delong flasks containing 25 m*l* of culture to which 5 to 10 mg of drug may be added. Preparative scale incubations are performed in multiple shaken flasks, or in stirred fermentors.

A screening process such as the one described above is used to identify microorganisms which are capable of providing metabolites of drug substrates. Although this is a simple thing to perform, it is the key step in successful studies on microbial transformations as a means of preparing mammalian drug metabolites. Great care must be placed on devising sensitive and reproducible analytical techniques which will give a true picture of the microbial transformation process. Optimization of detection systems involves determination of distribution coefficients of substrates and possible metabolites in extracting solvents, development of crisp and reproducible chromatographic systems capable of determining as little as 1% conversions of drugs to metabolites, and the selection of diagnostic spray reagents which will discriminate between drug metabolites and normal microbial secondary products which may coextract.

Microbial transformation experiments can be conducted in parallel with mammalian metabolic studies; or they may be conducted separately in order to obtain metabolites which will be considered as presumed mammalian metabolites. In either case, the great power of using microbial systems for conducting mammalian studies lies in the ease of scale-up to provide gram quantities of compounds for complete study.

B. The Biochemical Bases for Microorganisms as Models for Drug Metabolism Studies

1. Importance of Cytochrome P-450 Monooxygenases in Mammals

Of the three Phase I reactions in mammals, oxidative biotransformations are quantitatively of greatest importance. A tremendous explosion has occurred over the past 18 years regarding our understanding of the mechanisms of oxidation of all types of compounds. In Hayaishi's *Oxygenases* published in 1962,[33] much attention was paid to the gross aspects of oxygen metabolism. Since that time, an understanding of basic mechanisms of oxygen activation and of the physiological function of the oxygenases has been noted. These are summarized in recent works.[34-36]

Most oxidative Phase I reactions in mammals are catalyzed by cytochrome P-450-linked monooxygenases located primarily in the liver of mammals but occurring to some extent as well in the kidney, lung, gastrointestinal tract, spleen, and steroidogenic organs such as the adrenal cortex, testes, ovary, and placenta.[37] Considerable effort has gone into unravelling the electron transport chain associated with these relatively nonspecific enzyme systems.[35,36]

Cytochrome P-450 monooxygenases bind substrate and molecular oxygen and interact with reductases in a two-step sequence to activate the oxygen. Ultimately, one oxygen atom is incorporated into the substrate and the second is reduced to water. This series of events occurs in a cyclic process which is depicted in Figure 2. Formation of a P-450-xenobiotic-O_2 ternary complex helps to explain the selectivity imparted by the cytochrome monooxygenases. The interaction of NADPH-cytochrome P-450 reductase in a two-step (2-electron) sequence is also indicated in Figure 2. Furthermore, the

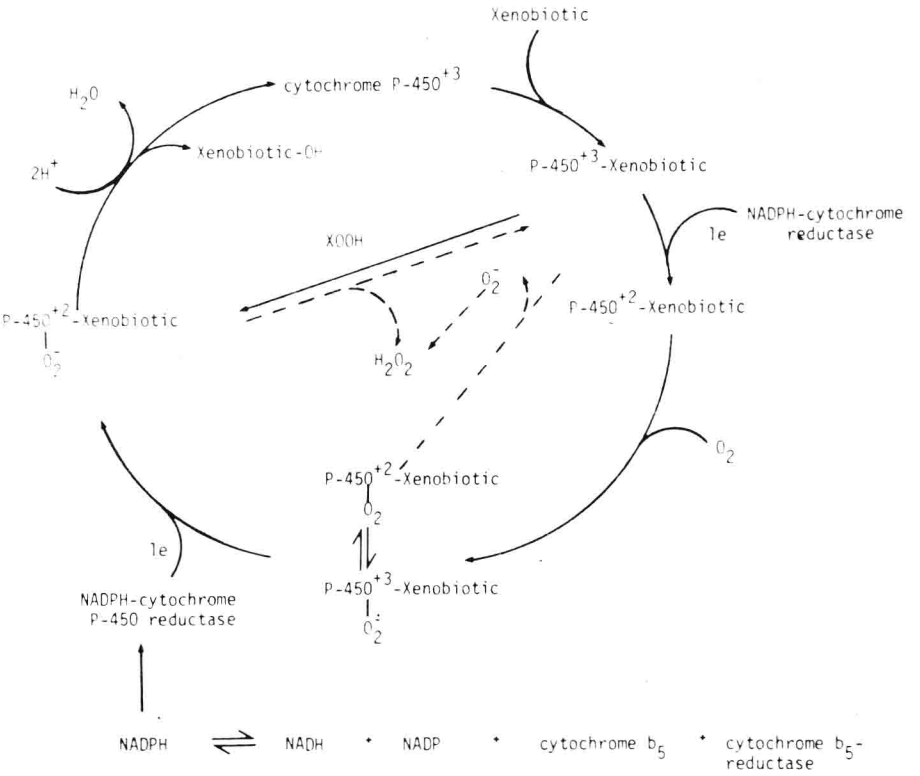

FIGURE 2. Oxidation of xenobiotics *via* cytochrome P-450-linked monooxygenases.

possible coupling of this reductive process with an NADH-mediated electron transfer as shown, explains the often suggested synergistic role for the latter pyridine-nucleotide as well as cytochrome b_5 and b_5-reductase.[38,39] A coupling/decoupling role for hydrogen peroxide has recently been proposed[35,40] as represented in Figure 2. Interestingly, NADPH (and NADPH-generating systems) can be replaced by hydrogen peroxide and a variety of organic peroxides in the oxidation of certain xenobiotics by microsomal mixtures[41] and certain purified cytochromes.[42] This is an intriguing observation which has not (to our knowledge) been duplicated in microorganisms. The effects of peroxides may support the postulated intermediary of the P-450^{+2}-O_2^- complex depicted in Figure 2.

Certain mechanistic similarities exist between mammalian and microbial P-450 monooxygenases and these are discussed in Section I.B.3.

Cytochrome P-450 monooxygenases are believed to mediate aliphatic and aromatic hydroxylations, *N*-, *O*-, *S*-dealkylations, and certain *S*- and *N*-oxidations in mammals. In general they can be visualized as mediating the attack of substrate by an electron deficient oxygen atom. In the case of aliphatic hydroxylations, this process can be thought of as an insertion reaction, while in aromatic hydroxylations, arene oxides primarily occur as intermediates.[43] In most cases, the hydroxyl-alkyl intermediates formed during *N*-, *O*- and *S*-dealkylations are so unstable that they spontaneously decompose to dealkylated substrate and a corresponding aldehyde.

2. Aromatic Hydroxylation in Microorganisms

Aromatic compounds are commonly converted into phenols by mammalian Phase I

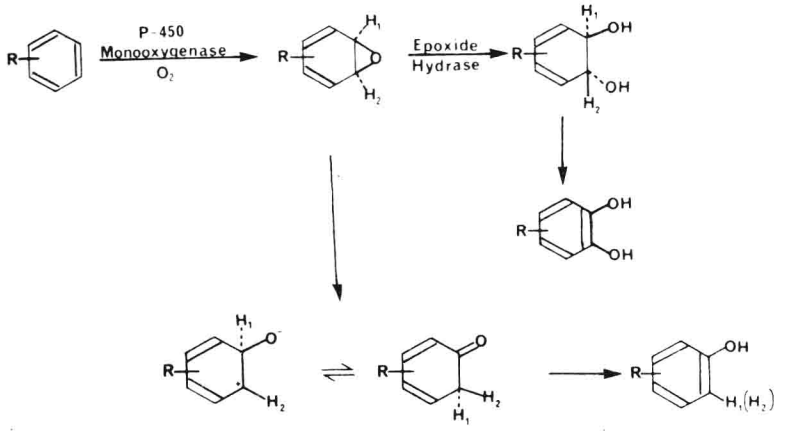

FIGURE 3. Typical monooxygenase mediated oxidation of substituted aromatic compounds.

transformation reactions, and phenols are also produced as major microbial metabolites. The similarities and differences between microbial and mammalian transformations of aromatic compounds have been considered in detail.[1,2] The striking similarities in the metabolizing systems, and the manner by which they hydroxylate aromatic substrates, were instrumental in the development of the microbial models of mammalian metabolism concept[1] and are reviewed in part here. Of necessity, this review of aromatic hydroxylations by microorganisms cannot be comprehensive, but it will serve to illustrate the development of the microbial models idea.

In mammals, aromatic hydroxylation patterns may be predicted by the rules of electrophilic substitution reactions. Electron-rich aromatic rings are readily hydroxylated, while electron deficient systems are hydroxylated sparingly or not at all. Hydroxylation reactions occur at positions predicted by the concepts of electrophilic substitution (i.e., aniline and anisole[43-46] are hydroxylated at *ortho*- and *para*- positions, while deactivated ring systems like benzoic acid and benzamide[46] are hydroxylated in the *meta*-position.) In di- and tri-substituted systems, hydroxylation is directed to positions predicted by a summation of substituent effects.[45,47]

Aromatic hydroxylation can be viewed as proceeding by a number of different mechanisms, but most available information suggests the involvement of an oxenoid mechanism with *arene*-oxide intermediates which rearrange nonenzymatically to phenolic metabolites.[48] This process is usually accompanied by the so-called "NIH-Shift",[43,44,49-51] a mechanism involving the migration of a substituent at the site of hydroxylation to an adjacent carbon atom as shown in Figure 3. The same enzymes forming arene oxides from aromatic substrates appear to be capable of forming epoxides from isolated and conjugated olefins.[48]

In many biological systems, cytochrome P-450 monooxygenases appear to be linked to epoxyhydrases which convert arene-oxide intermediates of compounds like naphthalene[48,52] into their corresponding *trans*-dihydrodiols. In fact, the occurrence of *trans*-dihydrodiol intermediates represents strong evidence for the involvement of arene-oxides derived from monooxygenase oxidation of aromatic compounds. The *trans*-dihydrodiols may be converted further into catechol products by the action of soluble hepatic dehydrogenases. Such catechol metabolites and their conjugates are important metabolic products of aromatic substrates like chlorobenzene.

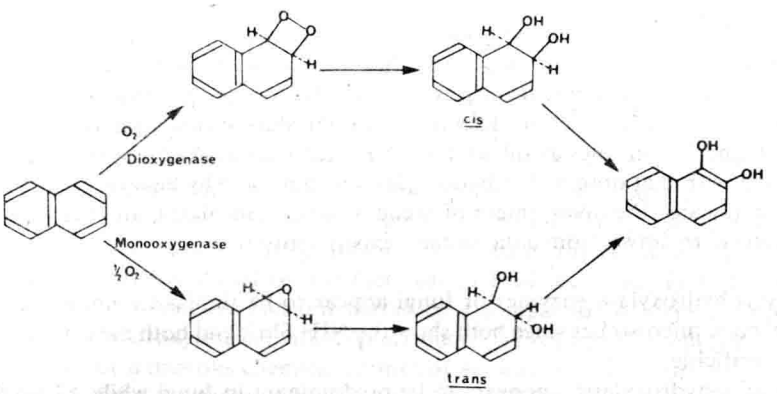

FIGURE 4. Monooxygenase *vs.* dioxygenase transformations of naphthalene.

The beautiful work by Daly, Jerina, and their associates has provided a number of useful gauges by which to measure the possible involvement of monooxygenases in aromatic hydroxylations by diverse metabolic systems. Lack of a primary isotope effect in enzymatic phenol formation (i.e., deuterated aromatic substrates); demonstration of the NIH-Shift; and *trans*-dihydrodiol formation are considered strong evidence for the presence of oxenoid monooxygenase activity.[43,44,53] These features of monooxygenases have been extremely valuable in differentiating monooxygenases from dioxygenases in microbial systems.

In bacteria, aromatic compounds were traditionally thought to be degraded solely by dioxygenase pathways.[54-57] A substrate such as naphthalene, for example, is converted through the hypothetical cyclic peroxy-intermediate into a dihydrodiol, and ultimately into the corresponding catechol (Figure 4). Bacterial systems frequently metabolize catechols further via ring cleavage reactions to CO_2 and water in the mineralization process. Jerina and co-workers[58] isolated and characterized one of the dihydrodiol intermediates clearly demonstrating that the reaction was dioxygenase mediated. The *cis*-arrangement of the diol system was evident by NMR spectral analysis. Prior to this time, a *trans*-dihydrodiol intermediate had only been suggested as being predominantly involved in the degradation of naphthalene and other aromatic compounds in microorganisms. Since then, other *cis*-dihydrodiol intermediates involved in the bacterial metabolism of aromatic hydrocarbons such as biphenyl,[58] benzo[a]pyrene[60] and benzo[a]anthracene,[61] and toluene,[62] and phenanthrene[63] have been characterized. Gibson and co-workers[61,64] have presented the interesting suggestion that quite possibly eukaryotic systems utilize monooxygenases in the metabolism of aromatic hydrocarbons, while the primary means for aromatic hydrocarbon metabolism in prokaryotic systems appears to involve dioxygenases. While at this time, the segregation of mono- vs. dioxygenase metabolic pathway utilization appears to hold, there are certainly exceptions. For example, the prokaryotic organism, *Pseudomonas* sp. (ATCC 11299a) adapted to phenylalanine affects hydroxylation of the 4-tritiated amino acid to 3-tritiated tyrosine involving migration and retention of 95% of the tritium label.[65] The corresponding dioxygenases have yet to be reported in mammals.

Much recent work implicates arene-oxide intermediates in the metabolism of aromatic hydrocarbons, particularly by eukaryotic fungal species in pathways directly analogous to those found in mammals.[1,2] The presence of microbial cytochrome P-450 monooxygenase enzyme systems has been clearly established in numerous cases,[4] and are reviewed in this chapter as well. The first clear evidence that such monooxygenases