11

Drug Disposition—Elimination

Drugs are eliminated from the body by *metabolism* and *excretion*. The liver is the major site of drug metabolism, but other tissues contain drug metabolizing enzymes and contribute to the biotransformation of certain drugs. The kidneys play a principal role in the excretion of drugs and/or their metabolites. Some drugs are excreted in the bile and may be eliminated in the feces. Drugs used for anesthesia are often excreted by the lungs.

This chapter concerns the basic principles of drug elimination. Emphasis is given to renal excretion of drugs and drug metabolism in the liver.

DRUG EXCRETION

Renal Excretion

The kidneys are involved in the elimination of virtually every drug or drug metabolite. Some drugs, such as gentamicin or cephalexin, are eliminated from the body almost solely by renal excretion in patients with normal renal function. Many more drugs are eliminated in part by the kidneys. Even when drug elimination from the body involves only biotransformation, the corresponding drug metabolites are usually cleared by the kidneys.

Renal Physiology. The renal excretion of a drug is a complex phenomenon involving one or more of the following processes: *glomerular filtration*, *active tubular secretion*, and *passive reabsorption*. These processes are depicted schematically in Figure 11–1. Depending on which one of these processes is dominant, renal clearance can be an important or negligible component of drug elimination.

The kidneys receive about 25% of cardiac output or 1.2 to 1.5 L of blood per minute. About 10% of this volume is filtered at the glomeruli. Therefore, about 130 ml of plasma water is filtered each minute. Although the pores of the glomerular capillaries are sufficiently large to permit the passage of most drug molecules, the glomeruli effectively restrict the passage of blood cells and plasma proteins. Accordingly, only free drug (drug that is not bound to plasma proteins) can be filtered.

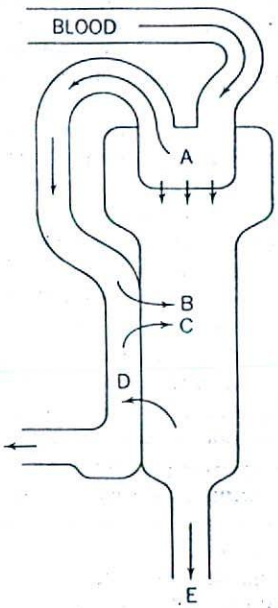


Fig. 11–1. Schematic representation of renal excretion of drugs depicting glomerular filtration of plasma water and unbound drug (A), active tubular secretion of organic acids (B) and bases (C), reabsorption of lipid-soluble drugs (D), and urinary excretion (E).

Although some 180 L of protein-free filtrate pass through the glomeruli each day, only about 1.5 L

is excreted as urine; the remainder is reabsorbed in the renal tubules. This often results in high urinary concentrations of certain solutes, particularly drugs that are not similarly reabsorbed. Many drugs, however, are efficiently reabsorbed from the distal portion of the nephron. In most instances, tubular reabsorption of drugs is a passive phenomenon. Nonionized, lipid-soluble drugs are rapidly and extensively reabsorbed, whereas polar compounds and ions are unable to diffuse across the renal epithelium and are excreted in the urine. For drugs that are principally eliminated by renal excretion, the more efficient the reabsorption of the drug, the longer is its biologic half-life.

The restrictive effect of plasma protein binding on glomerular filtration and the enormous capacity of the nephron to reabsorb certain solutes suggests that highly plasma protein-bound, lipid-soluble drugs would persist in the body for long periods of time after administration unless alternative elimination pathways were available. Of course, many of these drugs are subject to biotransformation in the liver or other tissues; others are subject to active tubular secretion at the proximal portion of the nephron. The fraction of drug in the blood that is not filtered may be effectively cleared by active tubular transport. The rate of tubular secretion is more closely a function of total rather than free drug concentration in blood or plasma and is independent of plasma protein binding.

Renal Clearance. The renal excretory mechanisms, filtration, reabsorption, and secretion, usually have the net effect of removing a constant fraction of the drug presented to the kidneys in renal arterial blood. The efficiency of renal excretion of a drug can be expressed in terms of a hypothetical volume of plasma that is completely *cleared* of that substance by the kidneys per unit time. This concept of *clearance* may be applied to any organ that secretes (e.g., biliary clearance) or metabolizes (e.g., hepatic clearance) a drug from the plasma. Renal clearance (Cl_R) is often a substantial fraction of the total clearance (Cl) of drug from the blood. For drugs that are essentially excreted unchanged in the urine, $Cl = Cl_R$.

Since renal clearance is a proportionality constant, relating the urinary excretion rate (dA_u/dt) of drug with its concentration (C) in blood or plasma, the following equation applies:

$$Cl_R = (dA_u/dt)/C \qquad (11\text{–}1)$$

Renal clearance is estimated by comparing the amount of drug (A_u) excreted over a short time interval (e.g., 1 hr) to the concentration of the drug in blood or plasma at the time corresponding to the midpoint of the urine collection interval.

Substances such as creatinine, the endogenous end product of creatine metabolism, or inulin, a carbohydrate, are eliminated by renal excretion but are not subject to either tubular secretion or reabsorption. Furthermore, these substances are not appreciably bound to plasma proteins. Therefore, the renal clearance of inulin or creatinine is an index of glomerular filtration rate (GFR).

In healthy individuals, the renal clearance of glucose is nearly zero because of efficient reabsorption in the renal tubules. On the other hand, certain substances are so efficiently excreted by the renal tubules that they are essentially cleared from the blood in a single pass through the kidneys. Para-aminohippuric acid (PAH) is handled in this manner. PAH is poorly lipid soluble, it does not penetrate erythrocytes, and it is not reabsorbed. Accordingly, the renal clearance of PAH is a measure of renal plasma flow rate (600 to 700 ml/min).

The renal clearance of a drug relative to GFR provides information on the mechanisms of renal excretion. Renal clearance values exceeding 130 ml/min are indicative of tubular secretion. The renal clearance of penicillin G (500 ml/min) exceeds the expected 52 ml/min calculated from the product of GFR (130 ml/min) and the fraction of drug in the plasma not bound to plasma proteins (0.4). Renal clearance values that are below 130 ml/min, even when adjusted for the degree of plasma protein binding, are indicative of tubular reabsorption. The renal clearance of phenytoin in man is about 5 ml/min. The fact that the drug is 90% bound to plasma proteins certainly contributes to this low value. Even when binding is taken into consideration, however, the renal clearance of free phenytoin (50 ml/min) is less than GFR, indicating reabsorption.

Secretion and reabsorption have opposite effects on renal clearance. When both processes are operative, presumptive evidence of either secretion or reabsorption, based on renal clearance values, does not rule out the other. Secretion may be operative even though the renal clearance of the drug is less than 130 ml/min. By the same token, reabsorption may occur with a drug having a renal clearance greater than 130 ml/min. It is also incorrect to assume that a drug with a renal clearance of about 130 ml/min is excreted simply by glo-

merular filtration. For example, sulfisoxazole undergoes tubular secretion and reabsorption in man but to a similar extent.[1]

Although estimates of renal clearance are informative with respect to excretion mechanisms, they give little information about the half-life of the drug. When a drug is eliminated solely by urinary excretion, the excretion rate (dA_u/dt) of the drug is given by:

$$dA_u/dt = KVC \qquad (11\text{–}2)$$

where K is the elimination rate constant of the drug, V is the apparent volume of distribution, and C is the drug concentration in blood or plasma. Accordingly, Equation 11–1 may be rewritten as follows:

$$Cl_R = \frac{KVC}{C} = KV \qquad (11\text{–}3)$$

Rearranging this expression, and recognizing that half-life ($t_{1/2}$) is equal to 0.693/K, it follows that:

$$t_{1/2} = \frac{0.693\ V}{Cl_R} \qquad (11\text{–}4)$$

Thus, the half-life of a drug is a function of both its clearance and volume of distribution. A drug may have a high clearance but still have a long half-life if V is also large.

Renal clearance also can be expressed in terms of the individual renal excretion processes, as follows:

$$Cl_R = (Cl_{RF} + Cl_{RS})\ (1 - FR) \qquad (11\text{–}5)$$

where Cl_{RF} is renal filtration clearance, Cl_{RS} is renal secretion clearance, and FR is the fraction of drug filtered and secreted that is reabsorbed. Renal filtration clearance (Cl_{RF}) is a function of GFR and plasma protein binding, i.e.,

$$Cl_{RF} = f_B\ GFR \qquad (11\text{–}6)$$

where f_B is the fraction of drug in the blood not bound to plasma proteins. GFR is usually estimated by determining creatinine clearance.

Drug secretion depends on the relative affinity of the drug for carrier proteins in the proximal tubule and plasma proteins, the rate of transport across the tubular membranes, and the rate of delivery of the drug to the site of secretion. These factors are included in the following equation:

$$Cl_{RS} = \frac{RBF\ f_B\ Cl_i}{RBF + f_B\ Cl_i} \qquad (11\text{–}7)$$

where RBF is renal blood flow and Cl_i is the intrinsic secretion clearance with respect to free drug.

Substituting Equations 11–6 and 11–7 for the appropriate terms in Equation 11–5, yields:

$$Cl_R = f_B \left[GFR + \frac{RBF\ Cl_i}{RBF + f_B\ Cl_i} \right] (1 - FR) \qquad (11\text{–}8)$$

Equation 11–8 is a general expression for the renal clearance of drugs. An expression incorporating urine pH may be substituted for FR for those cases where tubular reabsorption is pH-dependent.

When renal excretion is not blood flow rate-limited, then $RBF \gg f_B\ Cl_i$, and Equation 11–8 reduces to:

$$Cl_R = f_B\ (GFR + Cl_i)\ (1 - FR) \qquad (11\text{–}9)$$

If tubular reabsorption is negligible or blocked by changing urine pH (i.e., FR = 0), renal clearance is given by:

$$Cl_R = f_B\ (GFR + Cl_i) \qquad (11\text{–}10)$$

If tubular secretion is negligible or blocked by giving a competitive inhibitor like probenecid (i.e., $Cl_i = 0$), renal clearance is given by:

$$Cl_R = f_B\ GFR\ (1 - FR) \qquad (11\text{–}11)$$

Under the conditions described by Equations 11–9 to 11–11, renal clearance is sensitive to changes in plasma protein binding.

When renal excretion is blood flow rate-limited, then $RBF \ll f_B\ Cl_i$; Equation 11–8 reduces to:

$$Cl_R = (f_B\ GFR + RBF)\ (1 - FR) \qquad (11\text{–}12)$$

or

$$Cl_R = RBF\ (1 - FR) \qquad (11\text{–}13)$$

because $RBF \gg f_B\ GFR$. Under these conditions, renal clearance is sensitive to changes in blood flow rate.

Equations 11–6 through 11–11 indicate that there is usually a relationship between plasma protein binding and renal clearance. When binding is capacity limited, the apparent renal clearance of a drug may show pronounced concentration dependence. This has been demonstrated with an investigational cephalosporin antibiotic in the dog.[2]

Cefixime was given by intravenous bolus injection and plasma protein binding and renal clearance were determined periodically as the drug levels in plasma declined. Renal clearance was determined by dividing the amount of drug excreted in the urine per unit time (i.e., the excretion rate) by the total drug concentration in plasma at the midpoint of the urine collection interval. Drug levels ranged from 197 μg/ml at 0.25 hours to 33 μg/ml at 11 hours after administration.

Over this 6-fold drug concentration range, free fraction in plasma (expressed as a percentage) decreased from 34% to 10% and renal clearance decreased from 1.64 to 0.33 ml/min/kg. On the other hand, the renal clearance of unbound cefixime, calculated by dividing renal clearance by free fraction, was relatively constant (mean value, 3.3 ml/min/kg). These findings indicate that the profound decrease in renal clearance was largely the result of capacity-limited binding rather than nonlinear renal excretion.

Tubular Secretion. Tubular secretion is an active transport process whereby drug diffuses against a concentration gradient from the blood capillaries across the tubular membrane to the renal tubule. This active process accounts for the fact that certain drugs, like dicloxacillin, although extensively bound to plasma protein and not subject to hepatic metabolism, are rapidly eliminated. Plasma protein binding does not affect the rate of tubular secretion because there is rapid transport of unbound drug and rapid dissociation of the drug-protein complex.

The secretion process shares many of the characteristics of the specialized transport (absorption) systems of the intestine. The process exhibits some degree of structural specificity; transport systems specific for organic acids (e.g., thiazide diuretics) and organic bases (e.g., triamterene) have been identified. Each system is characterized by a maximum rate of transport (T_m) for a specific drug. In principle, tubular secretion is saturable; in practice, however, there are few examples of nonlinear renal excretion.

The tubular excretion of several beta-lactam antibiotics has been studied in healthy human subjects.[3] Each drug was infused intravenously at different rates to achieve a wide range of concentrations in plasma. Renal clearance of each antibiotic at each steady-state concentration was calculated for the non-plasma protein bound (free) fraction of the drug. Estimation of renal clearance in terms of unbound rather than total concentration was important because the plasma protein binding of one antibiotic, cloxacillin, was capacity limited; this was not the case for benzylpenicillin or cephradine. Tubular clearance was determined from the difference between renal clearance and glomerular filtration, assumed to be equal to creatinine clearance. Tubular reabsorption was considered negligible because high urine flow rates were maintained.

In each case, a plot of tubular excretion rate *versus* free drug concentration demonstrated a saturable process. Estimates of EC_{50}, the free drug concentration at which tubular excretion rate is 50% of maximum tubular excretion rate, were 5 to 10 mg/L for cloxacillin, 50 to 100 mg/L for benzylpenicillin, and 250 to 300 mg/L for cephradine. For cloxacillin, the results indicate that capacity-limited tubular secretion is of clinical interest. An EC_{50} of 6 mg/L corresponds to a total cloxacillin concentration of 54 mg/L, a level not unusual in clinical practice.

Another similarity of tubular secretion to active intestinal absorption is competitive inhibition of one drug by another. This characteristic has been used to prolong the half-life of drugs like penicillin that are eliminated to a considerable extent by tubular secretion. Probenecid, a weak organic acid, competitively inhibits the tubular secretion of penicillin G and other penicillins, and reduces the rate of urinary excretion. Probenecid has been used clinically to increase the duration of effect of penicillins. Parenteral penicillin G or ampicillin, in high doses, with probenecid is considered to be an effective treatment for gonorrhea.[4]

Many drugs are marketed as racemic mixtures. Although enantiomers have identical physical and chemical properties, the chiral macromolecules in the body are quite specific to the spatial arrangement of drug molecules. Consequently, stereospecific or stereoselective interactions between proteins or other macromolecules and drugs are common. These interactions result in stereoselective pharmacokinetics and pharmacodynamics of drugs.

In contrast to the many studies concerning stereoselective hepatic metabolism of drugs, few studies have examined whether stereoselective renal excretion of drugs occurs. Since tubular secretion appears to be a saturable, carrier-mediated process, it is reasonable to consider the possibility of stereoselectivity. Two systems are primarily respon-

sible for the active tubular secretion of drugs, one for organic anions and another for organic cations. Neither system has been carefully studied with respect to stereoselective tubular secretion.

Pindolol, a beta-blocker marketed as a racemic mixture, was used to study stereoselective renal clearance of organic cations.[5] Normal human subjects received an oral dose of racemic pindolol. A stereospecific assay method was used to measure the concentrations of d- and l-pindolol in plasma and urine. Renal clearance and other pharmacokinetic parameters of both enantiomers were calculated and compared.

The area under the drug concentration-time curve (AUC), half-life, and amount excreted in the urine were significantly greater for l- than for d-pindolol. Also, the renal clearance of l-pindolol was greater than that of d-pindolol in all subjects; mean values were 240 ml/min and 200 ml/min, respectively. Since binding to plasma proteins was not found to be stereoselective, differences in renal clearance between d- and l-pindolol probably reflect stereoselective renal transport.

Tubular Reabsorption. After undergoing secretion or glomerular filtration, most drugs are subject to tubular reabsorption. A large concentration gradient exists between drug in the renal tubules and free drug in the plasma, because of the efficient reabsorption of water. Tubular reabsorption of drugs is usually a passive process. The tubule membranes favor the transport of lipid-soluble drugs; compounds that are poorly lipid soluble or ionized are poorly reabsorbed. The reabsorption of drugs that are weak acids or bases depends on the pH of the tubular fluids.

Tubular Reabsorption and Urine pH. The pH of fluids in the proximal tubule approximates that of plasma (pH 7.4), whereas the pH in the distal tubule approximates that of urine, which may vary from 4.5 to 8.0; on the average, urine pH is 6.3. These extremes contrast with the narrow range of blood pH, 7.3 to 7.5. Accordingly, a large pH gradient may exist between blood and urine in the distal tubule.

Urine pH is affected by diet, drugs, and the condition of the patient. The pH of the urine also varies during the day. Respiratory and metabolic acidosis produce acid urine; respiratory and metabolic alkalosis produce alkaline urine. On the other hand, the urine is alkaline in renal tubular acidosis. Drugs like acetazolamide and sodium bicarbonate produce an alkaline urine; ammonium chloride and ascorbic acid produce an acid urine.

Since drug reabsorption takes place in the distal tubule, urine pH is assumed to indicate the pH at the site of reabsorption. According to the pH-partition hypothesis, acidification of the urine promotes the reabsorption of weak acids but retards the reabsorption of weak bases. The renal clearance of weak acids is increased if the urine is made alkaline because more drug is in the ionized form and cannot be reabsorbed. On the other hand, the renal clearance of weak bases is low in alkaline urine but may be increased dramatically if the urine is acidified.

The influence of pH on tubular reabsorption also depends on the pKa of the drug. Relatively strong acids or bases are virtually completely ionized over the entire range of urine pH and undergo little reabsorption. The critical range of pKa values for pH-dependent excretion is about 3.0 to 7.5 for acids and 7.5 to 10.5 for bases.[3]

The extent to which changes in urine pH alter the rate of drug elimination depends on the contribution of renal clearance to total clearance. Although weak acids like tolbutamide and warfarin are susceptible to pH-dependent changes in reabsorption, such changes have little effect on their elimination, which depends essentially on hepatic metabolism.

Studies to determine pH-dependent urinary excretion often maintain urine pH at the acid or alkaline extreme by continually administering either ammonium chloride or sodium bicarbonate during the course of the study. These studies are also useful for detecting renal mechanisms that are not evident under normal urine pH conditions. For example, in women subjects, a change in urine pH from 5.3 to 7.4 increases the renal clearance (corrected for protein binding) of sulfisoxazole from 92 ml/min to 187 ml/min.[1] Although sulfisoxazole renal clearance at the average value of urine pH is consistent with glomerular filtration, the data at the extremes of urine pH indicate that both tubular secretion and reabsorption contribute to the renal excretion of this sulfonamide.

The influence of urine pH on the elimination of sulfonamides has been studied by many investigators; often, the effects are considerable. Changes in urine pH from 5 to 8 have been shown to decrease the half-life of sulfaethidole in man from 11.4 to 4.2 hr.[7] The rate of elimination of sulfalene and sulfasymazine in healthy subjects was doubled

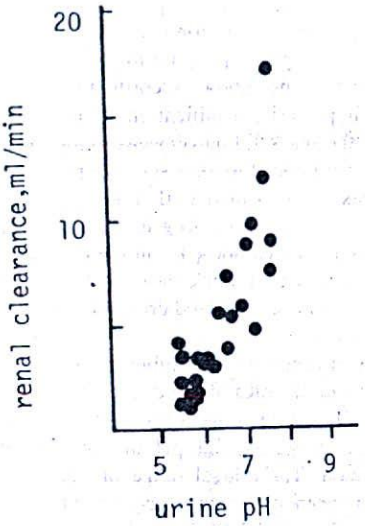


Fig. 11–2. Effect of urine pH on the renal clearance of sulfamethoxazole. (Data from Vree, T.B., et al.[9])

by administration of sodium bicarbonate, which increased urine pH from about 6 to 8.[8] Half-lives were decreased from 72 to 14 hr for sulfalene and from 32 to 7 hr for sulfasymazine. The effect of urine pH on the renal clearance of sulfamethoxazole is shown in Figure 11–2.[9]

Blood levels of salicylate commonly found in patients on high-dose aspirin therapy for rheumatoid arthritis fluctuate considerably with changes in urine pH. Patients concomitantly receiving antacids that alkalinize the urine may require higher doses of aspirin to maintain adequate blood levels of salicylate.

The renal clearance of methotrexate varies from 48 to 300 ml/min over a urine pH range of 5.5 to 8.3 in patients with various malignancies but normal renal function.[10] Preliminary data indicate that the increase in renal clearance, resulting from urinary alkalinization in these patients, is reflected by a shorter half-life of methotrexate.

pH-dependent renal excretion has also been demonstrated for many weak bases, including amphetamine, ephedrine, methadone, and fenfluramine. In some cases, the change in renal clearance also results in a change in the half-life of the drug. For example, the average half-life of pseudoephedrine in human subjects decreased from 13.4 to 4.7 hr when urine pH was reduced from 8 to 5.[11] Unanticipated toxicity after ordinary doses of pseudoephedrine in a patient with renal tubular acidosis was associated with high blood levels and low renal clearance, as a result of persistently alkaline urine.[12]

Large differences in elimination rate, as a function of urine pH, are found with amphetamine.[13] On the average, about a sevenfold difference in the total clearance of amphetamine from the plasma is evident when data from subjects with controlled acid urine are compared with data from the same subjects with uncontrolled urine pH.[14]

Urine pH also influences the fraction of drug excreted unchanged. For example, about 57% of a dose of amphetamine is excreted unchanged in subjects with acid urine (pH 4.5 to 5.6) compared to about 7% in subjects with alkaline urine (pH 7.1 to 8.0).[13] Renal excretion of unchanged drug accounts for about 90% of an oral dose of ephedrine in subjects with acid urine but only about 25% in subjects with alkaline urine.[15] Administration of amphetamine or related drugs with sodium bicarbonate not only enhances pharmacologic effects but also makes it much more difficult to detect unchanged drug in the urine of athletes who illicitly ingest stimulants to enhance performance.

The influence of urine pH on the pharmacokinetics of flecainide, an antiarrhythmic agent, has also been studied.[16] The cumulative urinary excretion following a 300 mg oral dose was 134 mg (45% of the dose) under acid conditions but only 22 mg (7% of the dose) under alkaline conditions. Renal clearance of flecainide in individual subjects ranged from 2 to 172 ml/min under alkaline conditions and from 98 to 968 ml/min under acid conditions.

Important differences in half-life and AUC were also observed. The mean half-life of flecainide was 33 hours under alkaline conditions and about 8 hours under acid conditions. The mean value for total AUC was more than 3 times greater under alkaline than under acid conditions. From a clinical toxicology point of view, efforts to acidify urine might be a useful therapeutic measure in patients with dangerously high serum levels of flecainide.

The occurrence of diurnal variation in urine pH, possibly related to decreased sensitivity of the respiratory center during sleep, is well known. Urine pH in most individuals is relatively low during sleep but increases after awakening. Accordingly, a corresponding diurnal cycle may occur in the rate of elimination of certain drugs. Clinical studies show that the mean half-life of sulfasymazine (pKa

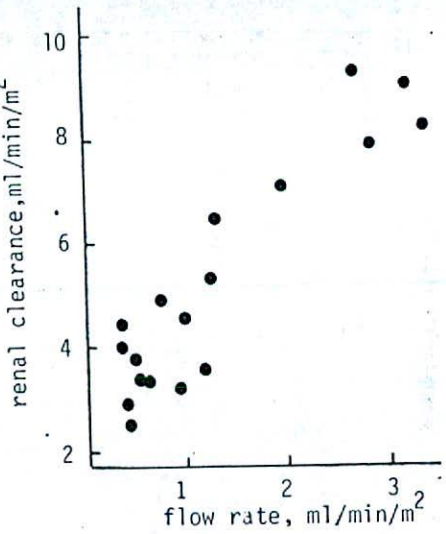


Fig. 11–3. Effect of urine flow rate on the renal clearance of theophylline. (Data from Levy, G., and Koysooko, R.[18])

= 5.5) during the night (35 hr) is about three times higher than during the day (13.5 hr).[17]

Tubular Reabsorption and Urine Flow Rate. Diuresis increases the renal clearance of drugs that are extensively reabsorbed, because it decreases the concentration gradient between the tubular fluid and the blood. The relationship between the renal clearance of theophylline and urine flow rate is shown in Figure 11–3.[18] Fluctuations in the excretion rate of chlorpheniramine[19] and pseudoephedrine[12] coincide with fluctuations in urine flow rate. Suppression of tubular reabsorption by altering urine pH and flow rate is the basis for the use of forced alkaline diuresis in the treatment of certain drug intoxications.

Active Tubular Reabsorption. Although the tubular reabsorption of most drugs is a passive process, there are some important exceptions. Lithium and fluoride appear to undergo active tubular reabsorption. Uric acid is thought to be reabsorbed by an active transport system that is inhibited by uricosuric drugs. The renal clearance of riboflavin in man increases with increasing vitamin concentrations in the plasma, suggesting capacity-limited tubular reabsorption.[20]

Crystalluria. In principle, a drug may be so concentrated in the renal tubules after water reabsorption that precipitation and kidney damage may occur. This phenomenon is termed *crystalluria.* It is of concern with drugs that are given in high doses and are excreted unchanged or converted to a metabolite with limited solubility in the urine. The use of certain sulfonamides has been associated with the formation of crystalline deposits of unchanged and/or acetylated drug in the kidney.[21] Crystalluria has also been observed in patients treated with large doses of ampicillin.[22] Precipitation can be avoided by assuring an adequate rate of urine flow. The minimum rate of urine flow may be calculated by dividing the excretion rate of drug or metabolite by its solubility in urine at a given pH.[23] A minimum urine flow rate of 190 ml/hr is required to prevent precipitation of sulfisoxazole in acidic urine (pH = 5); a flow of only 5 ml/hr is required if urine pH is 7. Hydration and alkalinization of urine during and after large doses of methotrexate are recommended procedures to prevent crystalluria.[10]

Biliary Excretion

A drug may be secreted by the liver cells into the bile and pass into the intestine. Some or most of the secreted drug may be reabsorbed in the small intestine and undergo *enterohepatic cycling;* the rest is excreted in the feces. This cycle may be repeated many times, until biotransformation, renal excretion, and fecal excretion ultimately eliminate the drug from the body. In this way, enterohepatic cycling may increase the persistence of drug in the body.

Often, biotransformation of a drug occurs in the liver, and a glucuronide or some other conjugate is secreted in the bile. In some instances, the polar metabolite cannot be reabsorbed, and fecal excretion occurs. In other cases, deconjugation takes place in the intestine, and the liberated parent drug may be absorbed.

Biliary excretion and renal tubular secretion share certain characteristics. Both are active, capacity-limited processes, subject to competitive inhibition. Concentrations of drug or metabolites in bile are often much higher than in plasma, consistent with an active transport mechanism. Many examples of competitive inhibition of biliary secretion have been reported.[24] Probenecid inhibits the biliary secretion of methotrexate in the rat; this inhibition is associated with increased toxicity.[25] A 25 mg/kg dose of methotrexate produces no mortality. The same dose given with a nontoxic dose of probenecid results in an 80% mortality rate.

In man, bile flow rate ranges from 0.5 to 0.8

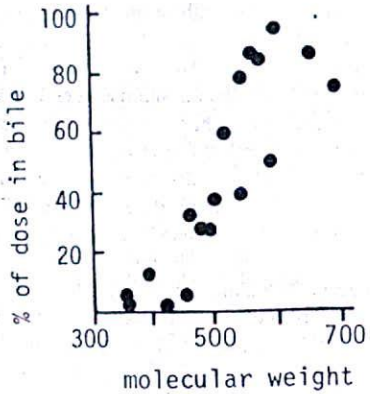


Fig. 11–4. The influence of molecular weight on the biliary excretion of various cephalosporins in the rat. (Data from Wright, W.E., and Line, V.D.[28])

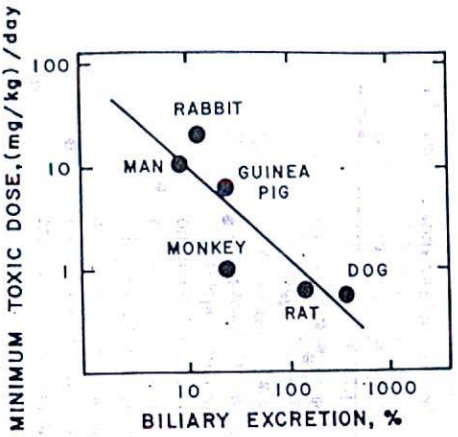


Fig. 11–5. Correlation between total (cumulative) biliary excretion (expressed as percent of dose) and the sensitivity to intestinal lesions in different species. (Data from Duggan, D.E., et al.[31])

ml/min. Factors affecting hepatic bile formation have been reviewed by Javitt.[26] Since the bile to plasma drug concentration ratio can approach 1000, biliary clearances of 500 ml/min or higher may be achieved. In most animal species, including man, drug excreted in the bile enters the intestine after storage in the gallbladder; the rat is unusual in that it has no gallbladder.

Seemingly, the most important factor influencing the excretion of a drug in bile is the molecular weight of the form of the compound excreted. Studies in the rat indicate that compounds having a molecular weight of less than about 300 tend to be excreted in urine, whereas compounds of molecular weight exceeding 300 are found in the bile in appreciable quantities.[27] Figure 11–4 shows the relationship between molecular weight and the biliary excretion of 18 cephalosporins in rats.[28] The molecular weight threshold for appreciable biliary excretion (i.e., > 5 to 10% of the dose) in man appears to be on the order of 400 to 500.[27]

Biliary excretion of drugs seems to be a more important elimination process in laboratory animals, including the rat and dog, than in man.[24] Its actual importance in man, however, is not clear, because information on the biliary excretion of drugs and metabolites in man is limited. Appreciable amounts of indocyanine green, digitoxin, cromolyn, erythromycin, and rifampin are excreted unchanged in the bile in man. Indomethacin, sulfobromophthalein, morphine, carbenoxolone, and estradiol also undergo biliary excretion in man, but largely in the form of conjugates.[29]

Biliary excretion is important in the elimination of rifampin. The half-life of the drug is about twice as long in patients with biliary obstruction as in patients without obstruction (5.7 hr vs 2.6 hr).[30] Unusually pronounced accumulation with repeated doses is found in patients with obstructive jaundice.

Biliary excretion of indomethacin and its conjugates appears to be an important if not a causative factor for intestinal lesions following indomethacin administration in many species.[31] The correlation between the cumulative biliary excretion of indomethacin and its conjugates and the sensitivity to intestinal lesions in different species is shown in Figure 11–5. Note that enterohepatic cycling in the dog and rat is so extensive that the intestine is exposed to more than 100% of the dose. Biliary excretion is also a factor in the elimination of sulindac, another nonsteroidal anti-inflammatory, in man.[32]

Recent investigations have been directed toward detecting enterohepatic cycling in man by interrupting the cycle in the intestine. For example, oral administration of cholestyramine, a nonabsorbable ion-exchange resin that strongly binds acid and neutral drugs, decreases the half-life of digitoxin from 6.0 to 4.5 days and reduces the effects of the drug on ECG values.[33] These results suggest that a significant fraction of the dose of digitoxin undergoes enterohepatic cycling; cholestyramine interrupts the cycle and promotes fecal excretion.

Cholestyramine has also been reported to decrease the half-life and anticoagulant effect and increase the clearance of warfarin in healthy human subjects.[34]

Oral administration of charcoal, a nonspecific adsorbent, is expected to produce results similar to those of cholestyramine. Charcoal administration decreases the half-life of the antileprotic drug dapsone from 20.5 to 10.8 hr.[35] Charcoal also enhances the elimination of phenobarbital, carbamazepine, and phenylbutazone in man.[36] Repeated doses of activated charcoal from 6 hr on after oral administration of a single dose of amitriptyline resulted in a significant decrease in half-life and total AUC of parent drug as well as of nortriptyline, its active metabolite.[37]

The results with nonabsorbable adsorbents are surprising, because they suggest that a far wider variety of drugs is cycled between the gut and systemic circulation in man than we suspected, based on our understanding of biliary excretion. An alternative explanation is that some drugs are secreted into the gut lumen by a nonbiliary mechanism and that this secretion is promoted by intestinal adsorbents. Studies with the toxic organochlorine pesticide chlordecone (Kepone) support this alternative mechanism.

Cholestyramine is useful in the treatment of chlordecone intoxication, because it increases the excretion of the pesticide in the stools.[38] These findings suggest that enterohepatic cycling of chlordecone is significant. Fecal excretion of chlordecone, however, is observed in man and rat even when bile is totally diverted from the small intestine.[39] Therefore, chlordecone must enter the human and rat small intestine by a nonbiliary mechanism as well as through bile. This alternative entry mechanism probably involves direct secretion or partitioning from the bloodstream to the lumen across the gut wall. This mechanism may apply to other drugs and chemicals.

Salivary Excretion

Transfer of drugs from blood to saliva depends on lipid solubility, pKa, and plasma protein binding. The concentration of a lipid-soluble nonionized drug in saliva approximates the free (unbound) drug concentration in plasma. Since the average pH of saliva (6.5) is lower than the pH of plasma (7.4), saliva/plasma free drug concentration ratios are less than unity for weak acids, and exceed unity for weak bases. Some drugs may be actively transported from blood to saliva. For example, the concentration of lithium in saliva of healthy subjects is reported to be 2 to 3 times higher than in plasma.[40] Metoprolol concentrations in saliva have been reported to be much higher than can be predicted from the pKa of the drug.[41]

Salivary excretion is of little quantitative importance in drug disposition; however, the salivary excretion of certain drugs may be of therapeutic interest. For example, the effectiveness of rifampin against meningococci harbored in the nasopharynx is thought to be related to the fact that salivary concentrations in patients receiving the drug exceed the minimum inhibitory concentration of rifampin for the carrier strains.[42] Salivary excretion of antibiotics may be a cause of lingua nigra or black hairy tongue in patients receiving these drugs.[43] Gingival hyperplasia in epileptics may be related to salivary excretion of phenytoin.

The relatively constant saliva/plasma drug concentration ratio for certain drugs has created interest in the use of drug concentration in saliva as an indicator of total or free drug concentration in plasma. Good to excellent correlations between drug concentrations in saliva and plasma or serum have been reported for antipyrine, theophylline, lithium, salicylate, phenytoin, phenacetin, carbamazepine, sulfapyridine, caffeine, ethanol, diazepam, phenobarbital, primidone, quinidine, acetaminophen, tolbutamide, and digoxin.

Although many reports suggest little variability in saliva-plasma concentration ratios among subjects, other reports are less encouraging. Large differences among relatively sick patients and within individual patients in saliva/plasma procainamide concentration ratios have been reported;[44] ratios ranged from 0.3 to 8.8. One patient had a ratio of 7.2 on one occasion and 2.8 on another. In general, the variability in ratios was related to variability in saliva pH, which ranged from 6.2 to 8.0. Patients with relatively low saliva pH values had relatively high saliva/plasma procainamide concentration ratios. These findings are consistent with the fact that procainamide is a weak base, with a pKa of 9.4.

The relationship between saliva/plasma drug concentration ratio, pKa, pH, and binding is expressed in the following equations:

$$\frac{C_s}{C_p} = \frac{1 + 10^{(pH_s - pKa)}}{1 + 10^{(pH_p - pKa)}} \times \frac{f_p}{f_s} \quad (11\text{–}14)$$

which applies to weak acids, and

$$\frac{C_s}{C_p} = \frac{1 + 10^{(pKa - pH_s)}}{1 + 10^{(pKa - pH_p)}} \times \frac{f_p}{f_s} \quad (11\text{–}15)$$

which applies to weak bases. The terms C_s and C_p refer to drug concentrations, pH_s and pH_p refer to pH, and f_p and f_s refer to free fractions in saliva and plasma. No drug binding has been reported in saliva; therefore, $f_s = 1$. The saliva to plasma concentration ratio of tolbutamide, a weak acid, has been calculated to be 0.012, based on the following values: pKa = 5.4, pH_s = 6.5, pH_p = 7.4, f_p = 0.09, and f_s = 1.0. This estimate agrees with experimental observations.[45] The difference in pH between saliva and plasma results in a saliva level that is much smaller than free tolbutamide concentration in plasma. The saliva/plasma concentration ratios of tolbutamide and chlorpropamide (weak acids), and procainamide and meperidine (weak bases) are sensitive to saliva pH over a wide range of values, from 6.0 to 8.0.[46] The saliva/plasma concentration ratio of phenobarbital is relatively independent of saliva pH up to pH 7.

Secretion of Drugs into Milk

The excretion of drugs in breast milk has received considerable attention.[47–49,50] Drugs ingested by a lactating mother must be expected to appear in her milk and be ingested by a breast-feeding infant.

Distribution into milk has been studied in some detail in goats and cows. These studies suggest that drug transfer between milk and plasma occurs by passive diffusion, consistent with pH-partition theory. Milk is generally more acidic than plasma, so weak bases tend to concentrate there, whereas weak acids tend to have milk-to-plasma (M/P) concentration ratios less than one.

Milk is a complex fluid with high fat and protein levels, but composition varies widely among species. This has hampered the development of animal models. A useful approach, which takes into account milk and plasma pH, milk and plasma protein binding, and milk fat partitioning, has been proposed for predicting M/P ratios in breast-feeding women.[51] The model was developed by assuming that only the unbound, un-ionized form of a drug located in the aqueous phases of blood and milk can diffuse across mammary membranes and that no carrier-mediated transfer occurs. Under these condition, at steady state, un-ionized free drug concentration in plasma equals un-ionized free drug concentration in skim milk, the aqueous phase of milk.

Drug concentration in skim milk (C_{SM}) is related to concentration in whole milk (C_M) as follows:

$$C_{SM} = (S/M)\, C_M \quad (11\text{–}16)$$

where S/M refers to the skim-to-whole milk drug concentration ratio.

Only that fraction of the drug in the aqueous phase not bound to milk proteins (f_{SM}) and un-ionized ($f_{SM\ un}$) is available for diffusion. Therefore,

C_{SM}, unbound, un-ionized

$$= (f_{SM})(f_{SM\ un})(S/M)\, C_M \quad (11\text{–}17)$$

A similar expression can be written for the un-ionized, free drug concentration in plasma, i.e.,

C_P, unbound, un-ionized

$$= (f_P)(f_{P\ un})\, C_P \quad (11\text{–}18)$$

Therefore, at steady state,

$$(f_{SM})(f_{SM\ un})(S/M)\, C_M = (f_P)(f_{P\ un})\, C_P \quad (11\text{–}19)$$

and the milk-to-plasma drug concentration ratio is given by:

$$M/P = C_M/C_P$$

$$= (f_P)(f_{P\ un}) / (f_{SM})(f_{SM\ un})(S/M) \quad (11\text{–}20)$$

Fleishaker et al.[51] used this approach, adding drugs to milk and plasma in vitro, to predict the distribution of diazepam, phenytoin, and propranolol in breast milk from lactating women. Single milk and serum samples were collected in the morning from each woman participating in the study. Milk was obtained by completely emptying the breast using an electric pump. After collection, pH and fat content were determined. Skim milk was prepared by centrifuging whole milk. Serum and skim milk protein binding was determined by equilibrium dialysis. S/M ratios were estimated after incubation of whole milk with drug for 1 hr.

The test drugs were bound to a smaller extent in skim milk than in serum. For example, at phenytoin concentrations ordinarily found in plasma during treatment with the drug, binding was 86% in serum but only 42% in milk. The S/M ratio was less than one for each drug indicating significant partitioning into milk fat. Diazepam showed the highest affinity with an S/M ratio of only 0.22.

The values of S/M, free fraction in skim milk

and plasma, and fraction of drug un-ionized in skim milk and plasma (estimated by assuming a plasma pH of 7.4 and a milk pH of 7.1) for each drug were used to calculate M/P ratios, according to Equation 11–20. Estimates were 0.16 for diazepam, 0.50 for propranolol, and 0.30 for phenytoin, in good agreement with published in vivo values found in nursing mothers.

The lowest M/P ratio was found with diazepam because it is the most extensively bound to plasma proteins (99.7%). If plasma protein binding were ignored, the M/P ratio would be about 1.0 rather than 0.16. Binding to milk proteins and partitioning into milk fat are also important. If these factors were ignored, the M/P ratio would be 0.013. Only when all factors are taken into account are reasonable predictions obtained.

Using the M/P ratio and the measured or calculated average steady-state drug concentration in maternal plasma (C_{ss}), one can predict the average drug concentration in milk and the amount of drug that will be ingested by the infant. Steady-state levels in the mother may be calculated as follows:

$$C_{ss} = DR \times F/Cl \qquad (11–21)$$

where DR is the dosing rate (i.e., the daily dose divided by 24 hours), F is the bioavailability of the drug, and Cl is drug clearance in the mother.

Average drug concentration in milk is the product of C_{ss} and M/P. The dose of drug ingested by the infant is the product of average drug concentration in milk and the volume of milk consumed. However, more drug will be ingested if breast feeding coincides with peak drug concentration in the mother, and less drug will be ingested if nursing takes place immediately before the mother's next dose. An average infant consumes about 150 ml of milk per kg of body weight per day. The ingested dose can then be compared, on a mg/kg basis, to the usual adult dose to assess the level of risk.

One commentary on breast feeding concluded that most drugs taken by a nursing mother will be excreted in her milk, but in amounts that are unlikely to harm the infant. Theophylline is a likely example. An average steady-state theophylline concentration of 15 μg/ml in the plasma of a nursing mother gives rise to an average level of about 12 μg/ml in breast milk.[52] If the infant nursed 1 liter of milk per day, about 12 mg of theophylline would be ingested or 2 mg/kg/day for a 6-kg infant. This amount of theophylline is relatively small compared with the usual initial adult dose of 4 mg/kg every 8 to 12 hr and is unlikely to cause harm.

A direct comparison of the infant dose with the usual adult dose may be misleading. Drug elimination in the infant may be comparatively poor because of immature renal function and incomplete development of hepatic microsomal enzymes. One report suggests that an adjustment factor accounting for the likely impairment of drug clearance in the infant is needed to assess the relative risk of drug ingestion in the breast-feeding infant.[50] These investigators suggest that a 3-day old term infant ingesting one-half the maternal dose in breast milk, on a mg/kg or mg/M² basis, must be assumed to have 50% greater exposure to the drug because of reduced clearance.

A review dealing specifically with psychotropic drugs in breast milk states:[53]

> With our present inadequate knowledge, it is difficult to prepare a list of drugs that are safe or are harmful to the breast-fed infant. However, we do know that drugs such as diazepam, lithium, bromides, reserpine and opium alkaloids are to be avoided and that barbiturates, haloperidol, and penfluridol should be administered (to the mother) with caution.

The Medical Letter on Drugs and Therapeutics updated information on drugs in breast milk in 1979.[54] It concluded as follows:

> Wherever possible, nursing mothers should not take drugs. Mothers who must take antithyroid drugs (especially radioactive iodine), lithium, chloramphenicol and probably most anticancer drugs should not nurse. The safety of many other drugs for use during nursing is not known.

A committee on drugs[54a] concluded in 1983 that the following agents are contraindicated during breast feeding: amethopterin, cyclophosphamide, and perhaps other cytotoxic agents because of possible immune suppression, association with carcinogenesis, and unknown effects on growth, bromocriptine because it suppresses lactation, and methimazole and thiouracil because of potential effects on the infant's thyroid function. Cimetidine, clemastine, ergotamine, gold salts, and phenindione were also cited as contraindicated. Metronidazole and various radiopharmaceuticals were listed as drugs that require temporary cessation of breast feeding for 1 to 14 days to allow elimination of the dose.

DRUG METABOLISM

Drug metabolism or biotransformation refers to the biochemical (enzymatic) conversion of a drug

to another chemical form. Many tissues in the body are capable of metabolizing drugs, but most drugs are mainly metabolized in the liver by enzymes localized in hepatic microsomes, a cellular fraction derived from the endoplasmic reticulum.

Drug-metabolizing enzymes oxidize, reduce, hydrolyze, or conjugate compounds. Reduction, oxidation, and hydrolytic reactions (Phase I pathways) result in metabolites with functional groups (e.g., hydroxyl, amine, or carboxyl) that can be conjugated (Phase II pathways). In man the most common conjugations of drugs or metabolites occur with acetate, sulfate, glycine, or glucuronic acid. Examples of the more important drug metabolism pathways in man are given in Table 11–1.

Most oxidative processes take place in liver microsomes. They require reduced nicotinamide phosphate (NADPH), molecular oxygen, and a complex of enzymes in the endoplasmic reticulum; the terminal oxidizing enzyme is cytochrome P-450, a hemeprotein. Many drugs, as well as steroid hormones, are oxidized by this microsomal system. Oxidation of certain drugs, such as alcohols and xanthines, may be catalyzed by nonmicrosomal enzymes; ethanol, mercaptopurine, and azothioprine are examples.

Reduction is a relatively uncommon pathway of drug metabolism. Azo dyes, used as food coloring, are reduced to form amines, both in the liver and by intestinal flora. Sulfasalazine is also cleaved by intestinal bacteria to form aminosalicylate, the active component, and sulfapyridine. Prednisone and cortisone are also reduced to active metabolites, prednisolone and hydrocortisone.

Digoxin is metabolized by anaerobic intestinal bacteria in the lower gastrointestinal tract to cardioinactive compounds called *digoxin reduction products*.[55] Digoxin inactivation by this pathway affects the bioavailability of the cardiac glycoside and results in increased dosage requirements for some patients. Factors found to increase inactivation include rapid gastric emptying and hypermotility as well as the administration of slowly dissolving dosage forms of digoxin. In such cases, a significant fraction of the dose reaches the lower bowel and is subject to reduction and inactivation.

Other investigators observed that although the oral anticoagulant acenocoumarol is efficiently metabolized by intestinal flora to its inactive amino metabolite, this is not a clinical problem because the drug is rapidly absorbed from the upper gastrointestinal tract after oral administration of commercial tablets and never reaches sites in the gut with a high density of microflora. On the other hand, appreciable amounts of reduced metabolites are recovered in the urine when acenocoumarol is given in a slowly-dissolving dosage form.[56]

Hydrolysis of esters and amides is a common pathway of drug metabolism. The liver microsomes contain nonspecific esterases, as do other tissues and plasma.

Glucuronide formation is the most common conjugation process of drug metabolism. It involves the reaction between uridine diphosphate glucuronic acid (UDPG) and drugs containing hydroxyl, carboxyl, or amine groups. The reaction is mediated by the microsomal enzyme glucuronyltransferase. Glucuronides are water soluble acids that are easily excreted in urine and bile. Some ester glucuronides are labile and can be hydrolyzed in urine or plasma to parent drug. High blood levels of clofibrate in patients with renal disease are the result of accumulation and hydrolysis of the glucuronide conjugate in the plasma.[57]

Aromatic acids are sometimes converted to glycine conjugates. The acids are activated by combining with ATP to form coenzyme A derivatives before conjugation with glycine. The conversion of benzoic acid to hippuric acid and salicylic acid to salicyluric acid are examples of this metabolic pathway.

Many amine compounds, including sulfonamides, isoniazid, dapsone, hydralazine, and procainamide, are metabolized to their acetyl derivative by acetylcoenzyme A and acetyltransferase.

Conjugation with sulfate is a common pathway of metabolism of hydroxy compounds, particularly steroids. The sulfate donor is 3′-phosphoadenosine-5′-phosphosulfate (PAPS). Sulfate conjugates, like glucuronides, have a high renal clearance.

Drugs subject to biotransformation usually produce several metabolites. In some instances, dozens of metabolites originate from the administration of a single drug (e.g., chlorpromazine). Metabolites can arise from parallel or consecutive pathways. For example, meperidine is simultaneously metabolized to normeperidine (N-dealkylation) and meperidinic acid (ester hydrolysis); salicylate is simultaneously conjugated with glycine and glucuronic acid. On the other hand, phenytoin is largely metabolized to 5-phenyl-5-hydroxyphenylhydantoin (aromatic hydroxylation), which in turn is conjugated with glucuronic acid; phenacetin is oxi-

Table 11–1. Metabolic Pathways for Drugs in Man

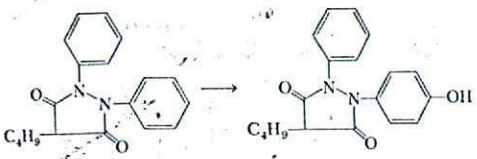
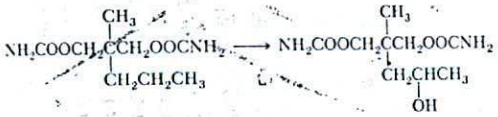
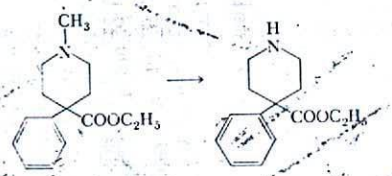
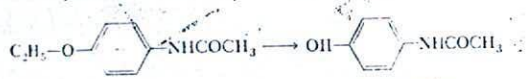
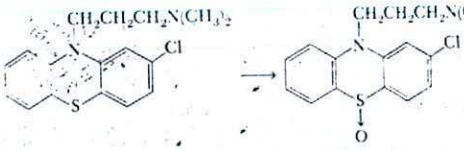
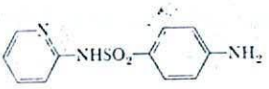
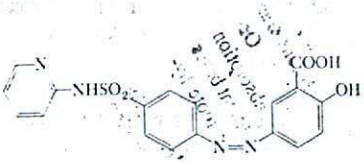
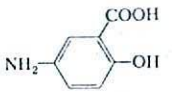
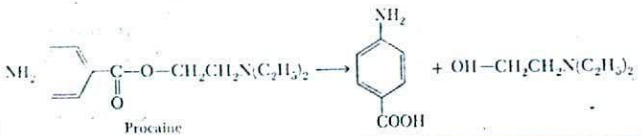
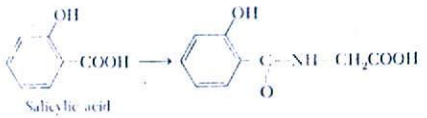
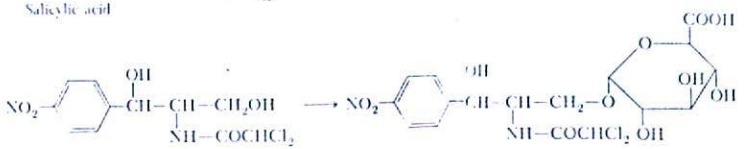
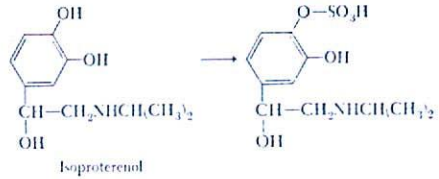
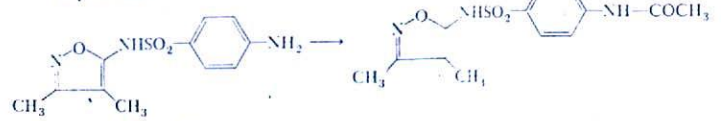
Type of reaction	Example
1. Oxidation	
a. Aromatic hydroxylation	 Phenylbutazone
b. Aliphatic hydroxylation	 Meprobamate
c. Oxidative N-dealkylation	 Meperidine
d. Oxidative O-dealkylation	 Phenacetin Acetaminophen
e. S-oxidation	 Chlorpromazine
2. Reduction	 Sulfapyridine  Sulfasalazine  5-Aminosalicylate
3. Hydrolysis	 Procaine

Table 11–1. (Cont.)

Type of reaction	Example
1. Conjugation a. Glycine	 Salicylic acid
b. Glucuronic acid	 Chloramphenicol
c. Sulfate	 Isoproterenol
d. Acetylation	 Sulfisoxazole

dized (dealkylation) to acetaminophen, which in turn is sulfated and glucuronidated.

Induction and Inhibition of Drug Metabolizing Enzymes

Microsomal drug metabolism can be stimulated by a large number of drugs and chemicals by a process known as enzyme induction.[58] Microsomal enzyme induction is a complex process associated with an increase in liver weight, proliferation of the endoplasmic reticulum, and increases in microsomal protein and cytochrome P-450. Elevated levels of cytochrome P-450 are the result of increased synthesis.

At least two kinds of inducers have been found, exemplified by phenobarbital and polycyclic aromatic hydrocarbons. Differences between these kinds of inducers have been discussed by Conney.[58] Phenobarbital-type inducers stimulate a wide range of metabolic pathways in liver microsomes, including oxidation, reduction, and glucuronide formation. Polycyclic aromatic hydrocarbons, such as 3-methylcholanthrene or cigarette smoke, stimulate a more limited group of metabolic reactions. Phenobarbital enhances the metabolism of meperidine to normeperidine but polycyclic hydrocarbons do not. Cigarette smoke stimulates the metabolism of theophylline but phenobarbital does not.

The effect of smoking on estrogen metabolism was studied in postmenopausal women treated for 1 year with different doses of oral estradiol. Estrogen levels were lower in smokers than nonsmokers, particularly so in subjects receiving high-dose estradiol (4 mg). In this group, serum levels of estrone and estradiol in smokers were only 50% of those in nonsmokers. Moreover, a significant inverse correlation was found between the number of cigarettes smoked daily and the changes in the levels of serum estrone and estradiol. This report suggests that increased hepatic metabolism results in lower estrogen levels among postmenopausal smokers, which may contribute to the reported increased risk of osteoporosis among smokers.[59]

Some drugs induce microsomal enzymes that play a role in their own metabolism. This phenomenon has been called self-induction or autoinduction. Carbamazepine, a widely used anticonvulsant, is a well-known example. The metabolic clearance of carbamazepine increases on continu-

ous administration until the microsomal enzymes are fully induced.

Rapid development of enhanced clearance after high-dose cyclophosphamide, possibly indicative of autoinduction, has also been reported.[60] The mean clearance of cyclophosphamide was observed to increase from 93 ml/min on the first day of treatment to 178 ml/min on the second day. This was associated with an increase in the mean clearance of coadministered dexamethasone from 369 ml/min to 526 ml/min. An increased rate of formation of phosphoramide mustard, an active metabolite of cyclophosphamide, with higher peak concentrations was also seen. These results suggest that high-dose cyclophosphamide causes an increase in its own clearance through an apparent induction of hepatic-metabolizing enzymes, detectable 24 hours after initial exposure to cyclophosphamide.

Today, far more drug metabolism and interaction studies are concerned with inhibition of microsomal enzymes rather than induction. Certain drugs, including monoamine oxidase inhibitors, such as isocarboxazid, phenelzine, and tranylcypromine, and xanthine oxidase inhibitors, such as allopurinol, are used clinically to inhibit specific enzyme systems. Usually, these drugs are not as specific as we wish, and more than one enzyme system is inhibited. This lack of specificity applies to many drugs. The antiulcer drug cimetidine is recognized as one of the most potent and comprehensive inhibitors of microsomal drug metabolism used in clinical medicine.

The mechanisms by which drugs produce enzyme inhibition are poorly understood. Possibilities include:[61] (1) substrate competition (two drugs competing for the same enzyme); (2) competitive or noncompetitive inhibition (a substance, which is not necessarily a substrate, reduces the affinity of the enzyme for its substrate); (3) product inhibition (the product of the enzyme reaction, the metabolite, competes with the substrate); and (4) repression (the amount of enzyme is reduced, either by decreased formation or increased destruction).

Ordinarily, the administration of a drug with an inhibitor of drug-metabolizing enzymes signals a potentially undesirable drug interaction, occasionally requiring a reduction in the dose of the drug to avoid dangerously high blood levels. Sometimes, inhibition may be used to advantage to improve the delivery or extend the persistence of a drug.

A well recognized example is the combination of levodopa and carbidopa. This combination is considered the treatment of choice by most neurologists when symptoms of Parkinson's disease significantly interfere with normal daily activities. Carbidopa is a dopa decarboxylase inhibitor that does not cross the blood-brain barrier and, therefore, does not prevent the conversion of levodopa to dopamine in the central nervous system. By preventing the extracerebral metabolism of levodopa, carbidopa increases the amount available in the brain for decarboxylation to dopamine. This serves to enhance the therapeutic response and reduce adverse events caused by peripheral effects of dopamine and other catecholamines. This combination increases the plasma concentration of levodopa, reduces dosage requirements by about 75%, and significantly decreases the incidence of nausea and vomiting.

Cytarabine (Ara-C) must be given by intravenous infusion for the treatment of lymphatic cancers because absorption from the gastrointestinal tract is poor. Rectal administration results in a bioavailability of only 6%. Poor bioavailability may be related in part to inactivation of cytarabine to Ara-U by deaminase enzymes in the lower bowel and/or in the liver. With this in mind, investigators administered rectal cytarabine with 3,4,5,6-tetrahydrouridine (THU), which inhibits deamination. This combination resulted in nearly a 4-fold increase in the blood levels of cytarabine. The investigators concluded that a suppository containing cytarabine and THU may be a useful alternative to slow iv infusion of the drug.[62]

Imipenem is the first of a new class of beta-lactam antibiotics called carbapenems. These compounds have broader activity against bacteria than even the third-generation cephalosporins. Studies early in the development of imipenem indicated that the beta-lactam ring was hydrolyzed by a renal dehydropeptidase enzyme in the brush border of the kidney. Seemingly associated with this process was the occurrence of nephrotoxicity in several animal species. The simultaneous administration of cilastatin, a dehydropeptidase inhibitor with no antibacterial activity, eliminated the nephrotoxicity potential. The commercial dosage form contains imipenem and cilastatin in a 1:1 ratio. Cilastatin has no effect on the pharmacokinetics of imipenem but does increase the fraction of the dose excreted unchanged in the urine.[63]

Active Metabolites

Metabolites usually differ considerably from the parent drug with respect to pharmacologic effects and disposition. Most are rapidly eliminated and have little pharmacologic activity, but some play an important role in the effects of the drug.

Oxyphenbutazone (from phenylbutazone), acetaminophen (from phenacetin), nortriptyline (from amitriptyline), morphine (from codeine), prednisolone (from prednisone), mesoridazine (from thioridazine), and desipramine (from imipramine) are examples of metabolites that have been used as drugs in their own right. Primidone, an anticonvulsant, is appreciably metabolized to phenobarbital, which undoubtedly contributes to the efficacy of this drug in the treatment of seizures. Several of the metabolites of diazepam, including nordiazepam and oxazepam, are active, as is the acetyl metabolite of procainamide.

The activity of some drugs may reside wholly in one or more metabolites. Drugs that require bioactivation to be useful are sometimes called prodrugs. Cyclophosphamide is an example. It was developed with the intention of slowly releasing phosphoramide mustard, one of several active metabolites, in order to prolong the cytotoxic effects of the mustard. Levodopa is an inactive precursor of dopamine. It is used because, unlike dopamine, levodopa can cross the blood-brain barrier and then undergo decarboxylation to form the active drug.

Prednisone and clorazepate are also prodrugs but not very useful ones. Prednisone is not active per se but is the precursor of prednisolone. Prednisolone, however, is formed so rapidly after the administration of prednisone that there is no difference in clinical use in giving prednisolone or prednisone. Clorazepate is a precursor of N-desmethyl diazepam (nordiazepam). Ordinarily, rapid and complete conversion of clorazepate to nordiazepam occurs in gastric acid after oral administration. However, since the elimination half-life of nordiazepam is several days, it is difficult to understand why clorazepate should be used instead of nordiazepam, other than to avoid patent infringement.

Some active metabolites exert the same kind of pharmacologic effect as the parent compound. N-acetylprocainamide is an antiarrhythmic agent, acetaminophen like phenacetin is an analgesic, and nortriptyline and desipramine are antidepressants as well as metabolites of amitriptyline and imipramine, respectively.

The major pathway for the biotransformation of morphine is hepatic glucuronidation to morphine-3-glucuronide and to a lesser extent to morphine-6-glucuronide (M6G). The results of recent studies point to the potential significance of M6G in the clinical effects of morphine. Persistent narcotic effects in the presence of M6G accumulation in patients with renal failure provide circumstantial evidence for the metabolite's clinical activity. More directly, significant analgesic activity has been found after iv administration of M6G to cancer patients.[64] No morphine or morphine-3-glucuronide was detected in plasma at any time after administration.

While encainide is an effective antiarrhythmic agent, there is considerable evidence to suggest that active metabolites mediate most of the effects seen during long-term treatment. In most patients concentrations of the active metabolites *O*-desmethyl encainide (ODE) and 3-methoxy-*O*-desmethyl encainide (MODE) are higher than those of encainide. Moreover, the correlations between antiarrhythmic activity and either ODE or MODE plasma levels are much stronger than with plasma levels of encainide itself.

In perhaps 5 to 10% of patients, however, who appear to be genetically poor metabolizers of encainide and certain other drugs, mean plasma encainide concentrations are unusually high while low or negligible levels of ODE and MODE are seen. In these patients, the long-term effects of encainide may be mediated largely by the parent drug.[65]

In some cases, active metabolites appear to have a mechanism of action different from the parent drug. For example, normeperidine is much less potent as an analgesic but more potent as a central nervous system stimulant and convulsant than meperidine, the parent drug. Normeperidine is thought to be largely responsible for the adverse effects of meperidine.

Some investigators hold that the cardiotoxicity of doxorubicin is more closely related to its major metabolite doxorubicinol than to the parent drug itself.[66] The reduced metabolite was markedly more potent than parent drug at compromising both systolic and diastolic cardiac function in isolated dog tissue. On the other hand, the cytotoxicity of doxorubicinol in tumor cell lines was far less than doxorubicin. The investigators suggested that in-

hibition of aldoketo reductases that catalyze the reduction of doxorubicin to doxorubicinol would result in more drug to kill cancer cells but less metabolite to compromise cardiac function.

A clue as to the source of drug toxicity—metabolite or parent—may be obtained in some instances by administering the parent with an inhibitor of drug metabolism. A more favorable safety profile implicates one or more metabolites. Cyproheptadine, a drug with both antihistaminic and antiserotonergic activity, damages the insulin-secreting cells of the pancreas to produce a diabetic state. Administration of an inhibitor of drug metabolism effectively protects against the insulin loss induced by cyproheptadine.[67] These findings suggest that a metabolite may be involved in the pancreatic beta-cell toxicity of cyproheptadine.

Sometimes, the formation of relatively small amounts of a reactive metabolite is singularly responsible for toxic effects of the drug.[68] Large doses of acetaminophen are hepatotoxic because of the reaction of a minor metabolite with liver proteins. Metabolites of halothane are also reactive, combining with phospholipids and proteins of the endoplasmic reticulum in the liver. Methoxyflurane and, to a lesser extent, enflurane produce renal toxicity because of the liberation of inorganic fluoride.

In a small number of patients, perhaps as few as 1 in 10,000, treatment with anticonvulsant medication results in a hypersensitivity syndrome usually requiring discontinuance of therapy. Typically, the reaction is delayed in onset after initiation of drug therapy and can cause severe morbidity or even result in death.

The low incidence of anticonvulsant hypersensitivity suggests that this risk may be secondary to a genetic defect in drug metabolism. With this in mind, investigators have studied aromatic anticonvulsants to help understand the pathogenesis of idiosyncratic reactions.[69]

Phenytoin, phenobarbital, and carbamazepine are metabolized to hydroxylated aromatic compounds. Reactive aromatic epoxides called arene oxides are intermediates in this process. Arene oxides may bind to cellular macromolecules, interfering with cell function and initiating immunologic response. Epoxide hydrolases are cellular enzymes critical for the detoxification of arene oxides. Studies have suggested that genetically-deficient detoxification of arene oxides might predispose patients to toxicity.

Lymphocytes from patients with suspected hypersensitivity to anticonvulsants were incubated in vitro with metabolites of phenytoin, phenobarbital, or carbamazepine, generated by hepatic microsomes from enzyme-induced mice. Healthy human subjects never exposed to anticonvulsant drugs and patients with seizure disorders chronically treated with anticonvulsants without immunologically-based adverse effects provided the control lymphocytes.

Defining cytotoxicity as the percentage of dead cells above baseline, the investigators determined that the toxic effects of drug metabolites on lymphocytes from control subjects were $< 1\%$ for phenytoin and phenobarbital and 3.6% for carbamazepine. Toxicity of the drug metabolites to cells from patients with suspected hypersensitivity differed significantly from controls: 13.5% for phenytoin, 13.3% for phenobarbital, and 20.6% for carbamazepine.

The correlation of clinical response to treatment with anticonvulsants with in vitro lymphocyte results was impressive. All 34 patients who had hypersensitivity reactions to phenytoin, also had positive in vitro results (i.e., true positives). One patient treated with phenytoin with no adverse effects, had a negative in vitro test (true negative). Results were similar for carbamazepine: 25 true positives and 2 true negatives. For patients treated with phenobarbital, there were 21 true positives and 3 true negatives, but two false positives and one false negative were also obtained.

The investigators concluded that "the in vitro lymphocyte toxicity assay provides a model for the investigation of some hypersensitivity reactions. Our work suggests that it may aid in diagnosis and in the prediction of adverse reactions."[69]

The offspring of women with epilepsy treated during pregnancy have a higher incidence of congenital malformations than do those born to women with untreated epilepsy or women without epilepsy. A major risk factor for this high incidence of malformation is the administration of valproic acid in combination with other anticonvulsants, particularly phenytoin and carbamazepine.[70] Recent evidence suggests that valproic acid inhibits epoxide hydrolase, the microsomal enzyme required to detoxify unstable, reactive arene oxide metabolites, including those formed in the oxidative metabolism of phenytoin and carbamazepine.[71] These findings support the recommendation that combination drug

therapy with valproic acid should be avoided during pregnancy.

Disposition of Metabolites

Although important exceptions exist, most biotransformations result in metabolites that are considerably less pharmacologically active than the parent compounds. Most metabolites are also more polar than their precursors. Distribution of certain metabolites, such as glucuronide and sulfate conjugates, tends to be limited to the extracellular space. The apparent volume of distribution of a metabolite is usually less than that of the parent drug. Metabolites are excreted in the urine more readily than their precursors because often they are not subject to tubular reabsorption.

Renal excretion plays a major role in the elimination of metabolites. Considerable accumulation of drug metabolites is found in patients with renal impairment. Steady-state levels of propranolol glucuronide, 4-hydroxypropranol glucuronide, and naphthoxylactic acid, the principal metabolites of propranolol in plasma, in uremic patients are 20 to 30 times as high as in patients with normal renal function.[72] Some active metabolites may also accumulate in patients with renal failure.[73] Examples include the metabolites of allopurinol, procainamide, and clofibrate.

Metabolite Kinetics

The amount of a metabolite in the body at any time after administration of parent drug is a function of its formation rate and its elimination rate. Either step in this process may be rate limiting. When the elimination rate constant of the drug is smaller than that of the metabolite, metabolite levels decline in parallel with levels of parent drug (i.e., the half-life of both the drug and metabolite appear to be the same). When administered as such, the real elimination half-life of the metabolite may be found to be much shorter than that of the parent drug, but when it is formed from parent drug, the apparent half-life of the metabolite is never less than that of the parent.

When the second step in the process is rate limiting (i.e., the elimination rate constant of the metabolite is smaller than that of the drug), levels of drug and metabolite do not decline in parallel. The half-life of the metabolite is always greater than that of the drug and is the same whether administered or formed.

When metabolite levels are formation rate-limited, the metabolite is cleared so quickly that the amount in the body is kept quite low, always lower than the amount of parent drug. When metabolite levels are elimination rate-limited, it is likely that metabolite will accumulate on repetitive dosing of the parent drug and steady-state levels will be higher than those of the parent.

Sometimes, a pharmacokinetic study will find that the apparent half-life of a metabolite is equal to that of the parent drug, suggesting formation-rate limited elimination, but metabolite concentrations in plasma are higher than those of parent drug. This appears to be contradictory because formation-rate limited means that only small amounts of metabolite are in the body at any time. However, if the metabolite has a much smaller volume of distribution than the parent drug, this small amount of metabolite may result in a higher blood level than will a larger amount of parent drug.

After administration of drug, the total area under the plasma level-time curve (AUC) can be calculated for both metabolite and drug. The ratio of these AUC values is as follows:

$$\mathrm{AUC(m)/AUC} = \mathrm{Cl_f/Cl(m)} \quad (11\text{–}22)$$

where AUC(m) is the area for the metabolite, AUC is the area for the drug, Cl_f is the formation clearance for converting the drug to the particular metabolite, and Cl(m) is the elimination clearance of the metabolite.

Substituting fm (Cl) for Cl_f, where fm is fraction of the dose of drug converted to the metabolite and Cl is drug clearance, yields

$$\frac{\mathrm{AUC(m)}}{\mathrm{AUC}} = \frac{\mathrm{fm\ Clearance\ of\ drug}}{\mathrm{Clearance\ of\ metabolite}} \quad (11\text{–}23)$$

After a single dose of parent drug, AUC, AUC(m), and drug clearance can be calculated. Sometimes, fm may also be estimated, from urinary excretion data. When this is possible, the clearance of the metabolite may be calculated by means of Equation 11–23.

When the entire dose of a drug, or nearly so, can be accounted for by drug-related compounds in the urine, then fm is the amount of the particular metabolite excreted in the urine divided by the dose. Often, urinary recovery is far less than the administered dose. This may result from alternative excretion pathways (e.g., bile) or the formation and excretion of unrecognized metabolites. Some investigators have estimated fm from these data by comparing the amount of the particular metabolite excreted in the urine to the total amount of drug-

related material in the urine. Error may be introduced by this approach. For example, if a metabolite were eliminated by both biliary and urinary excretion, the amount recovered in the urine would be less than the amount formed and both fm and metabolite clearance would be miscalculated.

The mean residence time of a metabolite can usually be calculated after intravenous or oral administration of parent drug. After iv drug, the apparent mean residence time of metabolite ($MRT_{M/P}$) is given by

$$MRT_{M/P} = MRT + MRT_M \quad (11\text{–}24)$$

where MRT is the mean residence time of the drug and MRT_M is the mean residence time of the metabolite. On rearrangement,

$$MRT_M = MRT_{M/P} - MRT \quad (11\text{–}25)$$

After oral administration,

$$MRT_M = MRT_{M/P(ORAL)} - MRT_{(ORAL)} \quad (11\text{–}26)$$

where $MRT_{M/P(ORAL)}$ is the apparent mean residence time of metabolite after oral administration of parent drug and $MRT_{(ORAL)}$ is apparent mean residence time of drug after oral administration.

If a drug distributes rapidly, so that a one-compartment model applies, the half-life of a metabolite can be estimated from mean residence time. Under these conditions, the following applies to parent drug and metabolite, respectively.

$$MRT = 1.44\ t_{1/2}(drug) \quad (11\text{–}27)$$

and

$$MRT_{M/P} = 1.44\ [t_{1/2}(drug) + t_{1/2}(M)] \quad (11\text{–}28)$$

The mean residence time for a metabolite is always larger than the mean residence time of its precursor; the difference between them is an estimate of the true half-life of the metabolite, i.e.,

$$t_{1/2}(M) = [MRT_{M/P} - MRT]/\ 1.44 \quad (11\text{–}29)$$

Species Differences

Drug metabolism studies in laboratory animals are often useful in suggesting likely drug metabolites to be found in man. Some species may be more useful than others. A survey of drug metabolism studies in laboratory animals and man, covering 32 compounds, among which were amphetamines, arylacetic acids, and sulfonamides, concluded that the rhesus monkey resembles man most closely in terms of urinary metabolite patterns; patterns in the dog and rat were much less useful for predicting results in man.[74] For example, sulfadimethoxine is mainly acetylated in the rat, but in man the major urinary metabolite is a glucuronide. Scores of similar examples can be found.

Species differences in the activity of microsomal enzyme systems are usually so great as to render the results of studies in laboratory animals meaningless as a guide for assessing the duration of drug activity in man. Table 11–2 shows the duration of effect of the skeletal muscle relaxant, carisoprodol, in different laboratory animals.[75] A range of 0.2 to 21.5 hr was observed in response to the same mg/kg dose. Interestingly, brain or serum concentrations of carisoprodol at the end of paralysis were similar in all species.

Many drugs are metabolized much more rapidly in the rat or dog than in man. A certain dose of a drug may have fleeting activity in a test animal but elicit an adequate response in man. These differences are of concern to those involved in the development of new drugs, because present methods of drug screening may overlook potentially useful drugs. These considerations are also important for the toxicologic evaluation of drugs in laboratory animals.

Stereoselective Drug Metabolism

Chemical synthesis of a drug with an asymmetric or chiral center usually results in two enantiomers,

Table 11–2. Species Differences in the Hepatic Oxidation (μmoles/g liver per 30 min) and Half-Life (hr), Duration of Paralysis (hr), and Brain and Serum Concentrations (μg/g or μg/ml) at the End of Paralysis of Carisoprodol After a 200 mg/kg Single Intraperitoneal Dose*

Species	Hepatic oxidation	Half-life	Paralysis	Concentration	
				Brain	Serum
Mouse	0.60	0.3	0.2	112	130
Guinea pig	0.44	0.8	0.5	105	129
Rat (male)	0.42	0.8	0.5	90	105
(female)	0.16	2.4	1.6	108	128
Rabbit	0.07	7.5	5.4	93	112
Cat	0.02	36	21.5	113	135

*Data from Kato, R.[75]

mirror images that cannot be superimposed on one another. This 50:50 mixture is called a racemate. In contrast, enzymes and receptor sites are stereoselective. Therefore, stereoisomers often exhibit pronounced differences in pharmacologic and toxicologic properties. Furthermore, various aspects of drug disposition (e.g., binding, metabolism, renal excretion) also exhibit stereoselectivity.

Despite the important differences in both pharmacologic and pharmacokinetic properties that may exist between two enantiomers, less than 20% of all racemic drugs are marketed as preparations containing only one isomer. In most cases, stereospecific synthesis is difficult and separation of the enantiomers is a major challenge. Often, a racemate will contain an "active" and "inactive" enantiomer. The "inactive" enantiomer, however, may not be an inactive compound. It may contribute partially to overall drug effect, be an antagonist, or have actions at other receptors resulting in undesirable side effects.

Warfarin is used as a racemic mixture of R- and S-warfarin. The S-enantiomer is about 5 times more potent than the R-enantiomer and is eliminated more rapidly. The metabolism of warfarin is highly stereoselective. The major metabolic route of S-warfarin is oxidation through ring hydroxylation of the coumarin nucleus to form primarily 7-hydroxy-S-warfarin. R-warfarin is primarily metabolized by oxidation to 6-hydroxy-R-warfarin and by reduction to R,S-warfarin alcohols.[76]

Studies in young adults have shown that l-hexobarbital is eliminated more rapidly than d-hexobarbital.[77] More recently, the pharmacokinetics of the hexobarbital enantiomers were compared in young (mean 23 years) and elderly adults (mean 68 years).[78] In each group, the apparent clearance of l-hexobarbital was considerably larger than that of d-hexobarbital. In the younger subjects, a mean value of 16.9 ml/min per kg was determined for l-hexobarbital compared with a mean value of 1.9 ml/min per kg for d-hexobarbital.

The apparent clearance of d-hexobarbital was nearly the same in young and elderly subjects. In contrast, the apparent clearance of l-hexobarbital was about twice as high in young subjects as in elderly subjects (16.9 versus 8.2 ml/min per kg). This is the first demonstration of age-related preferential decline in metabolism of one enantiomer over another for any racemic drug in animals or humans.

The (+)-isomer of amphetamine is metabolized more rapidly than the (−)-isomer.[79] When urine pH is maintained above 7, the half-life of the (+)-isomer is about 16 hr, whereas that of the (−)-isomer is about 26 hr. When the urine is acidified by administration of ammonium chloride, so that metabolism plays a smaller role in the overall elimination of amphetamine, half-life differences between the isomers almost disappear.

Differences in rates of metabolism have also been observed with the enantiomers of propranolol[80,81] and ibuprofen.[82] Propranolol is used clinically as an equal mixture of S- and R-enantiomers. S-Propranolol is about 100 times more potent as a beta-blocker than the R-enantiomer and is believed to be largely responsible for the clinical effects of the racemic drug. Repeated dosing of the racemate resulted in higher steady-state levels of S-propranolol than of R-propranolol, suggesting that propranolol undergoes stereoselective metabolism.[81]

For most 2-arylpropionic acid nonsteroidal antiinflammatory drugs (NSAIDs), the S-enantiomer is the active species. Only one of these drugs, naproxen, is available as the S-enantiomer; all the others are racemic mixtures. Interest in their stereochemistry was aroused by observations that the differences in potency between the two enantiomers in in vitro tests of antiinflammatory activity were much greater than in in vivo tests. For example, S-ibuprofen is 160 times more potent than R-ibuprofen in the inhibition of prostaglandin synthetase but has only about 50% greater potency than the R-enantiomer in an acetylcholine writhing test in mice or a pain threshold test in rats.[83]

This inquiry led to the discovery of a novel stereospecific pathway for the metabolism of the inactive R-enantiomers. These enantiomers undergo a stereospecific metabolic inversion, which progressively transforms the inactive enantiomers to the pharmacologically active S-enantiomers. This inversion has been shown for ibuprofen and benoxaprofen and may occur with ketoprofen and fenoprofen.[84] No inversion has been observed with indoprofen; preliminary studies suggest that carprofen may also exhibit little, if any, inversion.[83] Quantitatively, a 50% inversion of R- to S-benoxaprofen has been observed in human subjects, and nearly two thirds of R-ibuprofen has been found to be stereospecifically inverted to S-ibuprofen.

Lam[84] points out that even when the rate of inversion is rapid and the extent of inversion is considerable, the inactive R-enantiomer serves no ther-

apeutic purpose and is, at best, merely a prodrug for the active S-enantiomer. Differences in the rate and extent of inversion in patients is a potential source of variability in therapeutic response to NSAIDs and allows for variable amounts of possibly toxic metabolites to be formed from the inactive R-enantiomer. Administration of the S-enantiomer would remove such variability and increase the therapeutic ratio.

In another study, boys with attention-deficit disorder were given single doses of dl-methylphenidate.[85] Drug levels in plasma were determined with an enantioselective assay method. In all 6 children, plasma levels of d-methylphenidate were at least 5-fold greater than those of the l-enantiomer. Mean AUC values were 24.5 ng-hr/ml for the d-form and 3.8 ng-hr/ml for the l-form. The plasma level-time curve for total methylphenidate ($d+l$) is almost superimposed on that of the d-enantiomer because the contribution of the l-enantiomer to the total level is very small.

Nicoumalone, like warfarin, is a racemic, coumarin-type oral anticoagulant, available in the UK and other countries but not in the U.S. It is the second most commonly prescribed oral anticoagulant in the United Kingdom and is the most widely used oral anticoagulant in continental Europe. Preliminary studies indicated important differences in both pharmacokinetics and pharmacodynamics when each enantiomer of nicoumalone was given separately. More recently, a stereospecific assay for nicoumalone has been developed, permitting more detailed study. The kinetics of the individual enantiomers were determined after a single oral dose of the racemic mixture to 3 healthy human subjects.[86]

Dramatic differences were observed: mean apparent clearance was 21 ml/min for the R-enantiomer and 292 ml/min for the S-enantiomer; mean half-life was about 7 hr for the R-form and about 1 hr for the S-form. The protein binding of the enantiomers of nicoumalone was also found to be different. Percent unbound was 1.0 for the S-enantiomer and 0.7 for the R-form. Further evaluation of the data suggest that about 30% of an oral dose of S-nicoumalone would be lost to first-pass metabolism, whereas only 3% of an oral dose of R-nicoumalone would be similarly affected.

Verapamil, a widely used calcium channel blocker, is also subject to stereoselective first-pass metabolism.[87] Suspicions of an extensive first-pass effect were raised when clinicians realized that an iv dose of 5 to 10 mg was effective in terminating various supraventricular tachyarrhythmias, whereas an oral dose of 80 to 160 mg was needed to elicit an effect comparable to that seen after iv administration. The reason for this, at least in part, is that despite its almost complete absorption, the bioavailability of verapamil is only 20 to 30% because of extensive presystemic hepatic elimination. Based on a bioavailability of 20 to 30%, however, an oral dose of 25 to 50 mg should be sufficient to elicit a response equivalent to a 5 to 10 mg iv dose.

Analysis of the concentration-effect relationship revealed the reason for this seeming discrepancy. The same concentration of verapamil (i.e., dl-verapamil) is more effective after intravenous than after oral administration. On average, verapamil plasma levels 3 times higher were needed after oral administration to produce the same prolongation on the ECG as after iv administration. This is contrary to all precepts of pharmacokinetics.

The standard analytical method for assaying verapamil measures both the d- and l-forms. l-Verapamil, however, is about 8–10 times more potent than d-verapamil. Therefore, the most plausible explanation for this anomaly is that the more active l-isomer is preferentially metabolized during hepatic first-pass metabolism. It follows that a given plasma level after iv administration will be richer in the more potent l-verapamil than after oral administration and thus produce a greater pharmacologic effect.

It has been demonstrated that after oral administration, the d-enantiomer has a bioavailability of 50%, about 2.5 times greater than that of l-verapamil. The d- to l- isomer ratio of plasma verapamil after iv administration is about 2, whereas after oral administration the ratio is about 5. These differences in the d- to l-enantiomer ratio of plasma verapamil in relation to route of administration explain the observed differences in the concentration-effect relationships.

Labetalol is sometimes claimed to provide alpha- and beta-blocking activity in a single drug and is indicated for the treatment of high blood pressure. In fact, labetalol has two asymmetric centers and is therefore a mixture of four diastereomers. The alpha- and beta-blocking activities are not distributed uniformly among the four isomers. The R,R-isomer of labetalol, also called dilevalol, is primarily responsible for the beta-adrenergic receptor blocking activity, but has only weak alpha-activity.

The SR-isomer produces most of the alpha-blocking activity. Of the four diastereomers, only dilevalol has an antihypertensive effect in spontaneously hypertensive rats comparable to that of labetalol. Although the two inactive isomers are without toxicity, some clinicians believe it would be preferable to use only the active RR-isomer to lessen the potential for interactions among the isomers or with other drugs.[84]

Ariens[88] caustically observed that "too often, and without it being noticed, data in the scientific literature on mixtures of stereoisomers, racemates, are presented as if only one compound were involved. This neglect of stereochemical aspects of drug action, including metabolism, excretion, etc. notwithstanding, computerized curve fitting, generation of extensive tables with pharmacokinetic constants, and postulation of complex multicompartment systems, degrades many pharmacokinetic studies to expensive 'highly sophisticated pseudoscientific nonsense.' "

A report from Australia[89] described a theoretical analysis to illustrate the potential which exists for misinterpretation of drug disposition and plasma drug concentration-effect data generated for a racemic drug using a nonstereoselective assay. The investigators demonstrated convincingly that the use of a nonselective analytical method can lead to the collection of data which may be both quantitatively and qualitatively inaccurate with respect to the individual enantiomers. For example, the clearance of the unresolved drug (i.e., the sum of the R- and S-enantiomers) may indicate nonlinear pharmacokinetics, even though the kinetics of the enantiomers are concentration- and time-independent. We would never think of carrying out a pharmacokinetic analysis on levels of radioactivity in plasma after administration of a labeled drug. The same caution should be applied to the pharmacokinetic analysis of data obtained after administration of a racemate.

Capacity-Limited Metabolism

The rate of an enzymatic process, like biotransformation, can usually be described by the Michaelis-Menten equation:

$$\text{Rate of metabolism} = \frac{V_{max}C}{K_m + C} \quad (11\text{–}30)$$

where C is the drug concentration in the plasma, V_{max} is the maximum production rate of metabolite, and K_m is the Michaelis constant. The constant V_{max} is a function of the total amount of metabolizing enzyme; $1/K_m$ reflects the affinity between drug (substrate) and enzyme. Operationally, K_m is the drug concentration at which the rate of metabolism is one half of the maximum.

Experience suggests that the usual dose of most drugs results in plasma concentrations that are much smaller than the K_m values associated with their metabolism. Since $C \ll K_m$, it follows from Equation 11–30 that:

$$\text{Rate of metabolism} = \frac{V_{max}}{K_m} C = k_m C \quad (11\text{–}31)$$

where k_m is the apparent first-order metabolic rate constant. Accordingly, the elimination of most drugs that are eliminated totally or in part by biotransformation can be described by first-order kinetics.

Some drugs, including ethanol, salicylate, and phenytoin, have one or more K_m values that are comparable to or less than their usual concentrations in the plasma following therapeutic doses. One study suggests that the apparent K_m for phenytoin in patients is as low as 4 μg/ml.[90] The usual desired concentration range for phenytoin in treating seizures is 10 to 20 μg/ml. Calculations based on studies in normal adults suggest that the K_m values (expressed in terms of amount of drug in the body) for the glycine conjugation and for the acyl glucuronidation of salicylate are about 340 and 640 mg, respectively.[91] Considerably larger amounts of salicylate are found in patients taking aspirin or other salicylate preparations for rheumatoid arthritis or rheumatic fever.

The elimination of drugs like phenytoin or salicylate cannot be described by first-order kinetics; their pharmacokinetics are said to be *nonlinear*. The relative rate of elimination is slower at higher concentrations than at lower concentrations of drug in the plasma. In other words, it takes longer to decrease a high drug concentration by 50% than to decrease a low concentration by the same percentage. Because the apparent half-life (actually, the time required to decrease the peak concentration of drug in blood or plasma by 50%) of these drugs increases with increasing dose, their elimination is said to be *dose-dependent*. Strictly speaking, however, Michaelis-Menten elimination is *concentration-dependent* rather than dose-dependent.

Drugs manifesting dose-dependent elimination present unusual challenges in therapeutic manage-

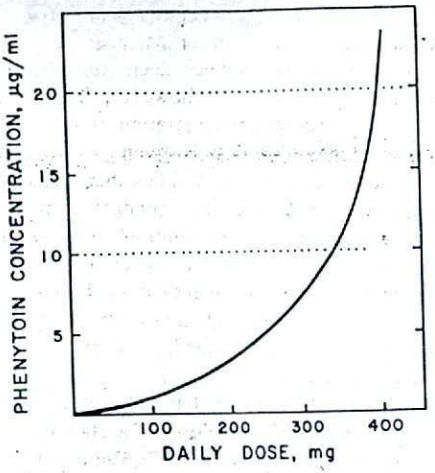


Fig. 11–6. Relationship between steady-state serum concentration of phenytoin and daily dose. (Data from Mawer, G.E., et al.[90])

ment, because steady-state concentrations change disproportionately with changes in dose. For example, a 50% increase in the daily dose of aspirin produces about a 300% increase in the concentration of salicylate in the plasma.[92] The nonlinear relationship between steady-state concentration and dose also explains why a relatively small (20%) increase in the daily dose of aspirin can produce a pronounced therapeutic response in patients with rheumatic fever or acute arthritis who have not responded to the lower dose.[93]

The unusual pharmacokinetics of salicylate elimination presents a dilemma in the design of rational dosage regimens. The problem is particularly serious in patients with rheumatoid arthritis, because effective salicylate therapy requires the use of doses that result in near-toxic levels. Small changes in dose can produce a change in blood concentrations from ineffective levels to levels causing serious adverse effects. It is significant that the most severe instances of aspirin intoxication result from therapeutic overdosage,[94] and that more children die of therapeutic than of accidental aspirin poisoning.[95]

Serious problems may occur when ethotoin or phenytoin is used in the treatment of seizures. Increasing the daily dose of ethotoin 2-fold (from 30 to 60 mg/kg) produced, on the average, a 3-fold change in steady-state plasma concentrations in epileptic patients.[96] In one patient, this dosage change produced a 7-fold increase in steady-state levels.

The theoretical curve relating steady-state serum concentrations to daily dose of phenytoin, derived from studies in 15 epileptic patients, is shown in Figure 11–6.[90] The curve rises steeply when the daily dose exceeds 340 mg. The steepness of the curve is illustrated by a case report of a female patient with grand mal epilepsy.[90] Serum concentrations of 7.6 and 4.6 µg/ml were observed on 2 consecutive visits while she was taking 300 mg of phenytoin daily. These levels are subtherapeutic in many adult patients. Between the visits she had a major convulsion. The maintenance dose was increased to 350 mg daily. About 5 weeks later she returned to the clinic complaining of ataxia, a common sign of phenytoin intoxication. Her serum phenytoin concentration had risen to 27 µg/ml, a level at which adverse effects of the drug are frequent. In this patient, a modest (17%) increase in the daily dose of phenytoin produced about a 5-fold change in steady-state serum concentration of the drug.

Dosage adjustments for theophylline therapy have traditionally been based on a linear pharmacokinetic model, whereby a change in dose at steady state would result in a proportional change in the serum concentration of theophylline. Increasingly, however, investigators have suggested that the pharmacokinetics of theophylline following therapeutic doses may not be linear in certain patients. Children treated aggressively with serum theophylline levels close to 20 µg/ml, for example, have been found to become toxic during a bout of influenza, which only modestly decreased the clearance of theophylline.

In view of these reports, investigators in Japan[97] determined the incidence and implications of capacity-limited elimination in pediatric and adult patients with chronic asthma receiving a sustained-release form of theophylline as principal therapy. Patients in whom at least two steady-state concentration measurements were obtained at two or more different doses were selected. Nonlinearity was defined as a percent change in plasma levels exceeding the change in dose by at least 50%.

Nonlinear elimination was observed in 40% of the children and 42% of the adults. In these patients, the mean maximum elimination rate (V_{max}) was significantly greater in children than in adults, 32 *versus* 22 mg/kg per day, indicating that the metabolic capacity for theophylline is greater in children than in adults. Also, V_{max} values were significantly correlated with age. Mean values for

K_m, on the other hand, were similar in the two groups, 13.0 and 12.9 μg/ml, respectively, and individual K_m values were independent of age. These values for K_m are well within the therapeutic concentration range of theophylline (usually considered to be 10 to 20 μg/ml).

Nonlinear pharmacokinetic characteristics have also been reported for propranolol. Steady-state levels were higher when a daily dose of 80 mg was given as 40 mg twice daily than when given as 20 mg 4 times daily.[98] The 24-hour steady-state AUC values were 340 ng-hr/ml after dosing 4 times a day and 446 ng-hr/ml after dosing twice a day, a difference in apparent bioavailability of 30%. These findings suggest nonlinear first-pass metabolism of propranolol. In general, the bioavailability of first-pass drugs that obey Michaelis-Menten kinetics will be sensitive to rate of drug input.[99] This helps us to understand why the relative bioavailability of slow-release dosage forms of propranolol is only about 50% compared with conventional tablets given 3 times a day.

Propafenone is an investigational antiarrhythmic agent that markedly slows conduction. It is eliminated almost entirely by hepatic metabolism. Propafenone is very well absorbed but bioavailability is incomplete, because of first-pass metabolism, and dose-dependence.[100] When the oral dose was increased from 150 to 450 mg, peak concentration of propafenone in serum increased by a factor of six. In other subjects, a 3-fold increase in dose from 300 to 900 mg/day produced a 10-fold increase in steady-state propafenone serum concentrations.

Nicardipine, a potent, orally active vasodilator, related to nifedipine, is under investigation for use in hypertension, angina, and cerebrovascular disease. In one study, oral doses of nicardipine ranging from 10 to 40 mg every 8 hours were given to healthy subjects. The steady-state bioavailability of nicardipine was found to be dose-dependent and averaged 19% at 10 mg, 22% at 20 mg, 28% at 30 mg, and 38% at 40 mg. The investigator cautioned that if one wished to increase the oral dose higher than 40 mg every 8 hours, such increases should be very conservative and plasma concentrations should be monitored.[101]

Other Examples of Nonlinear Metabolism

Certain drugs display nonlinear pharmacokinetics that are not consistent with Michaelis-Menten kinetics. These drugs display dose-dependent or time-dependent pharmacokinetics rather than concentration-dependent pharmacokinetics.

Concentration-dependent or Michaelis-Menten kinetics means that clearance decreases with increasing drug concentration; however, drug clearance at a given drug concentration is the same whether a high or low dose is given.

Dose-dependent kinetics implies that clearance changes with dose rather than concentration. For example, clearance of acetaminophen from the blood at any concentration is lower following a toxic dose than after a therapeutic dose. This occurs because high doses of acetaminophen cause hepatotoxicity, which reduces the liver's ability to metabolize the drug.

Dose-dependent kinetics have also been found in laboratory animals with doses of acetaminophen that do not cause hepatotoxicity.[102] The underlying mechanism involves depletion of the sulfate pool in the body, which is only slowly restored. Sulfate conjugation plays a principal role in the elimination of acetaminophen: a reduction in the rate of acetaminophen sulfate formation reduces the overall rate of elimination of the drug.

Product inhibition of drug metabolism can also produce dose-dependent kinetics.[103] Studies in laboratory animals with phenytoin and phenylbutazone indicate that certain hydroxylated metabolites can inhibit the metabolism of their precursors. In man, however, evidence of product inhibition is scant, possibly because safety considerations limit the administration of drug metabolites. Diazepam, however, provides an opportunity for investigation because several of its metabolites are used as drugs in their own right. It has been found that nordiazepam inhibits the metabolism of diazepam.[104]

Unusual, nonlinear pharmacokinetic characteristics have also been observed with nitroglycerin.[105] As nitroglycerin iv infusion rates were increased from 10 to 40 μg/min, the steady-state concentration in plasma increased disproportionately, from 0.4 to 4.2 ng/ml. However, when the infusion rate was then decreased to 10 μg/min, steady-state concentrations of nitroglycerin were always higher during the second 10 μg/min infusion than during the first 10 μg/min infusion. On average, steady-state levels were 1.0 ng/ml during the second low-dose infusion compared with 0.4 ng/ml during the first.

The investigators pointed out that high concentrations of the dinitrate metabolites accumulate after administration of nitroglycerin. These high

metabolite levels may inhibit the clearance of the parent drug (end-product inhibition). Alternatively, capacity-limited binding of nitroglycerin to blood vessels at or near the infusion site would also explain the unusual results.[105]

Time-dependent kinetics means that the clearance of the drug changes with continuous administration of the same dose, whether or not there is significant accumulation of the drug on multiple dosing. For example, the elimination of diazepam is slower after multiple dosing than following a single dose.[106] This change in clearance appears to be a consequence of the considerable accumulation of nordiazepam on multiple dosing of diazepam and of nordiazepam's ability to inhibit the metabolism of diazepam.[104]

Time-dependent pharmacokinetics is also observed with drugs that stimulate their own metabolism. The steady-state concentration of drugs with linear pharmacokinetics can be predicted from data obtained after a single dose because clearance is assumed to remain constant throughout treatment. If a drug is subject to autoinduction, however, clearance is higher after multiple doses than following a single dose. Studies with the anticonvulsant carbamazepine illustrate this point.[107]

Carbamazepine was given to patients as a single oral dose; 1 week later the patients received the drug 3 times a day for 2 to 3 weeks. The half-life of carbamazepine was shorter in all patients after multiple doses (21 hr, on the average) than after the initial single dose (36 hr, on the average). The average steady-state concentration predicted from the single dose data was higher (17 μg/ml) than the steady-state levels observed during treatment (8 μg/ml). The results are consistent with self-induction of microsomal enzymes; the clearance of carbamazepine doubles on repeated dosing. Similar results have been observed in the rhesus monkey during constant rate intravenous infusion of carbamazepine (Fig. 11–7).[108]

There is also evidence that salicylate metabolism is autoinduced.[109] Healthy subjects receiving 3.9 g aspirin per day show maximum salicylate concentrations in plasma after about 4 days; thereafter, salicylate levels decline by about 25 to 30% despite a constant daily dose. The results may be a consequence of self-induction of a metabolic pathway (e.g., salicylurate formation).[110]

Another kind of time-dependent pharmacokinetics, as yet unexplained, has been observed with certain drugs having no obvious similarities in chemical structure but sharing the common characteristic of a high hepatic clearance; each of these drugs shows a large first-pass effect after oral administration because of presystemic hepatic metabolism. Propranolol accumulates during continued oral administration to a greater extent than predicted from its half-life and area under the curve (AUC) after a single oral dose.[111] Presystemic extraction decreases from 78% after a single dose to 66% at steady state following 80 mg given every 8 hr. In other words, the systemic availability of propranolol increases from 22 to 34% during multiple dosing; steady-state levels are about 50% greater than expected.

During chronic oral treatment of hypertensive patients with labetalol, an α,β-adrenoceptor block-

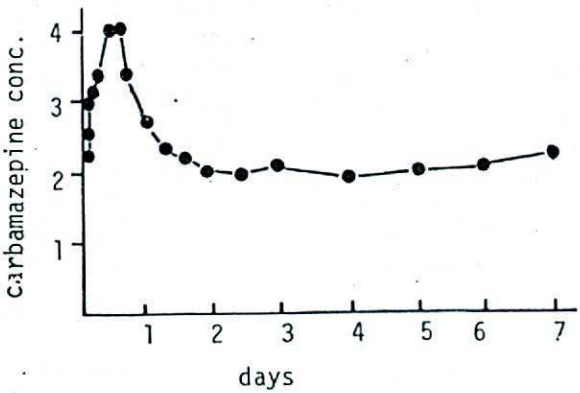


Fig. 11–7. Carbamazepine concentrations in serum (μg/ml) in a monkey during constant rate intravenous infusion of the drug. (Data from Pitlick, W.H., and Levy, R.H.[108])

ing drug, observed mean steady-state levels in patients were almost twice those predicted from a single oral dose.[112] Unexpected accumulation at steady state has also been observed with the analgesic propoxyphene.[113]

In patients with atrial fibrillation, oral and intravenous single dose studies with verapamil, a calcium channel blocker, indicate that only 35% of the oral dose is available; about two thirds of the dose is subject to first-pass hepatic metabolism. The first-pass metabolism of oral verapamil decreases considerably on multiple dosing; mean verapamil concentration in plasma at steady state was nearly twice the value predicted from the single-dose studies.[114,115]

Nonlinear accumulation of verapamil does not occur on intravenous administration. Steady-state concentrations of verapamil following constant-rate intravenous infusion were similar to those predicted from single-dose intravenous studies.[114] On the other hand, the antiarrhythmic drug lidocaine, which is also subject to a large first-pass effect on oral administration, does show clinically significant, nonlinear accumulation during continuous constant-rate intravenous infusion.[116,117]

Some believe that the decline in lidocaine clearance during continuous intravenous infusion may be related to inhibition by monoethylglycinexylidide (MEGX), a metabolite of lidocaine. In support of this hypothesis, investigators in Scotland found that the clearance of lidocaine when given with MEGX decreased from 970 ml/min to 800 ml/min.[118]

The nonlinear pharmacokinetics of drugs subject to first-pass hepatic metabolism presents a confusing picture at this time. Not all drugs display this characteristic. For example, the antidepressant nortriptyline, which is subject to significant presystemic hepatic metabolism, accumulates in a highly predictable manner on multiple oral dosing.[119] Some drugs (e.g., verapamil) show nonlinearity on oral dosing but not on intravenous dosing. Lidocaine shows nonlinear accumulation on intravenous dosing, but has not been studied on oral dosing. Saturation of a high affinity, low capacity enzyme system or binding site in the liver may explain some of the results, but more than one mechanism is likely.

Extrahepatic Metabolism

Many tissues other than the liver contain microsomal and soluble drug metabolizing enzymes, but for the most part their role in drug disposition is poorly understood.

The liver seems to be the only consistently important organ for drug metabolism, as it relates to the elimination of drugs from the body. The drug metabolizing activity of certain organs, including the skin, the gut, and the lungs, is of general interest, however, because their anatomic position permits them to exert a kind of first-pass effect that limits drug availability to sites of pharmacologic effect.

The epidermis can carry out several metabolic reactions including glucuronide conjugation. There is evidence for the cutaneous metabolism of adrenal steroids, hydrocortisone, and fluorouracil. Recent in vitro studies indicate that the antiviral drug vidarabine is extensively metabolized in skin.[120] Drug metabolism in skin could decrease the potency and duration of effects of topically applied drugs intended to act locally, or produce a first-pass effect for drugs intended for systemic effects.

The most important extrahepatic site of drug metabolism is the gastrointestinal tract. Certain drugs may be extensively conjugated after oral administration by enzymes in the intestinal epithelium. The consequence of this presystemic metabolism is incomplete bioavailability.

Intestinal metabolism explains why isoproterenol is 1000 times less active after oral than after intravenous administration, despite the fact that there is little difference in the total urinary recovery of drug-related material after equal intravenous and oral doses of radiolabeled drug. Oral administration of isoproterenol in man produces a pattern of metabolism that differs markedly from that seen after intravenous dosing.[121] After intravenous dosing, unchanged isoproterenol accounts for more than 60% of the urinary radioactivity; the remainder is present as free or conjugated 3-0-methyl isoproterenol. In contrast, the major metabolite in plasma and urine after oral administration is a sulfate conjugate of isoproterenol that is formed in the intestinal wall.

Similar differences in metabolic pattern in man, as a function of route of administration, have been reported for terbutaline.[122] After intravenous administration, unchanged terbutaline accounts for 70 to 90% of the urinary radioactivity. The remainder is present as a sulfate conjugate of terbutaline. On the other hand, the sulfate conjugate accounts for about 70% of drug-related material in the urine after oral administration. Sulfate conjugation in the

gut wall during absorption of terbutaline is a likely explanation for this difference.

Drug metabolism studies in man suggest that at least two other bronchodilator drugs are metabolized in the intestine after oral administration. A sulfate conjugate accounts for about 50% of the total urinary radioactivity after oral administration of rimiterol but for only 2% after intravenous administration.[123] A similar difference with respect to the extent of formation of sulfate conjugate after oral and intravenous administration has been found with isoetharine.[124]

Albuterol (salbutamol), the most widely used beta$_2$-agonist in the world, is also subject to gut metabolism after oral administration.[125] After iv administration of albuterol, urinary excretion of unchanged drug accounts for 64% of the dose and the sulfate conjugate accounts for 12%. With oral administration, bioavailability was 50% and urinary excretion of unchanged drug and sulfate conjugate accounted for 32% and 48% of the dose, respectively. The incomplete bioavailability suggests that oral albuterol is subject to presystemic metabolism. The markedly different composition of drug-related material after iv and oral albuterol suggests that presystemic metabolism occurs in the gastrointestinal mucosa.

The low plasma morphine levels observed in man after oral administration compared to those that result from parenteral administration are probably due to the rapid conjugation of the drug with glucuronic acid in the cells of the intestinal mucosa during absorption.[126]

The relatively high activity of β-glucuronidase in the intestine may contribute to the duration of effect of drugs that undergo biliary secretion in the form of glucuronide conjugates. The conjugates may be hydrolyzed, thereby promoting reabsorption and enterohepatic cycling of parent drug.

Esterases in the intestine appear to contribute to the less than complete bioavailability of aspirin after oral administration.[127] These enzymes may also be important for the bioactivation of drugs that are given in the form of ester prodrugs.

The intestinal flora in the distal small intestine and colon metabolize certain poorly absorbed drugs or drugs that are subject to biliary excretion. Intestinal bacteria play an important role in the metabolism of sulfasalazine in man.[128] The drug consists of 5-aminosalicylate in azo linkage with sulfapyridine (see Table 11–1). On oral administration, sulfasalazine is partially absorbed during its transit through the small intestine and is excreted unchanged in the urine. The unabsorbed portion of the dose enters the cecum and colon where the molecule is cleaved at the azo linkage by bacteria. Most of the sulfapyridine thus formed is absorbed and subsequently metabolized. The fate of the 5-aminosalicylate moiety is probably similar. The cleavage is believed to be essential for the therapeutic effects of the drug, to liberate 5-aminosalicylate.

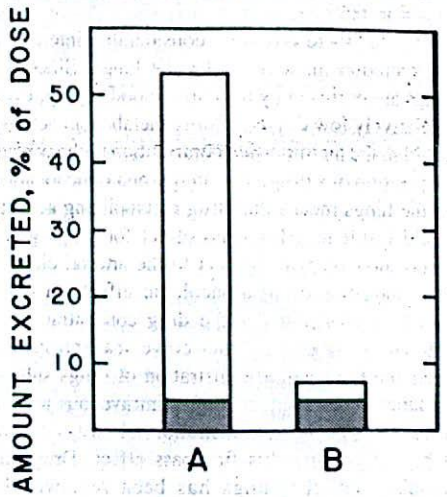


Fig. 11–8. Urinary excretion of sulfasalazine (shaded area) and its metabolite sulfapyridine (open area) in a patient before (A) and after (B) colectomy. (Data from Schroder, H., Lewkonia, R.M., and Price Evans, D.A.[129])

The importance of microflora metabolism in the intestine to the disposition of sulfasalazine is evident from the results of studies in a patient before and after total colectomy (Fig. 11–8). The excretion of unchanged sulfasalazine in urine was unaffected by the surgery. The effect on sulfapyridine, however, was dramatic; its excretion in urine decreased from about 50 to 3% of the dose.[129] It is also likely that far less 5-aminosalicylate was formed in this patient.

Sulindac is a nonsteroidal antiinflammatory drug that undergoes conversion to a sulfide compound, which is 5 times more potent than sulindac, and an inactive sulfone. Studies in patients with surgical ileostomies suggest that gut microflora are important for the reduction of sulindac to sulfide.[130] It appears that about 50% of the active sulfide metabolite found in normal subjects given sulindac is

formed by gut bacteria mostly from sulindac excreted in the bile.

Of late, there has been considerable interest in the metabolism of drugs by the lungs. Since the lungs are perfused by the entire blood supply, even a relatively low degree of drug metabolism activity could make an important contribution to the overall disposition of a drug. The unique anatomic location of the lungs means that drug metabolizing activity could result in a first-pass effect for drugs given intravenously, with respect to the arterial circulation and sites of pharmacologic effect. In other words, the area under the drug concentration in arterial blood versus time curve may be greater after intra-arterial administration of drugs subject to lung metabolism than after intravenous administration. Direct administration of drugs to the lungs may avoid this first-pass effect. Drug metabolism by the lungs has been reviewed by Brown,[131] and more recently by Roth and Wiersma.[132] The contribution of lung metabolism to the clearance of drugs from the blood has been clearly presented by Collins and Dedrick and associates.[133,134]

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