

Review

SPECIES SIMILARITIES AND DIFFERENCES IN PHARMACOKINETICS

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ABSTRACT:

One of the fundamental challenges drug metabolism scientists face in drug discovery and development is the extrapolation of metabolic data and risk assessment from animals to humans. Although it is generally believed that data from animal studies can be reasonably extrapolated to humans with the application of appropriate pharmacokinetic principles, there are certainly some limitations. The purpose of this review is to show some species similarities and differences in absorption, distribution, metabolism, and excretion, with an attempt to address the questions of whether animal data can be extrapolated and what the limitations are. The underlying mechanisms responsible for these similarities and differences are discussed and examples given for illustration. In addition, the concepts of allometric and physiological models for extrapolation of kinetic data are briefly highlighted.

In conclusion, although pharmacokinetics has been advanced greatly in recent years, it is not yet possible to predict all of the pharmacokinetic parameters of a drug in humans from animal studies. Nevertheless, under certain well-defined conditions, it

may be possible to make reasonably good predictions. For example, the intrinsic absorption of a given drug across the wall of the gastrointestinal tract is probably similar among species, because the nature of the biomembrane of epithelial cells is similar in mammals and because the absorption process is basically an interaction between the drug and the biomembrane. However, other factors, such as pH-dependent solubility and first-pass metabolism that affect the absorption, may differ from species to species resulting in species differences in absorption.

Predicting the renal excretion of drugs in humans has been relatively successful using the glomerular filtration rate ratio between humans and animals. Similarly, if the elimination of a drug is mainly by the liver and the rate of elimination is limited by hepatic blood flow, one could predict the clearance of the drug in humans by the hepatic blood flow. However, biochemical parameters, such as protein binding and drug metabolism, are less predictable. These parameters vary considerably among species.

Drug development is an extremely costly, time-consuming, and complicated endeavor. Recent surveys indicate that the average new chemical entity taken to market in the United States requires ~10–12 years of research and costs more than \$300 million. Much of the cost and time are expended on collecting data concerning the efficacy and safety of the drug. Because of the ethical constraints, relevant pharmacological and toxicological assessments have to be extensively studied in laboratory animals before first drug administration in humans. Although the validity of animal testing to predict efficacy and safety in humans has been questioned, it is generally believed that data from animal studies can be reasonably extrapolated to humans with the application of appropriate pharmacokinetic principles.

Pharmacokinetics not only support drug safety and efficacy evaluation during drug development, but also play a very important role in

drug discovery. Many drug companies now include pharmacokinetics as part of their screening criteria. A drug is not worth developing, even if it is the most potent compound in an *in vitro* pharmacological test, if it is poorly absorbed or is so rapidly eliminated that it shows no activity *in vivo*. Thus, key criteria for the selection of a drug candidate are good oral absorption and appropriate biological half-life. At the early stage of drug discovery, there is usually a very small quantity of each compound available. This limits kinetic studies to one or two animal species. Therefore, the selection of animal species and experimental design of studies are important to provide a reliable prediction of drug absorption and elimination in humans. After clinical studies show a drug to be pharmacokinetically unsatisfactory, it is very costly to begin again with a new compound.

The purpose of this review is to show some species similarities and differences in ADME¹, with an attempt to address the question of whether animal data can be extrapolated to humans. Whenever possible, the underlying mechanisms responsible for these similarities and differences will be discussed and examples given for illustration. In addition, the concepts of allometric and physiological models for extrapolation of kinetic data and some basic theoretical considerations of pharmacokinetics will be briefly highlighted.

Biological Similarities

From an evolutionary point of view, all mammals are similar, because they originate from a common ancestor, yet they differentiate because of their dissimilar environmental adaptations. Humans share with other mammals many similarities in anatomy, physiology, and

¹ Abbreviations used are: ADME, absorption, distribution, metabolism, and excretion; AUC, area under the plasma concentration-time curve; V_d , volume of distribution; CL, elimination clearance; CL_{int} , hepatic clearance; f_p , unbound fraction of drug in plasma; CL_{int} , intrinsic metabolic clearance; CL_R , renal clearance; GFR, glomerular filtration rate; CL_{UR} , renal clearance of unbound drug; f_u , unbound fraction of drug in tissue; V_d , volume of distribution of unbound drug; HIV, human immunodeficiency virus; AIDS, acquired immune deficiency syndrome; C_{max} , maximal concentration; CCK_B, brain cholecystokinin receptor; PEG, polyethylene glycol; NK-1, neurokinin receptor.

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biochemistry (1), whereas the most obvious differences are their size and shape. Many anatomical and physiological parameters can be described as exponential functions of body weight, using the allometric equation expressed as (2, 3):

$$Y = aW^b, \quad (1)$$

where Y is an anatomical or physiological parameter, W is the body weight, a is the allometric coefficient, and b is the allometric exponent.

The allometric equation for liver weight (kg) as a function of total body weight (kg) is $0.037 W^{0.85}$ (4). The liver is 4.5% of the total body weight in a 250 g rat and 2% in a 70 kg human. Similarly, the allometric equation for kidneys (g) as a function of total body mass (g) is $0.021 W^{0.85}$ (5). The kidneys are 0.9% of total body weight in a 250 g rat and only 0.4% in a 70 kg human. Although both the liver and kidney play important roles in the elimination of drugs, the concept of relative organ size must be taken into consideration when species differences in drug elimination are compared. The relative organ size decreases as animal size increases. Furthermore, the relative amount of hepatic enzymes, such as cytochrome P450 (6, 7) and relative number of nephrons/g weight of kidney tissues (8) are higher in small animals than humans. Collectively, we would expect small animals to eliminate drugs more rapidly than human beings when compared on a weight-normalized basis.

Physiological parameters also change as a function of total body weight in an allometric manner. For example, the allometric relationship between the blood circulation time (sec) and total body weight (kg) is $21 W^{0.21}$ (9). The blood circulation time is 15 sec in a 250 g rat and 50 sec in a 70 kg human. This means that a drug molecule circulates the body 4 times/min in the rat and only 1 time/min in humans. Similarly, both relative hepatic and renal blood flow (ml/min/kg) decrease as the animal size increases in an allometric manner. The exponent values are 0.89 for hepatic blood flow (4) and 0.77 for renal blood flow (10). Thus, the smaller species deliver drugs more frequently and faster to the eliminating organs, and eliminate the drugs, particularly those with high clearances, more rapidly. However, some physiological parameters, such as body temperature ($36^\circ\text{--}38^\circ\text{C}$), hematocrit (40–45%), and serum albumin concentration (3–4 g/dl) are relatively constant among animals and are independent of animal size (11).

Biochemistry provides countless examples of similarities between species, of which one of the most instructive is the structure of cytochrome P450s. Cytochrome P450s appear to be derived from a single ancestral gene, ~1.36 billion years ago (12). To date, at least 10 cytochrome P450 gene families have been identified in mammals. Although all members of this superfamily possess highly conserved regions of amino acid residues, there are small changes in the primary amino acid sequences of the cytochrome P450s across species. It is known that even small changes in the amino acid sequences can give rise to profound differences in substrate specificity. Similarly, although structural and functional homologies of serum albumin exist among species, the amino acid sequences are not identical between human and animal species. Therefore, unlike anatomical and physiological parameters, there is no distinct allometric relationship between the biochemical parameters (protein binding and enzyme activity) and body weight.

Physiological and Allometric Models

Two well-known methods (the physiological and allometric models) have been used to predict the pharmacokinetics of drugs in humans from data in animals.

The physiological model offers a mechanistic approach, wherein organs of interest are arranged according to organ size and blood flow

to each organ (13–16). Biochemical parameters, such as plasma protein binding, enzymatic kinetic parameters, and excretion clearance determined *in vitro* and *in vivo*, are often incorporated into the model. Using this approach, the kinetic behavior of drugs in humans can be predicted.

In contrast, the allometric approach is more empirical, examining the relationships between body size, time, and kinetic parameters without necessarily understanding the underlying mechanisms (17, 18). A linear regression of a log–log plot of the kinetic parameters vs. the body weight of the animal species produces an allometric relationship from which a particular kinetic parameter in humans can be calculated. Generally, a good allometric relationship is expected for the kinetic parameters reflecting anatomical and physiological functions, but not for the parameters involving protein binding and biotransformation processes.

Although both physiological and allometric methods are of academic interest, they are not practical and have only limited use in drug discovery and development. The former approach tends to be costly and time-consuming, with frequent failures in obtaining all the parameters needed. The latter is also costly and time-consuming, because it requires the kinetic data from at least four or five species to define the allometric relationship properly. Often, these kinetic parameters are not available before the initial clinical studies, which is the time when an accurate prediction of human pharmacokinetics is most needed. In fact, most of the reported literature successfully showing the animal-human prediction, particularly those based on an allometric approach, are essentially hindsight and not predictive.

In practice, the selection of a drug candidate for further development in humans relies heavily on the limited information of pharmacokinetics obtained from one or two animal species and on earlier experience gained from similar drugs. Thus, an understanding of the underlying mechanisms for the species differences in pharmacokinetics is important for the interpretation of data.

Theoretical Considerations

Absorption. Oral bioavailability of a drug is defined as the fraction of an oral dose of the drug that reaches the systemic circulation. Because the entire blood supply of the upper gastrointestinal tract passes through the liver before reaching the systemic circulation, the drug may be metabolized by the liver and the gut wall. Mathematically, the bioavailability (F) can be described as:

$$F = f_G \cdot f_H \cdot f_{\text{abs}}, \quad (2)$$

where f_{abs} is the fraction of drug absorbed, and f_G and f_H are the fractions of drug not cleared by the gut wall and liver, respectively, during the first passage of absorption. The loss of drug before its reaching the systemic circulation is referred to as the first-pass effect.

After oral administration, the plasma concentration (C) and AUC of a drug for a linear one-compartment system can be expressed as:

$$C = \frac{K_a \cdot F \cdot \text{dose}}{V_d(K_a - K_{\text{el}})} (e^{-K_{\text{el}} \cdot t} - e^{-K_a \cdot t}) \quad (3)$$

and

$$\text{AUC} = \frac{F \cdot \text{dose}}{V_d \cdot K_{\text{el}}} = \frac{F \cdot \text{dose}}{CL}, \quad (4)$$

where K_a and K_{el} are the first-order rate constants for absorption and elimination (19). It is evident that the plasma concentration of drugs after oral administration is affected not only by the rate and extent of absorption (K_a and F), but also by the elimination (K_{el}) and V_d , whereas the AUC is only influenced by clearance and bioavailability.

Differences between species in response to the administered doses can, therefore, be explained in terms of the processes that govern the ADME.

Elimination Clearance. Elimination of drugs occurs in specific organs, usually the liver and the kidney. If the elimination is mainly by the liver, the CL is approximately equal to CL_H that can be expressed as:

$$CL \approx CL_H = \frac{Q \cdot f_p \cdot CL_{int}}{Q + f_p \cdot CL_{int}}, \quad (5)$$

where Q is the hepatic blood flow, and CL_{int} is a measure of the intracellular removal of drug, described by the Michaelis-Menten equation:

$$CL_{int} = V_{max}/K_M, \quad (6)$$

where V_{max} is the capacity of the enzyme (or carrier) system, K_M is the Michaelis constant.

For low clearance drugs, $Q \gg f_p \cdot CL_{int}$, the CL is directly related to f_p and CL_{int} as shown in eq. 7.

$$CL \approx CL_H \approx f_p \cdot CL_{int}. \quad (7)$$

This means that plasma protein binding and metabolism activity are the major determinants for the elimination of low clearance drugs. As previously described, there is no clear allometric relationship between metabolism activity (or protein binding) and body weight. Therefore, it is not expected that the elimination of low clearance drugs has a good allometric relationship with body weight across species. The extrapolation of antipyrine metabolism to humans failed. The intrinsic clearance of antipyrine in humans, a measurement of hepatic oxidative metabolism of this drug, is only one-seventh of that which would be predicted from other species using the allometric approach (18).

For high clearance drugs, $f_p \cdot CL_{int} \gg Q$ and the CL is limited by hepatic blood flow.

$$CL \approx CL_H \approx Q. \quad (8)$$

Because hepatic blood flow is shown to have an allometric relationship with body weight (4), it is expected that the elimination of high clearance drugs can show an allometric relationship with body weight. The best-studied example is propranolol. This drug is mainly eliminated by the liver, and the CL is so rapid that it is limited by hepatic blood flow. The CL is 90 ml/min/kg for the rat, 34 ml/min/kg for the dog, 18 ml/min/kg for the monkey, and 15 ml/min/kg for the man (20). The CL value in each species approximates their hepatic blood flow rate, suggesting that the CL of propranolol in humans can be extrapolated from animal data using the allometric approach.

If the drug is eliminated mainly by the kidney, the CL is approximately equal to CL_R . Renal handling of drugs usually involves three processes: glomerular filtration, renal tubular secretion, and reabsorption from the renal tubular lumen. Glomerular filtration is a passive process and is a function of the unbound concentration of drug in plasma. The relationship between CL_R and these processes can be expressed as:

$$CL_R = f_p \cdot GFR + CL_s - CL_{ra}, \quad (9)$$

where CL_s and CL_{ra} are secretion clearance and reabsorption clearance, respectively.

Rearrangement of eq. 9 yields:

$$CL_R/f_p = CL_{uR} = GFR + (CL_s - CL_{ra})/f_p. \quad (10)$$

When CL_{uR} is greater than GFR, this means that renal tubular secretion of the drugs occurs. Conversely, when CL_{uR} is less than GFR,

reabsorption of the drug occurs. It is known that renal physiology has a good allometric relationship with the body weight (17, 18). Thus, the renal excretion of drugs in humans can be extrapolated from animal data using the allometric relationship. Ceftriaxime (21, 22) and aztreonam (23) are good examples. Both antibiotics are renally excreted and show low protein binding. The pharmacokinetics and plasma concentration profiles of these antibiotics in humans have been successfully extrapolated from data in animals using the allometric approach. Similar to ceftriaxime and aztreonam, the plasma clearance of methotrexate also has shown a good allometric relationship among six mammals, including humans (18). Methotrexate, a widely used anticancer agent, is mainly excreted as unchanged drug in the urine and has low protein binding (24).

Volume of Distribution. It is generally believed that only the unbound drug can diffuse across membranes that restrict distribution from vascular compartment to tissue compartment and *vice versa*. Therefore, changes in drug protein binding in plasma and tissue can affect the distribution of drugs in the body. Kinetically, drug distribution is often expressed in terms of the V_d , which is usually defined as the ratio of amount of drug in the body to the blood or plasma concentration.

Because the V_d usually has no direct physiological meaning and does not refer to a real volume, this parameter may be useful in certain clinical situations. It may help predict the usefulness of dialysis in intoxicated patients or patients with renal dysfunction, and it is also useful in calculating the plasma concentration after intravenous administration of drugs that are required to be given parenterally.

Although ~60% of total body albumin is located in the interstitial fluid of the extravascular space, V_d is mathematically expressed as (25):

$$V_d = V_p(1 + R_{EI}) + f_p \cdot V_p \left(\frac{V_E}{V_p} - R_{EI} \right) + V_T \frac{f_p}{f_i}, \quad (11)$$

where R_{EI} is the ratio of the amount of protein in the extracellular fluids outside the plasma to that in plasma, and V_E is the extracellular space less the plasma volume. The values of V_T , V_E , and V_p are ~0.45, 0.17, and 0.04 liters/kg, respectively, and R_{EI} is considered to be 1.5 (26).

Thus, eq. 11 can be simplified to:

$$V_d(\text{liters/kg}) = 0.1 + 0.11 f_p + 0.45 \cdot \frac{f_p}{f_i}. \quad (12)$$

When a drug is not bound to plasma and tissue proteins ($f_p = f_i = 1$), the V_d will approximate the volume of body water (0.66 liters/kg). Antipyrine is an example of such a drug that has very low binding to plasma and tissue proteins (27). When a drug is extensively bound to plasma protein ($f_p \rightarrow 0$), the smallest V_d that a drug can have is 0.1 liters/kg. The V_d of most nonsteroidal antiinflammatory drugs ranges from 0.1 to 0.2 liters/kg (28). On the other hand, when a drug is extensively bound to tissue protein ($f_i \rightarrow 0$), it may have a extremely large V_d . Examples of such drugs include chlorpromazine (21 liters/kg), nortriptyline (20 liters/kg), and desipramine (42 liters/kg) (29). There is no upper limit of the V_d . As a matter of fact, the V_d of chloroquine is estimated to be as high as 800 liters/kg (30, 31).

Rearrangement of eq. 12 yields

$$V_d/f_p = 0.11 + \frac{0.1}{f_p} + \frac{0.45}{f_i}. \quad (13)$$

As indicated in the equation, usually a change in f_i has a greater effect than f_p on V_d . Because there is no allometric relationship between protein binding and body weight, it will be difficult to project the V_d

and V_d of drugs in humans from data in animals.

When a drug has very low binding to plasma and tissue proteins, or when a drug only distributes extracellularly, the V_d of the drug reflects total body water or extracellular water. In these cases, the V_d in humans can be readily predicted from data in animals, because both the total body water and extracellular water (liters/kg) decrease as animal size increases in an allometric manner (17). The allometric exponent values are 0.89 for the total body water and 0.68 for the extracellular water.

Half-Life. Most drugs are administered in a fixed dose given at regular intervals to achieve therapeutic objectives. Half-life of drugs in plasma is one of the important factors that influence the selection of a dosage regimen. By knowing the half-life of a drug, one can predict the degree of accumulation of a given dosing regimen and the time to reach steady-state concentration (19).

There are some drugs are given less frequently than their half-lives. For example, histamine H_2 -receptor antagonists have half-lives in the order of 2 hr, but are usually given twice or once a day (32). Virtually no accumulation of these drugs in plasma was observed after repeated dosing. On the other hand, many drugs are given more frequently than their half-lives. For these drugs, the half-life is >24 hr, but they are given either several times a day or once a day. Thus, drug accumulation in plasma is expected to be substantial. Benzodiazepines are this class of drugs (33, 34).

Kinetically, a drug's half-life is proportional to its volume of distribution, but inversely to its CL . For low clearance drugs, the plasma half-life ($t_{1/2}$) can be expressed as (28):

$$t_{1/2} = \frac{(1 - H + K_p H) \left[0.07 + 0.076 f_p + 0.31 \frac{f_p}{f_i} \right]}{f_p \cdot CL_{int}}, \quad (14)$$

where H is the hematocrit and K_p is the partition coefficient of drug between red blood cells and plasma. It is evident that the half-life of low clearance drugs is determined by binding of drugs to plasma and tissue proteins, as well as intrinsic metabolic activity.

Because the half-life of a drug is determined by its V_d and CL , it is difficult to predict the half-life in humans from data in animals. Mordenti (35) has successfully predicted the half-life of ceftizoxime in humans using the allometric approaches; however, this is only valid for drugs that are renally excreted and have low protein binding. For this class of drugs, the CL and V_d reflect, more or less, the renal function and total body water, respectively. For drugs that are eliminated mainly by metabolism, such as propranolol, there is no allometric relationship for the half-life. The clearance of propranolol is ~ 90 ml/min/kg for the rat, 34 ml/min/kg for the dog, and 18 ml/min/kg for the monkey, but these species have a similar half-life of 30–40 min (20).

Even when a drug is mainly eliminated by renal excretion, the half-life is still unpredictable if protein binding is involved. Digoxin is a good example. The elimination of digoxin is mainly by renal excretion; 70–90% of the administered dose appears unchanged in the urine (36). Although the total clearance of digoxin is similar in the range of 4–6 ml/min/kg in four animal species (rats, cats, dogs, and humans), the half-life varied dramatically from 2.5 hr in the rat to 58 hr in the cat, with that of 39 hr in humans being in-between, because of marked differences in the volume of distribution among species (37).

Species Similarities and Differences in Pharmacokinetics

Because body size is so different between species, the dose must be normalized. In most pharmacological and toxicological studies, the dose is given as mg/kg. This means that the dose is proportional to

body mass. However, in certain clinical situations, the equivalent dose is normalized by body surface area (mg/m^2) (38–40).

In comparing the pharmacokinetics in different animal species, it is common to normalize the data on the basis of body weight or body surface area, but data can also be normalized to kidney weight in the case of renally excreted drugs, or liver weight for drugs excreted in the bile, or the amount of cytochrome P450 for drug metabolism. In addition, data can be expressed on an absolute basis, such as the fraction of unbound drug in plasma, the extent of absorption, and bioavailability.

Absorption. Absorption is a very complicated process. The causes that influence the rate and extent of absorption can be categorized as physiological and physicochemical factors. The former factors include gastric and intestinal transit time, blood flow rate, and pH. The latter factors are the drug's intrinsic properties, such as pK_a , molecular size, lipophilicity, and solubility (41, 42). The former factors are species-dependent, whereas the latter ones are clearly species-independent. Because the nature of the biomembrane of intestinal epithelial cells is similar across species and because the absorptive process (simple diffusion) is basically an interaction between the drug and the biomembrane (43, 44), the intrinsic absorption of drugs across the wall of the gastrointestinal tract is probably quite similar among species.

Two principal factors that determine the site of absorption are residence time and absorptive surface area. Generally, the upper region of small intestine (duodenum/jejunum) is the major site of absorption, because of the large surface area provided by epithelial folding and the villous structures of the absorptive cells. Gastric-emptying times in laboratory animals are related to their stomach volume and body size. During the fasting state, the gastric-emptying time for drugs in solution is ~ 10 min for the rat, 30 min for the rabbit, 40–50 min for the dog, and 1 hr for human (45–47). Transit time of the small intestine also varies among species. It is not surprising that the species-dependent gastric-emptying and intestinal transit times can result in differences in the rate and/or extent of drug absorption. In addition, the laboratory animals can be divided, according to their dietary habits, into herbivorous (rabbit, guinea pig, rat, and mouse), carnivorous (dog and cat), and omnivorous (pig and monkey) species. Diet can also affect the absorption of drugs among species, particularly in humans where the diet is so variable.

Furthermore, species differences in gastric and intestinal pH may also contribute to the differences in drug absorption. The rate of gastric acid secretion is known to be widely different among species. For examples, rats and humans are good acid secretors, and cats and dogs are poor secretors (48–54). When the solubility of a drug is pH-dependent, species differences in absorption of the drug are expected.

L-735,524 (fig. 1) is a good example for pH-dependent absorption. L-735,524, a potent and specific inhibitor of HIV protease, is currently under investigation for the treatment of AIDS. The aqueous solubility is pH-dependent, 60 mg/ml at pH 3.5 and <0.03 mg/ml at pH 5. When L-735,524 was given orally as a suspension in 0.5% methylcellulose (pH 6.5) at a dose of 10 mg/kg, the bioavailability was 15.8% for the rat and 16% for the dog at the fasted state. When the same dose of L-735,524 was given as a solution in 0.05 M citric acid (pH 2.5), the bioavailability was 72% in the fasted dog and 23% in the fasted rat (55). These results have demonstrated a profound species difference in drug absorption.

The blood clearance of L-735,524 was 79 and 11 ml/min/kg for rats and dogs, respectively. From the clearance data, an extensive first-pass effect is expected in rats, but not in dogs. In a separate study, the hepatic first-pass extraction in rats was estimated to be $\sim 70\%$ by comparing the concentrations in the systemic circulation during portal

or femoral vein infusions at steady-state. It was concluded that the low bioavailability in rats was mainly attributed to an extensive first-pass effect. When taking the hepatic first-pass effect into account, the extent of absorption of L-735,524 in the rat was estimated to be ~53% for the methylcellulose suspension and 77% for the citric acid solution.

The high bioavailability (72%) in dogs from the citric acid solution supports the argument that there was no extensive first-pass metabolism in this species. The marked differences in bioavailability in dogs between the citric acid solution and methylcellulose suspension are most likely the result of the pH-dependent solubility of L-735,524 and the low basal gastric acid secretion in this species.

The basal acid secretion in dogs is ~0.1 $\mu\text{mol}/\text{min}/\text{kg}$, which is <1% of the maximal capacity of 18 $\mu\text{mol}/\text{min}/\text{kg}$. Ehrlein and Pröve (56) and Feldman and Grossman (57) have shown that the duodenal pH (~7.0) in dogs remained relatively constant after receiving saline. Thus, one may expect that the duodenal pH in dogs will be low (pH <3) after receiving the citric acid solution, but high (pH $\geq$ 6) after receiving the methylcellulose suspension.

In contrast to the dog, the rat is a good gastric acid secretor (48, 52). The basal secretion is ~30% of the maximal capacity (30 $\mu\text{mol}/\text{min}/\text{kg}$). It is conceivable that the pH of the methylcellulose suspension will be reduced in the duodenum of rats after mixing the gastric juice. The less marked differences in the bioavailability between the methylcellulose suspension and citric acid solution suggest that the differences in duodenal pH in rats were small after administration of these two formulations.

These results suggest that gastric pH may play an important role in the absorption of L-735,524 and that pH-dependent solubility can explain the species difference in the absorption of L-735,524. To test this hypothesis, the drug was given orally to dogs as a 100-mg capsule 1 hr after feeding or without food. Both C_{max} and AUC were higher in the fed state than in the fasted state by a factor of 3 to 4. The increased absorption of L-735,524 in dogs is believed to be caused by the stimulation of gastric acid secretion by the food and the consequent lowering of pH and enhancement of the solubility of the drug. Ehrlein and Pröve (56) have shown that the duodenal pH in dogs decreased from 7 to 2 within 60 min after receiving a mashed potato meal. Furthermore, pretreatment of rats with famotidine, a potent H_2 -receptor antagonist, caused substantial decreases in both C_{max} and AUC after the oral administration of L-735,524 in methylcellulose suspension, but not in citric acid solution. The decreased absorption in rats is most likely caused by a decrease in the gastric acid secretion caused by famotidine (58).

After understanding the underlying mechanisms for the low bioavailability in rats and dogs, we predicted that L-735,524 would be well absorbed in humans, because humans normally have a high gastric acid secretion and that the first-pass effect of the drug would be insignificant in humans. As expected, when L-735,524 was given orally as capsules to AIDS patients (600 mg; 10 mg/kg), the plasma profiles were similar to those in dogs receiving the same dose (10 mg/kg) in citric acid. The C_{max} and AUC in humans were 10 μM and 19 $\mu\text{M}\cdot\text{hr}$, respectively (provided by Dr. K. C. Yeh, Merck Research Laboratories), and the corresponding values for dogs were 11.5 μM and 12.5 $\mu\text{M}\cdot\text{hr}$.

Another appropriate example that shows species similarity in drug absorption is L-365,260 (fig. 1), a potent CCK_B receptor antagonist for the treatment of anxiety. This drug has a very poor aqueous solubility (~2 $\mu\text{g}/\text{ml}$), and when given orally as a suspension in 0.5% methylcellulose, bioavailability was 14% for the rat, 9% for the dog, and 2% for the monkey (59). The poor bioavailability in all species studied

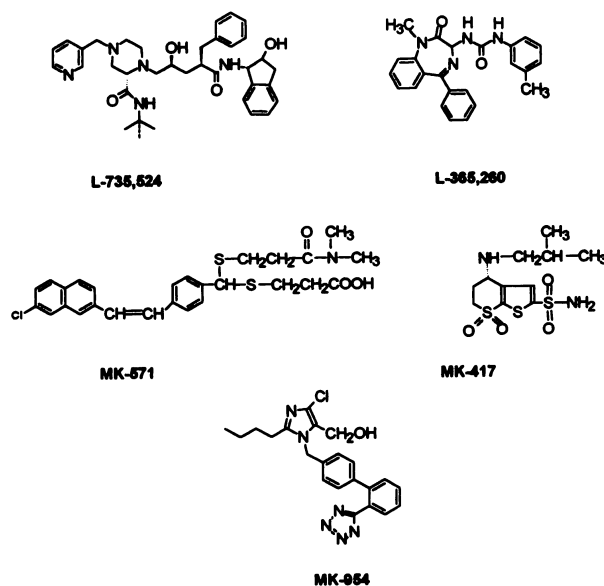


FIG. 1. Chemical structures of Merck's investigational compounds.

could be attributed to poor absorption or extensive first-pass metabolism. To examine the possible first-pass effect, the hepatic first-pass extraction in rats and dogs was determined by comparing the concentrations in the systemic circulation during portal and femoral vein infusion. The hepatic first-pass metabolism was low and estimated to be 0.3 for the rat and 0.15 for the dog. Thus, the limited bioavailability of L-365,260 is more likely to be attributable to its poor absorption as a result of its poor aqueous solubility.

When L-365,260 was given orally as a solution in PEG 600 to rats and dogs, the bioavailability was increased 3- to 4-fold in rats and 8- to 9-fold in dogs. Although the underlying mechanism for the improved absorption is unknown, PEG 600 may have exerted a cosolubilizing effect to maintain a higher drug concentration in solution in the gastrointestinal tract, resulting in a better absorption. Because L-365,260 absorption was improved in both rats and dogs by using PEG 600, good absorption of L-365,260 is expected in humans if the drug can be dosed in PEG 600 solution. With this information at hand, L-365,260 was dosed in capsules containing PEG 600 in the subsequent clinical studies. The C_{max} and AUC are comparable in the dog and humans when compared on a basis of weight-normalized dose, indicating a reasonably good absorption in humans. The C_{max} and AUC were 2.3 $\mu\text{g}/\text{ml}$ and 450 $\mu\text{g}\cdot\text{min}/\text{ml}$ for the dogs and 0.50 $\mu\text{g}/\text{ml}$ and 148 $\mu\text{g}\cdot\text{min}/\text{ml}$ for normal human subjects, when the same dose (50 mg) of L-365,260 in PEG capsules was given orally to dogs (12 kg) and normal volunteers (70 kg).²

Drugs pass the gastrointestinal lumen into the blood stream via two routes: 1) transcellularly, in which the drugs are transported into and through epithelial cells into the bloodstream; and 2) paracellularly, in which drugs reach the bloodstream via the tight junctions between the epithelial cells. These junctions are permeable to water, electrolytes, and small molecules (<150 Da); however, the capacity is severely restricted by the number of aqueous pores available (60). The intercellular spaces occupy only ~0.01% of the total surface area of the epithelium. Alendronate, an antiosteolytic agent under investigation in the treatment of a number of bone disorders, is poorly absorbed after

² J. H. Lin, unpublished data.

oral administration. The bioavailability was 0.9% for the rat, 1.8% for the dog, and 1.7% for the monkey (61). The poor absorption is most likely attributed to its poor lipophilicity that prohibits transcellular transport across the epithelial cell membrane, and to its relatively large molecular size (250 Da) that hinders paracellular transport. Lipophilicity of drugs is usually measured by the octanol/buffer partition ratio. The octanol/buffer partition ratio of alendronate was estimated to be 0.0017 and independent of pH values ranging from 2 to 11. Alendronate has 5 pK_a values (0.8–12.2) and is completely ionized at this pH range. Because the anatomical structure of epithelial cells is similar in animals and humans, and because alendronate can only be absorbed by paracellular transport, one would anticipate that the absorption of alendronate in humans will be very low. As expected, the bioavailability of orally administered alendronate was <1% in normal healthy volunteers (Dr. A. Porras, Merck Research Laboratories).

Although oral absorption is expected to be different among species because of their differences in gastrointestinal physiology, the prediction of absorption in humans has been reasonably successful after appropriate application of pharmacokinetics and careful examination of the underlying mechanisms, as in the previously cited three examples. In a survey, Clark and Smith (62) have shown that the fraction of absorption for a large variety of drugs is remarkably consistent between animals species and humans; however, the bioavailability differs substantially among species, presumably a result of species differences in the magnitude of first-pass metabolism. This survey implies that the intrinsic absorption of most drugs is similar across species. Although the dog is believed to be a useful model, particularly for oral dosage form testing, because of its anatomical similarity to human and its relatively easy to receive different formulations (63, 47), there is still a large discrepancy in bioavailability of drugs between dogs and humans in some cases, as the example of L-735,524. Generally, the rat has a high hepatic drug-metabolizing activity. If the rat is used as a model, it is important to distinguish whether the low bioavailability is caused by poor absorption or by an extensive first-pass effect, or a combination of both.

Protein Binding and Tissue Distribution. It has long been known that the extent of binding of drugs to plasma proteins differs considerably among animal species. The observed species differences in plasma binding may reflect differences in the affinity or the number of binding sites on the protein molecule. Albumin, the major drug binding protein in plasma, is composed of a single polypeptide chain of ~590 amino acids. Its main function is to regulate the osmotic pressure of blood. Although structural and functional homologies of albumin exist among species, there are small changes in the amino acid sequences between human and animal species (64–66). As shown in the section of theoretical considerations, differences in plasma binding can independently affect both distribution and elimination of the drug, particularly for drugs with low clearance, such as diflunisal, MK-571, and MK-417.

Diflunisal, a nonsteroidal antiinflammatory drug, is extensively bound to plasma protein and eliminated mainly by conjugation as ester and ether glucuronides in humans and rats (67, 68). Although diflunisal exhibits concentration-dependent pharmacokinetics in both rats and humans, the overall kinetic behavior of the drug is different in these two species. The apparent plasma clearance of diflunisal in rats remains relatively constant over the therapeutic concentration range of 50–150 $\mu\text{g/ml}$ (150–450 μM) (69), whereas the clearance in humans decreases with concentration over this same range (70). In contrast, *in vitro* binding studies have demonstrated that diflunisal shows nonlinear plasma protein binding in rats over the therapeutic concentration range, but the unbound fraction of diflunisal in plasma

remains unchanged in humans over this range (71). The differences in the kinetic behavior of diflunisal in rats and humans over the therapeutic concentration range cannot be solely explained by the species differences in plasma protein binding of the drug. A detailed kinetic study has demonstrated that lack of changes in plasma clearance in rats is a consequence of the opposing effects of saturable metabolism and saturable plasma protein binding (69), whereas the nonlinear *CL* in humans is likely attributed to the saturable metabolism alone. Further studies with serum albumin reveal that the number of binding sites for diflunisal is different between human and rat albumin, whereas the affinity for both albumin is comparable. The number of binding sites and association constants are ~ 3 and $4.8 \times 10^5 \text{ M}^{-1}$ for human serum albumin, and 1 and $4.2 \times 10^5 \text{ M}^{-1}$ for rat serum albumin (71). Consistent with this, displacement studies with the drug binding site markers specific to albumin ($[^{14}\text{C}]$ diazepam, $[^{14}\text{C}]$ warfarin, and $[^3\text{H}]$ digitoxin) suggest that diflunisal binds to three discrete binding sites (warfarin, digitoxin, and diazepam) on human serum albumin, but only one site (warfarin) on rat serum albumin (71). Although the albumin concentration in plasma (500–600 μM) is similar between humans and rats, these results strongly suggest that a much higher drug concentration is required to saturate plasma protein binding of diflunisal in humans, because of a larger number of binding sites.

Plasma binding of MK-571 (fig. 1), a potent leukotriene D_4 antagonist, has been studied in 12 mammalian species (72). The binding of MK-571 enantiomers to plasma protein is extensive, stereoselective, and species-dependent. In some species, the *S*-enantiomer binds to a greater extent than the *R*-enantiomer; in others, the *R*-enantiomer binds more extensively and in still other species there is no stereoselectivity. For both enantiomers, the unbound fraction in plasma differs by a factor of 8 among the species studied. Further studies with serum albumin suggest that this protein is the major component for MK-571 enantiomer binding and that the species differences in stereoselective binding that are caused by differences in association constants are linked to structural differences in the albumin molecule (72). The *R*-enantiomer of MK-571 is bound to rat plasma to a greater extent than the *S*-enantiomer, whereas in dog and monkey plasma the reverse is true. The elimination clearance of the enantiomers is related to the stereoselective plasma protein binding; that with the greater unbound fraction being cleared more rapidly (73). Although MK-571 is a low clearance drug, the stereoselective elimination can be, at least partly, explained by plasma protein binding, as indicated by eq. 7.

MK-417 (fig. 1) binding to carbonic anhydrase is another example where clearance can be affected. Carbonic anhydrase, a zinc metalloenzyme, is primarily present in red blood cells of all mammals, accounting for >90% of the enzyme in the body. MK-417 is a potent carbonic anhydrase inhibitor used as a topical ocular hypotensive agent for the treatment of glaucoma. The pharmacokinetic parameters of MK-417 remained unchanged when the dose was increased from 0.05 to 0.2 mg/kg iv, whereas the blood clearance and V_d increased substantially when the dose exceeded 2 mg/kg iv (74, 75). The dose-dependent pharmacokinetics of MK-417 is caused by the saturation of protein binding of the limited carbonic anhydrase in blood cells (76).

The binding parameters (np and K_d) shown in table 1 indicate that the binding of MK-417 to erythrocytes is extensive and species-dependent (77, 78). Under linear conditions (0.05 mg/kg iv), the elimination kinetics are also species-dependent. Both blood clearance and half-life were roughly related to their binding data, smaller np/K_d values being associated with enhanced elimination. However, binding data alone cannot quantitatively explain the species differences in the elimination kinetics of MK-417. For example, the difference of np/K_d between rats and dogs was only 2.5-fold, and the differences in *CL*

TABLE 1

Pharmacokinetic and binding data of MK-417 in rats, rabbits, and dogs^a

Species	Erythrocyte Binding			Intravenous (0.05 mg/kg)		
	<i>n</i> _p	<i>K</i> _d	<i>n</i> _p / <i>K</i> _d	<i>CL</i>	<i>V</i> _d	<i>t</i> _{1/2}
	μM	μM		ml/hr/kg	ml/kg	hr
Rat	101.3 ± 36.1	0.509 ± 0.22	199	1.29 ± 0.09	82.8 ± 4.3	45.7 ± 3.9
Rabbit	65.1 ± 6.9	0.282 ± 0.063	231	1.15 ± 0.20	86.4 ± 5.4	65.3 ± 6.0
Dog	35.7 ± 6.5	0.073 ± 0.029	489	0.0380 ± 0.008	102 ± 11.6	1859 ± 367
Human	24.5 ± 4.8	0.023 ± 0.003	1065	—	—	—

Values are mean ± SD. *n*_p, maximal binding capacity; *K*_d, dissociation constant; *t*_{1/2}, terminal half-life.^a Data modified from refs. 77 and 78.

and half-life were 33- and 40-fold, respectively. Other factors, such as metabolism (*CL*_{int}), may also contribute to the overall species differences in the elimination kinetics.

Based on binding data in human erythrocytes and the kinetic parameters from laboratory animals (table 1), one might expect that MK-417 would be cleared very slowly, similarly to that in the dog with a prolonged half-life of 2–3 months. After instillation of MK-417 (0.04 mg/kg) into the eyes of patients, the half-life in blood was only ~2 weeks, much shorter than expected (Dr. G. A. Winchell, Merck Research Laboratories). As shown in eqs. 7 and 14, for low clearance drugs, such as MK-417, *CL* and half-life are dependent not only on the blood binding, but also on metabolic activity. Thus, it is difficult to predict the elimination in humans from animal data, when both protein binding and metabolism are involved in the elimination.

In the case of alendronate, the drug is taken up by bone tissue to the extent of 60–70% of an intravenous dose (30–40% is excreted by the kidneys), and renal excretion is the only route of elimination of the drug (79–81). Both the binding of alendronate to plasma protein and bone uptake are species-dependent (82). The drug is highly bound to rat plasma, but not to dog plasma. The unbound fraction is ~0.04 for the rat and 0.5 for the dog. The apparent uptake clearance (*CL*_{up}) by tibia in dogs and rats is 0.075 and 0.18 ml/min/g bone tissue, respectively. Like other organs, uptake of alendronate by bone tissue is controlled by plasma flow (*Q*), *f*_p, and *CL*_{int}, as described by the following equation:

$$CL_{up} = Q(1 - e^{-f_p CL_{int}/Q}). \quad (15)$$

By applying the equation, the *CL*_{int} was estimated to be 10 ml/min/g for the rat and 0.3 ml/min/g for the dog. Although there is a pronounced species difference in the *CL*_{int} by a factor of 30, the apparent *CL*_{up} in rats is only 2- to 3-fold greater than that in dogs, as a result of the opposite effect of the marked species differences in plasma protein binding of alendronate.

As discussed in the theoretical section, *V*_d is an important determinant for *C*_{max} and half-life. Therefore, it is also important to predict *V*_d before the initial clinical studies. Fichtl *et al.* (83) reported that there were striking species differences in plasma protein binding and *V*_d of propranolol. The values for the *V*_d varied by >20-fold being lowest in monkeys and highest in rabbits. However, *V*_f was virtually the same in all species (fig. 2). Consistent with this, Sawada *et al.* (84) reported that the *V*_f values of 10 basic drugs are quite similar among species, including humans.

Fichtl *et al.* (83) proposed that the species similarity of *V*_f for propranolol is probably caused by the similar extent of tissue binding among the species. Evidence of species similarity in tissue binding has been presented by Fichtl and Schulmann (85). For a series of 44 drugs differing widely in their physicochemical properties, a close correlation was observed in the tissue binding for the individual drugs

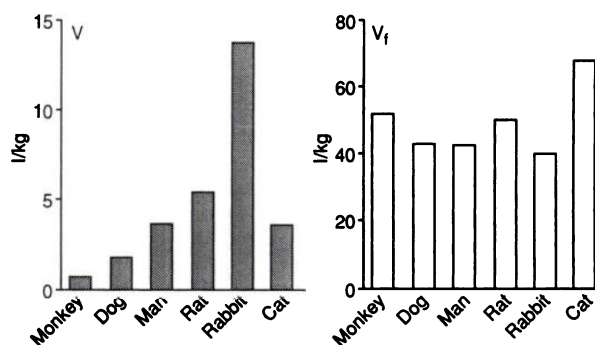


FIG. 2. Apparent volume of distribution of propranolol in different species (left).

When distribution volume of unbound is calculated (right), species differences largely disappear. (After Fichtl *et al.*, 1987; reproduced with permission from the publisher).

between the different species, when binding to rat, rabbit, and human muscle tissues was compared. Based on these results, they propose that the *V*_f of drugs should be similar in humans and other species. Therefore, with the knowledge of *V*_f from laboratory animals and *f*_p from human plasma protein binding determined *in vitro*, one can predict the *V*_d in humans before the initial clinical studies. Unfortunately, this approach is not valid for all drugs. Boxenbaum (86) compared the pharmacokinetic parameters for 12 benzodiazepines in dogs and humans. Eight of the 12 benzodiazepines had quite different *V*_f values between the dog and human, the difference being as much as 7-fold for lorazepam (table 2). The large species differences in the *V*_f values were also reported for β-lactam antibiotics (table 3) (87). As indicated in eq. 13, the *V*_f of a drug depends on both the *f*_p and *f*_u. Even if the *f*_u of the drug is truly similar among species, species differences in the *V*_f is still to be expected, because the *f*_p can still be species-dependent. Thus, the species similarity in the *V*_f of propranolol observed (fig. 2) by Fichtl *et al.* (83) could be fortuitous.

As demonstrated in the previous examples, it is quite difficult to predict the protein binding and *V*_d of drugs in humans from data of animals.

Metabolism. After ingestion, drugs are excreted either unchanged or chemically modified. Drug metabolism is generally divided into phase I and phase II reactions. Phase I metabolism generally adds or exposes polar functional groups on lipophilic drugs, and phase II metabolism occurs when a functional group reacts with an endogenous substrate to yield a conjugated metabolite that is readily excreted from the body through urine and/or bile.

From an evolutionary standpoint, it is not surprising that similarities

TABLE 2
Pharmacokinetic parameters of benzodiazepines in dogs and humans^a

Species	$t_{1/2}$	CL_m	V_d	f_p	V_f
	hr	ml/min/kg	liters/kg		liters/kg
Bromazepam					
Man	12.0	0.77	0.900	0.477	1.9
Dog	6.1	3.79	1.97	0.477	4.1
Chlordiazepoxide					
Man	9.38	0.564	0.466	0.0605	7.7
Dog	2.33	6.76	2.54	0.0640	39.6
Chlornordiazepam					
Man	79.5	0.434	2.75	0.0454	60.6
Dog	9.02	5.28	5.21	0.0679	76.7
Clonazepam					
Man	35.8	1.64	5.48	0.134	40.9
Dog	1.58	11.2	1.55	0.130	11.9
Demoxypam					
Man	30.0	0.283	0.703	0.217	3.2
Dog	14.5	1.29	1.59	0.331	4.8
Diazepam					
Man	36.0	0.672	1.91	0.0134	142.5
Dog	7.62	35.0	23.6	0.040	590.0
Flunitrazepam					
Man	13.5	3.92	4.58	0.220	20.8
Dog	1.32	73.1	13.0	0.180	72.2
Lorazepam					
Man	12.4	1.56	1.67	0.105	15.9
Dog	1.98	40.7	11.3	0.105	107.6
Medazepam					
Man	5.13	2.64	1.17	0.007	167.1
Dog	0.987	106	14.8	0.0150	986.6
Nordiazepam					
Man	45.5	0.205	0.807	0.034	23.7
Dog	1.87	14.8	2.38	0.041	58.0
Oxazepam					
Man	7.33	1.06	0.673	0.0427	15.8
Dog	0.683	31.7	1.88	0.105	17.9
Triazolam					
Man	2.25	6.24	1.26	0.110	11.5
Dog	0.917	17.6	1.40	0.120	11.7

$t_{1/2}$, half-life; CL_m , metabolic clearance.

^a Data modified from ref. 86.

occur in drug-metabolizing enzymes across species. Not only are there frequently identical enzyme systems, but also primary sequence similarity between species for specific enzyme proteins. Examples of this include cytochrome P450 and UDP-glucuronosyltransferase isozymes. During the past 10 years, greater insight has been obtained about the species similarity of the enzyme systems. The reader is referred to the excellent review of these topics (88–91). In spite of the high degree of structural homology, the orthologous isozymes show subtle differences in a small number of amino acid residues. These small changes in the primary amino acid sequence can result in profound differences in substrate specificity (92). Although P4503A4 and P4503A5 share >85% amino and sequence homology, their catalytic properties seem to be qualitatively and quantitatively different. For example, P4503A4 catalyzes erythromycin *N*-demethylation 10-fold faster than does P4503A5 (93). Furthermore, P4503A4 metabolizes cyclosporine A to two hydroxylated metabolites (M1 and M17) and one methylated metabolite (M21), whereas P4503A5 does not metabolize cyclosporine A to M17 or M21, but produces the M1 metabolite (94).

Carboxylesterase hydrolyzes drugs containing ester bonds or amide linkages, and plays an important role in drug metabolism. This en-

zyme is widely distributed in the tissues of mammals (95). Immunological studies have shown that there are multiple isozymes of hepatic microsomal carboxylesterases in various animal species (96–99). Although the isozymes from various species show considerable similarities in physical and immunochemical properties, there are small differences in amino acid sequences. Hosokawa *et al.* (100) have compared the amino acid sequences and substrate specificity of purified carboxylesterases from liver microsomes of mice, hamsters, rats, guinea pigs, rabbits, and monkeys. Although there was high homology (80–95%) in identified amino acid residues, all of the carboxylesterases had a different first amino acid at the amino-terminal, and the substrate specificity was considerably different.

Perhaps the best-known instance of species differences in drug metabolism is the inability of the cat to form glucuronides of certain foreign compounds (101). Similarly, the dog has little or no ability to form *N*-acetylated metabolites of some compounds (102). Although it was originally thought that UDP-glucuronosyltransferase and *N*-acetyltransferases were genetically deficient in these species, it is now clear that the species defect in cats and dogs is substrate-dependent. It has been known for many years that cats form bilirubin glucuronides

TABLE 3

Renal excretion and V_f of β -lactam antibiotics in various species^a

Species	V_f ml/kg	CL_{ur} ml/min/kg	V_f ml/kg	CL_{ur} ml/min/kg
Cefotetan		Moxalactam		
Mouse	455	30.9	389	13.8
Rat	357	9.51	541	15.1
Rabbit	483	12.0	531	10.0
Dog	408	3.46	358	3.61
Monkey	1285	7.81	306	2.89
Human	1478	4.26	345	3.13
Cefmetazole		Cefpiramide		
Mouse	566	36.5	1178	23.5
Rat	875	14.3	488	6.21
Rabbit	536	20.2	1135	10.0
Dog	313	5.23	648	5.17
Monkey	726	12.9	1467	3.41
Human	953	10.3	3000	1.87
Cefoperazone		Cefazolin		
Mouse	362	—	458	15.8
Rat	385	4.18	3082	67.4
Rabbit	955	9.15	645	19.4
Dog	372	4.35	375	4.36
Monkey	782	3.39	571	6.82
Human	818	1.51	823	6.17

^a Data modified from ref. 87.

(103). Also, the dog formed *N*-acetylated metabolite of cilastatin, a potent inhibitor of renal dehydropeptidase I (104). The capacity of *N*-acetylation for cilastatin was in the order of rats > dogs > humans.

The *in vitro* metabolism of MK-954 (Dup 753) (fig. 1), a potent nonpeptide angiotensin II receptor antagonist, has been studied in incubations with liver slice preparations from rats, monkeys, and humans (105). Metabolism of MK-954 is qualitatively and quantitatively different among the species. In the rat, the primary route of metabolism is oxidative, leading to either monohydroxylated or oxidized (carboxylic acid) metabolites, whereas in monkeys, glucuronidation of the tetrazole moiety predominates. The metabolism of MK-954 by human liver slices, however, is not dominated by a single metabolic pathway, as with rats and monkeys, but is characterized by an approximately equal formation of both oxidized and glucuronidated metabolites. The investigators suggest that the observed short duration of action of the drug in the monkey may be caused by the low formation rate of the carboxylic acid metabolite in this species. This pharmacologically active carboxylic acid metabolite has a much longer half-life than the parent drug in all species studied. These studies with MK-954 have demonstrated the usefulness of *in vitro* models in drug metabolism, including species comparisons and metabolite identification.

Stevens *et al.* (6) recently compared phase I and phase II hepatic drug metabolism activities using human and monkey liver microsomes. Of the eight P450-dependent activities measured, only *N*-nitrosodimethylamine *N*-demethylase activity is not significantly different in the two species. Coumarin 7-hydroxylase activity is greater in the human than in the monkey, whereas erythromycin *N*-demethylase, benzphetamine *N*-demethylase, pentoxyresorufin *O*-dealkylase, ethoxycoumarin *O*-deethylase, and ethoxyresorufin *O*-deethylase activities are significantly greater in monkey microsomes. Of the seven microsomal and cytosolic phase II activities measured, only 17 α -ethynyl estradiol glucuronidation

is significantly higher in the human. These results clearly show that the metabolic capacities of the human and rhesus monkey drug-metabolizing enzymes are different.

The pharmacokinetic parameters, disposition half-life, and metabolic clearance of 12 benzodiazepines were compared in dogs and humans (86). As indicated in table 2, all benzodiazepines are metabolized more rapidly in dogs than in humans. The differences in metabolic clearance between these two species range from 3-fold for triazolam to 72-fold for nordiazepam.

Flurbiprofen, a 2-arylpropionic acid nonsteroidal antiinflammatory drug, exists as racemate and is used as such. Like other 2-arylpropionic acid drugs (106, 107), the antiinflammatory activity of flurbiprofen is believed to be caused by the *S*-enantiomer only. A unique characteristic of the metabolism of this class of drugs is the unidirectional inversion of the *R*- to the *S*-enantiomer. Inversion of *R*-flurbiprofen to its optical antipode is species-dependent. The fraction of inversion (*Fi*) is complete in the guinea pig (*Fi* = 1.0), incomplete in the dog (*Fi* = 0.39), and very low in the rat and gerbil (*Fi* $\leq$ 0.05) (108). Similar to the rodent species, the inversion of flurbiprofen is also insignificant in humans (109).

It is clear that extrapolation of drug metabolism from animals to humans is the most difficult one of the ADME processes. The difficulty of projecting drug metabolism to humans is further complicated by the situation that animal studies are normally conducted in an inbred strain of that species. Thus, the metabolism data in animal species are generally less variable, ~2- to 3-fold (6, 110). On the other hand, there are great interindividual differences in drug metabolism in patients caused by environmental and genetical factors, as well as the underlying disease (111). Interindividual variations in the levels and activities of cytochrome P450 enzymes have been investigated in human liver microsomes (112, 113). For some isozymes, the differences are as great as 50-fold. Therefore, it is very difficult to predict human metabolism.

Fortunately, the increased availability of human livers and the advanced knowledge of human drug metabolism have permitted the use of *in vitro* studies to predict the *in vivo* metabolism of drugs in humans, even before the initial clinical studies. Over the last several years, a body of research has evolved using human tissue for earlier drug metabolism studies. Under certain well-defined conditions, it is possible to extrapolate *in vitro* metabolism to *in vivo* situations (114, 115). There are many examples of good correlation between *in vitro* and *in vivo* drug metabolism, not only in qualitative but also in quantitative aspects. The antipyretic agent, ethoxybenzamide, is exclusively metabolized to salicylamide by rat hepatic microsomes. The *in vitro* enzymatic kinetic parameters, V_{max} and K_M values of 3.46 μ mol/min/kg and 0.378 mM are in good agreement with those (3.77 μ mol/min/kg and 0.192 mM) obtained *in vivo* by application of a two-compartment model (116). Felodipine, a calcium channel blocker, is primarily metabolized to its pyridine analog in rats, dogs, and humans. The hepatic intrinsic clearance (V_{max}/K_M) of the drug obtained from *in vitro* studies with liver microsomes was 16 liters/hr for the rat, 39 liters/hr for the dog, and 259 liters/hr for the human, and agreed well with those observed *in vivo*; the corresponding values were 6.2 liters/hr, 88 liters/hr, and 321 liters/hr (117). Similarly, a good *in vitro* and *in vivo* correlation of cytarabine hydrochloride has been reported by Dedrick *et al.* (118).

Excretion. Drugs and their metabolites are usually eliminated from the body via the urine or bile or sometimes both. The relative contribution of biliary and urinary excretion to the overall elimination of drugs depends on the nature of the drug and the animal species. Generally, biliary excretion predominates in drugs with a relatively large molecular weight (>300) (119). Studies with organic anions and

TABLE 4
Biliary excretion in various species of compounds of molecular size in the range of 300–800^a

	Methylene-Disalicylic Acid ^b	Succinyl-Sulfathiazole ^b	Stilbestrol Glucuronide ^b	Sulfadimethoxine N'-Glucuronide ^b	Phenolphthalein Glucuronide ^b	Indocyanine Green ^b	Bromsulfophthalein ^c
Mol. wt.	288	355	445	487	495	775	794
% of Dose Excreted in Bile							
Rat	54	29	95	43	54	82	82
Dog	65	20	65	43	81	97	54–97
Rabbit	5	1	32	10	13	94	—
Guinea pig	4	1	20	12	6	—	92
Monkey	—	0.2	—	—	9	—	—
Human	—	—	—	—	—	High	61

^a Data modified from ref. 113.

^b Excreted as unchanged compound in bile.

^c Excreted in bile as glutathione conjugate (mol. wt. 1020).

cations reveal that hepatic carriers preferentially recognize lipophilic drugs with a charged or a hydrophilic group (120, 121). Although it is clear that molecular size, polarity, and structural features are important determinants in biliary excretion of drugs, the relative importance of these factors is unclear. One striking feature of many drugs excreted in bile is that their structures are amphipathic in character (*i.e.* they contain both polar and nonpolar groups). Many lipophilic compounds are excreted into bile at a higher rate after conjugation with glutathione or glucuronic acid, presumably because the reaction not only increases molecular weight but also adds a polar group.

The amount of an organic chemical that is excreted in bile varies widely among species. In general, the mouse, rat, and dog are good biliary excretors, and the rabbit, guinea pig, monkey, and human are relatively poor (119). Table 4 shows biliary excretion in various species of compounds with molecular size in the range of 300–800. As indicated in table 4, it seems that the species differences in biliary excretion become less marked when the molecular size exceeds 700. The underlying mechanism for the species difference is at present obscure. Species differences in hepatic blood flow and bile flow do not seem to correlate with biliary excretion of compounds (122).

The GFR values vary considerably among species ranging from 1.8 ml/min/kg for humans to 10 ml/min/kg for mice (table 5). As can be seen in the table, the species differences in GFR are mainly attributable to their relative number of glomeruli (or nephrons) per kg body weight (8). The mouse has the largest relative number of nephrons and the largest GFR. Because it is generally believed that the GFR accurately reflects the renal function, one can predict CL_R of drugs in humans by using the ratio of the GFR values in animals (rats or dogs) to that in humans. In other words, the ratio of GFR should well reflect the ratio of CL_R of drugs between animals and humans.

Famotidine, a H_2 -receptor antagonist, is mainly excreted by the kidneys in rats with a CL_R of 28 ml/min/kg (123). The renal clearance in humans can be predicted to be ~6.2 ml/min/kg, because the GFR is 4.5-fold greater in rats than in humans (table 5). The predicted value is in good agreement with the observed value of 4.44 ml/min/kg (124). Enalaprilat and lisinopril, angiotensin-converting enzyme inhibitors, are exclusively excreted by the kidneys. Their CL_R in rats are 18 and 11 ml/min/kg, respectively (125). With the same approach, the CL_R for these two drugs in humans is expected to be 4 and 2.4 ml/min/kg, respectively, which are also in good agreement with the observed data (3.1 and 1.7 ml/min/kg) (Dr. M. R. Dobrinska, Merck Research Laboratory). Sawada *et al.* (87) have compared the CL_R of six β -lactam antibiotics in six different species, including humans (table 3).

TABLE 5
Comparative physiology of glomerular filtration^a

Species	Body Wt.	GFR	No. of Glomeruli/Kg Body Wt.
	kg	ml/min/kg	
Mouse	0.02	10.0	5.9×10^5
Rat	0.265	8.7	2.9×10^5
Rabbit	2.5	4.8	1.6×10^5
Dog	10	4.0	0.9×10^5
Monkey ^b	5	2.0	—
Human	70	1.8	0.29×10^5

^a Data modified from ref. 8.

^b Data obtained from ref. 11.

Again, the prediction of CL_{uR} from rats to humans is reasonably good with the exceptions of cefmetazole and cefazolin. The ratios of CL_{uR} between rats and humans for cefmetan, cefoperazone, moxalactam, and cefpiramide are 2.2, 6.0, 4.8, and 3.3, respectively, compared with the GFR ratio of 4.5 (table 5). The ratios are 1.4 for cefmetazole and 10.9 for cefazolin. These results suggest that for many drugs it may be possible to predict the renal excretion data from animals to humans.

Extrapolation of Efficacy and Safety Data

Drugs exert wanted and unwanted pharmacological effects, which are determined, on the one hand, by their intrinsic sensitivity and, on the other hand, by their access to their site of action (biophase). The intrinsic sensitivity is directly related to the affinity and number of receptors, and the drug concentration at biophase is dependent on a number of processes, including absorption, distribution, biotransformation, and excretion.

Although there is evidence that the properties of many receptors—such as CCK_B receptors (126), angiotensin subtype AT_1 receptors (127), benzodiazepine receptors (128), and histamine H_2 -receptors (32)—are similar among species, a great number of examples have shown that the affinity of various receptors is different between animals. Mesulergine shows approximately a 50-fold higher affinity for the rat serotonin 5-HT₂ receptor than for the human receptor. Comparison of the deduced amino acid sequences of cDNA clones encoding the human and rat 5-HT₂ receptors reveals that a single naturally occurring amino acid change between the human and the rat 5-HT₂ receptors makes a major contribution to their pharmacological

difference (129). When the three classes of compounds—heterosteroids, cyanines, and modified peptides—were tested for their ability to displace ^{125}I -Bolton-Hunter-substance P binding from the NK-1 receptor, a distinct species-dependent structure-activity relationship was observed, providing evidence for species differences in NK-1 receptors in rats, guinea pigs, and humans (130). On the other hand, *in vitro* studies with rat, rabbit, and dog myocardium have shown that there are marked species differences in the number of myocardial α - and β -adrenergic receptors without major differences in the affinity of the receptor (131). Similar to the nonspecific binding to plasma and tissue proteins, binding of drugs to receptors is also species-dependent, and the species differences are generally unpredictable.

The toxicity of many drugs is an extension of their pharmacological action. This type of toxicity is specific and directly related to the intrinsic sensitivity of receptors, as well as drug concentration at biophase. Digitoxin toxicity is a good example of this type of toxicity. Most available evidence indicates that species differences in digitoxin toxicity are related to species differences in the sensitivity of the cation-transport enzyme Na^+/K^+ -ATPase and to the species differences in pharmacokinetics of digitoxin resulting in different drug concentration that inhibits this enzyme (132–134).

For some other drugs, toxicity occurs when sufficient drug and/or toxic metabolites are present to exert toxic effects on cells. This type of toxicity is less specific and depends mainly on pharmacokinetic factors. Anticancer drugs are good examples of this kind of toxicity. Quantitative comparison of toxicity and maximum tolerated dose of 18 anticancer agents in mouse, rat, hamster, dog, monkey, and human gives a strong correlation between species (135). For most of anticancer agents, the interspecies differences in toxic doses is mainly caused by pharmacokinetics [i.e. factors that determine the plasma AUC (136, 137)]. As proposed by Collins *et al.* (138), plasma AUC is now commonly used as a guideline for the dose escalation from the LD_{50} in mice to the maximal tolerated dose in cancer patients in the development of anticancer agents.

Considering possible pharmacokinetic and pharmacodynamic species differences, extrapolation of drug efficacy and toxicity from animals to humans is extremely complicated and difficult, if not impossible. In addition to the relationship between drug concentration and intensity of pharmacological effect, many relevant variables—such as formation of pharmacologically active or toxic metabolites, environmental factors and underlying diseases—must also be taken into consideration.

Conclusions

Although pharmacokinetics have advanced greatly in recent years, it is not yet possible to predict all the pharmacokinetic parameters of a drug in humans from animal studies. Nevertheless, under certain well-defined conditions, it may be possible to make reasonably good predictions. For example, the intrinsic absorption of a given drug across the wall of the gastrointestinal tract is probably similar among species, because the nature of the biomembrane of epithelial cells is similar in mammals and because the absorption process is basically an interaction between the drug and the biomembrane. However, the rate-limiting steps and factors, such as pH-dependent solubility and first-pass metabolism that affect the absorption, may differ from species to species resulting in species differences in absorption. It is, therefore, important to consider all factors that may effect absorption/bioavailability in explaining differences between the species.

Predicting the renal excretion of drugs in humans has been relatively successful using the GFR ratio between humans and animals. Similarly, if the elimination of a drug is mainly by the liver and the rate of elimination is limited by hepatic blood flow, one could predict the clearance of the drug in humans by the hepatic blood flow, as in the example of propranolol.

However, biochemical parameters, such as protein binding and drug metabolism, are less predictable. These parameters vary considerably among species. Fortunately, *in vitro* methodology has been well established and can replace animal studies in predicting plasma protein binding and enzymatic biotransformation of drugs in humans.

In his article entitled, "Of Mice, Microsomes and Men" published 30 years ago, Brodie (139) wrote:

Nature has raised an enormous barrier to drug development by assigning the drug-metabolizing enzymes to various species in astonishingly diverse amount. So great are these differences that it is often a matter of pure luck that animal experiments lead to clinical useful drugs.

Although scientists continue to search for an animal species in which the elimination rate and metabolic pattern of drugs are consistently the same as in humans (140, 141), we have not come very far in the past 30 years. It is very unlikely that an animal species will ever be found that closely resembles humans in ADME. We should therefore accept the fact that there are great interspecies differences and accept the concept that the human is a unique mammal. Furthermore, it is clear that animal pharmacokinetics does not constitute an objective *per se* for drugs intended for human use, but is necessary to clarify all aspects of drug safety and efficacy that cannot be determined in humans for ethical reasons. Thus, we should concentrate on elucidating the underlying mechanisms responsible for the species differences in drug action and elimination and on interpreting the significance of these differences.

References

1. A. C. Economos: Gravity, metabolic rate and body size of mammals. *Physiologist* **22**, 571–572 (1979).
2. S. J. Gould: Allometry and size in ontogeny and phylogeny. *Biol. Rev.* **41**, 587–640 (1966).
3. W. A. Calder III: "Size, Function, and Life History." Harvard University Press, Cambridge, MA, 1984.
4. H. Boxenbaum: Interspecies variation in liver weight, hepatic blood flow and antipyrine intrinsic clearance in extrapolation of data to benzodiazepines and phenytoin. *J. Pharmacokin. Biopharmacol.* **8**, 165–176 (1980).
5. E. F. Adolph: Quantitative relation in the physiological constitution of mammals. *Science* **109**, 579–585 (1949).
6. J. C. Stevens, L. A. Shipley, J. R. Cashman, M. Vandenbranden, and S. A. Wrighton: Comparison of human and rhesus monkey *in vitro* phase I and phase II hepatic drug metabolism activities. *Drug Metab. Dispos.* **21**, 753–760 (1993).
7. A. D. Rodrigues, J. L. Ferrero, M. T. Amann, G. A. Rotert, S. P. Cepa, B. W. Surber, J. M. Machinist, N. R. Tich, J. P. Sullivan, D. S. Garvey, M. Fitzgerald, and S. P. Arneric: The *in vitro* hepatic metabolism of AB-418, a cholinergic channel activator, in rats, dogs cynomolgous monkeys and humans. *Drug Metab. Dispos.* **22**, 788–789 (1994).
8. E. M. Renkin, J. P. Gilmore: Glomerular filtration. In "Handbook of Physiology-Renal Physiology" (J. Orlogg and R. W. Berliner, eds.), chap. 9, pp. 185–248. Williams & Wilkins, Baltimore, MD, 1973.
9. W. R. Stahl: Scaling of respiratory variables in mammals. *J. Appl. Physiol.* **22**, 453–460 (1967).
10. N. A. Edwards: Scaling of renal function in mammals. *Comp. Biochem. Physiol.* **52A**, 63–66 (1975).
11. B. Davies and T. Morris: Physiological parameters in laboratory animals and humans. *Pharm. Res.* **10**, 1093–1095 (1993).
12. D. W. Nebert and F. J. Gonzalez: The P-450 gene superfamily. In "Principles, Mechanisms and Biological Consequences of Induction" (K. Ruckpaul and H. Rein, eds.), chap. 2, pp. 35–61. Taylor & Francis, London, UK, 1990.
13. K. B. Bischoff, R. L. Dedrick, D. S. Zaharko, and J. A. Longstreth: Methotrexate pharmacokinetics. *J. Pharm. Sci.* **60**, 1128–1133 (1971).

14. J. H. Lin, Y. Sugiyama, S. Awazu, and M. Hanano: Physiological pharmacokinetics of ethoxybenzamide based on biochemical data obtained *in vitro* as well as on physiological data. *J. Pharmacokinet. Biopharm.* **10**, 649–661 (1982).
15. J. H. Lin, Y. Sugiyama, M. Hanano, and S. Awazu: Effect of product inhibition on elimination kinetics of ethoxybenzamide in rabbits. Analysis by physiological pharmacokinetic model. *Drug Metab. Dispos.* **12**, 253–256 (1984).
16. R. L. Dedrick: Animal scale-up. In "Pharmacology and Pharmacokinetics" (T. Teorell, R. L. Dedrick, and P. G. Condliffe, eds.), pp. 117–145. Plenum Press, New York, 1974.
17. W. R. Chappell, and J. Mordenti: Extrapolation of toxicological and pharmacological data from animals to humans. In "Advances in Drug Research" (B. Testa, ed.), vol. 20, pp. 1–116. Academic Press, London, UK, 1991.
18. H. Boxenbaum, and R. W. D'Souza: Interspecies pharmacokinetic scaling, biological design and neoteny. In "Advances in Drug Research" (B. Testa, ed.), vol. 19, pp. 139–196. Academic Press, London, UK, 1990.
19. M. Gibaldi and D. Perrier: "Pharmacokinetics," 2nd ed. Marcel Dekker, New York, 1982.
20. G. H. Evans, A. S. Nies, and D. G. Shand: The disposition of propranolol. III. Decreased half-life and volume of distribution as a result of plasma binding in man, monkey, dog and rat. *J. Pharmacol. Exp. Ther.* **186**, 114–122 (1973).
21. J. Mordenti: Pharmacokinetic scale-up: accurate prediction of human pharmacokinetic profiles from animal data. *J. Pharm. Sci.* **74**, 1097–1099 (1985).
22. T. Murakawa, H. Sakamoto, S. Fukada, S. Nakamoto, T. Hirose, N. Itoh, and M. Nishida: Pharmacokinetics of ceftizoxime in animals after parenteral dosing. *Antimicrob. Agents Chemother.* **17**, 157–164 (1980).
23. E. Swabb, and D. Bonner: Prediction of aztreonam pharmacokinetics in humans based on data from animal. *J. Pharmacokinet. Biopharm.* **11**, 215–223 (1983).
24. W. E. Evans, W. R. Crom, and J. C. Yalowich: Methotrexate. In "Applied Pharmacokinetics: Principles of Therapeutic Drug Monitoring" (W. E. Evans, J. J. Schentag, and W. J. Jusko, eds.), chap. 30, pp. 1009–1056. Applied Therapeutics, Inc., Spokane, WA, 1986.
25. S. Øie, and Tozer, T. N. Tozer: Effects of altered plasma protein binding on apparent volume of distribution. *J. Pharm. Sci.* **68**, 1203–1205 (1979).
26. A. C. Guyton: "Textbook of Medical Physiology." Saunders Publishing Co., Philadelphia, PA, 1976.
27. R. Soberman, B. B. Brodie, B. B. Levy, J. Axelrod, and V. Hollander: The use of antipyrine in the measurement of total body water in man. *J. Biol. Chem.* **179**, 31–42 (1949).
28. J. H. Lin, D. M. Cocchetto, and D. E. Duggan: Protein binding as a primary determinant of the clinical pharmacokinetic properties of non-steroidal anti-inflammatory drugs. *Clin. Pharmacokinet.* **12**, 402–432 (1987).
29. T. N. Tozer: Concepts basic to pharmacokinetics. *Pharmacol. Ther.* **12**, 109–131 (1981).
30. E. O. Titus: Recent developments in the understanding of the pharmacokinetics and mechanism of action of chloroquine. *Ther. Drug Monit.* **11**, 369–379 (1989).
31. M. Frisk-Holmberg, Y. Bergqvist, E. Termond, and B. Domeij-Nyberg: The single dose kinetics of chloroquine and its major metabolite desethyl chloroquine in healthy subjects. *Eur. J. Clin. Pharmacol.* **26**, 521–530 (1984).
32. J. H. Lin: Pharmacokinetic and pharmacodynamic properties of histamine H_2 -receptor antagonists: relationship between intrinsic potency and effective plasma concentrations. *Clin. Pharmacokinet.* **20**, 218–236 (1991).
33. S. Caccia, and S. Garattini: Formation of active metabolites of psychotropic drugs: an updated review of their significance. *Clin. Pharmacokinet.* **18**, 434–459 (1990).
34. D. J. Greenblatt, M. Divoll, D. R. Abernethy, H. R. Ochs, and R. I. Shader: Clinical pharmacokinetics of the newer benzodiazepines. *Clin. Pharmacokinet.* **8**, 233–252 (1983).
35. J. Mordenti: Forecasting cephalosporin and monobactam antibiotic half-lives in humans from data collected in laboratory animals. *Antimicrob. Agents Chemother.* **27**, 887–891 (1985).
36. R. H. Reuning, and D. R. Geraets: Digoxin. In "Applied Pharmacokinetics: Principles of Therapeutic Drug Monitoring" (W. E. Evans, J. J. Schentag, and W. J. Jusko, eds.), chap. 18, pp. 570–623. Applied Therapeutics, Inc., Spokane, WA, 1986.
37. W. A. Ritschel: *In vivo* animal models for bioavailability assessment. *S.T.P. Pharm.* **3**, 125–141 (1987).
38. W. R. Chappell: In "Trace Substances in Environmental Health" (C. Hemphill, ed.), vol. XIX, pp. 326–337. University of Missouri, Columbia, MO, 1985.
39. E. J. Freireich, E. A. Gehan, D. P. Rall, L. H. Schmidt, and H. E. Skipper: Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey and man. *Cancer. Chemother. Rep.* **50**, 219–244 (1966).
40. J. D. Crawford, M. E. Terry, and G. M. Rourke: Simplification of drug dose calculation by application of the surface area principle. *Pediatrics* **5**, 783–796 (1950).
41. W. I. Higuchi, N. F. H. Ho, J. Y. Park, and I. Komiya: Rate-limiting steps and factors in drug absorption. In "Drug Absorption" (L. F. Prescott and W. S. Nimmo, eds.), pp. 35–60. ADIS Press, Sydney, Australia, 1981.
42. N. F. H. Ho, J. Y. Park, P. F. Ni, and W. I. Higuchi: Advancing quantitative and mechanistic approaches in interfacing gastrointestinal drug absorption studies in animals and humans. In "Animals Models for Oral Drug Delivery in Man: *In Situ* and *In Vivo* Approaches" (W. Crouthamel and A. C. Sarapu, eds.), p. 27. American Pharmaceutical Association, Washington, D.C., 1983.
43. W. D. Stein: Simple diffusion of nonelectrolytes and ions In "Channels, Carriers, and Pumps: An Introduction to Membrane Transport" (W. D. Stein, ed.), chap. 2, pp. 29–71. Academic Press, New York, 1990.
44. C. G. Wilson, C. Washington, and N. Washington: Overview of epithelial barriers and drug transport. In "Physiological Pharmacokinetics: Biological Barriers to Drug Absorption" (C. G. Wilson and N. Washington, eds.), chap. 1, pp. 11–20. Ellis Horwood Limited, Chichester, UK, 1989.
45. J. Watanabe, H. Okabe, T. Ichibashi, K. Mizajiri, H. Yamada, and R. Yamamoto: Gastric emptying rate constants after oral administration of drug solution to mice, rats and rabbits. *Chem. Pharm. Bull.* **25**, 2147–2185 (1977).
46. H. Mizuta, Y. Kawazoe, and K. Ogawa: Gastrointestinal absorption of chlorothiazide: evaluation of a method using salicylazosulfapyridine and acetaminophen as the marker compounds for determination of the gastrointestinal transit time in the dog. *Chem. Pharm. Bull.* **38**, 2810–2813 (1990).
47. J. B. Dressman: Comparison of canine and human gastrointestinal physiology. *Pharm. Res.* **3**, 123–131 (1986).
48. H. T. Debas: Peripheral regulation of gastric acid secretion. In "Physiology of the Gastrointestinal Tract" (L. R. Johnson, ed.), chap. 31, pp. 931–945. Raven Press, New York, 1987.
49. B. I. Hirschowitz: Apparent kinetics of histamine dose-responsive gastric water and electrolyte secretion in the dog. *Gastroenterology* **54**, 514–522 (1968).
50. T. N. Pappas, H. T. Debas, Y. Goto, and I. L. Taylor: Peptide YY inhibits meal-stimulated pancreatic and gastric secretion. *Am. J. Physiol.* **248**, G118–123 (1985).
51. E. Emas, and M. I. Grossman: Response of Heidenhain pouch to histamine, gastric and feeding before and after truncal vagotomy in dogs. *Scand. J. Gastroenterol.* **4**, 497–503 (1969).
52. H. Petersen, and M. I. Grossman: Stimulation of gastric acid secretion by dimaprit in unanesthetized rats. *Agents Actions* **8**, 566–567 (1978).
53. K. G. Wormsley, and M. E. Grossman: Maximal histalog test in control subjects and patients with peptic ulcer. *Gut* **6**, 427–435 (1965).
54. W. Wyrwicka, and R. Garcia: Effect of restraint on gastric secretion in cats. *Pavlov. J. Biol. Sci.* **14**, 249–253 (1979).
55. J. H. Lin, I.-W. Chen, K. J. Vastag, and D. Ostovic: pH-dependent oral absorption of L-735,524, a potent HIV protease inhibitors in rats and dogs. *Drug Metab. Dispos.* **23**, 730–735 (1995).

56. H. J. Ehrlein and J. Pröve: Effect of viscosity of test meals on gastric emptying in dogs. *J. Exp. Physiol.* **67**, 419–425 (1982).
57. E. J. Feldman, and M. I. Grossman: Liver extract and its free amino acid equally stimulate acid secretion. *Am. J. Physiol.* **239**, G493–496 (1980).
58. R. G. Pendleton, M. L. Torchiana, C. Chung, P. Cook, S. Siese, and B. V. Clineschmidt: Studies on MK-208 (YM-11170) a new, slowly dissociable H_2 -receptor antagonist. *Arch. Int. Pharmacodyn.* **266**, 4–16 (1983).
59. I.-W. Chen, J. M. Dorley, H. G. Ramjit, S. M. Pitzenberger, and J. H. Lin: Physiological disposition and metabolism of L-365,260, a potent antagonist of brain cholecystokinin receptor, in laboratory animals. *Drug Metab. Dispos.* **20**, 390–395 (1992).
60. J. L. Madara, and J. R. Pappenheimer: Structural basis for physiological regulation of paracellular pathway in intestinal epithelia. *J. Memb. Biol.* **100**, 149–164 (1987).
61. J. H. Lin, D. E. Duggan, I.-W. Chen, and R. L. Ellsworth: Physiological disposition of alendronate, a potent antioleolytic bisphosphonate, in laboratory animals. *Drug Metab. Dispos.* **19**, 926–932 (1991).
62. B. Clark, and D. A. Smith: Pharmacokinetics and toxicity testing. *Crit. Rev. Toxicol.* **12**, 343–385 (1984).
63. W. Crouthamel, and I. Bekersky: Preclinical evaluation of new drug candidates and drug delivery systems in dogs. In "Animal Models for Oral Drug Delivery in Man: *In Situ* and *In Vivo* Approaches" (W. Crouthamel and A. Sarapy, eds.), p. 107. *American Pharmaceutical Association*, Washington, D.C., 1983.
64. U. Kragh-Hansen: Molecular aspects of ligand binding to serum albumin. *Pharmacol. Rev.* **33**, 17–53 (1981).
65. W. M. Callan and F. W. Sunderman, Jr.: Species variations in binding of ^{65}Ni (II) by serum albumin. *Res. Commun. Chem. Pathol. Pharmacol.* **5**, 459–472 (1973).
66. S. Garten, and W. D. Wosilait: Comparative study of the binding of coumarin anticoagulants and serum albumins. *Biochem. Pharmacol.* **20**, 1661–1668 (1971).
67. D. J. Tocco, G. D. Breault, A. G. Zacchei, S. L. Steelman, and C. V. Perrier: Physiological disposition and metabolism of 5'-(2'-4'-difluorophenyl) salicylic acid, a new salicylate. *Drug Metab. Dispos.* **3**, 453–466 (1975).
68. J. H. Lin, E. H. Ulm, and D. E. Duggan: Possible mechanisms for reduced plasma clearance of diflunisal in rat experimental renal failure. *J. Pharmacol. Exp. Ther.* **238**, 978–984 (1986).
69. J. H. Lin, K. F. Hooke, K. C. Yeh, and D. E. Duggan: Dose-dependent pharmacokinetics in rats. Dual effects of protein binding and metabolism. *J. Pharmacol. Exp. Ther.* **235**, 402–406 (1985).
70. P. J. Meffin, D. M. Brooks, J. Bertouch, J. R. Veenendaal, and B. J. Harrington: Diflunisal disposition and hypouricemic response in osteoarthritis. *Clin. Pharmacol. Ther.* **33**, 813–821 (1983).
71. J. H. Lin: Species differences in protein binding of diflunisal. *Drug Metab. Dispos.* **17**, 221–223 (1989).
72. J. H. Lin, F. A. deLuna, E. H. Ulm, and D. J. Tocco: Species-dependent enantioselective plasma protein binding of MK-571, a potent leukotriene D_4 antagonist. *Drug Metab. Dispos.* **18**, 484–487 (1990).
73. D. J. Tocco, F. A. deLuna, A. E. W. Duncan, J. H. Hsieh, and J. H. Lin: Interspecies differences in stereoselective protein binding and clearance of MK-571. *Drug Metab. Dispos.* **18**, 388–392 (1990).
74. J. H. Lin, I.-W. Chen, E. H. Elm, J. R. Gehret, and D. E. Duggan: Dose-dependent pharmacokinetics of MK-417, a potent carbonic anhydrase inhibitor, in rabbits following single and multiple doses. *Drug Metab. Dispos.* **18**, 836–841 (1990).
75. J. H. Lin, E. H. Ulm, and L. E. Los: Dose-dependent stereopharmacokinetics of 5,6-dihydro-4H-4(isobutylamino)thieno(2,3-B) thiopyran-2-sulfonamide-7,7-dioxide, a potent carbonic anhydrase inhibitor, in rats. *Drug Metab. Dispos.* **19**, 233–238 (1991).
76. J. H. Lin: Dose-dependent pharmacokinetics: experimental observations and theoretical considerations. *Biopharm. Drug Dispos.* **15**, 1–31 (1994).
77. J. H. Lin; I.-W. Chen, and T.-H. Lin: Species-dependent stereopharmacokinetics of MK-927, a potent carbonic anhydrase inhibitor. *Drug Metab. Dispos.* **19**, 816–822 (1991).
78. J. H. Lin, T.-H. Lin, and H. Cheng: Uptake and stereoselective binding of the enantiomers of MK-927, a potent carbonic anhydrase inhibitor, by human erythrocytes *in vitro*. *Pharm. Res.* **9**, 339–344 (1992).
79. J. H. Lin, I.-W. Chen, and D. E. Duggan: Effect of dose, sex and age on disposition of alendronate, a potent antioleolytic agent, in rats. *Drug Metab. Dispos.* **20**, 473–478 (1992).
80. J. H. Lin, I.-W. Chen, F. A. deLuna, and M. Hichens: Renal handling of alendronate in rats: an uncharacterized renal transport system. *Drug Metab. Dispos.* **20**, 608–613 (1992).
81. J. H. Lin, I.-W. Chen, F. A. deLuna, and M. Hichens: Role of calcium in plasma protein binding and renal handling of alendronate in hypo- and hypercalcemic rats. *J. Pharmacol. Exp. Ther.* **267**, 670–675 (1993).
82. J. H. Lin, I.-W. Chen, and F. A. deLuna: Nonlinear kinetics of alendronate: plasma protein binding and bone uptake. *Drug. Metab. Dispos.* **22**, 400–405 (1994).
83. B. Fichtl, A. V. Nieciecki, and K. Walter: Tissue binding versus plasma binding of drugs: general principles and pharmacokinetic consequences. In "Advances in Drug Research" (B. Testa, ed.), vol. 20, pp. 117–166. Academic Press, London, UK, 1991.
84. Y. Sawada, M. Hanano, Y. Sugiyama, H. Harashima, and T. Iga: Prediction of the volumes of distribution of basic drugs in humans based on data from animals. *J. Pharmacokin. Biopharm.* **12**, 587–596 (1984).
85. B. Fichtl, and G. Schulmann: Relationships between plasma- and tissue-binding of drugs. In "Protein Binding and Drug Transport" (J. P. Tillement, and E. Lindenlaub, eds.), pp. 255–271. Schattauer, New York, 1986.
86. H. Boxenbaum: Comparative pharmacokinetics of benzodiazepines in dog and man. *J. Pharmacokin. Biopharm.* **10**, 411–426 (1982).
87. Y. Sawada, M. Hanano, Y. Sugiyama, and I. Iga: Prediction of the disposition of β -lactam antibiotics in humans from pharmacokinetic parameters in animals. *J. Pharmacokin. Biopharm.* **12**, 241–261 (1984).
88. F. P. Guengerich, (ed.): "Mammalian Cytochromes P450." CRC Press, Boca Raton, FL, 1987.
89. C. J. Henderson, and C. R. Wolf: Molecular analysis of cytochrome P-450s in the CYP2 gene family. In "Progress in Drug Metabolism" (G. G. Gibson, ed.), chap. 3, pp. 73–139. Taylor & Francis, London, UK, 1992.
90. C. J. Mulder, M. W. H. Coughtrie, and B. Burchell: Glucuronidation. In "Conjugation Reactions in Drug Metabolism" (G. J. Mulder, ed.), chap. 4, pp. 51–105. Taylor & Francis, London, UK, 1990.
91. M. W. H. Coughtrie: Role of molecular biology in the structural and functional characterization of UDP-glucuronosyltransferase. In "Progress in Drug Metabolism" (G. G. Gibson, ed.), pp. 35–72. Taylor & Francis, London, UK, 1992.
92. L. P. Lindberg, and M. Negishi: Alteration of mouse cytochrome P450_{coh} substrate specificity by mutation of a single amino-acid residue. *Nature* **339**, 632–634 (1989).
93. S. A. Wrighton, W. R. Brian, M. A. Sari, M. Iwasaki, F. P. Guengerich, J. L. Raucy, D. T. Molowa, and M. Vanderbranden: Studies on the expression and metabolic capabilities of human liver cytochrome P450III_{A5} (HLP3). *Mol. Pharmacol.* **38**, 207–213 (1990).
94. T. Aoyama, S. Yamano, D. J. Waxman, D. P. Lapenson, U. A. Meyer, V. Fischer, R. Tyndale, T. Inaba, T. Kalow, H. V. Gelboin, and F. J. Gonzalez: Cytochrome P-450 hPCN3, a novel cytochrome P-450III_A gene product that is differentially expressed in adult human liver. cDNA and deduced amino acid sequence and distinct specificities of cDNA-expressed hPCN1 and hPCN3 for the metabolism of steroid hormones and cyclosporine. *J. Biol. Chem.* **264**, 10388–10395 (1989).
95. T. Satoh: Role of carboxylesterases in xenobiotic metabolism. In "Reviews in Biochemical Toxicology" (E. Hodgson, J. R. Bend, and R. M. Philot, eds.), vol. 8, pp. 155–181. Elsevier, Amsterdam, Holland, 1987.
96. K. Moroi, and T. Satoh: Comparison between procaine and isocarboxazid metabolism *in vitro* by a liver microsomal amidase-esterase. *Biochem. Pharmacol.* **24**, 1517–1522 (1975).
97. R. Mentlein, and E. Heymann: Hydrolysis of ester and amide-type drugs by the purified isozymes of nonspecific carboxylesterase from rat liver. *Biochem. Pharmacol.* **33**, 1243–1247 (1984).
98. W. Junge, and E. Heymann: Characterization of isozymes of pig liver carboxylesterase II kinetic studies. *Eur. J. Biochem.* **95**, 519–525 (1979).

99. S. K. Miller, A. R. Main, and R. S. Rush: Purification and physical properties of oligomeric and monomeric carboxylesterase from rabbit liver. *J. Biol. Chem.* **255**, 7161–7167 (1980).
100. M. Hosokawa, T. Maki, and T. Satoh: Characterization of molecular species of liver microsomal carboxylesterases of several animal species and human. *Arch. Biochem. Biophys.* **277**, 219–227 (1990).
101. D. Robison, and R. T. Williams: Do cats form glucuronidates? *Biochem. J.* **68**, 23P (1956).
102. W. W. Weber, G. N. Levy, and D. W. Hein: Acetylation. In "Conjugation Reactions in Drug Metabolism" (G. J. Mulder, ed.), pp. 163–191. Taylor & Francis, London, UK, 1990.
103. K. P. M. Heirwegh: Formation, metabolism and significance of bilirubin 1X-glycosides. In "Conjugation Reactions in Drug Biotransformation" (A. Aitio, ed.), pp. 67–76. Elsevier, Amsterdam, Holland, 1987.
104. J. H. Lin, I.-W. Chen, and E. H. Ulm: Dose-dependent kinetics of cilastatin in laboratory animals. *Drug Metab. Dispos.* **17**, 426–432 (1989).
105. R. A. Stearns, R. R. Miller, G. A. Doss, P. K. Chakravarty, A. Rosegay, G. J. Gatto, and S.-H. L. Chiu: The metabolism of Dup 753, a nonpeptide angiotensin II receptor antagonist by rat, monkey and human liver slices. *Drug Metab. Dispos.* **20**, 281–287 (1992).
106. T. Y. Shen: Perspectives in non-steroidal antiinflammatory agents. *Angew. Chem. Int. Ed.* **11**, 460–472 (1972).
107. K. M. Williams: Enantiomers in arthritic disorders. *Pharmacol. Ther.* **46**, 273–295 (1990).
108. S. Manzel-Soglowek, G. Geisslinger, W. S. Beck, and K. Brune: Variability of inversion of (R)-flurbiprofen in different species. *J. Pharm. Sci.* **81**, 888–891 (1992).
109. F. Jamali, B. W. Berry, M. R. Tehrani, and S. A. Russell: Stereoselective pharmacokinetics of flurbiprofen in humans and rats. *J. Pharm. Sci.* **77**, 666–669 (1988).
110. E. C. Eberhart, B. Gemzik, M. R. Halvorson, and A. Parkinson: Species differences in the toxicity and cytochrome P-450 111A-dependent metabolism of digitoxin. *Mol. Pharmacol.* **40**, 859–867 (1991).
111. F. Sjöquist: Interindividual differences in drug responses: an overview. In "Variability in Drug Therapy Description, Estimation, and Control" (M. Rowland, L. B. Sheiner, and J. L. Steimer, eds.), pp. 1–10. Raven Press, New York, 1985.
112. T. Shimada, H. Yamazaki, M. Mimura, Y. Inui, and F. P. Guengerich: Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J. Pharmacol. Exp. Ther.* **270**, 414–423 (1994).
113. F. P. Guengerich, and C. G. Turvy: Comparison of levels of several human microsomal cytochrome P-450 enzymes and epoxide hydrolase in normal and disease states using immunochemical analysis of surgical liver samples. *J. Pharmacol. Exp. Ther.* **256**, 1189–1194 (1991).
114. M. Hanano, Y. Sawada, T. Iga, and Y. Sugiyama: The integration of *in vitro* data with physiological data. In "Topics in Pharmaceutical Sciences." (D. D. Breimer, and P. Speiser, eds.), pp. 63–77. Elsevier Science Publisher B. V., Amsterdam, Holland, 1987.
115. Y. Sugiyama, Y. Sawada, T. Iga, and M. Manano: Reconstruction of *in vivo* metabolism from *in vitro* data. In "Xenobiotic Metabolism and Disposition" (R. Kato, R. W. Estabrook, and M. N. Cayen, eds.), pp. 225–235. Taylor & Francis, London, UK, 1989.
116. J. H. Lin, M. Hayashi, S. Awazu, and M. Hanano: Correlation between *in vitro* and *in vivo* drug metabolism rate: oxidation of ethoxybenzamide in rat. *J. Pharmacokinet. Biopharm.* **6**, 327–337 (1978).
117. C. Bäärnhielm, H. Dahlbäck, and I. Skanberg: *In vivo* pharmacokinetics of felodipine predicted from *in vitro* studies in rat, dog and man. *Acta Pharmacol. Toxicol.* **59**, 113–122 (1986).
118. R. L. Dedrick, D. D. Forrester, and D. H. W. Ho: *In vitro-in vivo* correlation of drug metabolism—deamination of 1- β -D-arabino-furanosylcytosine. *Biochem. Pharmacol.* **21**, 1–16 (1972).
119. R. L. Smith: Excretion of drugs in bile. In "Handbook of Experimental Pharmacology. Volume XXVIII: Concepts in Biochemical Pharmacology" (B. B. Brodie, and J. R. Gillette, eds.), pp. 354–389. Springer-Verlag, Berlin, Germany, 1971.
120. D. K. F. Meijer, C. Neef, and G. M. M. Groothuis: Carrier-mediated transport in the handling of drugs by the liver. In "Topics in Pharmaceutical Sciences" (D. D. Breimer, and P. Speiser, eds.), pp. 167–189. Elsevier Science Publisher, B. V., Amsterdam, Holland, 1983.
121. D. F. K. Meijer, W. E. M. Mol, M. Müller, and G. Kurz: Carrier-mediated transport in the hepatic distribution and elimination of drugs, with special reference to the category of organic cations. *J. Pharmacokinet. Biopharm.* **18**, 35–70 (1990).
122. R. L. Smith: "The Excretory Function of Bile: The Elimination of Drugs and Toxic Substances in Bile." Chapman and Hall, London, UK, 1973.
123. J. H. Lin, L. E. Los, E. H. Ulm, and D. E. Duggan: Urinary excretion kinetics of famotidine in rats. *Drug Metab. Dispos.* **15**, 212–216 (1987).
124. J. H. Lin, A. N. Chremos, K. C. Yeh, J. Antonello, and G. A. Hessey II: Effects of age and chronic renal failure on the urinary excretion kinetics of famotidine in man. *Eur. J. Clin. Pharmacol.* **34**, 41–46 (1988).
125. J. H. Lin, I.-W. Chen, E. H. Ulm, and D. E. Duggan: Differential renal handling of angiotensin-converting enzyme inhibitors: enalaprilat and lisinopril in rats. *Drug Metab. Dispos.* **16**, 392–296 (1988).
126. V. J. Lotti, and S. L. Chang: A new potent and selective non-peptide gastrin antagonist and brain cholecystokinin receptor (CCK_B) ligand: L-365,260. *Eur. J. Pharmacol.* **162**, 273–280 (1989).
127. R. S. L. Chang, and V. J. Lotti: Angiotensin receptor subtypes in rat, rabbit and monkey tissues: relative distribution and species dependency. *Life Sci.* **00**, 1485–1490 (1991).
128. K. Ito, Y. Yamada, K. Nakamura, Y. Sawada, and T. Iga: Classification of benzodiazepine hypnotics in humans based on receptor occupancy theory. *J. Pharmacokinet. Biopharm.* **21**, 31–41 (1993).
129. H.-T. Kao, N. Adham, M. A. Oslen, R. L. Weinshank, T. A. Branchek, and P. R. Hartig: Site-directed mutagenesis of a single residue changes the binding properties of the serotonin 5-HT₂ receptor from a human to a rat pharmacology. *FEBS Lett.* **307**, 324–328 (1992).
130. K. C. Appell, B. J. Fragale, J. Loscig, S. Singh, and B. Tomczuk: Antagonists that demonstrate species differences in neurokinin-1 receptors. *J. Pharmacol. Exp. Ther.* **41**, 772–778 (1992).
131. A. Mukherjee, Z. Haghani, J. Brady, L. Bush, W. McBride, L. M. Buja, and J. T. Willerson: Differences in myocardial α and β -adrenergic receptor numbers in different species. *Am. J. Physiol.* **245**, H957–H961 (1983).
132. G. T. Okita: Species differences in duration of action of cardiac glycosides. *Fed. Proc.* **26**, 1125–1130 (1967).
133. J. C. Allen, and A. Schwartz: A possible biochemical explanation for the insensitivity of the rat to cardiac glycosides. *J. Pharmacol. Exp. Ther.* **168**, 42–46 (1969).
134. R. S. Gupta, A. Chopra, and D. K. Stetsko: Cellular basis for the species differences in the sensitivity to cardiac glycosides (digitalis). *J. Cell. Physiol.* **127**, 197–206 (1986).
135. E. J. Freireich, E. A. Gehan, D. P. Rall, L. H. Schmidt, and H. E. Skipper: Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey and man. *Cancer Chemother. Rep.* **50**, 219–244 (1966).
136. A. Gescher, and D. R. Newell: The role of pharmacokinetics and metabolism studies in anticancer drug discovery and development. In "Progress in Drug Metabolism" (G. G. Gibson, ed.), chap. 7, pp. 263–294. Taylor & Francis, London, UK, 1992.
137. G. Powis, M. M. Ames, and J. S. Kovach: Dose-dependent pharmacokinetics and cancer chemotherapy. *Cancer Chemother. Pharmacol.* **6**, 1–9 (1981).
138. J. M. Collins, D. S. Zaharko, R. L. Dedrick, and B. A. Chabner: Potential roles for preclinical pharmacology in phase I clinical trials. *Cancer Treat. Rep.* **70**, 73–80 (1986).
139. B. B. Brodie: Of mice, microsomes and men. *Pharmacologist* **6**, 12–26 (1964).
140. J. Caldwell: The current status of attempts to predict species differences in drug metabolism. *Drug Metab. Rev.* **12**, 221–237 (1981).
141. R. L. Smith, and J. Caldwell: Drug metabolism in non-human primates. In "Drug Metabolism from Microbe to Man" (D. V. Parke, and R. L. Smith, eds.), p. 331. Taylor & Francis, London, UK, 1976.