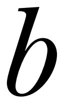
The Physiological Basis of Veterinary Clinical Pharmacology

J. Desmond Baggot, MVM, PhD, DSc, FRCVS

Formerly Professor of Preclinical Veterinary Studies, Faculty of Veterinary Science, University of Zimbabwe, Harare and Professor of Clinical Pharmacology, School of Veterinary Medicine, University of California, Davis.



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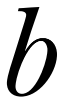

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Preface

Material for this monograph has been collected over the past 25 years. The book is, in some respects, an update of *Principles of Drug Disposition in Domestic Animals* which was published in 1977, but it is more broad in scope. References to selected pre-1975 papers are included because of their inherent value.

The diversity of species in which drugs are used for clinical purposes together with the emphasis placed on the various classes of drugs distinguishes veterinary from human pharmacology. Physiological characteristics of different species essentially reflect adaptations that evolved over centuries to promote survival of the existing species. Even though each species is unique, the pattern of most physiological processes in the species within a taxonomical class can be described in mathematical terms. Species differences in the response to fixed doses or dosage regimens of drugs generally have a physiological or biochemical basis. An uncharacteristic response to dosage of a drug in animals of a particular species often warrants investigative research on the underlying mechanism to which the observed effect could be attributed.

Pharmacokinetic parameters are most useful for quantifying species differences in the bioavailability and disposition of drugs, and for calculating therapeutic dosage regimens. An assumption made in dosage calculations, which appears to be generally valid, is that the same range of therapeutic plasma concentrations is applicable to eutherian mammalian species. Disease states and pharmacokinetic-based drug interactions can alter the disposition of a drug to an extent that modification of usual dosage is required for safety and efficacy of the drug. The formulation of dosage forms determines not only the route of administration but also the clinical efficacy of a drug. Because residues of drugs and drug metabolites in the tissues and edible products of food-producing animals are unacceptable, drugs should be formulated as preparations that will be efficacious and will not prolong the persistence of residues. In formulating veterinary dosage forms, meagre consideration has been given to differences in the pharmacodynamic activity and to species variations in the bioavailability and disposition of the enantiomers of chiral drugs. Application of interspecies allometric scaling of major pharmacokinetic parameters is useful at the pre-clinical stage of drug development and may identify non-conforming species with regard to the disposition of commercially available drugs. It is well established that drug dosage cannot be extrapolated between different classes of animals (mammals, birds, fishes, reptiles). At the present

time there is insufficient information available on the pharmacodynamic activity and pharmacokinetic behaviour of drugs in marsupial species to comment on the feasibility of extrapolating dosage from eutherian to marsupial mammals. The conservation of exotic animals requires protection of their natural habitats from human intrusion as the various adaptations that characterize different species have evolved in concert with their habitats.

Veterinary clinical pharmacology is an integrative discipline with the general objective of providing the requisite information for judicious selection of drug preparations for use in animals at dosages that will alleviate discomfort and pain, avoid undesirable drug interactions and effectively treat animal diseases. The specific aim of drug therapy is to readjust disease-altered physiological and/or biochemical processes to the state that is normal for the animal species. The author hopes that this book will promote postgraduate research that will both contribute to advancement of veterinary clinical pharmacology and further the well-being of animals.

J. Desmond Baggot
Ballsbridge, Dublin

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Terms and Abbreviations

ACE	angiotensin-converting enzyme
AChE	acetylcholinesterase
AUC_{0-24}	area under the concentration-time curve measured from $t=0$ to $t=24\,h$
AUIC	area under the inhibitory plasma concentration – time curve (with reference to antimicrobial agents)
AUMC	area under the first moment of the plasma concentration – time curve, i.e. the area under the curve of the product of time and plasma concentration over the time-span zero to infinity
b.d.	twice daily
BSP	bromosulphalein
BUN	blood urea nitrogen
C_{max}	maximum concentration of a drug
CK	creatine kinase
Cl	clearance (L/h or mL/min)
D	dose (mg)
DDT	dichlorodiphenyl-trichloroethane
DEET	diethyltoluamide
E	extraction ratio
E_H	hepatic extraction ratio
ED_{50}	median effective dose (mg/kg)
F	systemic availability (extent of absorption)
f_b	fraction of bound drug
f_u	fraction of unbound drug
FMO	flavin-containing mono-oxygenase
GABA	γ-aminobutyric acid
GFR	glomerular filtration rate
HPLC	high performance liquid chromatography
IBR	infectious bovine rhinotracheitis
ICG	indocyanine green
i.m.	intramuscular
i.o.	intraosseous
i.p.	intraperitoneal
i.v.	intravenous
k_a	absorption rate constant

k_d	disposition rate constant
K_M	Michaelis constant
LD_{50}	median lethal dose (mg/kg)
LOQ	limit of quantification
M	molar
MAT	mean absorption time
MIC_{90}	minimum inhibitory concentration required to prevent visible growth of 90% of a bacterial species *in vitro*
MLP	maximum life-span potential
MRT	mean residence time
n	number
NS	not significant
NSAIDs	non-steroidal anti-inflammatory drugs
OTC	oxytetracycline
OTC-C	conventional oxytetracycline
OTC-LA	long-acting oxytetracycline
P	probability
P_{Cr}	creatinine concentration in plasma
PASME	post-antibiotic sub-minimum inhibitory concentration effect
PCB	polychlorinated biphenyl
PCV	packed cell volume
pH	negative logarithm of the hydrogen ion concentration
pK_a	negative logarithm of the acidic ionization/dissociation constant
p.o.	*per os* (by mouth)
p.r.	*per rectum* (rectal administration)
Q	blood flow (L/h)
R_0	infusion rate required to produce steady-state plasma concentration
s.c.	subcutaneous
SD	standard deviation
SEM	standard error of the mean
t	time
$t_{\frac{1}{2}}$	half-life (i.v. administration of a drug)
U_{Cr}	creatinine concentration in urine
V	total volume of urine formed during collection period
$V_{d(area)}$	volume of distribution (L)
$V_{d(ass)}$	volume of distribution at steady-state (L)
V_{max}	maximum reaction velocity
α	absorption-rate constant
β	elimination-rate constant
β-agonists	
β-antagonists	

Author's Note

The values of pharmacokinetic terms for drugs mentioned in this monograph are average values in the various animal species, while drug doses and dosage regimens are based on average values and agree reasonably well with those usually recommended. Some emphasis is placed on veterinary dosage forms since they influence the clinical efficacy of drugs to a greater degree than is generally appreciated. Advancement of veterinary clinical pharmacology rests both on elucidating the physiological and/or biochemical basis of species variations in response to dosage of drugs and on the development of dosage forms that will most effectively deliver the drugs to their sites of action without producing adverse effects. Keen observation and attention to detail are requirements of animal management in general and of veterinary clinical pharmacology in particular.

Chapter 1
The Pharmacokinetic Basis of Species Variations in Drug Disposition

Introduction

The diversity of species in which drugs are used and studied distinguishes veterinary from human pharmacology. Another difference, which relates to clinical indications, is the emphasis placed on the various classes of drugs.

An understanding of the complex relationship between the dose of a drug and the clinically observed pharmacological effect can generally be obtained by linking the pharmacokinetic (PK) behaviour with information on pharmacodynamic (PD) activity (Fig. 1.1) (Holford & Sheiner, 1981).

Fig. 1.1 Schematic representation of the dose–effect relationship.

The plasma drug concentration profile occupies a central role between the dose administered and the characteristic pharmacological effect(s) produced by the drug. An inherent assumption is that the drug concentration in plasma is related to the concentration at the site of action, which can rarely be measured *in vivo*. The requirement for species differences in the dose (mg/kg) or dosage rate (dose/dosage interval) of a pharmacological agent may be attributed to variation between species in pharmacokinetic behaviour or pharmacodynamic activity, or both, of the drug. Whether a systemically acting drug produces a therapeutic or toxic effect is mainly determined by size of the dose when a single dose is administered or the dosage rate when multiple doses are administered at a constant dosage interval.

Drugs (pharmacological agents) act by modifying pre-existent physiological or biochemical processes in the body. The mechanisms of action of drugs appear to be the same in mammalian species. The clinical utility of pharmacokinetics relies on the premise that a range of therapeutic plasma concentrations can be defined for each pharmacological agent; some examples are given

in Table 1.1. The pharmacodynamic properties (affinity and efficacy) of a drug are embodied in the therapeutic concentration range. There is substantial evidence to support the hypothesis that the therapeutic concentration range is the same for human beings and domestic animals. The calculation of a dosage regimen (dose and dosage interval) for a drug preparation is based upon a knowledge of the therapeutic concentration range and the pharmacokinetic parameters that describe bioavailability and disposition of the drug. Species differences in the dosage regimen for a drug preparation can generally, but not always, be attributed to variation between species in pharmacokinetic behaviour of the drug.

Table 1.1 Principal pharmacological effect and range of therapeutic plasma concentrations of some drugs.

Drug	Pharmacological effect	Therapeutic concentrations
Quinidine	Anti-arrythmic	2–6 μg/mL
Procainamide	Anti-arrythmic	6–14 μg/mL
Lignocaine (lidocaine)	Anti-arrythmic	1.5–5 μg/mL
Propranolol	Anti-hypertensive	20–80 ng/mL
Verapamil	Anti-arrythmic	80–320 ng/mL
Digoxin	Positive inotropic	0.6–2.4 ng/mL
Phenobarbitone	Anticonvulsant	10–25 μg/mL
Pethidine (meperidine)	Analgesic	0.4–0.7 μg/mL
Theophylline	Bronchodilator	6–16 μg/mL

Plasma concentration profile

Following the administration of a single dose of a drug preparation (dosage form), the factors that influence the plasma drug concentration profile include: the size of the dose (mg/kg), the formulation and route of administration of the drug preparation, the extent of both plasma protein binding and extravascular (tissue) distribution, and the rate of elimination (which refers to biotransformation and excretion) of the drug. The significant variable associated with oral, intramuscular or subcutaneous administration, namely bioavailability (i.e. the rate and extent of drug absorption into the systemic circulation), can be discounted by administering the drug intravenously as a parenteral solution (if available). It is only when a drug is administered intravenously that complete systemic availability (100% absorption of the dose) can be assumed.

Some intravenous anaesthetic agents

Pharmacokinetic studies of intravenous anaesthetic agents provide useful information for comparative purposes. Following the intravenous injection of a

single dose (25 mg/kg) of pentobarbital sodium to goats and dogs, the plasma concentration–time curves (plotted on arithmetic coordinates, Fig. 1.2) show that the various reflexes return and the animals of both species awaken from anaesthesia at the same plasma pentobarbitone concentrations, but at widely different times after drug administration (Davis *et al.*, 1973). The difference in the duration of anaesthetic effect is related to species variation in the rate of biotransformation (hepatic microsomal oxidation) of pentobarbitone.

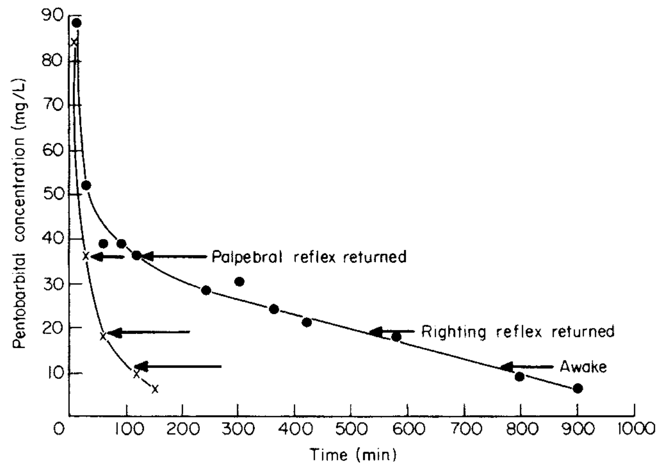

Fig. 1.2 Curves showing the decline in plasma concentrations of pentobarbital in goats (x—x) and dogs (•—•) following the intravenous injection of a single dose (25 mg/kg) of pentobarbital sodium. Arrows indicate the plasma pentobarbital concentrations (and related times) at which the various reflexes return and the animals of both species awaken from anaesthesia. (Reproduced with permission from Davis *et al.* (1973).)

The systemic clearance and the half-life of thiopentone, administered as an intravenous bolus dose, significantly differ between sheep and dogs. However, both species, as well as cats and human beings, awaken from anaesthesia at the same plasma thiopentone concentration (20 μg/mL). It is mainly redistribution of thiopentone from the highly perfused tissues (including the central nervous system (CNS)) to less well perfused tissues (such as skeletal muscle) and ultimately body fat, rather than elimination by hepatic biotransformation, that determines the duration of anaesthetic effect (Fig. 1.3) (Brodie *et al.*, 1952). Compared with mixed-breed dogs, Greyhounds and probably other lean breeds of hound (such as Whippet, Saluki and Afghan) recover more slowly from thiobarbiturate (thiopental and thiamylal) induced anaesthesia and show intermittent struggling and relapses into sleep during the recovery period. The slower and less smooth recovery of Greyhounds may be largely attributed to the lower body fat content, as a percentage of body weight, and partly to slower dose-dependent hepatic biotransformation of thiobarbiturates. Between 2 and 8 h after intravenous administration, the plasma concentrations of thiopental

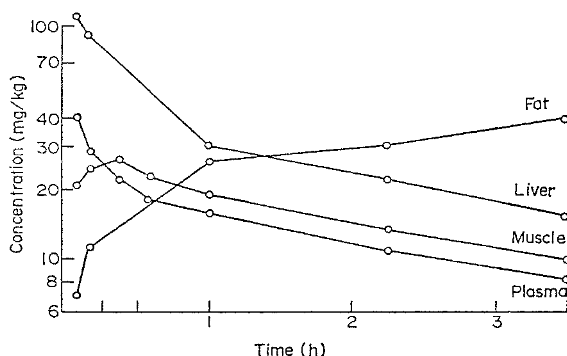

Fig. 1.3 Concentrations of thiopentone in various tissues and plasma of a dog after the intravenous administration of 25 mg/kg. (Reproduced with permission from Brodie *et al.* (1952).)

and thiamylal are significantly higher in Greyhounds than in mixed-breed dogs (Fig. 1.4) (Sams *et al.*, 1985). Premedication with acepromazine (0.25 mg/kg, i.m.) generally delays the time of awakening from thiopentone anaesthesia, although there is wide individual variation (Baggot *et al.*, 1984). The delayed awakening may have a pharmacodynamic rather than pharmacokinetic basis, or could be due to the sedative effect of the acepromazine.

Propofol, a highly lipophilic intravenous anaesthetic, rapidly induces anaesthesia of ultra-short duration in goats, dogs and human beings. Both redistribution and biotransformation of the drug contribute to the brief duration of anaesthetic effect. Even though the disposition kinetics of propofol differ among species and between mixed-breed dogs and Greyhounds (Zoran *et al.*, 1993), the blood propofol concentration at which dogs and seemingly goats return to the sternal position and human beings regain consciousness appears to be the same (1 µg/mL). The systemic clearance, expressed as mL/min·kg, of propofol exceeds hepatic blood flow in all species, particularly in goats (*vide infra*, Table 1.14). It can be concluded that another organ (the lungs) or extrahepatic tissue contributes to the metabolism, which takes place by conjugation reactions (glucuronide and sulphate synthesis), of propofol.

Ketamine, a dissociative anaesthetic, is administered as a racemic mixture (present in the parenteral preparation) and is initially metabolized by the liver to *N*-desmethylketamine (metabolite I), which in part is converted by oxidation to the cyclohexene (metabolite II) (Fig. 1.5). The major metabolites found in urine are glucuronide conjugates that are formed subsequent to hydroxylation of the cyclohexanone ring. As the enantiomers differ in anaesthetic potency and the enantioselectively formed (metabolite I has approximately 10% activity of the parent drug) interpretation of the relationship between the anaesthetic effect and disposition of ketamine is complicated. On a pharmacodynamic basis, the *S*(+) enantiomer is three times as potent as the *R*(−) enantiomer (Marietta *et al.*, 1977; Deleforge *et al.*, 1991), while the enantiomer that undergoes *N*-demethylation (hepatic microsomal reaction) differs between species (Delatour *et al.*, 1991). Based on the observed minimum anaesthetic

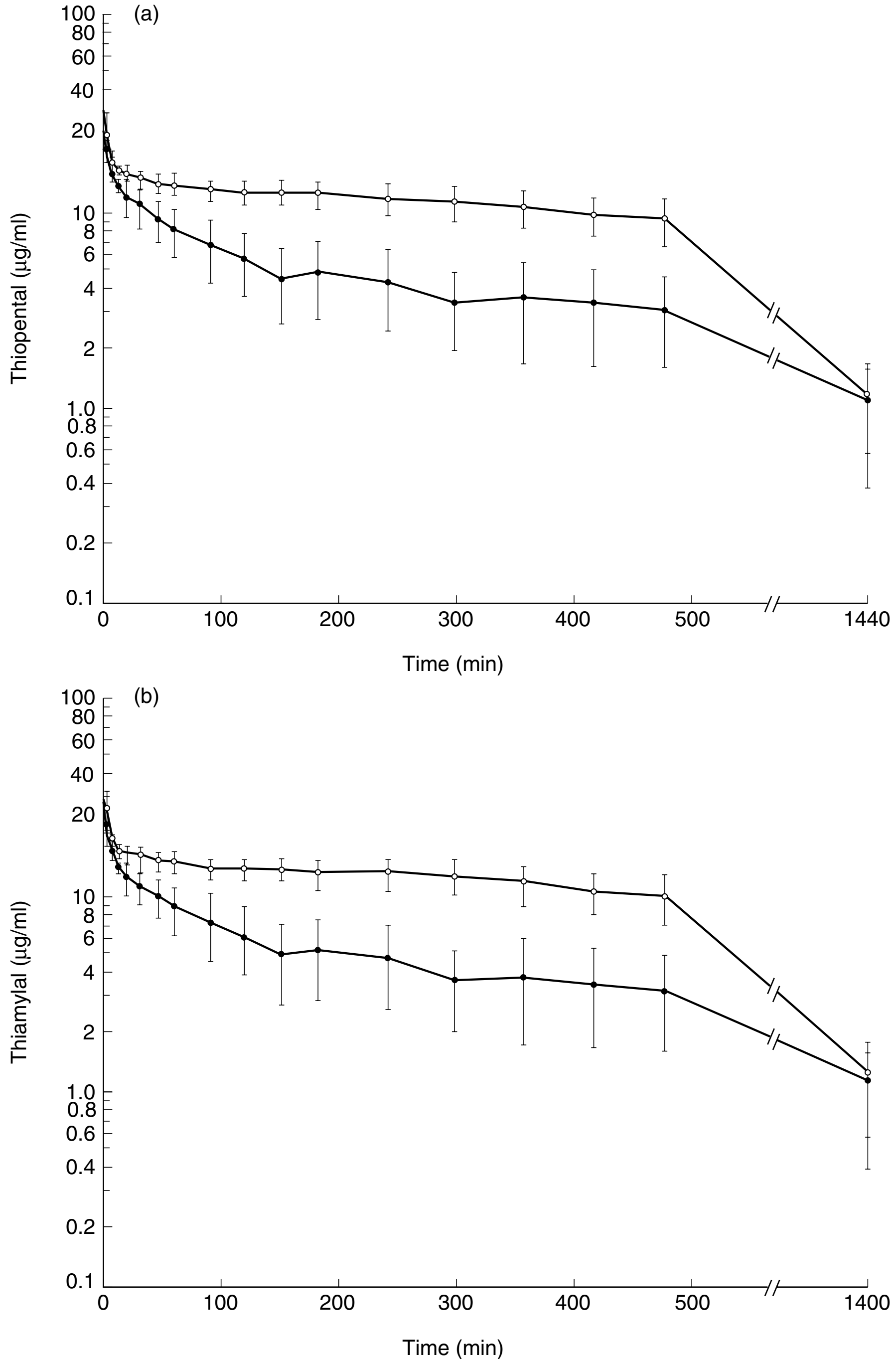

Fig. 1.4 Comparison of plasma thiobarbiturate concentration–time curves in Greyhounds and mixed-breed dogs following the intravenous administration of single doses (15 mg/kg) of thiopental and thiamylal (a) —Plasma thiopental concentrations in Greyhound (○—○) and mixed-breed dogs (●—●) after being given 15 mg of thiopental/kg, iv; mean ± SD. (b) —Plasma thiamylal concentrations in Greyhound (○—○) and mixed-breed dogs (●—●) after being given 15 mg of thiamylal/kg, iv; mean ± SD. (Reproduced with permission from Sams *et al*., (1985).)

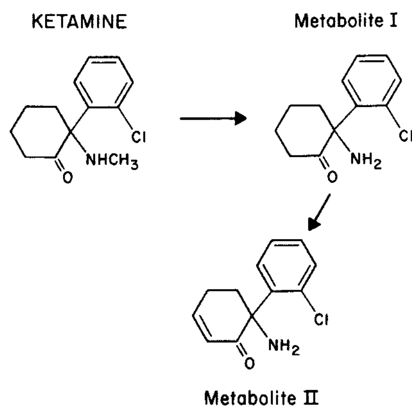

Fig. 1.5 Initial biotransformation (oxidative reactions) of ketamine. Both the parent drug and, to a lesser extent (10%), metabolite I are pharmacologically active.

concentration of ketamine in plasma (2 μg/mL), the duration of anaesthesia produced by a single intravenous dose relates mainly to distribution and partly, depending on the size of the dose, to biotransformation of the drug. The half-life of ketamine is shorter in domestic animals (sheep, 0.5 h; horses, 0.7 h; cattle, 0.9 h; dogs, 1 h; cats, 1.1 h), apart from pigs (2.3 h), than in human beings (2.5 h).

Species variations in dosage

Low dose requirements (relative to dogs) of xylazine (α_2-adrenoceptor agonist) for cattle and morphine (mainly μ-opioid agonist) for cats may be attributed to higher sensitivity of receptor sites in the central nervous system of the susceptible species to these drugs (Table 1.2). Brahman cattle appear to be even more sensitive than other breeds of cattle to xylazine, while the sedative dose for Isle of Rhum red deer (off the west coast of Scotland), although similar to that for cattle (0.1–0.2 mg/kg, i.m.), is one-tenth of the sedative dose required for mainland red deer (Fletcher, 1974). In giraffes, xylazine should not be used alone but it could be used in conjunction with etorphine; whenever the use of etorphine is intended, the narcotic antagonist diprenorphine should be available for administration. Certain breeds of dog (notably, the Basset Hound, Great Dane and Irish Setter) appear to be susceptible to bloat, probably due to aerophagia, some hours after xylazine administration.

The idiosyncratic toxicity, manifested by neurological effects, shown by a subpopulation of (rough-haired) Collies to ivermectin ($\geqslant$ 100 μg/kg, p.o.) may be attributed to a breed-related compromised blood–brain barrier (Tranquilli *et al.*, 1989), since γ-aminobutyric acid receptors that mediate neurotransmission are confined to the CNS in mammalian species. The pharmacokinetic behaviour of ivermectin does not differ between ‘ivermectin-sensitive’ and

Table 1.2 Species variations in drug dosage.

Drug (route of administration)	Animal species	Dose (mg/kg)	Dosage interval (h)
Single dose			
Xylazine hydrochloride (i.v.)	Dog	1.0	
	Cat	1.0	
	Horse	0.75	
	Cattle	0.075	
Morphine sulphate (i.m.)	Dog	1.0	
	Cat	0.1	
Succinylcholine chloride (i.v.)	Dog	0.3	
	Cat	1.0	
	Horse	0.1	
	Cattle	0.02	
Multiple doses			
Aspirin tablet(s) (p.o.)	Dog	10	12
	Cat	10	48
	Cow	100	12
Conventional aminophylline tablets	Dog	10	8
	Horse	5	12
Sustained-release anhydrous theophylline tablets (p.o.)	Dog	20	12
	Horse	15	24

normal Collies. A similar adverse effect has been observed in Murray Gray cattle, an Australian breed. The use of ivermectin is contra-indicated in Chelonians (tortoises, terrapins and turtles) and crocodiles.

The wide variation among species in the intravenous dose of succinylcholine (suxamethonium) required to produce a similar degree of neuromuscular blockade (depolarizing type) may be attributed to differences in the activity of pseudocholinerase, the enzyme that hydrolyses the drug. In ruminant species, at least 80% of whole blood cholinesterase activity is associated with the erythrocytes, which may account for the lower dose requirement for cattle (0.02 mg/kg) than for horses (0.1 mg/kg) and cats (1 mg/kg). Blood cholinesterase activity resides mainly in the plasma (pseudocholinesterase) of cats and horses. The eightfold longer duration of the neuromuscular blocking effect produced by succinylcholine in the rat compared with the cat was attributed to defective hydrolysis of the drug by plasma cholinesterase in the former species (Derkx *et al.*, 1971). Because succinylcholine does not produce analgesia, this drug should only be used in conjunction with an anaesthetic agent whenever a surgical procedure is to be performed.

Species differences in pharmacokinetic behaviour are far more common than in pharmacodynamic activity of drugs, but often only become evident

following the administration of multiple doses at a fixed dosage interval. The long dosage interval for aspirin (acetylsalicylic acid) in cats compared with dogs is related to the slow rate of synthesis of the glucuronide conjugate, due to the relative deficiency in cats of hepatic microsomal glucuronyl transferase activity. Glucuronide synthesis is the principal metabolic pathway for salicylate elimination; following the intravenous administration of sodium salicylate, the half-life of salicylate is 25–35 h in cats (and is dose-dependent) compared with 8.6 h in dogs and 1 h in horses. Low activity of glucuronyl transferase appears to be characteristic of Felidae, as it applies not only to the domestic cat (*Felis catus*) but also to the lion (*Panthera leo*), African civet (*Viverra civetta*) and forest genet (*Genetta pardina*) (French *et al.*, 1974). Taxonomically the civet and genet belong to the Viverridae family. The combination of slow formation of the glucuronide conjugate and the accumulation of a reactive metabolite, formed by an alternative metabolic pathway, to a level that exceeds the capacity of hepatic glutathione conjugation accounts for the toxicity of paracetamol (acetaminophen) in cats. Because feline haemoglobin is particularly susceptible to oxidative damage, methaemoglobinaemia consistently occurs in paracetamol toxicity. Parenteral preparations containing benzyl alcohol (as a preservative) should not be administered to cats; benzyl benzoate lotion should not be applied to cats. The relatively long dosage interval (12 h) for aspirin (100 mg/kg, p.o.) in cows is related to slow absorption of salicylate from the reticulo-rumen rather than hepatic metabolism, which takes place rapidly in cattle.

Therapeutic (i.e. safe and clinically effective) dosage regimens for conventional (immediate-release) aminophylline tablets are 10 mg/kg at 8-h intervals for dogs and 5 mg/kg at 12-h intervals for horses. These oral dosage regimens will maintain plasma theophylline concentrations within the therapeutic range (6–16 μg/mL) and produce the desired pharmacological effect (bronchodilation). The systemic availability of theophylline, administered as conventional aminophylline tablets, exceeds 90% in both species. Dosage intervals can be extended to 12 h for dogs and 24 h for horses by administering appropriate doses of sustained-release anhydrous theophylline tablets. A longer dosage interval for the horse than for the dog is unusual for a lipid-soluble drug, since most lipid-soluble drugs are more rapidly metabolized by the liver of horses than of dogs. Even though the optimum oral dosage regimen for metronidazole, which is indicated for the treatment of anaerobic infections (e.g. pleuropneumonia, liver abscesses, peritonitis), is the same (15–20 mg/kg administered at 8 h dosage intervals) for dogs and horses, it is usual to use a 12 h dosage interval for horses based on economic considerations and convenience of drug administration.

A dosage interval of 12 h appears to be appropriate to use in dogs for oral sustained-release anhydrous theophylline tablets (Koritz *et al.*, 1986) and oral sustained-release morphine sulphate tablets (Dohoo *et al.*, 1994). Following oral administration of these sustained-release dosage forms, the average systemic availability of theophylline is 76% and of morphine is 21%. Gastro-

intestinal transit time in monogastric species makes it unlikely that oral sustained-release dosage forms would provide drug for absorption for longer than 24 h. Controlled-release ruminal boluses for use in cattle, due to their retention in the reticulo-rumen, either continuously release drug (generally an anthelmintic) into ruminal fluid over a prolonged period (e.g. the ivermectin ruminal bolus, 135 days; fenbendazole ruminal bolus, up to 140 days; moranel tartrate ruminal bolus, at least 90 days) or intermittently release pulse doses at a regular (approximately 3-week) interval (e.g. oxfendazole ruminal bolus for cattle). The higher dosage requirement of some anthelmintics (e.g. benzimidazole carbamates, clorsulon) for cattle and goats than for sheep, although based solely on clinical efficacy, is probably related to more rapid hepatic metabolism of these drugs in cattle and goats. Because of the more rapid elimination of closantel in goats than in sheep, a suggested interval for repeated doses of the anthelmintic to prevent reinfection with benzimidazole-resistant *Haemonchus contortus* is 30 days for goats and 50 days for sheep (Hennessy *et al.*, 1993).

Species variations in drug disposition

Disposition is the term used to describe the simultaneous effects of distribution and elimination, that is, the processes that occur subsequent to the absorption of a drug. The factors that influence drug disposition include: the chemical nature and physicochemical properties of the drug, the extent and avidity of binding to plasma proteins and extravascular macromolecules (tissue components), the extent of extravascular distribution and, in ruminant animals, operation of the ion-trapping effect in ruminal fluid, blood flow to the organs of elimination (usually the liver and kidneys), the activity of drug-metabolizing enzymes (particularly those associated with hepatic microsomal-mediated metabolic pathways), and the efficiency of excretion (mainly renal) mechanisms. The most important pharmacokinetic parameters describing the disposition of a drug are the systemic (body) clearance (Cl_B), which measures the ability of the body to eliminate the drug, and the volume of distribution (V_d), which denotes the 'apparent' space in the body available to contain the drug. The half-life ($t_{1/2}$) expresses the overall rate of drug elimination, while the mean residence time (*MRT*), which is the statistical moment analogy to half-life, represents the average time the number of drug molecules introduced reside in the body.

Species variations in the disposition of a drug may be due to differences in the apparent volume of distribution or the rate of elimination of the drug. The apparent volume of distribution of most lipophilic organic bases (e.g. ketamine, ivermectin, macrolide antibiotics) is larger in ruminant than in monogastric species. However, differences in the rate of elimination, particularly of drugs that undergo extensive hepatic biotransformation, generally account for species variations in drug disposition.

Drug elimination processes

There are two distinct processes by which drugs are eliminated from the body: biotransformation (metabolism) and excretion; although both processes are involved in the elimination of the majority of drugs, either may predominate. Elimination is ultimately responsible for terminating the action of drugs. The mechanism(s) of elimination is determined by the molecular structure and chemical nature of the drug and by the same physicochemical properties as influence drug distribution. Biotransformation converts drugs to metabolites which are generally less active than the parent drug or inactive, more polar, less lipid-soluble and suitable for rapid removal from the body by renal excretion and, to some extent, biliary excretion.

Biotransformation

Biotransformation is the principal mechanism of elimination for lipid-soluble drugs and other foreign chemical substances (xenobiotics). Because of its high content of drug-metabolizing enzymes and rich blood supply (26–29% of cardiac output), the liver is the principal organ of drug biotransformation. The hepatic microsomal enzymes, which are associated with the smooth-surfaced (devoid of ribosomes) endoplasmic reticulum, mediate a variety of oxidative reactions and glucuronide conjugation (synthesis). Some of the enzyme-catalysed reactions involved with the biotransformation of drugs are utilized in the formation and subsequent metabolism (to facilitate excretion) of certain endogenous substances, such as steroid hormones, fatty acids, prostaglandins, leukotrienes, bile acids and bilirubin. Sites of drug biotransformation, in addition to the liver, include the lungs, kidneys, blood plasma, forestomach (in ruminant species), intestinal microorganisms, intestinal mucosa (orally administered drugs) and epidermis (topically applied drugs). The most likely major metabolic pathway for a drug can often be predicted, based on a knowledge of the functional group(s) in the molecule (Table 1.3). The general pattern of drug biotransformation is usually biphasic. The first phase comprises oxidative, reductive and hydrolytic reactions, while the second phase consists of the conjugation or synthetic reactions (Fig. 1.6) (Williams, 1967). The enzyme systems involved in phase I reactions are located primarily in the endoplasmic reticulum, while the enzymes involved in conjugation reactions (referred to as transferring enzymes) are mainly cytosolic.

Microsomal oxidative reactions constitute the most prominent phase I biotransformation pathway for a wide variety of structurally unrelated drugs (Table 1.4). Some drugs (e.g. amphetamine, diazepam, propranolol, lignocaine) simultaneously undergo more than one type of microsomal-mediated oxidative reaction. Microsomal enzymes are located primarily in liver cells, where they are associated with the smooth-surface (without ribosomes) endoplasmic reticulum (Fouts, 1961). Lipid solubility is a prerequisite for drug access to the

Table 1.3 Probable biotransformation pathways for drugs.

Functional group	Biotransformation pathways
Aromatic ring	Hydroxylation
Hydroxyl	
Aliphatic	Chain oxidation; glucuronic acid conjugation; sulphate conjugation (to a lesser extent)
Aromatic	Glucuronic acid conjugation; sulphate conjugation; methylation; ring hydroxylation
Carboxyl	
Aliphatic	Glucuronic acid conjugation
Aromatic	Ring hydroxylation; glucuronic acid conjugation; glycine conjugation
Primary amines	
Aliphatic	Deamination
Aromatic	Acetylation; glucuronic acid conjugation; methylation; sulphate conjugation; ring hydroxylation
Sulphydryl	Glucuronic acid conjugation; methylation; oxidation
Ester linkage	Hydrolysis
Amide bond	Hydrolysis

Source: Baggot (1977).

site where microsomal oxidation takes place. Microsomal drug oxidations require cytochrome P450 enzymes (haemoproteins that are localized in the smooth endoplasmic reticulum and exist in several forms), the closely associated NADPH–cytochrome P450 reductase, NADPH (reduced nicotinamide adenine dinucleotide phosphate) and molecular oxygen. The ability of the microsomal drug-metabolizing enzymes (mixed function oxidase, or cytochrome P450 mono-oxygenase, system) to catalyse various oxidative reactions may be ascribed to a common mechanism, hydroxylation (Brodie *et al.*, 1958; Gillette, 1963, 1966). The steps involved in microsomal oxidation are shown schematically (Fig. 1.7). The drug (or xenobiotic) substance reacts with the oxidized (Fe^{3+}) form of cytochrome P450 to form an enzyme–substrate complex (step 1). NADPH donates an electron to the flavoprotein (NADPH–cytochrome

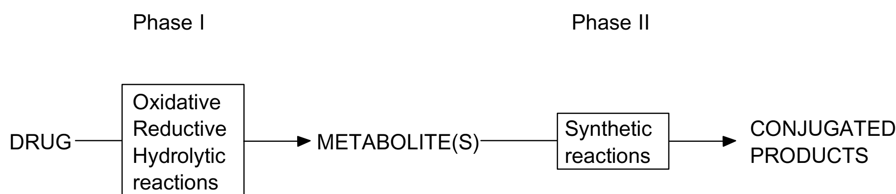

Fig. 1.6 General pattern of drug metabolism.

Table 1.4 Oxidative reactions catalysed by microsomal enzyme systems.

Oxidative reaction	Drug substrate
Aromatic hydroxylation	Amphetamine Phenobarbitone Phenytoin Phenylbutazone Propranolol
Side chain (aliphatic) oxidation	Ibuprofen Meprobamate Pentobarbitone Phenylbutazone
Oxidative dealkylation	
O-dealkylation	Codeine Griseofulvin
N-dealkylation	Diazepam Lignocaine Morphine Caffeine Theophylline
Oxidative deamination	Amphetamine Diazepam
Desulphuration (replacement of S by O)	Parathion Thiopentone
Sulphoxidation (S-oxidation)	Chlorpromazine Cimetidine

P450) reductase which, in turn, reduces the oxidized cytochrome P450–drug complex (step 2). A second electron is introduced from NADPH via the same flavoprotein reductase which serves to reduce molecular oxygen (O_2) and to form an 'activated oxygen'–cytochrome P450–drug intermediate (step 3). One atom of oxygen is transferred to the drug substrate to form the oxidized drug (hydroxylated product) and the second oxygen atom is released as water (step 4). Upon release of the oxidized drug, the oxidized form of cytochrome P450 is regenerated. The substrate specificity of this enzymatic reaction (cycle) is very low. High solubility in lipid is the only property that the wide variety of structurally unrelated drugs that serve as substrates for microsomal oxidation have in common.

The many isoenzymes of cytochrome P450 that exist are classified into gene families on the basis of their amino acid sequence. Of the twelve families that have been identified in mammalian species, three families (CYP1, CYP2 and CYP3) encode the enzymes involved in the majority of drug biotransformations, while the other families are involved in the metabolism of endogenous

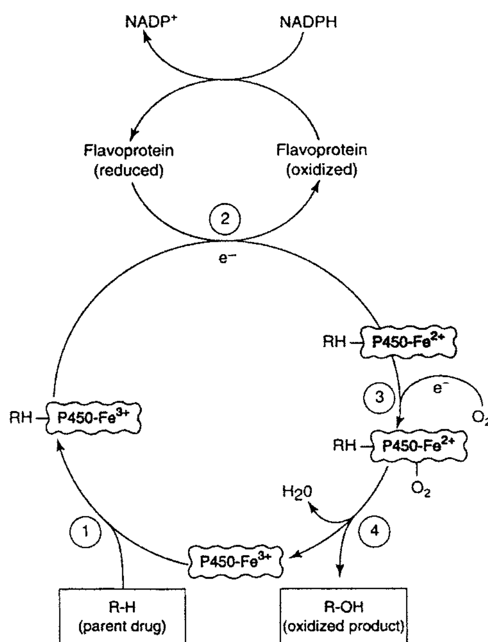

Fig. 1.7 Cytochrome P450 cycle in drug oxidations: RH, parent drug; ROH, oxidized metabolite; e^-, electron. (Reproduced with permission from Correia (1992).)

substances. The major subfamily of cytochrome P450 isoenzymes involved in biotransformation of drugs is CYP3A (the terminal capital letter denotes the subfamily) and is expressed at significant levels extrahepatically. The subfamily CYP1A is expressed at low levels in the skin but, in common with CYP3A, the activity of the isoenzymes can be induced (or inhibited) by a variety of drugs and xenobiotics.

Based on *in vitro* studies of hepatic microsomal-catalysed oxidative reactions, using liver specimens from a variety of animal species including man, and marker substrates for the various reactions, it can be concluded that neither the measured levels of cytochrome P450 and cytochrome b_5 nor the activity of NADPH–cytochrome *c* reductase accounts for species variations in the capacity of oxidative reactions (McManus and Ilett, 1976; Dalvi *et al.*, 1987; Souhaili-El-Amri *et al.*, 1986). However, species variations could be attributed to differences in values of the kinetic parameters (Michaelis constant, K_M, and the maximum reaction velocity, V_{max}) associated with individual reactions.

Oxidative reactions catalysed by non-microsomal enzymes are less varied than those mediated by cytochrome P450 mono-oxygenases but are important in the metabolism of some drugs (e.g. isoproterenol, methylxanthines, methimazole, ethanol, chloral hydrate), endogenous substances (adrenalin, histamine) and naturally occurring compounds (vitamin A). Theophylline and caffeine (methylxanthines) simultaneously undergo microsomal cytochrome

P450-mediated *N*-dealkylation and non-microsomal-catalysed oxidation. A flavin-containing mono-oxygenase catalyses the reversible oxidation of parent benzimidazole sulphide anthelmintics to their sulphoxide (active) metabolites, whereas cytochrome P450 mono-oxygenases catalyse oxidation of sulphoxides to the corresponding sulphone (inactive) metabolites. Whilst most reductive reactions are catalysed by non-microsomal enzymes, some are catalysed by cytochrome P450 enzymes, generally under conditions of low oxygen tension. Drugs that contain a nitro group (e.g. chloramphenicol, nitroimidazoles, nitroxynil) and azo-compounds (prontosil) are reduced to amines. Ruminal micro-organisms efficiently perform reductive reactions and are capable of inactivating orally administered drugs containing a nitro group.

Hydrolysis is a phase I biotransformation reaction that is limited to drugs with an ester (—COO—) or an amide (—CONH—) linkage. Examples of drugs that are esters include acetylsalicylic acid (aspirin; non-steroidal anti-inflammatory drug), diphenoxylate (synthetic opioid antidiarrhoeal agent), succinylcholine (depolarizing neuromuscular blocking drug) and procaine (local anaesthetic). The esterases are found in the blood plasma, liver and other tissues, primarily in the non-microsomal soluble fraction. Hydrolytic conversion of aspirin to salicylic acid (which is pharmacologically active) takes place rapidly, but conjugation of salicylate with glucuronic acid (a phase II reaction) occurs very slowly in cats compared to other domestic animal species. The wide variation between species in the dose (mg/kg) of succinylcholine may be attributed to differences in the activity of plasma pseudocholinesterase, the enzyme that hydrolyses the drug.

The amidases, which hydrolyse amide linkages, are non-microsomal enzymes and are found principally in the soluble fraction of the liver. Procainamide (anti-arrythmic) is the amide analogue of procaine. Most amides are hydrolysed more slowly than the corresponding esters. In the horse, the half-life of procainamide is 3.5 h compared with 0.85 h for procaine. Because hydrolysis of the amide bond takes place relatively slowly, drugs containing this bond are likely to undergo biotransformation by simultaneously occurring alternative pathways. Procainamide is converted to *N*-acetylprocainamide (acetylation is a phase II conjugation reaction), which curiously has a longer half-life (6.3 h in the horse) than the parent drug. As acetylation of primary aromatic amino groups does not take place in dogs, over 50% of an administered dose of procainamide is excreted unchanged in the urine. Lignocaine (lidocaine), a local anaesthetic and anti-arrythmic drug, undergoes biotransformation by simultaneously occurring hepatic microsomal oxidative reactions (aromatic hydroxylation and *N*-dealkylation) and hydrolysis of the amide bond (Fig. 1.8) (Keenaghan & Boyes, 1972). A portion of the phase I metabolites formed undergo conjugation (phase II reaction) before excretion in the urine. The rates of the different biotransformation reactions pertaining to a drug determine the relative amounts (fraction of dose) of the metabolites that are formed; these may vary widely between species.

Phase I biotransformation reactions usually convert the parent drug to a

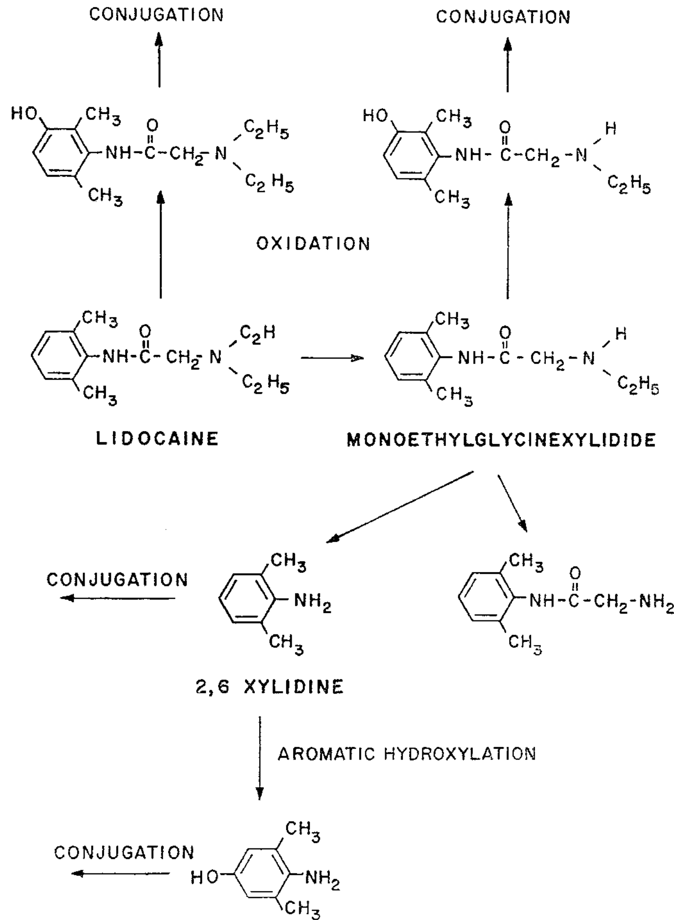

Fig. 1.8 The metabolic fate of Lidocaine (lignocaine). (Reproduced with permission from Keenaghan & Boyes (1972).)

more polar metabolite by introducing or unmasking a functional group, such as hydroxyl (—OH), carboxyl (—COOH), amino ($—NH_2$), or sulphydryl (—SH). If the phase I metabolites are sufficiently polar, they may be readily excreted. Otherwise, the acquired functional group enables conjugation with endogenous substances, such as glucuronic acid, acetic acid, sulphate (derived from sulphur-containing amino acids), or an amino acid (methionine, glycine), to take place. The conjugates (phase II metabolites) formed are almost invariably highly polar and rapidly excreted by the kidney (in urine) and the liver (in bile). A decrease in lipid solubility does not necessarily mean an increase in water solubility, which is the situation with some acetylated sulphonamides (e.g. sulphathiazole) particularly under acidic conditions such as occur in the urine of carnivorous species. The phase I metabolites may be either pharmacologically inactive or have modified (generally decreased) activity. Examples of