

Renal Drug Metabolism

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I. Introduction

A. History

The kidneys have important physiological functions including maintenance of water and electrolyte balance, synthesis, metabolism and secretion of hormones, and excretion of the waste products from metabolism. In addition, the kidneys play a major role in the excretion of drugs, hormones, and xenobiotics. Mechanisms involved in the transport of drugs in the proximal tubule in the secretory direction have been amply reviewed (Bessighir and Roch-Ramel, 1988; Pritchard and Miller, 1993). Reabsorptive transport for organic compounds, particularly amino acids (Zelikovic and Chesney, 1989; Silbernagl, 1992) and choline (Acara and Rennick, 1973; Acara et al., 1979) also have been studied. The concepts associated with pH dependence in the nonionic passive back diffusion of drugs are well-described (Roch-Ramel et al., 1992). However, the role of the kidney in the metabolism of both endogenous and exogenous compounds has not received appropriate attention.

Most of the current knowledge about drug metabolism is based on studies in which the liver was the experimental organ. It is now clear that the kidney actively metabolizes many drugs, hormones, and xenobiotics (Anders, 1980; Bock et al., 1990). In some cases, certain biotransformations occur at a faster rate in the kidney than in the liver; e.g., glycination of benzoic acid (Poon and Pang, 1995). Bowsher et al. (1983) found histamine N-methyltransferase activity to be higher in concentration in rat renal tissue than in any other organ. Gamma glutamyl transferase activity in mammalian tissues is at its highest in the kidney (Goldbarg et al., 1960; see Section III.F.).

The heterogeneity of the kidney makes it important to define the regional distribution of enzyme systems on a cellular and subcellular level. The human kidney has two distinct regions: an outer cortical region and the inner medullary region. The medulla is divided into several pyramids, the base of which is at the corticomedullary junction and the apex of which approaches the renal pelvis, forming a papilla. This heterogeneity is caused by three successive excretory systems that develop during embryonic development; and the latter two, the mesonephros and metanephros, contribute to the formation of the kidney. The ureteric bud, a specialized structure of the mesonephric duct, gives rise to the collecting ducts, calyces, pelvis, and ureter. The metane-

phros gives rise to the glomerulus, proximal, and distal tubules. Whereas most studies have been performed either in whole kidney or in cortical tissue, biotransformations have also been identified in the medullary region (Toback et al., 1977a; Lohr and Acara, 1990).

Information accumulated over the past 20 years demonstrates a large capacity for metabolism in the kidney, leading to activation or inactivation of numerous compounds and providing a major route for drug disposition. In addition, the metabolic products produced by the kidney may exert significant toxic effects. The pattern of blood flow through the kidney, the acidity of the urine, and the urinary concentrating mechanism provide an environment that facilitates the concentration of particular compounds in the medullary/papillary zone of the kidney, and sometimes even, their precipitation (e.g., uric acid) with resultant damage. Such reactions will be presented in a general way because the action of toxins on the kidney is beyond the scope of this review.

In this review, various methods will be described that have been used to study renal metabolism of drugs, xenobiotics, hormones, and endogenous compounds. The various types of metabolic reactions that occur in the kidney will be presented along with the compounds that occupy those particular routes. The contribution of the particular metabolic pathways to the direction of movement of metabolite, into blood or into urine, provides an interrelationship between transport and metabolism.

B. Methodology

Several different methods have been used to study the role of the kidney in the metabolism of drugs and xenobiotics. These vary from *in vivo* techniques, such as clearance and the Sperber chicken preparation, to *in vitro* studies of metabolism using organelles such as mitochondria and microsomes and molecular biology in which genes encoding specific enzymes of metabolism have been identified. Each technique has contributed different information regarding the way in which compounds are handled by the kidney.

1. Clearance. Historically, the contribution of the kidney to the elimination of a particular drug was measured as renal clearance (Moller et al., 1928). The term "clearance" must be defined because it is being used to describe an ever increasing number of functional equations. Renal clearance as described by Homer Smith (1956) is "the volume of plasma required to supply the quantity X excreted in urine each minute" or the volume of plasma completely cleared of that substance in 1 minute time. However, these two definitions are not the same because what is "cleared" may not necessarily appear in the urine.

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Clearance when it is applied to the kidney, is generally defined by the equation: $C_x = U_x \cdot V/P_x$ where U_x is the concentration of compound (x) in the urine, P_x is the concentration in the plasma, V is the urine flow rate and the result (C_x) expressed in volume per unit time, e.g., ml/min.

An important point is that this equation provides information on the amount of substance appearing in the urine but does not account for the portion of that substance that undergoes metabolism or synthesis in the kidney. When, as is frequently done, the Cl_x is related to glomerular filtration rate, a value <1 is taken to indicate a reabsorptive component or removal from tubular fluid. The disappearance of compound can be attributed to metabolism as well as reabsorption. Thus, the term "renal clearance" may be thought of as a general expression of the removal of compound by all the routes of the kidney as in $(A-V/A)Q$ (the concentration of compound in the renal artery (A) minus the concentration of compound in the renal vein (V) divided by the concentration in the renal artery times renal blood flow (Q)). "Urinary clearance" is that clearance associated with compound appearing in the urine. The difference between these two clearance terms is attributable to storage and metabolism (Acara, 1992).

Clearance methods, developed to quantify renal function, were the first methods used to study in vivo renal metabolism (Toretti and Weiner, 1976; Tucker, 1981). Metabolism may be detected during infusion of a radiolabeled compound when the label in the urine is identified as something other than the original compound (Quebbemann and Rennick, 1969; Acara and Rennick, 1972; Toback et al., 1977a,b).

^b Abbreviations: ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; AP, aminopyrine; BBM, brush border membrane; β -NF, β -naphthoflavone; BP, benzpyrene; CC β L, cysteine conjugate β -lyase; cDNA, complementary deoxyribonucleic acid; COMT, catecholamine-O-methyltransferase; CYP, cytochrome P450; DNA, deoxyribonucleic acid; mEH, microsomal epoxide hydroxylase; FAD, flavin adenine dinucleotide; FMO, flavin-containing monooxygenase; GFR, glomerular filtration rate; GSH, glutathione; GST, glutathione S-transferase; HMT, histamine N-methyltransferase; 5-HT, 5-hydroxytryptamine; LPS, lipopolysaccharide; MAO, monoamine oxidase; MB-COMT, membrane bound COMT; 3-MC, 3-methylcholanthrene; mEH, microsomal epoxide hydrolase; MFO, mixed function oxidase; mRNA, messenger ribonucleic acid; NAD, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide, reduced; NADPH, nicotinamide adenine dinucleotide phosphate; NAT, N-acetyltransferases; PABA, *para*-aminobenzoic acid; PAH, *p*-aminohippuric acid; PAPS, 3'-phosphoadenosine 5' phosphosulfate; PB, phenobarbital; PCB, polychlorinated biphenyl; PEA, phenylethylamine; PNMT, phenylethanolamine N-methyl-transferase; SADR, specific activity difference ratio; SAM, S-adenosylmethionine; SAR, specific activity ratio; sEH, cystolic (soluble) epoxide hydrolase; ST, sulfotransferase; STZ, streptozotocin; TEMT, cytosolic thioether-S-methyltransferase; TMT, thiol-methyltransferase; TPMT, soluble thiopurine-methyltransferase; TTCO, tetrachlorodibenzo-p-dioxin; UDP, uridine diphosphate; UDPGA, UDP-glucuronic acid; UDPGT, uridinediphosphoglucuronyl transferase; UGT, UDP-glucuronyltransferase.

Monitoring the fate of radiolabeled compounds after infusion into the renal artery has provided some insight into the kidney's capacity to metabolize. Diamond and Quebbemann (1981) demonstrated clearance of radiolabeled metabolite during steady-state infusion of radiolabeled metabolite (true clearance) and measurement of the clearance of unlabeled metabolite during steady-state infusion of radiolabeled metabolite and unlabeled precursor (apparent clearance). The difference between the apparent clearance and true clearance is the renal contribution to formation of urinary metabolite.

Tremaine, Diamond and Quebbemann (1984, 1985) developed another radioisotope technique, termed the specific activity difference ratio (SADR^b) technique. This involves the infusion of radiolabeled precursor as well as the infusion of unlabeled metabolites if not present endogenously. The technique permits the quantification of renal formation and excretion of several metabolites if they are excreted in measurable amounts. Specific activity ratio technique is a standard isotope dilution method. This is performed by a constant infusion of radiolabeled metabolite along with a constant infusion of unlabeled precursor. The specific radioactivities of metabolites in plasma and urine are subsequently measured. The ratio of the specific activity of metabolite in urine to that in plasma when subtracted from one indicates the fraction of urinary metabolite formed in the kidney and excreted; thus, determining the in vivo renal contribution to formation of a compound.

2. *Sperber technique in chickens*. Birds have a renal portal circulation, accessible through a leg vein, which permits the administration of substances to the ipsilateral kidney. Sperber (1946) demonstrated that when substrates transported by organic excretory transport carriers were infused into the leg vein, they were excreted in excess in the urine from the ipsilateral kidney. Substances entering the general circulation were excreted by both kidneys equally. Because the chicken has no bladder, ureters from either side may be isolated for urine collection (Campbell, 1960). Thus, when contralateral excretion is subtracted from ipsilateral excretion and blood flow through this system is considered, a value is obtained that describes the efficiency by which the compound is removed from the blood by the kidney. In measuring the excretion of the metabolites of an infused substance, those excreted in excess by the infused kidney represent the results of intrarenal metabolism.

An advantage of the Sperber technique include an in vivo system in which pico- to nanomole amounts of radioactive substrate may be studied. Because systemic contributions may be accounted for, it acts as an in vivo perfused kidney. The technique can be used to measure the metabolic capacity of the renal parenchyma and the effects of drugs and chemicals on the function of certain renal enzymes (Rennick, 1981).

Whereas urinary metabolites clearly reflect active biochemical pathways qualitatively, conversion rates are difficult to determine. The technique does not account for the reabsorptive route of the metabolites but only for that route that ends with the appearance of the metabolite in the urine.

3. *Isolated perfused kidney.* The isolated perfused kidney preparation permits the measurement of excretion, reabsorption, and renal metabolism. (Nishiitsutsuji-Uwo, 1967; Bowman, 1978; Nizet, 1978). Because the kidney is removed from the animal, the influence of other organs and tissues is not present. Renal clearance and urinary clearance may be determined for a given compound. As previously indicated, a large renal clearance associated with a low urinary clearance suggests a metabolic component. Kidneys may be perfused for up to 2 h and samples of perfusate and urine collected for appropriate analyses.

The kidney itself may be analyzed at the end of the experiment. Thus, the total disposition of substrate in urine, perfusate, and kidney, the direction of transport, and the associated metabolic routes can be measured, as can the effects of other agents on these compartments (Acara, 1979). The conversion of enalapril, an inhibitor of angiotensin converting enzyme, to its metabolite, enalaprilat, and the transport of drug and metabolite across the basolateral and luminal membranes using constant flow single pass and recirculating isolated perfused rat kidney preparations provided an intrinsic clearance for renal drug metabolism as well as identifying membrane transport steps (de Lannoy et al., 1990).

Glutathione content of isolated perfused kidney is consistently lower than that observed in vivo (Ross et al., 1980) and the maximum rates of drug metabolism may not be observed. Functional shortcomings include low glomerular filtration rate relative to perfusate flow and excessive delivery of filtrate to the distal tubule with decreased concentrating ability (Maack, 1980, 1986).

4. *Tissue Preparations.*

a. **KIDNEY SLICES.** Kidney slices have been used for the study of renal uptake and metabolism for decades (Forster, 1948). Kidneys are removed quickly after onset of anesthesia and kept chilled during slicing and until the start of incubation. Slices, thin enough to permit oxygen to reach all of the tissue, may be incubated for up to 2 h. At the termination of the experiment, slices are blotted on filter paper and weighed. Analyses of media and tissues for substrate and metabolites can reveal accumulation of substrate against a concentration gradient; as well as metabolic routes and intracellular and extracellular amounts of substrate and metabolites.

Phospholipid metabolism from (^{14}C)-choline was studied in mouse kidney slices during renal compensatory growth by Toback et al. (1974). Inner cortical slices were found to have enhanced synthesis of choline containing lipids during kidney regeneration (Toback et al., 1977b). Relative specific activities of enzymes have been studied

in dissected cortex, outer medulla, and inner medulla. Choline dehydrogenase activity was shown to increase in cortex and not change in the inner medulla during hypernatremia (Grossman and Hebert, 1989). Addition of dimethylaminoethanol, an analogue of choline and an inhibitor of choline oxidase, to isolated perfused kidneys as well as dissected tissue regions decreased betaine production from ^{14}C -choline (Lohr and Acara, 1990). Toback et al. (1977b) studied the phosphorylation of choline into choline phosphoglycerides in different kidney regions.

However, information regarding the direction of movement, i.e., reabsorptive versus excretory, is not obtained using kidney slices. Some controversy exists regarding whether or not tubules are collapsed. Uniformity of slices is important because substrate and oxygen should reach all cells. Sometimes the innermost cells of the slice are not exposed to the same concentrations as the surface cells (Foulkes, 1996). The reproducibility and viability of tissue slices were greatly improved by the more recent introduction of automated procedures to produce "precision cut" kidney slices (Smith et al., 1985).

b. **ISOLATED TUBULES.** Tubule segments may be obtained by several methods. Microdissection of large numbers of tubule segments may be performed after perfusion of the kidney with a collagenase containing buffer and incubation with collagenase. The tubule segments can then be used for metabolic studies in which the tubules are typically incubated with a radiolabeled compound. After incubation, the tubules can be separated from the medium either by centrifugation or rapid filtration. Specific nephron segments can be obtained by this method but the tissue yield is low. Development of microassays has permitted the study of enzyme activities in these segments (Endou, 1983a; Schlondorff, 1986).

Relatively homogeneous proximal tubule suspensions can be obtained in greater quantity by density gradient centrifugation, and, with oxygenation, remain viable for approximately 2 h. Obtaining suspensions of tubule segments other than proximal is more difficult because they constitute lower percentages of the kidney mass.

c. **CELL CULTURE.** Cultured cells have become a more popular tool for metabolic studies. In addition to primary cultures, there are now many continuous kidney cell lines (e.g., MDCK, LLC-PK1, OK, A6, JTC-12, BSC1) available for study (Handler and Burg, 1992).

The LLC-PK1, OK, and JTC-12 cell lines are of proximal tubular origin. Of these the LLC-PK1 cells, derived from the Hampshire pig, have been best characterized. They have characteristics such as Na-dependent glucose uptake, Na-dependent phosphate uptake, and Na-dependent amino acid uptake, and high activities of several brush border enzymes. The MDCK cell line is most characteristic of distal tubule, and the A6 cell line resembles the collecting duct.

Primary cultures of proximal renal tubules may also be grown for use in studies of renal drug metabolism. This may be accomplished with tubules obtained by microdissection or by macro separation techniques using rabbit, rat, dog or human kidney.

Metabolic studies of cells in culture are generally performed on cells which have reached confluence. The cells are rinsed with a buffer free of the metabolic substrate to be studied and then incubated for an appropriate time with substrate generally radiolabeled. The experiment is ended by aspirating the medium, rinsing the cells, and disrupting the cells with NaOH, scraping, or sonication. The cell fractions can then be further analyzed.

Fry and colleagues (1978) found that liver and kidney cells demonstrate similar conjugated metabolite patterns. Jones et al. (1979) found that the specific activities for formation of glucuronide and sulfate derivatives in the kidney were approximately 5% of those for liver cells, although formation of sulfhydryl derivatives was proportionately higher in kidney cells with paracetamol as substrate.

d. SUBCELLULAR FRACTIONS. Small (100 nm diameter) closed vesicles which form when cells are disrupted by homogenization and sediment at $100,000 \times g$ are identified as microsomes. They consist primarily of membranes of endoplasmic reticulum and have proven to be a valuable tool in studying synthetic and metabolic functions of the cell. Microsomes from kidney cortex have been used to study drug metabolism. After homogenization and centrifugation at low speed, the resulting supernatant is centrifuged at high speed for 60 min. The microsomal fraction is the pellet that is resuspended for use in drug metabolism studies. Substrates are added to the microsomal incubation medium to study rates of conversion to metabolites. Animals may be pretreated with various drugs and xenobiotics, and the effects of these compounds on microsomal enzyme activity determined *in vitro*. In addition to studying metabolism by use of microsomes, the cytosolic fraction (i.e., the supernatant from the microsomal fractionation) of cells can be isolated and used alone or in conjunction with the microsomal fraction.

5. *Molecular biology*. The increased use of molecular biology techniques in the past 15 years has heavily impacted on how we classify and identify proteins. When available, IUBMB enzyme numbers are given for the enzymes discussed in this review. However, many enzyme activities have only been studied in membrane fractions or using nucleic acid probes, and IUBMB numbers are not available. A parallel system based on genetic information has arisen. The advances in molecular biology have allowed isolated proteins to be cloned and sequenced. In many cases, especially caused by the ease of analysis and amplification of small amounts of nucleic acid materials, it is easier to study the genetic material rather than the protein. Genes are isolated in different organs and species on the basis of homology to known

genes whose enzymatic activities have been studied at the protein level. The transcriptional regulation of these genes can be studied and hypothetical protein sequences deduced. The explosion of molecular biology data has led to the same gene being isolated during studies of different phenotypes. To put some order into the system, there are organizations devoted to specific organisms: HUGO Gene Nomenclature Committee for human genes (accessed through <http://gdb.org/gdb>) and the Mouse Gene Nomenclature Committee for mouse genes (accessed through the Jackson Laboratory web site: <http://www.informatics.jax.org>). In addition, specific gene families have their own nomenclature organizations, such as the P450 Nomenclature Committee identified in the P450 section.

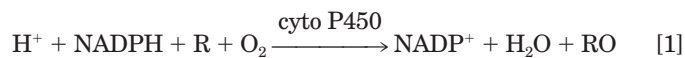
Molecular biology studies can never completely supersede the biochemical studies of isolated enzymes. The study of a gene isolated in the kidney as a homologue of a well-studied liver enzyme is extremely useful. However, it is not guaranteed that the enzyme encoded by that gene, even if it shows the same transcriptional regulation, has the same function in the kidney. The researcher is advised to check the gene banks for homologous genes, but to realize also that the strictly molecular biology studies do not indicate that the protein encoded by that gene has the implied function under physiological conditions in the organ of interest. With these caveats in mind, the authors of this review have attempted to use the most recent molecular biology designations of specific genes isolated in the kidney. Enzymatic activities of genes isolated in the kidney as homologues of liver genes are mentioned in the review, but they may be described more generally if their specific activity in the kidney has not been demonstrated.

II. Renal Pathways for the Biotransformation of Drugs

The authors have organized the description of the specific pathways by first presenting an overview of the general reaction, then a discussion of the specific enzyme involved, and finally the role of this reaction in kidney drug metabolism.

A. Cytochrome P450 Dependent Mixed Function Oxidase System

The most well-studied drug metabolism reaction in the kidney (as well as in the liver) is the cytochrome P450 (CYP) mixed function oxidase (MFO) reaction, which catalyzes the hydroxylation of a diverse group of drugs as shown below:



In the above equation, R is an oxidizable substrate and RO is the metabolite formed by the addition of oxygen.

The localization of P450 MFO in the kidney has been known since the early 1960s, and the early work in this area has been reviewed (Anders et al., 1980). Except for fatty acid hydroxylation (Oliw, 1994), which is found to have greater activity in the kidney than in the liver, it is clear that the renal metabolic contribution of the MFO system is much less than that of the liver.

There are multiple components of the MFO, and different proteins are described below. Cytochrome P450 is a heme containing enzyme that serves as the terminal oxidase component of the electron transfer system present in the endoplasmic reticulum. The usual second component of the system is the flavoprotein nicotinamide adenine dinucleotide phosphate (NADPH) dependent cytochrome P450 reductase that transfers reducing equivalents from NADPH to cytochrome P450. In addition, phospholipid is required for MFO activity. The lipid phosphatidylcholine appears to be required for the coupling of the cytochrome P450 to NADPH-dependent cytochrome P450 reductase. In addition, cytochrome b_5 and cytochrome b_5 reductase can also donate an electron from nicotinamide adenine dinucleotide, reduced (NADH) to cytochrome P450 (Guengerich, 1993).

In contrast to the wide range of cytochrome P450 proteins present in the cell, there appears to be a limited number of NADPH cytochrome P450 reductases. This enzyme contains 1 mole of flavin adenine dinucleotide (FAD) and 1 mole of flavin mononucleotide per mole of flavoprotein and is found in close association with cytochrome P450 in the endoplasmic reticulum. NADPH cytochrome P450 oxidoreductase (NADPH:ferricytochrome oxidoreductase, E.C. 1.6.2.4) has a Mr of 78.275 kDa and is found in close association with cytochrome P450 in the endoplasmic reticulum (O'Leary et al., 1996).

The enzyme activity of NADPH-cytochrome c reductase has been determined to be 34 and 77 nmol/mg protein/min in rabbit and mouse kidney, respectively (Litterst et al., 1975). Human kidney was found to have 10.9 nmol reduced product/mg protein/min (Jakobsson et al., 1978). These values range from 15 to 70% of that concentration found in the liver of the respective species.

Microsomal NADPH cytochrome c reductase activity was found in decreasing amounts from cortex to inner medulla (Zenser et al., 1978; Endou, 1983a,b). When isolated tubules were examined, the activity was greatest in the proximal tubule, although detectable in the glomerulus, distal tubule, and collecting tubule (Endou, 1983a,b). Induction by xylene and its isomers was observed in rat kidney by Toftgard and Nilsen (1982).

1. Cytochrome P450 in the kidney. The various cytochrome P450 proteins not only display different substrate activities, but they also display different regional and stereo selectivities so that the fate of a chemical in a tissue will be determined not only by the total cytochrome P450 concentration but also by the form(s) present in that tissue. There are a variety of oxidative reactions catalyzed by the cytochrome P450 system.

These include aliphatic hydroxylations, aromatic oxidation, alkene epoxidation, nitrogen dealkylation, oxidative deamination, oxygen dealkylation, nitrogen oxidation, oxidative desulfurization, oxidative dehalogenation and oxidative denitrication (Wislocki et al., 1980). Not all isoforms of cytochrome P450 have been identified in the kidney.

The study of the cytochrome P450 system has been aided by the agreement among the workers in this area on a common nomenclature for genes and gene products. The reader is referred to the P450 home page on the Internet for the latest information: <http://www.icgeb.trieste.it/p450/>. In 1989, Gonzalez summarized the current molecular biology data, while in 1990, Ioannides and Parke summarized the current protein work. The most current form of the nomenclature system, found in Nelson et al. (1996), will be given whenever possible. The reader is referred to this review for information on enzyme functions and species location of the P450 families and subfamilies. The italicized root symbol (CYP for human) will be followed by an Arabic number denoting the family, a letter representing the subfamily, and an Arabic number representing the gene within the subfamily. The gene products of the CYP genes, messenger ribonucleic acid (mRNA), and proteins will be referred to using all capital letters. Information from Nelson et al. (1993) was used to provide the new name replacing the old common names for P450 enzymes. However, much of the earlier work was done using enzyme assays or immunological techniques. Because it is not possible to verify that the P450 enzymes studied biochemically actually represent the enzymes encoded by the isolated genes common names will appear in this review in addition to the current nomenclature of Nelson et al. (1996).

The following protein isoforms of the CYP genes have been found in the kidney from studies of enzymes. CYP1A1 and CYP1A2 enzyme activities were found (Ioannides and Parke, 1990). CYP4A1 (P450_{LA ω}) was present in normal kidneys (Hardwick et al, 1987.) Untreated kidney was shown to have low CYP1A enzyme activity, which this was induced by polycyclic aromatic hydrocarbons, β -naphthoflavone, and 2-acetylaminoflourene (Ioannides and Parke, 1990).

Molecular biology techniques have helped to find cytochrome P450 genes in the kidney. Members of the CYP2 and CYP4 gene families were among the earliest genes found in the kidney (Gonzalez, 1989). CYP4A11 deoxyribonucleic acid (DNA) was cloned from a human kidney complementary deoxyribonucleic acid (cDNA) library independently by Palmer et al. (1993) and Imaoka et al. (1993). Messenger ribonucleic acid (mRNA) related to CYP4A11 was found in liver and kidney (with the highest abundance in the kidney) using Northern blot analysis and ribonuclease protection assays (Palmer, 1993). The CYP4A5, A6, and A7 genes have been found in rabbit kidney by Johnson et al. (1990), whereas the

rat *CYP4A2* was isolated by Kimura et al. (1989a,b). *CYP4A3* is also present in the kidney, and *CYP3A4* is expressed in 80% of human kidneys (Parkinson, 1996).

a. LOCALIZATION. The localization of microsomal cytochrome P450 in various regions of the kidney has been examined (table 1). P450 is found in highest concentration in the cortex, with smaller amounts in the outer medulla and more in the inner medulla in rabbits (Zenser et al., 1978; Armbrecht et al., 1979; Mohandas et al., 1981b). Using an isolated tubule preparation, Endou (1983b) investigated the distribution of P450 in rat and rabbit kidney. P450 was found to be localized to the proximal tubule, with the highest concentration in the S2 and S3 segments. At the same time using proximal and distal tubule suspensions and spectrophotometric means cytochrome P450 was found to be present in largest quantities in the proximal tubules (Cojocel et al., 1983). Other investigators subsequently confirmed the localization of cytochrome P450 to the proximal tubule (Foster et al., 1986). Immunochemical analysis has shown that P450IIE1 is located primarily in proximal tubules with some staining in distal tubules (Hu et al., 1993).

The study of the induction of various forms of P450 by benzpyrene (BP) and 3-methylcholanthrene (3-MC) have helped to establish that these enzymes exist in the kidney. BP induced a two-fold increase in rabbit kidney P450 in the S2 segment of the proximal tubule (Endou, 1983a).

In rabbits, a five-fold increase in cortical renal cytochrome P450 content was seen after exposure to 3-MC, whereas the outer medullary activity was only able to be identified after 3-MC treatment (Zenser et al., 1978; Armbrecht et al., 1979). Likewise, 3-MC caused induc-

TABLE 1
Cytochrome P450 content

Species	Location	Concentration (nmol/mg protein)	Reference(s)
Rat	Proximal tubule	0.014	Cojocel et al., 1983
Rat	Microsomes	0.14	Toftgard and Nilsen, 1982
Rat	Microsomes	0.15	Funae et al., 1985
Rat	Microsomes	0.09	Hasamura et al., 1983
Rat	Microsomes	0.07	McMartin et al., 1981
Rat	Microsomes	0.07	Jollie and Manes, 1985
Rat	Cortical microsomes	0.13	Orrhenius et al., 1973
Rat	Cortical microsomes	0.08	Kuo et al., 1982
Rabbit	Microsomes	0.18	Liem et al., 1980
Rabbit	Microsomes	0.10	Ding et al., 1986
Rabbit	Cortical microsomes	0.11	Kuo et al., 1982
Rabbit	Cortical microsomes	0.10	Mohandas et al., 1981b
Rabbit	Outer medulla microsomes	0.01	Mohandas et al., 1981b
Hamster	Cortical microsomes	0.8	Liehr et al., 1987
Hamster	Microsomes	0.25	Smith et al., 1986
Guinea pig	Microsomes	0.13	Smith et al., 1986

tion of renal microsomal P450 in rats (Endou, 1983b; Funae et al., 1985; Wilson et al., 1990). A 3-MC-inducible form of cytochrome P448 (*CYP4A7*), which catalyzed the hydroxylation of BP, has been isolated from the cortex of rabbit kidneys (Kusunose et al., 1989) and cytochrome P₁450 (*CYP1A1*), but not P_s450, was induced by 3-MC (Tuteja et al., 1985). In vivo hybridization of *CYP4A8* to rat kidney sections showed this DNA to be present in the outer stripe of the outer medulla or proximal tubule (Stromstedt et al., 1990).

2. *Drugs that induce cytochrome P450 proteins.* The following sections describe systems in which P450 protein have been reported to be induced by various drugs. The researcher is directed to these references for more specific information.

a. ANALGESICS. Oxidation of acetaminophen by P450 in the kidney was reported by Mohandas et al. (1981a,b). Immunohistochemical studies have been done that colocalized acetaminophen and *CYP2E1* protein in damaged kidney tissue after administration of acetaminophen to mice (Hart et al., 1995). Acetaminophen, a commonly used analgesic, induced nephrotoxicity in males or females treated with testosterone, which corresponded with the induction of renal *CYP2E1* protein in mice as shown by Western blot analysis and enzymatic activity (Hoivik et al., 1995). These results are consistent with metabolism of acetaminophen by *CYP2E1* protein causing toxicity. Note that nephrotoxicity has also been hypothesized to be related to prostaglandin synthesis activity in the kidney (cf section in lipid metabolism).

b. ANESTHETICS. In rats, ether anesthesia caused lowering of total CYP protein, *CYP1A*, and *CYP2B* activities, whereas increases in *CYP2E1* activity were found. These changes were more pronounced in fasted rats (Liu et al., 1993).

c. BARBITURATES. Isozymes of renal P450 have been found to be inducible by polycyclic aromatic compounds and barbiturates. Phenobarbital (PB) induced CYP enzymes in cortical microsomes from rabbit but not from rat (Kuo et al., 1982). Specifically *CYP1A1* and *A2* enzymes were induced by PB (Ioannides and Parke, 1990). Multiple forms of *CYP2B* and *CYP4B1* were shown to be induced in the kidney by PB using molecular biology studies (Ryan et al., 1993).

d. CHEMOTHERAPEUTIC AGENTS. It has been known for a while that cis-diaminedichloroplatinum caused an increase in CYP enzyme activity as measured by spectral methods (Jollie and Maines, 1985). Cis-platinum was found to specifically induce renal *CYP2C23* (Ohishi et al., 1994), and this immunological result was not correlated with any increase in lauric acid ω -hydroxylation activity.

e. ALCOHOL. The *CYP2E1* gene is an ethanol inducible form of P450 that metabolizes substrates such as ethanol, acetone, diethyl ether, *p*-nitrophenol, halothane, benzene, and *N*-nitrosodimethylamine, and is expressed in rabbit kidney (Khani et al., 1988). The protein en-

coded by CYP2E1 is sometimes referred to a P450 3a, P4502E1, or P450alc.

Alcohol was shown to induce P450 isozyme 3a (CYP2E1) in the kidney using antibodies to the liver protein. This increased expression accompanied a seven-fold increase in the isozyme 3a dependent rate of aniline and butanol metabolism in kidney microsomes (Ding et al., 1986). Ethanol has been found to induce specifically isozyme CYP2E1 in the kidney by 50 to 80% (Ueng et al., 1987) and 500% (Ding et al., 1986). Cyp2e-1 (the mouse ortholog of CYP2E1) is present in kidney and induced by alcohol (Thomas et al., 1987). In addition to alcohol CYP2E1 protein was induced by bacterial lipopolysaccharide (LPS) irritants. Bacterial LPS also induced CYP4A2 and 4A3 along with CYP2E1 (Sewer et al., 1997).

Inhalation of alcohol vapor was found to be the best way to induce the protein encoded by CYP2E1, as detected by immunoblotting, in the proximal convoluted tubule of the rat kidney. This result correlated with induction of chlorzoxazone hydroxylation in rat kidney microsomes (Zerilli et al., 1995). The half-life of immunoreactive CYP2E1 protein in kidney was found to be approximately 6 h (Roberts et al., 1994). The CYP2E1 protein and gene can also be induced by pyridine (Kim et al., 1992).

f. IMMUNOSUPPRESSIVE AGENTS. Total kidney cytochrome P450 levels were increased after exposure to cyclosporin, whereas hepatic P450 decreased (Mayer et al., 1989). Using Western blot analysis, Yoshimura et al. (1989) showed that cyclosporin A induced a protein in rat kidney that cross reacted to rabbit CYP2B protein. In rat renal microsomes obtained after treatment of the animal with cyclosporin A, immunoblotting with antibody to rabbit CYP2B4 protein did not show any increase of this protein. In addition, levels of cross reacting material to CYP4A5 (P450 kd) was also found after treatment (Yoshimura et al., 1993b). Western blots of the membrane protein from rat kidney obtained using antibodies to enzyme P450LM2, (CYP2B4) showed no induction of this protein by rapamycin. However, there was an increase in aminopyrine N-demethylase activity after rapamycin treatment (Yoshimura et al., 1993a). Cyclosporin A has been shown to induce CYP4A2 in rat kidney (Nakamura et al., 1994).

3. *Specific renal cytochrome P450 enzymes.* The following sections discuss representative types of reactions attributed to kidney P450 enzymes (CYP) by purification and enzyme assay. These studies have found differences in activities in P450 enzymes isolated from kidneys of different species. If a specific P450 protein designation has been reported in the cited article the correct CYP nomenclature is given.

a. AROMATIC OXIDATION. This common reaction for drugs and xenobiotics that contain a benzene ring has been shown to occur in kidney microsomes. The hydroxylation of BP has been examined in rat (Mayer et al.,

1989), rabbit (Zenser et al., 1978) hamster, and guinea pig renal microsomes (Smith et al., 1986). Likewise, the hydroxylation of aniline and biphenyl in various species was found to occur at low levels of activity (Litterst et al., 1975).

b. N-DEALKYLATION. N-dealkylation occurs in the metabolism of drugs containing an alkyl group attached to an amine. In all instances, its activity in the kidney is found to be equal to or less than that of the liver (Or-rhenius et al., 1973; Litterst et al., 1975). The most commonly used substrate, aminopyrine (AP), undergoes N-dealkylation in mouse, rat, rabbit, hamster, guinea pig, and human kidney. Using AP as substrate, catalytic activity of human renal cytochrome P450 was demonstrated in a reconstituted system using rat NADPH-cytochrome P450 reductase and rat cytochrome b₅ (Imaoka et al., 1990a).

Zenser et al. (1978) examined the demethylation of AP in microsomes from cortex, outer medulla, and inner medulla of rabbit kidney and found activity only in the cortex, which was approximately one-third that of liver. Pretreatment with 3-MC induced cortical activity and caused appearance of activity in the outer medulla (Zenser et al., 1978).

N-demethylation of benzphetamine was demonstrated in rabbit renal microsomes, but not in rat. The activity was induced three-fold by PB treatment (Kuo et al., 1982). Likewise, benzphetamine demethylation was induced by PB in renal microsomes from hamsters. Activity was 5 to 10% that seen in liver (Smith et al., 1986).

O-DEALKYLATION. Oxygen dealkylation occurs in the metabolism of drugs containing an ether group. Renal cytochrome P450 2C23 (k2), A8(k-4), and 4A2(k-5) have been shown to catalyze the O-deethylation of 7 ethoxycoumarin in a reconstituted system (Imaoka et al., 1990a). Enzyme activity was demonstrated in microsomes from rat and rabbit kidney. PB induced activity only in rabbits (Kuo et al., 1982) and a two-fold induction was seen with ethanol (Ueng et al., 1987). O-deethylation of 7-ethoxycoumarin and exthoxyresorufin in renal microsomes was approximately 15% that of liver in hamster, but much less in guinea pig (Smith et al., 1986). No induction of ethoxycoumarin deethylase by PB, polybrominated biphenyl or β -naphthoflavone (β -NF) occurred. Ethoxyresorufin deethylase activity in renal microsomes was less than 10% that of liver in both hamster and guinea pig and activity was induced by β -NF (Smith et al., 1986). Rat kidney activity was induced by xylene (Toftgard and Nilsen, 1982). Cytochrome b₅ independent O-dealkylation using 7-ethoxycoumarin was found in rat and human kidney (Imaoka et al., 1990a). Cojocel et al. (1983) reported O-deethylation of 7-ethoxycoumarin to be highest in the proximal tubule fraction of the kidney. O-dealkylation of ethoxyresorufin was shown in rat kidney microsomes exposed to the polychlorinated biphenyl (PCB), Aroclor, by Beebe et al. (1995) and by exposure to the pesticide Fenarimol (Paolini et al., 1996).

c. OXIDATIVE DEHALOGENATION. Halogenated organic compounds, such as general anesthetics, undergo oxidative dehalogenation to the corresponding alcohol or acid. Renal microsomes metabolize dichloromethane at a rate 5% that of liver microsomes (Kubic and Anders, 1975).

4. *Nondrug factors that affect cytochrome P450 enzymes in the kidney that may modulate kidney drug metabolism.* In addition to being induced by drugs, various cytochrome P450s are induced by endogenous substrates, hormones, toxins, and various metabolic states. The presence of these additional effectors of P450 metabolism could affect the metabolism of drugs in the kidney. Below are representatives of these nondrug modulators on P450 function and expression in the rabbit kidney.

a. ENDOGENOUS LIPID METABOLISM. MFO and therefore P450 enzymes have been well-documented as playing a role in fatty acid and steroid metabolism in the liver. The kidney has been recently shown to be a site of these metabolic reactions. Expression of CYP3A, which catalyzes the 6-hydroxylation of endogenous steroids, has been found in amphibian (A6) renal cells and rat kidney and human kidney microsomes (Schuetz et al., 1992). In immunochemistry, studies using antibodies raised to an adrenal cortex enzyme (steroid 21-hydroxylase), immunoreactive protein was localized in the distal, cortical, and medullary collecting tubules of the kidney. CYP21A1 (P450_{C21}) enzymatic activity had previously been reported in the bovine kidney (Sasano et al., 1988).

The kidney has many P450s that are involved in fatty acid metabolism. The general role of cytochrome P450 in the arachidonate cascade has been reviewed by Capdevila and colleagues (1992). Zenser recognized in 1979 that there were at least two separate pathways for arachidonic acid metabolism in the kidney. One pathway was the prostaglandin cyclooxygenase pathway, whereas a second pathway involved the NADPH-dependent mixed function oxidase P450 system. The action of the CYP4A family (arachidonic acid $\omega/\omega-1$ oxygenase activity) metabolism in the kidney has been related to hypertension (Laniado-Schwartzman et al., 1996; Makita et al., 1996) and the action of CYP2A, the arachidonic acid epoxygenase, in the kidney may also be related to disease states (Makita et al., 1996). Thus, these target enzymes could become the targets of drugs of the future.

The renal cytochrome P450 arachidonic acid system has been reviewed by Laniado-Schwartzman and Abrahams (1992). A gene encoding a putative rat kidney arachidonic acid epoxygenase was isolated from a kidney cDNA library. Overexpression of this gene in COS-1 cells produced a protein that catalyzed the NADPH-dependent metabolism of arachidonic acid with similar specificity to P450 2C23 (Karara et al., 1993).

A P450 isozyme, responsible for the oxygenation of polyunsaturated fatty acids, has been well-documented in the kidney (Oliw, 1994). Cytochrome P450K (CYP2C6) protein, catalyzing the hydroxylation of vari-

ous saturated fatty acids to the corresponding ω - and $\omega-1$ hydroxy derivatives has been studied in rat kidney cortex by spectral and enzymatic methods (Ellin et al., 1972).

The CYP4 gene family, expressed in rat kidney tissue, includes isoforms that catalyze the $\omega/(\omega-1)$ hydroxylation of prostaglandin A (CYP4A7) (Kusunose et al., 1989) and fatty acids (Imaoka et al., 1990b). Members of this family of CYP4A proteins have been shown to be induced in kidney by peroxisome proliferators such as di-(2-ethylhexyl)phthalate (Okita et al., 1993). In the rat kidney CYP4A1 mRNA was induced by methylofenapate (Bell et al., 1992) and CYP4A2 mRNA was induced by dehydroepiandrosterone, an adrenal steroid that is a peroxisome proliferator at high dosages (Webb et al., 1996).

b. TOXIN BIOTRANSFORMATION. The biotransformation of many toxins by P450 enzymes has been shown to occur in the kidney. Renal microsomal cytochrome P450 was induced by xylene in the rat kidney and studied as the O-deethylation of 7-ethoxyresorufin (Toftgard and Nilsen, 1982). Enzyme assays and immunoblots were used to demonstrate that dietary exposure to Aroclor 1254, a PCB, induced P450 1A2 (Beebe et al., 1995) whereas exposure to the pesticide, Fenarimol, induced CYP 1A1 (Paolini et al., 1996).

Not all toxic agents stimulate kidney P450 enzymes. The mutagenic compound, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) has been found in chlorinated drinking water and shown to inhibit kidney EROD (7-ethoxyresorufin-O-deethylase) activity. However, note that MX induced UDP glucuronosyltransferase activity in kidney (see glucuronidation section).

Studies have shown tissue specific expression of dioxin induced P450 enzymes. Form 6 (CYP1A1 protein in rabbit) was found to be induced by dioxin (TCDD) in rat kidney, whereas form 4 (CYP1A2 in humans) was found to be localized in the rabbit liver as measured by enzymatic assay (Liem et al., 1980). Using more sensitive immunohistochemistry, it was found that P-450 isoforms 4 and 6 stained proximal tubules and endothelium intensely after TCDD treatment.

The aromatic hydrocarbon receptor is a small cytoplasmic protein required for the induction of CYP1A1 at the transcriptional level. This protein has the highest affinity for TCDD and is sometimes called the Dioxin inhibitor. (Parkinson, 1996). The general action of the aromatic hydrocarbon receptor and its localization in the kidney has been reported by Ioannides and Parke (1990).

In rabbit kidney of untreated animals it was found that forms 2 and 3 were localized to proximal tubules (Dees et al., 1982). In addition, the P450 gene family, P450 1A1 has been found in kidney tissue after induction with TCDD (Gonzalez, 1989).

c. SEX HORMONES. As in other organs, sex related expression and metabolism of various xenobiotics and en-

ogenous substrates by P450 enzymes have been shown in the kidney (Hawke and Welch, 1985; Hu et al., 1993). Renal P450 enzymes have been induced both in males or testosterone treated females (Williams et al., 1986) and by estrogen (Liehr et al., 1987). Inducers such as 3-MC, PB, and purazole changed induction profiles of CYP2A by significantly increasing 7 α -hydroxylase activity in male kidney microsomes, whereas only PB increased activity in females (Hoivik et al., 1995; Pelkonen et al., 1994).

Male specific induction of the gene encoding P450 4A2 as tested by Northern blot analysis after treatment with the peroxisome proliferator, clofibrate, has been reported in the rat kidney (Sundseth and Waxman, 1992). Administration of androgens to female mice changed the mouse kidney pattern of expression of P450 enzymes from female to male in 8 days. Male mice missing the androgen receptor were not responsive to testosterone and maintained the female P450 kidney distribution. (Henderson and Wolf, 1991). CYP4A2, a P450 isozyme found in greater abundance in female rat kidney, was also induced by growth hormone under certain conditions (Imaoka et al., 1992).

d. FASTING, OBESITY, EXERCISE, AND DIABETES. The P450 content/g of kidney was increased by 50% over that of controls in the obese overfed rat (Corcoran and Salazar, 1988). Starvation has been shown by enzyme assay, spectrophotometric methods, and Western blots (Imaoka et al., 1990b) to increase activity of CYP4A2 (P450 K-5) extending early work showing starvation increased amounts of P450 and laurate- ω -oxidation activity in rat kidney microsomes (Hasumura et al., 1983; McMartin et al., 1981). In addition, exercise has been demonstrated to increase renal microsomal P450 by up to 60% in male rats and age to reduce it (Piatkowski et al., 1993).

In rats with streptozotocin (STZ)-induced diabetes, the P450 content was reduced by 50% (Del Villar et al., 1995). P450 2E1, 4A2, and 4A8 (K-4) in renal microsomes were induced by diabetes along with ω and ω -1 hydroxylation activity (Shimojo et al., 1993). It is possible that although total P450 content may decline, specific P450's may increase in STZ-induced diabetes.

B. N-Oxidation (Flavin-Containing Monooxygenases)

Flavin containing monooxygenases (FMOs) are found in liver, kidney, and lung and can oxidize the nucleophilic nitrogen, sulfur, and phosphorus heteroatom of a variety of xenobiotics. They require NADPH and O₂ and catalyze some of the same reactions as cytochrome P450. These are mostly detoxication reactions, and metabolites produced generally result from the chemical reaction between a peracid or peroxide. FMO plays a role in the N- and S-oxygenation of numerous xenobiotics.

FAD is reduced by NADPH and oxidized NADP⁺ remains bound to the enzyme, which then binds oxygen producing a relatively stable peroxide. During oxygenen-

ation, the 4a-hydroperoxyflavin is converted to 4a-hydroxyflavin and the flavin peroxide oxygen is transferred to the substrate.

cDNAs for five different FMOs (*FMO1*, *FMO2*, *FMO3*, *FMO4*, *FMO5*) have been cloned and sequenced. Each of these genes has been mapped to the long arm of chromosome 1. The open reading frames deduced from the DNA sequence of *FMO1*, 2, 3, and 5 contain between 532 and 535 amino acid residues and the calculated molecular mass is approximately 60 kDa. *FMO 4* is believed to encode 25 more amino acid residues. Each of these genes is expressed in a species and tissue specific manner in humans and other mammals. The kidney of mouse, rat, and human contains relatively high levels of FMO1, and FMO3 is high in the mouse and rat but not in the human kidney (Dolphin et al., 1991; Parkinson, 1996). The forms of FMO are distinct gene products with different physical properties and substrate specificities. Human FMOs 1 and 3 have been expressed in bacterial and insect systems, and the proteins found to be functionally active in catalyzing the N-oxidation of N-ethyl-N-methylaniline and pargyline (Phillips et al., 1995).

Many basic drugs, such as benadryl, imipramine, chlorpromazine, nicotine, morphine, methaqualone, methadone, and meperidine, form N-oxides. The chicken kidney was found to produce 7.2 $\mu\text{mol/hr/g}$ kidney of trimethylamine oxide from trimethyl amine in vivo (Acara et al., 1977). The same metabolism in chicken liver homogenates occurred at a rate of 9.3 $\mu\text{mol/hr/g}$ liver (Baker et al., 1963). After meperidine perfusion of the isolated rat kidney, meperidine N-oxide was identified by GC/MS as the major renal metabolite (Acara et al., 1981).

Vickers et al. (1996) found that N-oxidation was the major renal biotransformation pathway for the 5HT₃ antagonist, tropisetron. Although the overall contribution to tropisetron metabolism was very small, 2 to 12 pmol/hr/mg slice for human rat and dog kidney were comparable to human and rat liver (but not dog). In the kidney, the only metabolite formed of imipramine was its N-oxide (Lemoine et al., 1990). The kinetic analysis indicated an affinity of 7 mM for human liver microsomes versus 0.3 mM in kidney.

C. Alcohol Oxidation

The principle route of elimination of alcohol is by oxidation to the aldehyde and subsequently to the carboxylic acid. Alcohols can also be directly conjugated with glucuronic acid and metabolized by a microsomal P450 enzyme. Although 90% of ethanol metabolism occurs in the liver, the enzyme is ubiquitous and renal metabolism also plays a role in elimination.

Alcohol dehydrogenase (ADH) (E.C. 1.1.1.1) is a cytoplasmic NAD⁺ dependent zinc metalloenzyme that catalyzes the reaction oxidizing an alcohol to an aldehyde and reduces NAD⁺ to NADH. At this time, the human ADH family consists of seven genes, which have evolved

from a common ancestral gene. The genes encode proteins belonging to one of five classes of ADH isoenzymes based on structural and kinetic features. Class I (ADH1, ADH2, ADH3) has a low K_M for ethanol and is sensitive to inhibition by pyrazoles. Classes II (ADHP) and III (ADH5) have a higher K_M for ethanol, greater affinity for long chain alcohols, and are insensitive to pyrazole inhibition. Class IV (ADH7), isolated from rat stomach, has enzyme characteristics of class II but substantial structural differences (Pares et al., 1992). Class V (ADH6) has been described in liver and stomach (Yasunami et al., 1991).

Kidney ADH has been studied in several species (Moser et al., 1968). Five major isozymes were isolated from various tissues in baboons, with the kidney extract showing the highest activity of Class I isozymes termed ADH 1 and ADH 2 (Holmes et al., 1986). The specific activity of ADH from kidney extract with 5 mM ethanol as substrate was 476 nmol/min/g tissue, roughly one-third that seen in liver extract.

ADH mRNA was found to be present in the inner cortex and medulla of kidneys from female Wistar rats (Qulali et al., 1991). Treatment with estradiol induced ADH mRNA resulting in a three-fold increase in ADH activity. ADH activity of liver was 5 times that of kidney but showed no induction with estradiol. Subsequent studies localized estradiol-induced ADH mRNA only to kidney tubule cells and further elucidated the role of hormones in the control of rat kidney ADH (Qulali et al., 1993). Fasting, hyperthyroidism, and treatment of male rats with estradiol increased ADH activity. Androgens were found to induce ADH mRNA in mouse kidney (Felder et al., 1988; Tussey and Felder, 1989; Watson and Paigen, 1990) and although androgen treatment caused a difference in the transcription rate of mRNA in the kidney, liver ADH level was controlled posttranscriptionally.

D. Aldehyde Oxidation

Aldehydes are produced as intermediates in many biological reactions. The most common source of aldehyde in humans is acetaldehyde formed from the metabolism of ethanol. In addition, aldehyde formation may result from biogenic amine metabolism, amino acid metabolism, and lipid peroxidation of polyunsaturated fatty acids (Ambruziak and Pietruszko, 1993).

Aldehydes may be oxidized to their corresponding carboxylic acid by enzymes such as aldehyde dehydrogenase (ALDH) (E.C. 1.2.1.3), aldehyde oxidase, and xanthine oxidase. ALDH activity in the kidney was first described by Deitrich (1966). The overall activity of ALDH in the rat kidney varies from 20 to 80% of that in rat liver (Deitrich, 1966; Vasilou and Marselos, 1989; Dipple and Crabb, 1993). Because proximal tubule cells contain cytochrome P450 and ADH, the cells have the

potential to oxidize a variety of compounds to aldehydes that are potential cytotoxins. The presence of ALDH in these cells is thus beneficial.

Three major classes of ALDH (E.C.1.2.1.3) containing several isozymes have been described. Class 1 are cytosolic ALDHs that have been localized primarily in the liver and exhibit broad substrate specificity and a low K_M with acetaldehyde as a substrate. Class 2 ALDHs are mitochondrial enzymes that are present at significant levels in human (Agarwal et al., 1989), opossum (Holmes et al., 1991), rat (Dipple and Crabb, 1993), and mouse (Ront et al., 1987) kidney, as well as the liver. Class 2 ALDHs also show a low K_M for acetaldehyde and are active in aliphatic and biogenic amine metabolism. Class 3 ALDHs include the major corneal/stomach ALDH with a high K_M for acetaldehyde and have not been described in the kidney.

The genomic structure of the gene encoding human class 1 ALDH protein (Hsu et al., 1989) and class 2 ALDH protein (Hsu et al., 1988) have been described. Each has approximately 50 kb and consists of 13 coding exons separated by 12 introns. Human class 3 ALDH has also been cloned and sequenced. Recently several additional human ALDH genes have been identified but have not yet been assigned to gene classes. In particular, ALDH7 cDNA was cloned from human kidney tissue (Hsu et al., 1994). Information on ALDH genes may be obtained from the Internet at the "Vasilou Laboratory Home Page" (<http://www.uchsc.edu/sp/sp/alcdbase/alcdbase.html>).

An isozyme termed ALDH5 was found to be present in both cytosolic and mitochondrial fractions of many opossum organs including the kidney (Holmes et al., 1991). The isozyme ALDH4, a mitochondrial enzyme with a high K_M was found to have significant activity in kidney (Agarwal et al., 1989; Holmes et al., 1991). On purification, it was found to be identical with glutamate-semi-ALDH (E.C. 1.5.1.2) (Forte-McRobbie and Pietruszko, 1986). Isozymes have been shown to vary in time of appearance during development (Ront et al., 1987). A rat kidney ALDH isozyme that catalyzes the oxidation of retinol to retinoic acid has been isolated (Labrecque et al., 1995) and the cDNA encoding this protein has been cloned (Bhat et al., 1995).

Subcellular fractionation of proximal tubule fragments from rabbit kidney using propionaldehyde as a substrate showed ALDH activity to be bimodally distributed in the mitochondrial and cytosolic fractions. Kinetic characteristics suggested the presence of two isoenzymes (Hjelle et al., 1983).

3-MC induced ALDH activity in rat kidney using benzaldehyde/NADP as substrate but no change was seen after PB treatment (Vasilou and Marselos, 1989). It appears that the pattern of ALDH induction is dependent on the inducer.

E. Oxidative Deamination (Monoamine Oxidase)

Monoamine oxidase (MAO) amine:oxygen oxidoreductase (deaminating flavin-containing E.C.1.4.3.4.) catalyzes the oxidative metabolism of amines. MAO is tightly associated with the outer mitochondrial membrane. The reaction catalyzed by MAO consists of reductive and oxidative half reactions. In the reductive half reaction, the amine substrate is oxidized and the covalently attached FAD reduced to the hydroxyquinolone. In the second half-reaction, the FAD is reoxidized by oxygen with formation of H₂O₂ and an aldehyde (Weyler et al., 1990). The sum of the MAO partial reactions is:



The kinetics of oxidation by MAO appear to be substrate dependent. In most instances, the substrates are oxidized in a ping-pong mechanism, whereas in others there is a ternary mechanism.

There are two isoforms of MAO, designated A and B, which are widely distributed in mammalian tissues and function to breakdown not only biogenic amines but a wide variety of amines including aliphatic, aromatic, primary, secondary, and tertiary amines. The two forms were originally demonstrated through selective inhibition by clorgyline (MAO-A) (Johnston, 1968) and deprenyl (MAO-B) (Knoll and Magyar, 1972). Subsequently, they have been distinguished by differences in substrate preference (Lyles and Shaffer, 1979), tissue and cellular distribution (Weyler et al., 1990), immunological properties (Denny et al., 1983), and most recently determination of nucleotide sequences of cDNA (Bach et al., 1988). cDNA cloning of human liver monoamine oxidase A and B has determined that they are derived from separate genes. The MAO-A and MAO-B human genes are located next to each other on the human X chromosome. The A and B forms have subunits with molecular weights of 59.7 and 58.8 kDa, respectively with 70% sequence identity. The obligatory cofactor FAD is co-

valently bound to cysteine in the same pentapeptide sequence in both isoforms. The genes for monoamine oxidase A and B have identical exon-intron organization, suggesting a common ancestral gene (Grimsby et al., 1991).

The kidney contains high concentrations of both MAO-A and -B. Most of the activity has been shown to be in tubular cells and the enzyme is of particular importance in the metabolism of amines filtered by the kidney (Fernandes and Soares-da-Silva, 1990).

Table 2 shows the kinetic parameters of MAO from homogenates of human and rat kidney. In human kidney, the similar activities toward 5-hydroxytryptamine and phenylethylamine suggest that differences in V_{max} are caused by differences in concentration of the enzyme (Fernandes and Soares-da-Silva, 1992). The activity of MAO-A is similar in cortex and medulla, whereas that of MAO-B is higher in the cortex. Studies in rabbit renal proximal tubule using Western blot analysis and enzyme assays show MAO-B to be the predominant isoform. This isoform holds the I₂ imidazoline binding site, a regulator of MAO (Gargalidis-Mondanese et al., 1997). In rat tissue, MAO-A is the predominant form. The MAO activity of human kidney is approximately one-third that of liver (Vogel et al., 1983).

It has been demonstrated that in rat renal slices a substantial amount of newly formed dopamine is deaminated to 34-dihydroxyphenylacetic acid in renal tubular cells (Fernandes and Soares-da-Silva, 1990). The effect of aging on MAO activity in the kidney has been studied, with little change found in rabbits or mice (Feldman and Roche, 1978) but a small decrease in MAO-A and MAO-B activity was seen in aging rats (Lai et al., 1982).

F. Aldehyde and Ketone Reduction

Aldehydes and ketones are widely distributed and have several biological functions. In addition to ADH, there are several enzymes in the aldo-keto reductase

TABLE 2
Kidney monoamine oxidase

Species	Tissue	Enzyme	Substrate	V _{max} ^a	K _M ^b	Reference(s)	
Human	Cortex	MAO-A	5HT	142	302	Fernandes and Soares-da-Silva, 1992	
	Medulla	MAO-A	5HT	153	309	Fernandes and Soares-da-Silva, 1992	
	Cortex	MAO-B	5HT	166	58	Fernandes and Soares-da-Silva, 1992	
	Medulla	MAO-B	PEA	93	20	Fernandes and Soares-da-Silva, 1992	
	Whole kidney	MAO-A	5HT	53	392	Vogel et al., 1983	
	Whole kidney	MAO-B	PEA	117	374	Vogel et al., 1983	
	Whole kidney	MAO-A	5HT	353	218	Sullivan et al., 1986	
	Whole kidney	MAO-B	PEA	112	5	Sullivan et al., 1986	
	Whole kidney	MAO-A	Tyramine	456	183	Sullivan et al., 1986	
	Whole kidney	MAO-B	Tyramine	882	360	Sullivan et al., 1986	
	Whole kidney	MAO-A	Tryptamine	435	28	Sullivan et al., 1986	
	Whole kidney	MAO-B	Tryptamine	108	41	Sullivan et al., 1986	
	Whole kidney	MAO-A	5HT	145		Haenick, 1981	
	Whole kidney	MAO-B	PEA	32		Haenick, 1981	
	Rat	Cortex	MAO-A	5HT	62	220	Fernandes and Soares-da-Silva, 1992
		Medulla	MAO-A	5HT	59	214	Fernandes and Soares-da-Silva, 1992
Cortex		MAO-B	PEA	31	39	Fernandes and Soares-da-Silva, 1992	
Medulla		MAO-B	PEA	15	67	Fernandes and Soares-da-Silva, 1992	

^a V_{max} = nmoles/mg protein/hr; ^b K_M = 10⁻⁶ M.

family that may participate in the metabolism of aldehydes and ketones in the kidney. Aldose reductase (E.C. 1.1.1.21), aldehyde reductase (E.C. 1.1.1.2), and carbonyl reductase (E.C. 1.1.1.184), also referred to as ketone reductase, are members of this enzyme family that are found in relatively high amounts in the kidney. These enzymes reduce aldehydes and ketones to their corresponding alcohols. Aldose reductase has been found to metabolize only endogenous compounds in the kidney. Therefore it will not be discussed further in this review.

1. *Aldehyde reductase.* The aldehyde reductases are members of a superfamily of NADPH-dependent reductases that contribute to the metabolism of certain carbonyl compounds (Bohren et al., 1989). The enzymes are of monomeric structure and have broad substrate sensitivity in reducing xenobiotics and naturally occurring carbonyl compounds. The enzymes are widely distributed with kidney having the highest activity in most species (Bosron and Prairie, 1973), and a concentration of up to 0.5% of the total protein in tissue homogenates. Aldehyde reductase was first purified to homogeneity in pig kidney (Bosron and Prairie, 1972) and has been demonstrated to be active in the reduction of *p*-nitrobenzaldehyde, indole-3-acetaldehyde, DL-glyceraldehyde, and D-glucuronate (Flynn, 1986). The cDNA of human aldehyde reductase has been cloned and sequenced and encodes a protein with a deduced Mr of 37 kDa.

Immunohistochemical studies in rats have shown aldehyde reductase to be present in the kidney cortex, localized primarily to the proximal convoluted tubules (Terubayashi et al., 1989). Aldehyde reductases are characterized by inhibition by PB (Flynn, 1982).

2. *Ketone reductase.* Ketone reductase (also known as carbonyl reductase) is another member of the aldo-keto reductase superfamily that may reduce certain unoxidized carbonyl substances. Aromatic, acyclic, and unsaturated ketones may be reduced to free or conjugated alcohols. The best substrates are quinones (Wermuth et al., 1986). The various ketone reductases have common features such as ubiquitous tissue distribution, primarily cytosolic localization, and preference for NADPH as a coenzyme.

The enzymes are monomers with molecular weights of 28 to 40 kDa, and occur in multiple forms. This heterogeneity may be caused by autocatalytic reductive alkylation by 2-oxocarboxylic acid (Wermuth et al., 1993). The structure and gene sequence of human carbonyl reductase has now been identified and found to be a member of the short-chain dehydrogenase family (Wermuth et al., 1988).

In the human kidney, carbonyl reductase was found to be localized to the proximal convoluted and straight tubules (Wirth and Wermuth, 1992). The enzyme was found to be identical with a prostaglandin 9-ketoreductase from human and pig kidney (Shieber et al., 1992).

The enzyme purified from rabbit kidney reduces several drugs with a ketone group, such as acetohexamide,

befunolol, metapyrone, levobunolol, daunorubicin, haloperidol, moperone, trifluoperidol, and loxoprofen, along with several other aldehydes and ketones (Imamura et al., 1993; Higuchi et al., 1993). Dihydromorphinone reductase has been isolated from chicken and rabbit kidney (Roerig et al., 1976), warfarin reductase from human, rabbit, and rat kidney (Moreland and Hewick, 1975), and oxisuran, daunorubicin, and metapyrone reductase from rabbit and human kidney (Ahmed et al., 1979). The specific activities of renal reductase toward these substrates was generally high, second only to liver in terms of tissue distribution (Ahmed et al., 1979). Although generally found to be cytosolic enzymes, the microsomal fraction in mouse, rat, and guinea pig kidney had significant metapyrone reductase activity (Opperman et al., 1991). The enzymes have been found to be inhibited by quercetin (Felsted and Bachur, 1980) and by nonsteroidal drugs such as indomethacin (Higuchi et al., 1994).

3. *Other.* Other enzymes that have been shown to be involved in the reduction of xenobiotic carbonyl compounds in the kidney are dihydrodiol dehydrogenases and 11- β -hydroxysteroid dehydrogenase. Dihydrodiol dehydrogenase (E.C. 1.3.1.20) catalyzes the reduction of dicarbonyl compounds and some aldehydes in the presence of NADPH (Hara et al., 1989) and plays a role in the detoxification of endogenous ketoaldehydes such as methylglyoxal and 3-deoxyglucosone. Dimeric dihydrodiol dehydrogenase has been isolated from monkey kidney (Nakagawa et al., 1989). 11- β -hydroxysteroid dehydrogenase has been assayed in mouse kidney and displays xenobiotic carbonyl reductase activity toward the drug metapyrone as well as endogenous glucocorticoid 11-B-oxidoreduction (Maser et al., 1994).

G. Hydrolysis Mechanisms

1. *Ester and amide hydrolysis/carboxylesterase and amidase.* Carboxylesterases/amidases (E.C. 3.1.1.1) catalyze hydrolysis of carboxylesters, carboxyamides, and carboxythioesters, as seen below in equations 3 to 5, respectively (Heymann, 1980). The specificity of the carboxylesterase/amidase action depends on the nature of R, R', R''.

carboxylester:



carboxylamide:



carboxythioester:



Several chemicals have been shown to be detoxified by liver carboxylesterase, including insecticides (organophosphates, and carbamates), herbicides (esters of phe-

noxy acid and picinolic acid) and drugs. Common classes of drugs hydrolyzed by carboxylesterases include anesthetics (procaine, lidocaine), analgesics (acetyl salicylic acid, phenacetin), and antibiotics (chloramphenicol). Metabolic studies of these drugs have generally been performed using liver microsomes. Few have been studied in kidney tissue.

The carboxylesterases represent a multigene family. Using cDNA clones of various carboxylesterases, it has been shown that the sequences required for hydrolytic activity at the active sites of all the esterases tested including acetylcholinesterase are highly conserved (Sato and Hosokawa, 1995).

Because proteins classified as carboxylesterases have also been shown to be potential amidases, and vice versa, we will refer to this class of enzymes simply as carboxylesterases. As with several other enzymes, there are differences in classification schemes. In genetic nomenclature, each esterase is termed "Es" followed by a number. However, this is not available for most of the enzymes discussed in this review. A classification scheme introduced by Aldridge (1953) is based on the inhibition of certain carboxylesterases by paraoxon. The A-esterases (arylesterases) act preferentially on aromatic esters, use paraoxon as a substrate, and are inhibited by 4-chloromercuribenzoate or Hg^{2+} . The B-esterases (carboxylesterases or serine hydrolases) have aliphatic esters as the preferred substrate, are inhibited by paraoxon, and Hg^{2+} is without effect. The C-esterases (acetylesterases) act on acetyl esters, are not inhibited by paraoxon, and are activated by 4-chloromercuribenzoate or Hg^{2+} (Bergmann et al., 1957). Although these three classes (along with cholinesterase) have been described in renal tissue, the best studied in the kidney are the B-esterases (Ecobichon and Kalow, 1964), where there are relatively high amounts localized predominantly to the proximal tubule (Wienker et al., 1973; Bocking et al., 1976; Yan et al., 1994).

Ecobichon and Kalow (1964) first described aliesterase and acetylesterase activities present in human kidney. In further studying the species distribution of renal carboxylesterase activity, Ecobichon (1972) found that horse, dog, ox, guinea pig, and female mouse kidney tissue homogenates had activity comparable with that found in human kidney, approximately 10% of that in liver. Cat, rat, and male mouse kidneys had activities comparable with that seen in liver with α -naphthylacetate as substrate.

Carboxylesterases have been purified from kidneys of different species. A trimeric B-esterase with a Mr of approximately 160 kDa was purified from pig kidney (Heymann and Iglesia, 1974). A testosterone-dependent carboxylesterase termed esterase-9A, with a Mr of 45 to 51 kDa was purified from mouse kidney (Goepfing et al., 1978), which was later shown to be a component of the ES-20 trimer (DeLooze et al., 1985).

A carboxylesterase was purified from rat kidney, which was found to have a Mr of approximately 60 kDa and primarily localized to the microsomal fraction, although activity was also found in the cytosol. Maximum activity toward methylbutyrate occurred at a concentration of 200 mM at a pH of 8. This carboxylesterase catalyzed the hydrolysis of mono-acylglycerols and short-chain triacylglycerols but not long-chain triacylglycerols (Tsujita et al., 1988).

A rat kidney decarboxylase, termed hydrolase B, has been cloned and sequenced and found to be indistinguishable from rat liver hydrolase B (Yan et al., 1994). Immunocytochemical studies have shown the enzyme to be localized to the proximal tubule. It catalyzes the hydrolysis of *p*-nitrophenylacetate with low affinity and is insensitive to inhibition by phenylmethylsulfonyl fluoride. The active site had the catalytic triad composed of Ser²⁰³, His⁴⁴⁸, and Asp⁹⁷, or Glu²²⁸ (Morgan et al., 1994). Palmitoyl-coenzyme A hydrolase was subsequently found to have the same N-terminal amino acid sequence as hydrolase B (Tsujita and Okuda, 1993).

Carboxylesterase activity is found in the microsomal fraction of kidney homogenates (Ashour et al., 1987; Morgan et al., 1994; Yan et al., 1994). Electron microscopy of proximal tubule kidney cells showed that most of the B-esterase activity is localized in the endoplasmic reticulum, although some is seen on the periphery of the mitochondria. However, another study showed B-esterase localized to the mitochondria in mouse kidney (Bocking et al., 1976). Low levels of A-esterase activity have been demonstrated in the microsomal fraction of rat kidney (Pond et al., 1995).

The substrate specificity of kidney carboxylesterase has principally been studied using simple aliphatic esters, such as methyl-butyrates (Tsujita et al., 1988) and phenol esters such as *p*-nitrophenyl acetate (Ecobichon, 1972). Acetylsalicylic acid esterase activity has been demonstrated in rat kidney (Ali and Kaur, 1983).

The physiological regulation of kidney carboxylesterase appears to be similar to that of the liver. There are several testosterone-dependent esterases in male mouse kidney. Using *p*-nitrophenylacetate as a substrate, carboxylesterase activity was induced in rat kidney by the polycyclic hydrocarbons BP and 3-MC (Nousainen et al., 1984).

2. *Epoxide hydrolysis.* The epoxide hydrolases are a family of enzymes that hydrolyze various epoxides to their corresponding diols. There are four known principal epoxide hydrolases: microsomal epoxide hydrolase (mEH), cytosolic (soluble) epoxide hydrolase (sEH), cholesterol epoxide hydrolase, and leukotriene epoxide hydrolase.

Aromatic and olefinic compounds may be metabolized by MFOs to reactive electrophiles such as epoxides. These epoxides can be formed as metabolites of drugs such as cyproheptadine, carbamazepine, and protriptyline. The epoxides can then (a) rearrange to alcohols; (b)

be metabolized by glutathione transferase; or (c) be metabolized to transdihydrodiols by mEH (E.C.4.2.1.63). The latter product is usually nontoxic but may serve as a precursor to more reactive dihydrodiol epoxides.

mEH plays a more important role in the metabolism of xenobiotics. The mEH is in the same compartment as the epoxide producing monooxygenase system. It catalyzes the conversion of highly reactive arene and alkene oxides to the less reactive dihydrodiols by cleavage of the oxirane ring. The enzyme appears to be predominantly localized to the endoplasmic reticulum. The best substrates are oxiranes with 1 to 2 hydrophobic constituents.

The liver microsomal enzyme system has been sequenced (Heinemann and Ozols, 1984; Porter et al., 1986) and well characterized as to substrate specificity as well as enzyme properties and kinetics (Wixtrom and Hammock, 1985). Constitutive expression of mEH RNA was observed in rabbit kidney (Hassett et al., 1989). In addition to the mRNA characterized for one form of mEH in rat (mEH_b), two other mEH_b mRNAs that differed at the 5' end were isolated in rat liver that were the result of alternative mRNA splicing (Honscha et al., 1991), but only one mRNA was expressed in kidney. It is believed that all of the mRNAs encode the same protein. mEH has been found in kidney from rat, mouse, and hamster (Oesch et al., 1977; Oesch and Schmassman, 1978) and localized to proximal tubules and collecting ducts (Tsuda et al., 1987; Sheehan et al., 1991). In human kidney immunohistochemical studies demonstrated mEH to be localized primarily to proximal and distal tubule, but there was also staining in the collecting ducts and vascular endothelial cells (McKay et al., 1995). mEH activity in human kidney was found to be present at 5% that in liver (Pacifci et al., 1988a).

mEH is commonly increased by treatment with xenobiotics. In rat kidney, where a control level of 1.2 μg/mg protein of mEH was found, there was a two-fold increase after treatment with clofibrate (Moody et al., 1987). Treatment of rats with thiazole caused a six- to eight-fold increase in kidney mEH mRNA levels with a two-fold increase in mEH protein (Kim et al., 1994). Induction of rat proximal tubule epoxide hydrolase by administration of lead acetate has been shown in the absence of induction of the hepatic enzyme (Sheehan et al., 1991).

Whole kidney EH mRNA in rat was found to rise postpartum but remained stable after 35 days of age (Simmons and Kasper, 1989). As opposed to liver where there were age and gender related changes in mEH expression, microsomes from rat kidney showed no age-dependent change in either sex (Kim and Kim, 1992).

sEH has differing substrate specificity than the microsomal enzyme. Many compounds that are poor substrates for mEH are good substrates for sEH. These substrates include epoxides derived from polycyclic aromatic compounds, unsaturated fatty acids, and trans-

substituted styrene derivatives. Poorly metabolized epoxides are the best inhibitors, and induction studies suggest that sEH is peroxisomal in origin.

The cDNA encoding rat liver sEH has been characterized and expressed in *Escherichia coli* (Knehr et al., 1993). Human liver sEH cDNA encoding the sEH protein has also been isolated (Beetham et al., 1993). The human gene *EPHX2*, encoding the sEH protein has been localized to chromosome 8 and its structural organization determined. The gene consists of 19 exons and is approximately 40 kb (Sandberg and Meijer, 1996).

sEH has been detected in rat and mouse kidney tissues. In the rat, sEH activity using transstilbene oxide and transethylstyrene oxide as substrates was greater in kidney than liver (Schladt et al., 1986). The hypolipidemic agent, clofibrate, increased sEH two-fold.

sEH has been detected in mouse kidney (Kaur and Gill, 1985) at a level two-fold less than that of the liver enzyme. sEH activity was greater in liver than in kidneys of male and female mice and shown to increase with age (Pinot et al., 1995). There was greater activity of sEH in both the liver and kidneys of adult males than females and this sexual dimorphism was more pronounced in the kidneys (283% higher) than in the liver (55% higher). The same study showed that testosterone and clofibrate independently regulate sEH activity in vivo and that kidneys and liver respond differently to these agents.

Cholesterol 56-oxide hydrolase is a microsomal enzyme which catalyzes the hydroxylation of several steroid 56-oxides by trans-addition of water (Guenther, 1990). It has been identified in rat kidney.

Leukotriene A₄ hydrolase catalyzes the hydrolytic cleavage of the LTA₄ oxirane ring. It is a cytosolic enzyme that has been found to have significant activity in guinea pig kidney (Izumi et al., 1986).

III. Phase II: Synthetic Conjugation Pathways

In conjugation reactions, a xenobiotic is linked to an endogenous moiety through a functional group that may be present on the original drug or which results from a (phase I) reaction of oxidation, reduction or hydrolysis. In many conjugation reactions a proton, present in a hydroxyl, amino or carboxyl group, is replaced by the conjugating agent. In general, conjugated metabolites are highly water soluble and have no pharmacological activity. Glucuronidation, sulfation, acetylation and conjugation with glutathione or amino acids form the major phase II reactions.

A. Glucuronidation

One of the major routes of inactivation and elimination of xenobiotics, as well as certain endogenous compounds, is conjugation with uridine diphosphate (UDP) glucuronic acid (UDPGA). This reaction, catalyzed by UDP-glucuronyltransferases (UGT) (E.C. 2.4.1.17), results in compounds that generally are less

biologically active and more polar. The latter characteristic facilitates their excretion in bile and urine (Siest et al., 1987). Glucuronidation is an S_N2 reaction in which a nucleophile acceptor group on the substrate attacks an electrophilic C-1 atom of the glucuronic acid group. Thus, many electrophilic groups such as hydroxyl, carboxyl, sulfhydryl, or phenol can serve as an acceptor. N-glucuronides may be formed by certain nitrogen containing groups such as tertiary or aromatic amines.

Esterification of the hemiacetyl hydroxyl group of glucuronic acid to organic acids forms acyl or ester glucuronides. The acyl glucuronides, unlike glucuronides formed with phenols and alcohols, have a great susceptibility to nucleophilic substitution and intramolecular rearrangement (Faed, 1984). Although it has been thought for some time that phase II metabolites of drugs, such as the O- or N-glucuronides, are rapidly excreted, this is not true for the reactive acyl glucuronides (Spahn-Langguth and Benet, 1992). Nonsteroidal antiinflammatory drugs of the aryl-alkyl class with a high incidence of adverse drug reactions including nephritis and acute renal failure were removed from the market (e.g., benoxaprofen, indoprofen, alclufenac, ticynafen and ibufenac). It has been proposed that the formed acyl glucuronides acting as electrophiles and reacting with sulfhydryl and hydroxyl groups of cell macromolecules might be responsible for this toxicity (Spahn-Langguth and Benet, 1992).

Glucuronidation requires a sufficient supply of UDPGA and its concentration in the cytosol may determine the transferase activity. This may be more critical in extrahepatic tissues than in the liver. The concentration of UDPGA in the kidney has been found to be one-fifteenth that of liver in human tissues (Cappiello et al., 1991) and also substantially lower than in livers of guinea pig, rat, and mouse (Wong, 1977).

Once glucuronidation has occurred, whether in the liver, kidney, or other organs, the glucuronide conjugates are excreted either in bile or urine. The renal clearance of glucuronides has been studied in several animal species. Active tubular secretion has been demonstrated for certain glucuronide conjugates, such as those of catecholglucuronides in the chicken (Quebbemann and Rennick, 1969) and 1-naphtholglucuronide in the isolated perfused kidney of the rat (Redegeld and Noordhoek, 1986).

As with other drug metabolizing enzymes in the kidney, UGTs can be activated and induced by factors altering the metabolic state of the animal. For example, kidney microsomes of STZ diabetic rats have been shown to have a lower UGT substrate efficiency that was not reversed by insulin treatment (Del Villar et al., 1995).

Extrahepatic glucuronidation can easily be demonstrated in whole animal experiments (Cassidy and Houston 1980; Mazoit et al., 1990; Gray et al., 1992; and Vree et al., 1992). Much of this extrahepatic metabolism

is assumed to occur in the kidney. This review will focus on examples of UGTs that can definitely be localized to the kidney.

1. Isoforms. Molecular probes have recently been developed that have helped to characterize the large number of isoforms in the UGT gene family. Over 25 UGT cDNAs have been cloned and sequenced (Burchell, 1994). The human hepatic UGT cDNAs cloned have been classified into two families, UGT1 and UGT2. The product of the phenol/bilirubin UGT1 gene has many isoforms and is active in the glucuronidation of a variety of drugs. The product of the steroid/bile acid UGT2 gene catalyzes the glucuronidation of steroids, fatty acids,

TABLE 3
Glucuronidation

Therapeutic agents	
Acetaminophen	Hjelle et al., 1986
Azidothymidine (AZT)	Howe et al., 1992
Chloramphenicol	Hjelle et al., 1986
Frusamide	Vree et al., 1992
Harmol	Mulder et al., 1984
Harmol	Dawson et al., 1985
4-Methylumbelliferone	Aitio, 1974
4-Methylumbelliferone	Chowdhury et al., 1985
4-Methylumbelliferone	Mulder et al., 1985
4-Methylumbelliferone	Rush and Hook, 1984
Morphine	Watrous and Fujimoto, 1971
Morphine	Paul et al., 1989
Paracetamol	Jones et al., 1979
Paracetamol	Ross et al., 1980
Paracetamol	Emslie et al., 1981
Paracetamol	Bock et al., 1993
Propofol	LeGuellec et al., 1995
Ritodrine	Pacifici et al., 1993
Sulfamethazine	Nouws, 1988
Endogenous compounds	
Androsterone	Chowdhury et al., 1985
Bilirubin	Chowdhury et al., 1985
Bilirubin	Coughtrie et al., 1987
Bilirubin	Koster et al., 1986
Bilirubin	Mottino et al., 1991
Catechol	Rennick and Quebbemann, 1970
Diethylstilbesterol	Hjelle et al., 1983
Estradiol	Coughtrie et al., 1987
Estrone	Chowdhury et al., 1985
Estrone	Hjelle et al., 1983
Testosterone	Chowdhury et al., 1985
Enzyme-specific substrates	
1-Aminophenol	Litterst et al., 1975
3,6-Quinone	Koster et al., 1986
3,6-Quinol	Koster et al., 1986
1-Naphthol	Chowdhury et al., 1985
1-Naphthol	Diamond et al., 1982
1-Naphthol	Hjelle et al., 1987
1-Naphthol	Koster et al., 1986
1-Naphthol	Rush and Hook, 1984
4-Nitrophenol	Chowdhury et al., 1985
4-Nitrophenol	Diamond et al., 1982
4-Nitrophenol	Rush and Hook, 1984
4-Nitrothiophenol	Coughtrie et al., 1987
4-Nitrothiophenol	Diamond et al., 1982

and certain carboxylic drugs. Further development in this area will permit a unified nomenclature structure.

The different isoforms have different substrate specificities (see Section III.A.2.). They act on aglycones, principally those which have hydroxyl, carboxyl, sulfhydryl, or amino groups. Certain isozymes of UGT1 (previously called GT1) (i.e., UGT1*02) accept bulky substrates such as 4-hydroxybiphenyl morphine and chloramphenicol, whereas others (previously called GT2) (i.e., UGT1*6) accept flat planar substrates such as 1-naphthol, 3-hydroxy-BP. Using DNA probes to HP1 and HP4 human kidneys have been found to have mRNA that hybridized to the specific UGT1 subfamily. In these same experiments, UGT activity was demonstrated in kidney microsomes (Sutherland et al., 1993; Burchell et al., 1994).

Isozymes have also been distinguished on the basis of physiological criteria, by induction studies, and more recently by purification. As mentioned above, the principle enzyme forms involved in xenobiotic transformations have been denoted "GT1" (induced by 3-MC, TCDD, Aroclor 1254, and β -NF) and "GT2" (induced by PB). Coughtrie and colleagues (1987) purified rat kidney UGT, which was active toward 1-naphthol, 4-nitrophenol, phenol, bilirubin, and estradiol. Activity was induced after treatment of rats with β -NF but not after treatment with PB. Bilirubin UGT was specifically induced after treatment with clofibrate.

Differences in renal UGT isoforms between species are clearly apparent. For example, renal glucuronidation of morphine is found in microsomes from human fetal (Pacifi and Rane, 1982) and adult (Yue et al., 1988) kidney but not in rat kidney microsomes (Rush and Hook, 1984; Koster et al., 1986). Thus, human kidney contains the functional "GT2", not found in the rat. Using recombinant UGT1A1, (R)-naproxen was found to be a stereoselective substrate of rat UGT1A1 but not of the orthologous human UGT1A1 (Mouelhi et al., 1993). Induction of UGT activity by TCDD, as measured in kidney microsomes, has been shown to be species specific. In rat kidney, UGT was induced five-fold, whereas in rabbit and guinea pig kidney microsomes, no induction was seen (Hook et al., 1975).

2. Substrates and kinetics. The activity of UGT in kidney microsomes has been found to be substrate dependent (Chowdhury et al., 1985). Rates of glucuronidation of 1-naphthol and morphine are comparable in human tissue but the lower concentration of UDPGA in kidney provides evidence that the renal contribution to total glucuronidation may be limited in vivo (Capiello et al., 1991). There are a large number of xenobiotics as well as endogenous compounds that have been shown to undergo glucuronidation in the kidney (table 3).

Rat renal microsomal UGT toward 1-naphthol was fully activated by 0.03% deoxycholate whereas the hepatic enzyme required 0.05% deoxycholate. Activity was found to be 0.25 nmol/min/mg protein in kidney micro-

somes and 1.21 nmol/min/mg protein in liver (Rush and Hook, 1984).

Bilirubin glucuronidation rate in rat renal microsomes was found to be approximately one-half that of the liver in untreated control animals (Chowdhury, 1985; Coughtrie et al., 1987). A comparison of formation of codeine 6-glucuronide by Yue et al. (1990) showed that the rate for human liver microsomes was 0.77 nmol/mg/min whereas that for kidney microsomes was 14-fold less.

Kinetic studies of UGTs are difficult to compare because phospholipids had differential effects on microsomal preparations isolated from rat liver and kidney (Yokota et al., 1991). The activity of the hepatic enzyme toward naphthol was increased eight-fold by lysophosphatidylcholine but the renal activity increased only three-fold. Dilauroylphosphatidylcholine increased activity of the hepatic transferase two-fold but reduced that of the renal enzyme.

3. Induction of renal glucuronyl transferase. Most studies demonstrating induction of this enzyme in the kidney have been performed in the rat (table 4). Induction of UGT isozymes of 54 kDa and 56 kDa by 3-MC or A1254 was demonstrated in renal as well as hepatic microsomes (Koster et al., 1986). This increase in protein correlated with an increase in glucuronidation capacity for "GT1" substrates. Gel electrophoresis revealed protein bands of 54 and 55 kDa. In addition, immunoblot analyses with anti-rat liver uridinediphosphoglucuronyl transferase (UDPGT) revealed polypeptides of 56 and 54

TABLE 4
Induction of glucuronidation in the kidney

Inducer	Substrate	I/C ^a	Reference(s)
3,4 benzpyrene	O-aminophenol	1.0	Hanninen and Aitio, 1968
Cinchophen		3.2-4.6	Fry et al., 1978
Cinchophen	4-MUF	1.4	Hanninen and Aitio, 1968
3-MC	<i>p</i> -nitrophenol	1.6	Aitio, 1973
3-MC	<i>p</i> -nitrophenol	1.9	Yokota and Yuasa, 1990
3-MC	<i>p</i> -nitrophenol	1.5	Aitio et al., 1972
3-MC	O-aminophenol	1.0	Fry et al., 1978
3-MC	O-aminophenol	1.0	Hanninen and Aitio, 1968
3-MC	4-MUF	1.6	Yokota et al., 1989
3-MC	1-naphthol	2.0	Rush and Hook, 1984
B-naphthoflavone	<i>p</i> -nitrophenol	2.5	Rush and Hook, 1984
B-naphthoflavone	4-MUF	2.0	Rush and Hook, 1984
B-naphthoflavone	1-naphthol	2.0	Rush and Hook, 1984
B-naphthoflavone	4-hydroxybiphenyl	4.0	Yokota et al., 1989
B-naphthoflavone	Bilirubin	2.0	Coughtrie et al., 1987
B-naphthoflavone	Estradiol	1.6	Coughtrie et al., 1987
B-naphthoflavone	1-naphthol	3.0	Coughtrie et al., 1987
B-naphthoflavone	4-nitrophenol	3.0	Coughtrie et al., 1987
B-naphthoflavone	Phenol	3.0	Coughtrie et al., 1987
Phenobarbital	4-nitrophenol	1.0	Yokota and Yuasa, 1990
Phenobarbital	O-aminophenol	1.0	Hanninen and Aitio, 1968
Phenobarbital	1-naphthol	1.0	Yokota et al., 1989
Phenobarbital	O-aminophenol	1.0	Fry et al., 1978
Salicylic acid	O-aminophenol	1.8	Fry et al., 1978
Salicylic acid	O-aminophenol	1.8	Hanninen and Aitio, 1968
TCDD	4-MUF	1.4	Aitio and Parkki, 1978
TCDD	Paracetamol	1.8	Bock et al., 1993
TCDD	<i>p</i> -nitrophenol	5.4	Hook et al., 1975
Trans-stilbene	<i>p</i> -nitrophenol	2.5	Rush and Hook, 1984
Oxide	4-MUF	2.1	Rush and Hook, 1984

^a I/C = ratio of induced UGT activity to control UGT activity.

kDa that catalyzed glucuronidation of 1-naphthol and bilirubin. Yokota and colleagues (1989) have isolated UDPGT from rat kidney (termed "GT-2"), which immunologically corresponds to a form of UDPGT ("GT-1") purified from rat liver. It is inducible by 3-MC and has activity toward phenolic xenobiotics. In addition, they found that UDPGT activity toward phenolic xenobiotics and serotonin was enhanced several-fold by treatment of the animal with β -NF. They purified this form and found it to be a 54 kDa protein consisting of a polypeptide with high mannose oligosaccharide chains. The NH_2 terminal residues of this "GT-2" were found to differ by two residues in the final seven from the "GT-1" form. Kidney microsome UDPGT activity toward pNP was increased after induction by 3-MC but not by PB. An increase in kidney microsomal protein of 54 kDa, corresponding to GT-1, was recognized by immunoblotting (Yokota and Yuasa, 1990). Salicylic acid induces glucuronidation of o-aminophenol and catechol in the chicken kidney as seen using whole animal SADR studies (Diamond et al., 1982) and hexabromobiphenyl induces the glucuronidation of 4-methylumbelliferone in mouse kidney (Aitio et al., 1972).

4. Localization. With respect to localization within the kidney, UGT activity is primarily limited to the cortical region (Stevenson and Dutton, 1962; Rush and Hook, 1984), and the activity is greatest in cells of the proximal tubule (Hjelle et al., 1986). Renal UGT activity has been demonstrated in isolated tubule fragments (Fry et al., 1978) and isolated tubule cells (Jones et al., 1979) as well as the microsomes obtained from homogenization of renal cortical tissue. GT activity toward morphine has been demonstrated in human renal medullary tissue but at approximately half the level of that seen in the cortex (Yue et al., 1988). Because the proximal tubule cells are exposed to the initial filtrate through the glomerulus, it would be logical for them to play the primary role in xenobiotic conjugation. Within the individual cells, the UDP glucuronyltransferases (UGTs) are a family of membrane bound enzymes located in the endoplasmic reticulum. The enzyme, including the active site, is thought to be primarily within the lumen of the ER (Mulder, 1992).

5. Relative contribution of renal glucuronidation. Measurement of tissue activities showed that after the liver, the highest activity (one-third to one-half) of UDPGT is found in kidney (Krishna and Klotz, 1994). It is more difficult to determine the actual renal contribution to glucuronidation in vivo. Using the SADR technique, renal conjugation of pNP was studied in the chicken (Diamond and Quebbemann, 1981). Like humans, the chicken excreted pNP almost entirely in the urine. A minimum of 38% of pNP conjugation (sulfate and glucuronidation) was nephrogenic in origin at an infusion rate of 100 nmol/min/kg. At a 20-fold higher infusion rate, the percentage of renal contribution to conjugation dropped

markedly, indicating increased participation by other organs.

Studies examining in vivo conjugation of 1-naphthol and pNP in the rat revealed that renal metabolism accounted for a minimum of 20% of conjugation of either substrate in the urine, with a majority of the conjugation contributed by glucuronidation (Tremaine et al., 1984, 1985). Extrahepatic conjugation of harmol was found to be greater than that of the liver, but kidney localization for this activity was not specifically demonstrated (Mulder et al., 1984).

B. Sulfation

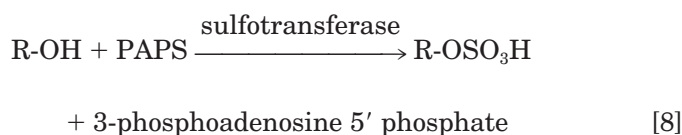
In addition to being a major conjugation pathway for phenols, sulfate contributes to the biotransformation of xenobiotic alcohols, amines, and thiols. It is also important in the metabolism of endogenous compounds such as neurotransmitters and steroid hormones. The resulting compounds are generally less active, more polar, and more readily excreted in the urine.

Sulfate conjugation is a multistep process. Inorganic sulfate is inert and must first be converted to adenosine-5' phosphosulfate (APS) and then to an activated form, 3'-phosphoadenosine 5' phosphosulfate (PAPS) by the following reactions:



The enzymes ATP-sulfurase and APS-kinase are present in the cytosol. PAPS is synthesized in all mammalian cells. The concentration is highest in the liver (Hazelton et al., 1985), but kidney cells synthesize significant amounts, with the concentration higher in the cortex than in the medulla (Hjelle et al., 1986).

The reaction by which sulfotransferases catalyze the transfer of a sulfonyl group from PAPS to the acceptor molecule (i.e., phenol) is shown in the following reaction:



These reactions are catalyzed by the enzyme, sulfotransferase (ST). The sulfotransferases are a family of soluble enzymes with different substrate specificities such as phenol ST, alcohol ST, amine-N-ST, and aryl-sulfate ST. Sulfotransferases have been isolated from a variety of organs including the kidney (Mulder and Jakoby, 1990), although the individual forms may not have been identified there.

Sulfate conjugation is regulated by (a) the sulfate concentration, (b) availability of inorganic sulfate, and (c) the synthesis of PAPS. Studies on the sulfate conju-

gation of 7-hydroxycoumarin by isolated kidney cells showed that inorganic sulfate was the most effective precursor for PAPS formation, but that cysteine, N-acetylcysteine, and glutathione could also be used (Dawson et al., 1983).

Capiello et al. (1989) showed that the concentration of PAPS in human kidney was 4.8 nmol/mg tissue, 21% that of the liver, and sulfotransferase activity was 0.034 nmol/min/mg. The conjugation of 2-naphthol by the kidney proceeded at 2% that of the liver. The reduced availability of PAPS in the kidney may reflect the small requirements for this substrate.

Sulfotransferase enzymes are members of a gene superfamily that includes phenol ST, hydroxysteroid ST, and flavonol ST. There are currently five human cytosolic ST enzymes: three phenol STs, a hydroxy steroid ST, and an estrogen ST. The cDNAs and genes have been cloned for each of these (Weinshilboum et al., 1997) and their chromosomal locations determined. At this time, 30 eukaryotic ST cDNAs have been reported. An ST enzyme classification system has been devised based on the primary amino acid sequence of the enzymes (Weinshilboum et al., 1997). Most of the cloning experiments have been performed using liver tissue. Recently, a cDNA of human ST, named *SULT1C1*, has been cloned and dot blot analysis of mRNA indicated that this cDNA was expressed in human kidney as well as other tissues (Her et al., 1997).

Overall, sulfate conjugation in the kidney, as well as in the liver, plays a lesser role quantitatively than glucuronidation in the metabolism of xenobiotics (Hjelle et al., 1986; Elbers et al., 1980; Redegeld et al., 1988). However, for certain compounds, such as harmol, renal sulfate conjugation predominates (Mulder et al., 1984). There has been relatively little study of renal sulfate conjugation compared with glucuronidation.

Sulfate of the steroids dehydroepiandrosterone and estrone, as well as the compound pNP was described by Holcenberg and Rosen (1965). Incubation of 17- β -estradiol with human renal slices produced estrone sulfate and estradiol-3-sulfate metabolites (Mellor and Hobkirk, 1975). Dehydroepiandrosterone sulfotransferase has been identified in the human fetus (Barker et al., 1994). Sulfate of the β -adrenoreceptor agonist ritodrine was found to be substantially higher in human fetal tissue than in adult kidney (Pacifici et al., 1993).

Pacifici et al. (1988b) demonstrated that sulfotransferase activity using 2-naphthol as substrate was present in the cytosolic fraction of human fetal as well as adult kidney. Activity in fetal kidney was twice as high as that of liver whereas adult kidney activity was 15-fold less than observed in the cytosolic fraction of adult liver. Naphthol was sulfated (as well as glucuronidated) by rabbit kidney (Hjelle et al., 1986). Incubation of human kidney cortex with biphenyl formed 4-hydroxyphenyl-sulfate at a rate one-tenth that of liver (Powis et al., 1987). 1-naphthol was metabolized to the sulfate and

glucuronide conjugate in the isolated perfused rat kidney as well as guinea pig tubule fragments (Redegeld et al., 1988; Schwenk and Locher, 1985).

Sulfotransferase activity in the kidney has been localized in cytosolic as well as membrane fractions of rabbit proximal tubules (Hjelle et al., 1986). Measurement of activity in homogenates of cortex and outer medulla, as well as in isolated proximal tubules of rabbit, showed greatest activity in the proximal tubule, which was 2.2-fold that of the cortex.

Using the Sperber technique in chickens, Quebbemann and Anders (1973) demonstrated renal tubule formation and excretion of sulfate (and glucuronide) conjugates of phenol and pNP. When infused into the saphenous vein at 1 nmol/min, 80% of the phenol and 50% of the pNP that reached the kidney were excreted in the urine. All the excreted phenol and pNP were identified as sulfate or glucuronide conjugates. Using the SADR technique, renal sulfate and glucuronide conjugation of pNP was studied in the chicken (Diamond and Quebbemann, 1981). pNP sulfate was found to be the major urinary metabolite, and approximately 38% of the pNP sulfate was nephrogenic at an infusion rate of 100 nmol/min/kg. Renal sulfate conjugation of 1-naphthol and pNP has been found to account for a minimum of 20% of the endogenously formed conjugates excreted in the urine of the rat.

Sulfate conjugation contributed to paracetamol metabolism in the isolated perfused kidney and hydroxybiphenyl conjugation in human kidney (Pacifici et al., 1991a). The maximal rate of 4-MUF conjugation as sulfate in kidney tubule fragments was 17% of that found in isolated hepatocytes (Fry et al., 1978). Formation of acetaminophen sulfate in isolated kidney cells was 5% that of liver, and was unchanged by treatment of rats with 3-MC (Jones et al., 1979).

C. Methylation

Methylation reactions are primarily involved in the metabolism of small endogenous compounds such as epinephrine, norepinephrine, dopamine, and histamine but also play a role in the metabolism of macromolecules such as nucleic acids and in the biotransformation of certain drugs. Unlike most other conjugative reactions, methylation leads to less polar compounds that may be less readily excreted from the body. N-, O-, and S-methyltransferases are present in the kidney. The cofactor, S-adenosylmethionine (SAM) is required as a methyl donor in reactions catalyzed by these enzymes. SAM is primarily formed by the condensation of ATP and L-methionine, and is present at varying levels in different tissues (Eloranta, 1977). In the rat, kidney tissue levels are approximately two-thirds that of the liver.

1. N-methylation. There are three N-methyltransferase enzymes that play a role in renal drug metabolism.

a. Histamine N-methyltransferase (HMT) (E.C. 2.1.1.8) is a cytoplasmic enzyme that requires SAM as a methyl donor and histamine as a methyl acceptor. The substrates for HMT are limited to histamine and similar amine compounds in which positions 12 and 3 are unsubstituted and there is a positive charge on the side chain. There are a large number of inhibitors of HMT including H1 and H2 receptor antagonists, diuretics, and local anesthetics (Tachibana et al., 1988).

Bowsher et al. (1983) found HMT activity in higher concentration in rat renal tissue than in any other organ. Interestingly, the renal artery contained a high level of this enzyme. The level of HMT in the kidney appears to control the urinary excretion rate of histamine in the rat (Snyder and Axelrod, 1965) and the dog (Lindell and Schayer, 1958). Purification of rat kidney HMT revealed a protein with a molecular weight of 33.4 to 35 kDa (Bowsher et al., 1983; Harvima et al., 1985). The cDNA of 1.3 kb encodes a protein of 295 amino acid residues with a calculated molecular weight of 34 kDa. After introduction of the cDNA, *Escherichia coli* cells expressed HMT activity with characteristics similar to the native enzyme (Takemura et al., 1992). More recently, human HMT cDNA has been cloned and expressed from human kidney (Girard et al., 1994). The amino acid sequence of this protein was 84% identical with the HMT from rat kidney. The partially purified HMT from the cytosol of transfected COS cells had properties similar to isolated renal cortical HMT. The structural organization and chromosomal localization of human HMT have subsequently been described (Aksoy et al., 1996).

HMT activity is higher in male rats and may be reduced by castration (Snyder and Axelrod, 1965). Significant increases in rat renal HMT activity have been observed after hydronephrosis (Barth et al., 1975). Pharmacogenetic studies showed minor strain variations in levels of renal enzyme activity among inbred mouse strains (Scott et al., 1991).

b. Phenylethanolamine N-methyl-transferase (PNMT) (E.C. 2.1.1.28) has been demonstrated in human kidney by histochemical staining and found to be located primarily in glomeruli, endothelial cells, and distal tubule cells (Kennedy et al., 1995). Unlike amine-N-methyltransferase, this enzyme is specific for its methyl acceptor substrates, requiring the presence of a phenylethanolamine compound. Its endogenous substrates include norepinephrine and epinephrine. PNMT cDNA was originally cloned from bovine adrenal medulla (Baetge et al., 1986). The cDNA was expressed in hamster kidney cells producing PNMT with a molecular mass of 21 kDa, which was enzymatically active. The amino acid sequence of human PNMT has been determined (Kaneda et al., 1988). The human enzyme consists of 282 amino acids and has a Mr of 30.9 kDa.

c. Amine-N-methyltransferase (E.C. 2.1.1.49), also termed indolethylamine N-methyltransferase, an en-

zyme that catalyzes the transfer of a methyl group from SAM to the amino group of indoleamines has been found in both human and rabbit kidney (Axelrod, 1962; Bhikharidis et al., 1975; Kennedy et al., 1995).

2. *O-methylation*. O-methylation of phenolic groups is important in the metabolism of neurotransmitters such as the catecholamines and structurally related drugs. Isoproterenol is metabolized to 3-O-methyl isoproterenol in the isolated perfused rat kidney (Szeffler and Acara, 1979). Catecholamine-O-methyltransferase (COMT) (E.C. 2.7.1.6) is present in cytosolic (S-COMT) and membrane bound (MB-COMT) forms that appear to have similar properties such as using SAM as the methyl donor and requiring the presence of Mg^{++} for activity. S-COMT is the predominant form, although MB-COMT has a higher affinity for catechol substrates. These forms have now been characterized and found to have differing structures at the N-terminus. COMT cDNAs have been isolated from several tissues and there appears to be only one gene for COMT in rat and human. This gene is regulated by two promoters, P₁ and P₂, with P₁ expressing S-COMT RNA and P₂ expressing MB-COMT mRNA (Tenhunen and Ulmanen, 1993).

Both forms of COMT have been described in kidney tissue. Using in situ hybridization histochemistry, Meister et al. (1993) detected S-COMT mRNA in the S3 segment of the proximal tubule and the thick ascending limb of Henle in adult rat and human kidney. The S-COMT mRNA was detected in prenatal rat kidney as early as 18 days. Another study using antiserum to recombinant rat COMT showed staining of proximal, distal, and collecting duct cells of rat kidney but no staining in glomeruli (Karkunen et al., 1994). MB-COMT was also found in kidney tissue (Nissinsen, 1984).

COMT catalyzes the transfer of a methyl group from SAM to a phenolic group of a catechol. Substrates include catecholamines, amino acids such as L-dopa, and certain alkaloids and steroids. S-adenosylhomocysteine and isosteres of catechol substrates inhibit COMT in vitro (Thakker and Creveling, 1990).

The enzyme has been isolated and partially purified from rat kidney (Darmont et al., 1976). Rat kidney COMT activity was found to be present at one-fourth the level found in liver (Weinshilboum et al., 1979) and at comparable levels in human kidney (Weinshilboum, 1978). Pharmacogenetic studies from inbred rat strains have revealed differences in inherited phenotype activity between strains (Weinshilboum et al., 1979). COMT activity is particularly high in newborn rat kidney as compared with other organs (Goldstein et al., 1980) and aging is associated with a decrease in COMT affinity for substrate (Vieira-Coelho and Soares-da-Silva, 1996).

3. *S-methylation*. Thiol methylation is important in the metabolism of many sulfhydryl drugs such as captopril, D-penicillamine, azathioprine, and 6-mercaptopurine (6MP). Thiol methyltransferases are important in

the detoxification of toxic thiols and are generally excreted as sulfoxides or sulfones. In addition, another important pathway for xenobiotic metabolism is termed the thiomethyl shunt, acting on compounds in which sulfur has been added from glutathione. It starts with the addition of glutathione followed by conversion to the cysteine conjugate. This cysteine conjugate is cleaved by cysteine conjugate β -lyase to pyruvate, ammonia, and thiol. The thiol is then methylated. Thus intermediates formed during mercapturic acid synthesis (described below in Section F.) may be diverted to undergo methylation and excretion rather than N-acetylation (Stevens and Bakke, 1990).

There are three enzymes with varied characteristics involved in S-methylation: microsomal thiol-methyltransferase (TMT), cytosolic thioether-S-methyltransferase (TEMT), and soluble thiopurine-methyltransferase (TPMT). Each of these enzymes have been found in kidney tissue and require SAM as a cofactor.

TMT is a membrane-bound enzyme that catalyzes D-methylation of aliphatic sulfhydryl compounds such as captopril and D-penicillamine. Microsomal TMT was found in the kidney by Fujita and Suzuoki (1973). The cDNA sequence of cytosolic thioether-S-methyltransferase has been determined and the enzyme purified (Warner et al., 1995). It has a molecular mass of 29.46 kDa. Both the nucleotide and amino sequences have significant homology with PNMT.

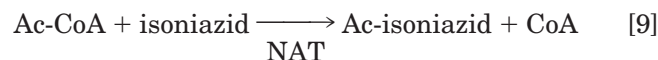
The role of thioether methyltransferase to methylate seleno, telluro, and thioethers to more water soluble onium ions suitable for urinary excretion was proposed by Mozier and Hoffman (1990). They also demonstrated that the renal activity of this enzyme was approximately one-third that of the liver for substrates tetrahydrothiophene and 2-hydroxyethyl ethyl sulfide and 1/100th for 2-chloroethyl ethyl sulfide. A similar relative value of one-third that of liver was found for human kidney methyltransferase activity for captopril (Pacifi et al., 1991b).

Thiopurine methyltransferase (TPMT) is a cytoplasmic enzyme that catalyzes the S-methylation of aromatic and heterocyclic sulfhydryl compounds such as 6MP and azathioprine preferentially. Soluble TPMT activity was described in the kidney by Remy (1963), who found the concentration to be much higher than that of the liver. Woodson and Weinshilboum (1983) purified TPMT from human kidney and found it to differ from TMT on the basis of subcellular distribution, substrate specificity, kinetic characteristics, and sensitivity to inhibition. Two TPMT isozymes have been purified from human renal tissue, both with molecular weights of approximately 35 kDa (Van Loon and Weinshilboum, 1990; Van Loon et al., 1992). Human TPMT cDNA has been cloned from one isoform using a T84 human colon carcinoma cell cDNA library. When expressed in COS-1 cells, this cDNA encoded a protein that expressed TPMT activity (Honchel et al., 1993). Kinetic analysis of the var-

ious substrates for human kidney TPMT have been reported (Deininger et al., 1994). Levels in human tissue including kidney are controlled by a common genetic polymorphism. Eighty-nine percent of people have high activity, 11% intermediate activity, and less than 1% low activity. The isozymes of TPMT isolated were not the basis for the genetic polymorphism (Van Loon and Weinshilboum, 1990).

D. Acetylation

Drugs and other foreign compounds that are acetylated in intact animals are either arylamines or hydrazines. Acetylation is the major metabolic route of arylamines such as isoniazid, sulfamethazine, *p*-aminobenzoic acid, hydralazine, procainamide, aminofluorene, and benzidine (Weber and Glowinski, 1980). N-acetylation of cysteine-S-conjugates is the final step in mercapturic acid synthesis and is discussed under that heading. The transfer of acetyl to the hydroxyl group of choline and the sulfhydryl group of coenzyme A are examples of endogenous acetylation but acetylation of these groups in xenobiotics is uncommon. N-acetyltransferases (NAT) catalyze acetylation by a double displacement (ping-pong) mechanism that is summarized below:



The reaction consists of two sequential steps: (a) transfer of the acetyl group from Ac-CoA with formation of an acetyl-enzyme intermediate, and (b) acetylation of the arylamine with regeneration of the substrate. Compounds such as N-ethylmaleide, iodacetate, and *p*-chloromercuribenzoate are irreversible inhibitors whereas compounds that are structurally similar to the substrates (i.e., salicylamide) are reversible inhibitors.

The human arylamine acetyltransferase genes that have been isolated from leukocyte DNA are termed NAT1 and NAT2 (Blum et al., 1990). Both encode proteins with an estimated Mr of 33.5 kDa. The coding regions of NAT1 and NAT2 have 87% nucleotide identity. Expression of NAT1 and NAT2 in monkey kidney COS-1 cells gave rise to functional proteins. Western blots detected proteins with an apparent Mr of 33 kDa for NAT1 and 31 kDa for NAT2 (Grant et al., 1991). The NAT2 product had identical molecular weight as NAT of human liver cytosol. NAT1 and NAT2 appear to be independently expressed.

Variability in human drug acetylation was noted 40 years ago with people being defined as rapid or slow acetylators based on their blood levels after administration of isoniazid. It has been more recently shown that this was caused by genetic variability, and that NAT2 is the locus for the polymorphism typified by the isoniazid-sulfamethazine-N-acetylation. NAT1 appears to have a discrete polymorphism associated with the acetylation of

p-aminobenzoate and *p*-aminosalicylate (Vatsis and Weber, 1994).

NAT activity has been identified in many tissues of various species. Booth (1966) found acetyltransferase activity in the soluble fraction of rat kidney homogenates to be less than that of liver and adrenal but higher than in other organs studied. Pacifici et al. (1986) used the cytosolic fraction from fetal or adult human kidneys and the arylamine, para-aminobenzoic acid (PABA), as substrate and found NAT activity to be 1.17 and 1.32 nmol/min/mg tissue respectively. This was one-third to one-half the activity found in the liver. No difference was detectable between the cortex and the medulla of the kidney. NAT activity was studied in tissues of inbred hamsters with a genetically defined Ac-CoA-dependent NAT polymorphism (homozygous rapid acetylators, homozygous slow acetylators, and heterozygous acetylators). PABA acetyl transferase activity was comparable in kidney and liver cytosols, with activity toward *p*-aminosalicylic acid, 2-aminofluorene, *p*-naphthylamine, and isoniazid significantly less in the kidney. In addition, there was a difference in kidney enzyme activity between the rapid acetylators and the slow acetylators for the compounds studied except for isoniazid (Hein et al., 1987a,b). An enzyme of polyamine degradation, spermine/spermidine N-acetyltransferase, was shown to be present predominantly in the distal tubules of rat kidney by in situ hybridization histochemistry (Bettuzzi et al., 1995).

Kidneys from male C57BL/6 and A/J acetylated PABA at twice the rate of kidneys from female mice. In addition, an increase in activity of NAT during development was found in male but not female mouse kidney. Testosterone increased NAT activity in females and estradiol decreased activity in males (Smolen et al., 1993).

E. Glutathione Conjugation

Glutathione (GSH) is synthesized from the amino acids glycine, L-cysteine, and glutamic acid. It is present at highest concentration in the liver, but kidney cells also have a level of 1 to 2 $\mu\text{mol/g}$ tissue. The concentration is higher in the cortex than in the medulla (Mohandas et al., 1984), and is present in cytosol, mitochondria, and nucleus. A sufficient supply of L-cysteine is essential for GSH synthesis.

GSH is present in the blood at a concentration of approximately 20 μM (Anderson and Meister, 1980), and correlates with the liver concentration. GSH is degraded by the proximal tubule of the kidney at both the luminal (Hahn et al., 1978) and basolateral membrane (Abbott, 1984). Nearly all GSH filtered is reabsorbed from the lumen of the proximal tubule.

GSH conjugation involves the formation of a thioether link between GSH and electrophilic compounds. This process usually facilitates detoxification and excretion but may be involved in the biosynthesis of certain compounds such as leukotriene C₄.

The glutathione S-transferase (GST) family of enzymes are the proteins involved in GSH conjugation in the following reaction where RX is an electrophilic compound.



These electrophilic compounds include epoxides, haloalkanes, nitroalkanes, alkenes, and aromatic halo- and nitro-compounds. Several different types of reactions have been shown to occur in vivo (Ketterer and Mulder, 1990), although not all have been demonstrated in kidney tissue. There may be nucleophilic displacement from a saturated carbon, as is seen in the conjugation of the chemotherapeutic agent melphalan. There may be nucleophilic displacement from an aromatic carbon as seen with the substrate 1-chloro-2,4-dinitrobenzene (CDNB). This has been shown to occur in rat kidney (Temellini et al., 1995). Reactions may occur with GSH by Michael addition, as seen with ethacrynic acid. A metabolite of the analgesic paracetamol has been shown to undergo glutathione conjugation in the isolated perfused kidney (Emslie et al., 1981). The leukotriene antagonist, Verkulast, has been shown to undergo GSH conjugation by Michael addition in rat kidney cytosol (Nicoll-Griffith et al., 1995). GSH may also react with xenobiotics containing strained oxirane rings, such as the BP metabolite BP-45-oxide.

Bond-forming reactions occur at the free cysteinyl thiol group of GSH. The GSH conjugates may then undergo further metabolism by any of a large number of pathways to various highly polar and water soluble metabolites that generally do not possess the biological activity of their precursors (Shaw and Blagbrough, 1989). In mammals, the most important of these are the mercapturic acids (*S*-substituted N-acetylcysteines) that are then excreted into urine or bile (Williams, 1967). Conjugation of potentially toxic electrophilic compounds with glutathione is an important detoxification pathway.

Several systems of nomenclature have been used by workers classifying human GST isoenzymes, and different systems are used for different species. Mannervik et al. (1992) proposed a classifying scheme in which each genetically distinct subunit has its own designation. There are five classes of soluble enzymes: α , μ , π , σ , and θ ; and two classes of microsomal enzyme: microsomal GST and leukotriene C₄ synthase. These classes have significant overlap in substrate specificities and are expressed in tissue specific patterns. Each class contains two or three subfamilies representing different subunit types. The separate families of GST have distinct gene structures. Class α genes isolated from humans are 11 to 12 kb, comprise seven exons, and are located on chromosome 6. Human μ genes are 5 kb in length, contain eight exons and are on chromosome 1. Class π genes are 3 kb

and contain seven exons on chromosome 11. Human α , μ , and θ families contain multiple genes but only one functional class π genes exists. The number of σ genes is unclear. The two membrane bound GSTs, microsomal GST and leukotriene C₄ synthase do not share sequence identity with the cytosolic enzymes or each other. Isozymes of classes α , μ , and π have been identified in kidney tissue.

These cytosolic GST isoenzymes have been purified, and all are homodimers or heterodimers containing two subunits, which function independently. Each subunit contains a GSH binding site (G-site) and a second substrate binding site (H-site). The substrate specificity can generally be determined by a small number of residues in the H-site.

There are at least 20 isoenzymes of GST in human tissue, but few of these have been specifically identified in kidney. In human kidney tissue, α , μ , and π class enzymes are present in significant amounts (Singh et al., 1987; Tateoka et al., 1987; Beckett et al., 1990). α -class GST represents 55 to 75% of total GST protein (Singh et al., 1987). Developmentally, the expression of α -class enzymes was up-regulated at 40 weeks of gestation, and localized primarily to the proximal tubules (Beckett et al., 1990). The levels of μ and π isozymes remained constant during development.

In the rat, α genes encode the Y_a, Y_c, and Y_k subunits; μ genes encode Y_b and Y_{b2}, and the π gene encodes Y_f. Immunoblotting has shown that all these enzymes are expressed in cortical homogenates, with a predominance of expression of α GST (Y_a). The medullary homogenates showed weaker staining. All enzymes studied showed most intense expression in the distal tubules (Davies et al., 1993).

In the rat, immunofluorescent analysis showed that the α -class enzyme was located primarily in the proximal tubule, whereas the π enzyme was in the distal convoluted tubule. Lesser amounts of μ were localized to the distal convoluted tubule (Di Ilio, 1991). Differential expression of isoenzymes from these three classes has been found in studies of rat (Guthenberg et al., 1985; Waxman et al., 1992; Johnson et al., 1992; Rozelle et al., 1993), hamster (Bogaards et al., 1992; Muse et al., 1994), and mouse kidney (Gupta et al., 1990). The specific activity toward individual substrates is also quite variable (Gupta et al., 1990; Bogaards et al., 1992). Methylation of rat kidney GST has been shown to inhibit GST activity toward CDNB. When isolated from human renal cortex, the GST activity toward CDNB was greater in women than men, consistent with previous findings in rats (Butera et al., 1990). This is thought to be caused by differences in subunit composition of the enzymes.

There are clear and significant differences between the renal and hepatic enzymes in their substrate specificities. Liver GST gene expression has been shown to be regulated by xenobiotics, and overexpression of GST has been demonstrated in cells resistant to anticancer drugs

(Pickett and Lu, 1989). Regulation of gene expression in the kidney by drugs has not been reported as yet.

Induction of GST in kidney tissues has been studied. Total GST activity against CDNB, trans-4-phenyl-3-buten-2-one (+PBO) and ethacrynic acid in kidney cytosol have been found to be inducible by ethoxyquin. The π -class subunit Y_p was increased four-fold and the μ -class Y_{b2} doubled after induction with ethoxyquin. PB and 3-MC had significant effects on liver but not kidney tissue (Derbel et al., 1993). Activity against CDNB was also increased in rat renal tissue after administration of the hydroxyl radical scavenger, dimethylthiourea.

Reduction of rat kidney GST activity has been seen after cisplatin therapy (Bompart et al., 1990). Conjugation with GSH is facilitated by the nucleophilic thiol of the L-cysteine residue. Studies have elucidated a pathway for the GSH-dependent bioactivation of nephrotoxic haloalkenes (Cummings and Prough, 1983; Anders et al., 1988) as a result of either their activation, leading to higher molecular reactivity, or to binding of the conjugate to a macromolecular receptor where the toxicity is mediated.

The enzyme believed to be responsible for the generation of reactive thiols from GSH conjugates of certain halogenated hydrocarbons, in the kidney, is the rat renal cysteine conjugate β -lyase (Blagbrough et al., 1986) or C-S lyase, which has been purified by Green and Odum (1985) (see Section II. H.). Disruption of the normal processing of GSH conjugates, either before the formation of the mercapturic acids or after their deacetylation, may result in the generation of reactive products from the cysteine conjugates which produce cytotoxicity, mutagenicity, and carcinogenicity. Some glutathione precursors of the cysteine conjugates have been associated with kidney cytotoxicity; 2-bromohydroquinone is nephrotoxic (Elfarrar and Anders, 1984) and the GSH conjugate of hexachloro-13-butadiene is mutagenic (Green and Odum, 1985). Pickett and Lu (1989) also showed that GSH conjugates of certain xenobiotics have been found to be toxic.

F. Mercapturic Acid Synthesis

Many GSH conjugates undergo further enzymatic modification by hydrolysis of the glutathione-S-conjugate at the γ -glutamyl bond. This reaction is catalyzed by the enzyme γ -glutamyl transferase (γ GT), (E.C.2.3.2.2). In addition to hydrolysis, γ GT can catalyze transpeptidation or autotranspeptidation. γ GT is an enzyme localized in the cell membrane of many cell types including kidney tubules. The kidney, in fact, has the highest activity in several mammals studied, including humans (Goldberg et al., 1960; Hinchman and Ballatori, 1990).

Initially, the biosynthesis of mercapturic acids (see fig. 1 below) involves conjugation of electrophilic xenobiotics with GSH. The next metabolic step is the hydrolysis of the glutathione conjugate (GSR) at the γ -glu-

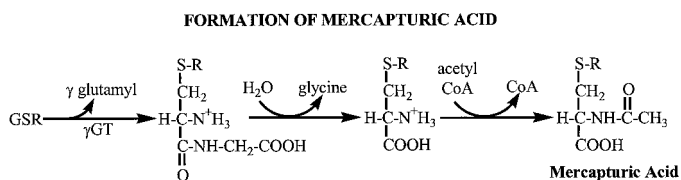


FIG. 1. Formation of mercapturic acid.

tamyl bond by γ GT. To reach the active site of γ GT, the GSR must be excreted from hepatic or renal cells. It is assumed that a carrier secreting GSH also accepts the GSH conjugate. GSH-S conjugates are hydrolyzed in the tubular lumen (Silbernagl et al., 1982). The γ -glutamyl peptides are not reabsorbed when the γ GT is blocked by acivicin. Mercapturic acid is then formed by the release of glycine by hydrolysis and acetate addition. The mercapturates may then be excreted in the urine by filtration and secretion.

Using the isolated perfused rat kidney, Davison et al. (1990) demonstrated that all the enzymes necessary for the catabolism of GSH conjugates to mercapturates were present within the kidney. Mercapturic acid synthesis is shown in fig. 1.

The renal γ GT enzyme consists of two nonidentical subunits encoded by a common mRNA. The precursor 61.8 kDa polypeptide is glycosylated in the Golgi and processed in two subunits: a light subunit with Mr 21 kDa and a heavy fragment of 41.8 kDa (Tate and Khadse, 1986). The catalytic site is on the light subunit. Variants in γ GT have been shown to have marked electrophoretic differences caused by their glycosylation. The structures of the human kidney γ GT have been determined (Yamashita et al., 1986). In comparing rat, cow, dog, and human kidney, Tate et al. (1988) found that renal transpeptidases shared many antigenic determinants, but differences were noted in their relative acceptor specificity attributable to subtle structural differences.

The activity of γ GT in mammalian tissues is at its highest in the kidney. This membrane bound glycoprotein is heavily concentrated on the brush border of the human proximal tubular cells, particularly in the pars recta (Endou et al., 1981). It is also found at a lower concentration in the antiluminal border of these tubules (Glenner et al., 1962; Curthoys and Lowry, 1973; Kugler et al., 1985; Shiozawa et al., 1989). At these sites the enzyme is oriented in the membranes so as to react with substrates present in the extracellular milieu (Li-Kam Wa et al., 1996). Histochemical analysis has revealed that γ GT is present in glomeruli (Sochor et al., 1980) and isolated microvessels (Dass et al., 1981). The active site of the enzyme appears to be located on the external surface of the brush-border membrane as demonstrated in vesicle studies (Horiuchi et al., 1978; Tsao and Curthoys, 1980).

The relative activity toward different substrates was determined by Tate and Meister (1974). Kinetic studies have shown that the rates of utilization of a γ -glutamyl

substrate via hydrolysis or transpeptidation depends on the pH (Cook and Peters, 1985) and on the presence of an acceptor substrate (McIntyre and Curthoys, 1980; Tate et al., 1988). Several classes of compounds have been shown to inhibit or stimulate the activity of γ GT (Meister and Tate, 1976).

The second reaction (fig. 1) in mercapturic acid formation after removal of the γ -glutamyl moiety of GSH is the hydrolysis of cysteinylglycine or its S-derivative. This is accomplished by peptidases that may be located intracellularly or bound to the plasma membrane. In the rat, a peptidase that was capable of hydrolyzing S-derived cysteinylglycine was found localized along with γ GT in the brush border membrane of the proximal tubule (Hughey et al., 1978). This peptidase, when partially purified, was found to have broad substrate specificities.

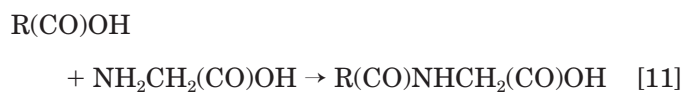
The final step (fig. 1) involves the acetylation of the S-substituted cysteines. The enzyme N-acetyltransferase is discussed in detail in Section III.D. This microsomal enzyme has been found to have its active site on the outer surface of the endoplasmic reticulum in rat kidney (Okajima et al., 1984). Cysteine conjugate N-acetyltransferases have been isolated and purified from rat (Duffel and Jakoby, 1982) and pig (Aigner et al., 1996) kidney.

The mercapturic acids are formed in both the liver and kidney (Inoue et al., 1982). In one study of rat kidney, clearance and microperfusion experiments with the precursor, S-benzyl-L-cysteine, demonstrated that 38% of the excreted mercapturic acid was acetylated in the kidney, primarily in the proximal tubule (Heuner et al., 1991). Studies of the disposition of bromosulfothalein-GSH conjugate in the isolated perfused rat kidney showed that the conjugate is metabolized by the kidney into two major metabolites: cysteinylglycine conjugate and diglutathione conjugate. The diglutathione conjugate is further metabolized to the dicysteinyglycine conjugate, one of the major urinary metabolites. The inhibition of γ GT with acivicin blocked the metabolism to cysteinylglycine and dicysteinyglycine conjugates (Snel et al., 1995). The metabolism of acetaminophen has also been studied in the isolated perfused kidney. The acetaminophen GSH conjugate was rapidly metabolized to 3-cysteinylacetaminophen and then more slowly to acetaminophen-3-mercapturate (Newton et al., 1986). It is interesting to note that the highest NAT activity is found in the proximal tubules in a distribution similar to that of γ GT and the peptidase for cysteinyl peptides (Hughey et al., 1978).

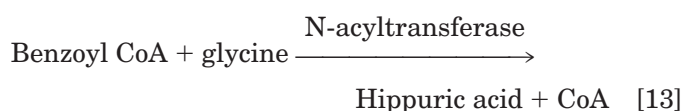
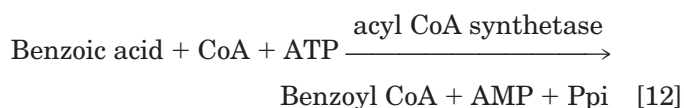
G. Amino Acid Conjugation

Amino acid conjugation reactions generally involve one of two amino acids, either glycine or glutamate with glycine being the more common (Smith and Williams, 1974). These reactions can occur with substrates containing a carboxyl or an alcohol moiety, especially substrates with aromatic groups. The acid or alcohol com-

bines with an amino acid to form an amide bond. The general reaction is shown below:



An example of amino acid conjugation is the formation of hippuric acid from benzoic acid and glycine. The resulting product (hippuric acid) is more polar in solution and possesses different excretion rates than the original substrate (benzoic acid). The enzymes required for amino acid conjugation are acyl-CoA synthetase and N-acyltransferase and they are present in liver and kidney, with some animals having higher activity in kidney (Krishna and Klotz, 1994).



In the kidney, these reactions take place in the mitochondria of cortical cells. The first step is catalyzed by the class of enzymes known as butyryl-CoA synthetases (EC 6.2.1.2, butyrate: CoA ligase (AMP-forming) (Ali and Qureshi, 1992). For benzoic acid conjugation, the benzoyl CoA synthetase has been shown to have a lower specific activity than the enzyme for reaction (13). This step may be inhibited by substances capable of forming CoA esters, such as valproic acid. The creation of the valproate CoA ester competes with benzoic acid conjugation for the available ATP, CoA, and enzyme (Gregus, 1993a). The second reaction is catalyzed by a series of enzymes known as glycine N-acyltransferases, which are known to act on several endogenous and exogenous substances. There are two distinct types of this enzyme corresponding to two distinct classes of substrate. The first of these is the arylacyl transferase (E.C.2.3.1.14), acting on arylacetic acids such as phenylacetylCoA, and the second is arylalkyl transferase (E.C.2.3.2.13), which acts on benzoic acid and its derivatives (Kelley and Vessey, 1993). There is evidence from studies in animals that these enzymes are more fully developed in neonates in the liver than in the kidney; whether this is true for humans is unknown (Ali and Qureshi, 1992).

Conjugation reactions can be limited by the amount of glycine available in the body or by the amount of enzyme available to catalyze the reaction. Whereas levels of glutamate do not seem to affect glutamate conjugations in that all reactions show saturation above a concentration of available substrate. Small organic acids such as benzoic and salicylic will be glycinated by the kidneys into hippuric and salicyluric acids respectively and then excreted. When this pathway works at maximal capac-

ity, the remainder of these drugs are glucuronidated (Vree et al., 1992).

1. *Glycine conjugation.* Glycine conjugation is the main metabolic pathway for salicylic acid. A small increase in the carbon side chain resulting in phenylacetic acid makes conjugation with glycine impossible and, in humans, conjugation is carried out with glutamine (Wan and Riegleman, 1972). Wan et al. (1972) demonstrated that the kidney contributed more to glycination than did the liver. Poon and Pang (1995) compared enzymatic constants for benzoic acid glycination in perfused rat kidney and liver and found the V_{\max} to be 195 versus 101 nmol/min/g and the K_m to be 5.3 and 12.0 μM respectively.

The role of glycine in establishing this maximal rate of benzoic acid conjugation can be established by measuring the increase in hippurate excretion after the administration of glycine. Even at the maximum rate of hippurate excretion, increasing the supply of glycine will result in an increase in excretion of hippurate (Quick, 1931). Gregus et al. (1993b) established that increases in the elimination of benzoic acid after coadministration of sodium benzoate and glycine depended upon the dose of sodium benzoate. The supply of glycine sets a maximal conjugation rate that can be reached with an optimal concentration of benzoate.

This is not the whole story. There are actually two factors that limit the rates of hippuric acid synthesis, namely depletion of Coenzyme A and the availability of glycine for conjugation. In the second reaction (13), glycine is consumed by conjugation, whereas CoA is regenerated each time a molecule of hippuric acid is synthesized. Once glycine levels fall low enough to limit the rate of reaction (13), CoA is trapped in the form of benzoyl CoA. This pool of activated intermediates effectively denies CoA to other metabolic processes; it also halts the production of more benzoyl CoA. In effect, this limits the supply of benzoyl CoA for reaction (13), placing a limit on the reaction rate (Gregus et al., 1992).

It was formerly believed that beyond the maximal rate of hippurate synthesis benzoic acid was shunted to another conjugation pathway with glucuronic acid (covered in III. A. Glucuronidation). More accurate techniques for the measurement of levels of excreted glucuronic acid conjugates have revealed that glucuronidation occurs at even very low levels of benzoic acid. Glucuronic acid conjugation with benzoic acid increases proportionally with benzoic acid concentration, showing no relationship with glycine availability or the resulting maximal rate of hippuric acid formation. Glucuronidation of benzoic acid to form hippuric acid is not a reserve pathway for benzoic acid elimination, but rather a second pathway operating completely independently of glycine conjugation (Schachter and Mauro, 1958).

Glycine plays the same role in the metabolism of salicylic acid via conjugation to salicyluric acid. The rate of salicylic acid elimination is roughly 20 times slower than

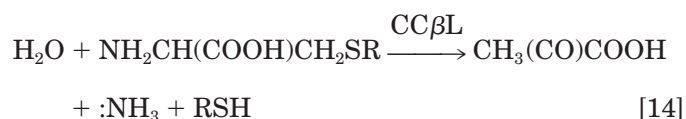
the rate for benzoic acid glycine conjugation. Liver metabolism is low, and practically all of the salicylic acid is formed by conjugation in the kidney. Saturation of salicylic acid conjugation does not occur caused by a shortage of glycine, as occurs in benzoic acid formation, but rather caused by the saturation of the enzymes involved (Wan and Riegleman, 1972).

Metabolism of PABA follows two pathways, either by acetylation to *p*-acetamidobenzoic acid (Section III. D.) and by glycine conjugation to *p*-aminohippuric acid. These reactions are localized to the kidney and liver. The quantitatively more important reaction is the acetylation (Wan et al., 1972).

2. *Glutamine conjugation.* Glutamine conjugation reactions are limited in the body to specific arylacetic acids. One such substance is phenylacetic acid, which can be converted in the kidney to indolacetylglutamine in various species of Old and New World monkeys and in humans (Smith and Williams, 1974).

H. Cysteine Conjugate β -Lyase

Cysteine conjugates of aromatic drugs are metabolized principally to the corresponding mercapturic acid. However, cysteine conjugates may also undergo a β -elimination (equation 14 below) or a transamination reaction catalyzed by the enzyme cysteine conjugate β -lyase (CC β L) (E.C. 4.4.1.13).



This enzyme will also catalyze transamination reactions. For example S-(12-dichlorovinyl)-L-cysteine can be transaminated to the 2-oxo-3-mercaptopropionate conjugate (Stevens et al., 1989). This enzyme also has been the focus of much study because of the fact that rather than resulting in detoxification, it may be responsible for the bioactivation of nephrotoxic cysteine and homocysteine conjugates (Elfarra and Anders, 1984; Monks et al., 1990). Liver and kidney are the principal sites of activity. CC β L activity was localized to the cystolic and mitochondrial fraction of rat kidney cortex (Stevens, 1985) and subsequently purified from both cystolic and mitochondrial sources (Stevens et al., 1988).

The cDNA for rat kidney CC β L has been sequenced and found to code for a protein of 48 kDa. The cDNA was transfected into COS-1 tissue culture cells with a resultant increase of 7- to 10-fold in cystolic β -lyase and glutaminase *K* activity (Perry et al., 1993).

In human kidney, β -lyase activity was found in cytosolic, mitochondrial, and microsomal fraction using S-(2-benzothiazolyl)-L-cysteine as the substrate, with the highest concentration found in the cytosolic fraction (Lash et al., 1990). The β -lyase activity copurified with cytosolic glutamine transaminase *K*, with total Mr of 85 kDa and with a 45 kDa subunit. The activity was inhibited

by aminooxyacetic acid, indicating that the enzyme contains pyridoxal phosphate. Human kidney CC β L was recently cloned and sequenced and found to have an overall 82% similarity in amino acid sequence with that of rat, with 90% similarity around the pyridoxal phosphate binding site. Expression of the cDNA in COS-1 cells produced a cystolic enzyme with CC β L and glutamine transaminase *K* activity (Perry et al., 1995).

Immunohistochemical studies showed that rat kidney CC β L was mainly localized to the proximal tubule (Jones et al., 1988) with one study demonstrating an increased concentration of enzyme in the pars recta (S3 segment), which displays a greater sensitivity to damage from nephrotoxic cysteine conjugates (MacFarlane et al., 1989).

The substrates studied using renal CC β L have principally been certain L-cysteine conjugates of aromatic compounds, such as S-(2-benzothiazolyl)-L-cysteine, and S-(1, 2-dichlorovinyl) L-cysteine. N-acetyl-S-(12,3,44-pentachloro-13-butadienyl)-L-cysteine induced CC β L activity in female rat kidney at low dosages, whereas at high dosages it suppressed activity (McFarlane et al., 1993). Alpha-ketoacids stimulated rat renal CC β L activity (Elfarra et al., 1987).

IV. Localization of Drug Metabolizing Enzymes in the Kidney

Those enzymes that have been localized by various studies using immunocytochemistry and enzyme activity measurements reported in this review are summarized here and appear in table 5 and fig. 2. Table 5 shows the distribution of enzymes in the kidney corresponding to fig. 2A. It appears that the cortical region is rich in metabolizing enzymes. Only in a few instances were the medullary zones studied. As seen in B of fig. 2, the proximal tubule is active in metabolizing drugs, although enzymes were found in all segments of the nephron. Many of these enzymes were localized to either the cytoplasmic or the microsomal fraction (C of fig. 2), whereas some appeared in both. These studies show the predominant distribution of drug metabolizing enzymes by regional, tubular, and cellular sites but may not be

TABLE 5
Distribution of drug metabolizing enzymes in kidney zones

Enzyme	Cortex	Medulla	Outer	Inner
N-acetyltransferase	✓	✓	—	—
Alcohol dehydrogenase	✓	✓	—	—
Aldose reductase	—	—	✓	✓
Aldehyde reductase	✓	—	—	—
Amino acid conjugation	✓	—	—	—
Cytochrome P450	✓ ^a	—	✓	—
Carboxylesterase	✓	—	—	—
Cysteine conjugate β -lyase	✓	—	—	—
Glutathione S-transferase	✓	✓	—	—
Monamine oxidase	✓ ^a	✓	—	—
NADPH-cytochrome c reductase	✓ ^a	—	—	✓
Steroid 21 hydroxylase	✓	✓	—	—
Sulfotransferase	✓	—	✓	—

^a Signifies greatest activity.

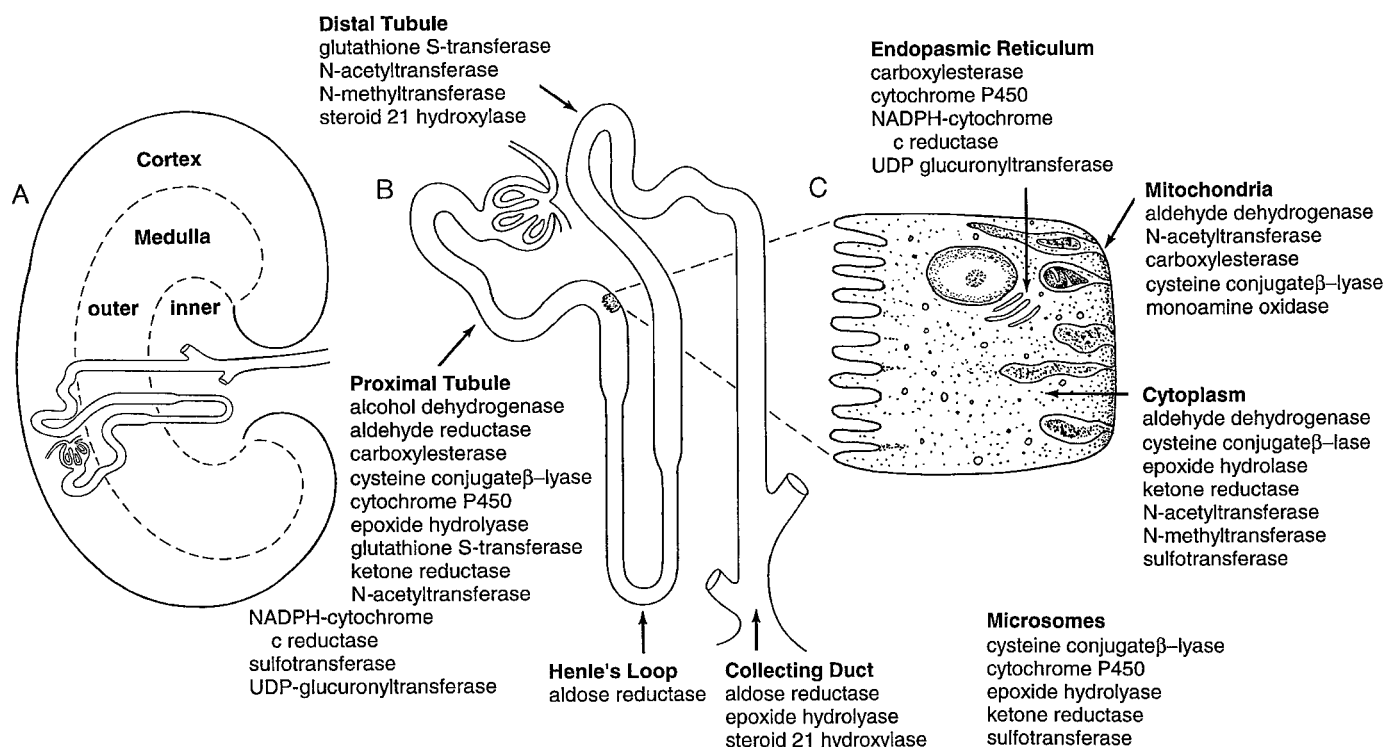


FIG. 2. Regional (A), tubular (B), and subcellular (C) localization of drug-metabolizing enzyme systems in the kidney.

exclusive because all regions were not systematically studied.

V. Effects of Renal Metabolism

Although the kidney will generally metabolize endogenous or exogenous chemicals to compounds with reduced biological activity, there are several instances in which metabolism will produce a toxic intermediate that may result in mutagenesis or cell necrosis. This topic has recently been reviewed (Anders and Dekant, 1994; Dekant et al., 1994; Spahn-Langguth and Benet, 1992).

Instances of metabolism to active metabolites have been demonstrated and can lead to beneficial effects. For example, the angiotensin-converting enzyme inhibitor, enalapril, is metabolized to enalaprilat, an active and polar dicarboxylic acid metabolite. The intrarenally formed metabolite either re-enters the circulation or undergoes excretion into the lumen (deLannoy et al., 1990).

Investigations of renal metabolism of drugs have led to the development of target organ-directed drug delivery systems in which systemic side effects of drugs are avoided. For example, Elfarra and Hwang (1993) demonstrated that the high concentration of renal β -lyase facilitated the conversion of S-(6-puriny)-L-cysteine to the antitumor and immunosuppressant drug 6MP by the kidney. This permits the accumulation of much higher concentrations at its target site in the kidney and avoids systemic toxic effects. Another example is the use of the high concentration of γ -glutamyl transpeptidase in the proximal tubule brush border to produce kidney

specific production of dopamine after the administration of the precursor γ -glutamyl dopa. The concentration of dopamine in mouse kidney after injection of γ -glutamyl dopa was five times higher than that seen after an equivalent dosage of dopamine was given (Wilk et al., 1978).

Metabolism of drugs and endogenous compounds can lead to alterations in either excretion or reabsorption depending on the pathway followed and the activity of the enzyme. The metabolite will determine the direction of movement. Amino acid conjugation results in loss of pharmacological activity and a compound more readily excreted into urine via tubular secretion mechanisms (Gregus, 1993a). Similarly, the formation of meperidine N-oxide contributed to a more rapid excretion of that drug by the isolated perfused kidney (Acara et al., 1981). If this route were not functioning as in renal failure, then other metabolites, such as normeperidine, might accumulate with attending effects. With the exception of methylation, most conjugative metabolisms lead to more polar compounds that may be excreted from the body rapidly. The activity of the metabolizing enzymes also plays a role. Choline for example will be metabolized to betaine, which is reabsorbed. However, when the choline oxidase enzyme is saturated, choline itself moves in the direction of excretion (Acara et al., 1979).

The most common ways in which the excretion of a substance is increased are for the drugs to become able to bind to the carriers for excretory transport and/or to become water soluble and filtered. Certain biotransformations contribute to these changes (Ullrich et al.,

1990). An OH group inserted into a benzene ring renders the molecule acceptable to the ρ -aminohippuric acid (PAH) transporter, if an electron-attracting group (NO_2^- , Cl^- , Br^- , HCO_3^-) is already present in the molecule. Esterification of carboxylates decreases their affinity to the PAH transporter. On the other hand, amino acids and oligopeptides such as glutathione become substrates of the organic cation transport system when they are esterified. Zwitterionic amino acids (except those that are too hydrophilic) become pure cations by esterification and pure anions by N-acetylation. However, N-acetylation of uncharged amino groups has little effect on affinity for the PAH transporter. Glycine conjugation increases the affinity of salicylate for the PAH transporter (Ullrich et al., 1987). The metabolism of an amine to its N-oxide leads to an increase in polarity and a drop in pKa. The semipolar N \rightarrow O linkage imparts to N-oxides a salt-like character that makes them readily soluble in water and only slightly soluble in organic solvents. The excretion of trimethyl amines by the renal tubule occurs predominantly as a result of the formation of an N-oxide by the renal tubular epithelial cells followed by movement into the tubule lumen.

Fig. 3 shows the possible pathways that may result during renal metabolism of a drug. Entry across either the brush border (BBM) or the basolateral membrane (BLM) is accompanied by biotransformation of the drug (A = organic anion; C = organic cation) to a metabolite (B or D, respectively). The metabolite may then move in the direction of reabsorption or secretion/excretion. In the upper section of the left panel, an organic anion such as salicylic acid can exchange with α -ketoglutarate across the BLM in a tertiary active transport step dependent on sodium α -ketoglutarate cotransport. Glycination of salicylic acid to e.g., salicyluric acid (indicated as B) provides a compound that more readily enters the urine. Other possibilities are that A remains unmetabo-

lized and is simply excreted as the administered drug; or B could be a different metabolite that is more likely to be reabsorbed. The lower left hand panel depicts reabsorption of an organic anion that may occur by anion exchange across the BBM as well as through cotransport with sodium. For example, pyrazinoate is cotransported into the tubule cell with sodium and is returned to the blood as pyrazinoate. Biotransformation of A to B could lead to a metabolite with a predominant pathway of either reabsorption or secretion. The right hand panel depicts possible pathways for disposition of organic cations. In the upper right of the panel an organic cation such as meperidine enters the renal tubule cell along its electrochemical gradient. It may be biotransformed to meperidine N-oxide (D), a more polar compound that enters the tubule fluid. Unmetabolized meperidine may exchange for a proton across the BBM or the metabolite may follow a reabsorptive route such as would be the case for the demethylated normeperidine. The lower half of the right hand panel depicts an organic cation (C), such as isoproterenol that can be reabsorbed across the BBM in exchange for a proton. Upon entry into the cell catechol-O-methyl transferase activity produces methylated isoproterenol that moves back into the blood. Some of the isoproterenol may itself cross the BLM and/or a more polar metabolite could enter the tubule fluid. In the isolated perfused rat kidney the fractional excretion of isoproterenol decreased as the amount of the 3-O-methyl metabolite increased (Szeffler and Acara, 1979).

In summary, although the liver plays a dominant role in drug metabolism, this review demonstrates that the kidney is metabolically active in the biotransformation of drugs. In some instances this role may, in fact, exceed that of the liver. The metabolic pathways of the kidney generally lead to a product that is more readily excreted in the urine, but in certain instances the metabolite may be reabsorbed and more active or toxic than the parent compound.

The metabolic pathways of the kidney should be considered in the administration of drugs, particularly to patients with renal impairment. Renal failure may affect the handling of drugs in several ways including drug distribution, bioavailability, and excretion. From this review, it is clear that the effects of alterations in kidney function on renal drug metabolism should also be taken into account in the determination of optimum drug therapy. Additional studies of renal drug metabolism of specific agents are needed to provide a better understanding of the role of the kidney in the disposition of drugs.

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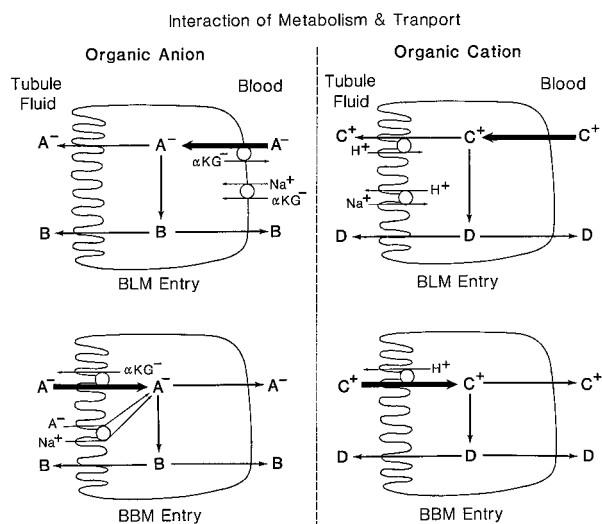


FIG. 3. Proposed interaction of transport and metabolism in the renal proximal tubule. Please see text for explanation of figure.

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