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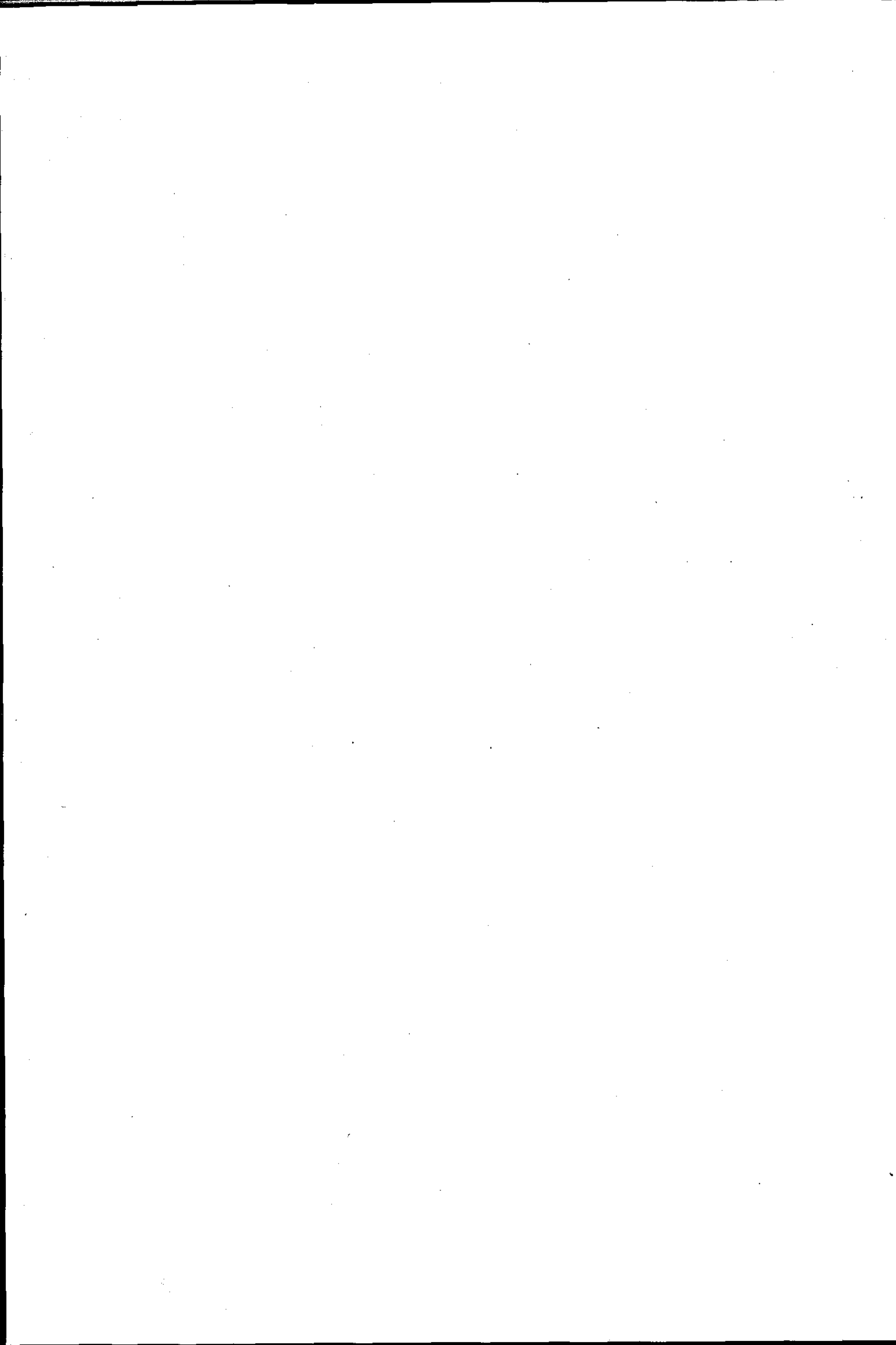

# COMPARATIVE PHARMACOKINETICS

# IN NON-HUMAN PRIMATES

by

EDWARD DOYLE

A Doctoral Thesis

submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy of the Loughborough University of Technology

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The fascination of what's difficult
Has dried the sap out of my veins, and rent
Spontaneous joy and natural content
Out of my heart.

W.B. Yeats

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## ABSTRACT

Several studies in the literature suggest that the biotransformation of foreign compounds in man is more similar to that in the non-human primate than to that in other laboratory animal species. However, published information about the pharmacokinetics of foreign compounds in non-human primates is limited and usually derived from one species, the rhesus monkey. In view of the often limited availability of this species for use in the development and safety evaluation of new drugs, pesticides, etc., it is necessary to search for potential replacement species for future use. This thesis compares the pharmacokinetics of seven well-known or commonly used drugs, i.e. antipyrine, diazepam, frusemide, ibuprofen, isosorbide dinitrate, paracetamol and pentobarbitone in the rhesus monkey, cynomolgus monkey and baboon with a view to defining further the suitability of these species for the study of newer substances. These three non-human primate species are those that are most frequently used in pharmacology and toxicology studies. The drugs were chosen for study because they are extensively used clinically and related human pharmacokinetic data are available for comparison.

Primary pharmacokinetic data are best obtained after intravenous doses when all the drug is injected into the bloodstream. Following intravenous injection of each compound alone at a dose level that would possibly be used in toxicology studies, plasma concentrations of drug were measured using established analytical techniques. The plasma concentration-time data was processed using non-linear least squares curve fitting techniques. Pharmacokinetic parameters were estimated using a two-compartment open model and in some cases, when appropriate, a one-compartment open model.

A statistical analysis of variance of pharmacokinetic parameters obtained for each drug showed that there were few significant pharmacokinetic differences ($P < 0.05$) between the rhesus monkey, the cynomolgus monkey and the baboon. In general, volumes of distribution adjusted for bodyweight differences, and half-lives of elimination were similar in the three non-human primate species studied. However,

the clearance of those compounds with low rates of systemic clearance, i.e. antipyrine, frusemide, ibuprofen and pentobarbitone was lower in the cynomolgus monkey than in the other two species. The clearance of those compounds with higher clearance rates, i.e. diazepam, isosorbide dinitrate and paracetamol, was similar in all three species of non-human primate.

A review of the literature of the pharmacokinetics of the compounds studied, in man and the commonly used laboratory animal species, reveals that, in general, the same pharmacokinetic model applies to the data for non-human primate species as for other species. With respect to volumes of distribution adjusted for bodyweight differences, the non-human primate is in general similar to man and to the commonly used laboratory animal species. In the non-human primate species, clearances adjusted for bodyweight differences tend to be similar to those in other commonly used laboratory animal species except in the rat. However, clearances of these drugs in humans appear to be lower than in animal species. Biological half-lives in the non-human primate species are closer to those in the laboratory animal species than to those in man. In particular, those compounds that have long biological half-lives in man ($t_{1/2} > 12$ hours) have considerably shorter half-lives in the non-human primate species studied and in other commonly used laboratory animal species cited in the literature.

Using pharmacokinetic criteria, and with respect to the compounds studied, it can be concluded that the cynomolgus monkey and the baboon are suitable alternatives to the rhesus monkey for drug evaluation studies. However, these non-human primates are pharmacokinetically more similar to other commonly used laboratory animal species than to man. Nevertheless, factors such as differences in the pattern and nature of biotransformation, distribution in target tissues and receptor and protein binding need also to be considered when selecting an animal model for man for use in the development and safety testing of drugs.

# CHAPTER 1

## GENERAL INTRODUCTION

Food additives, pesticides, pharmaceuticals, etc., are foreign to man in that they are non-nutrient. Such compounds could be hazardous if they were not rendered harmless by detoxication and thus eliminated (Williams, 1959).

The safety evaluation now required for such substances before man is exposed to them involves studying their effects in animals. Species such as the rat, dog and non-human primate are exposed to foreign substances for long periods and any toxic effects determined. Ideally, animal species used for laboratory testing would metabolise foreign compounds in the same way as man but unfortunately this is not so. There are many factors that can influence the fate of a compound in an organism, e.g. species, strain, age, sex, stress and disease, and species is one of the more important.

The subject of species variations in biotransformation has been reviewed many times (Williams, 1959; 1974; 1978; Hathway, 1970; Moore, 1972; Smith and Williams, 1974; Baty, 1975; 1977; 1979; Smith and Caldwell, 1977). No one animal species has been found in which the metabolism of all compounds is the same as that in man. The best that can be achieved is a compromise in which data obtained from different animal species is extrapolated to man (Smith, 1966; Burns, 1968; Conney et al, 1974; Gillette, 1976).

As non-human primates are closest to man in evolutionary status, these species might be considered good models for man. However, because of the expense and difficulty of their maintenance, non-human primate species have not been studied as thoroughly as they deserve. Indeed information on the pharmacokinetics of many well known drugs in non-human primate species is lacking. Reported pharmacokinetic data on drugs studied in non-human primate species are discussed in the appropriate sections of this thesis.

Wagner (1968) has stated that the purpose of pharmacokinetics is to study the time course of drug and metabolite concentrations and amounts in the biological fluids, tissues, excreta and to relate them to the time-course of drug action (pharmacological and toxic effects).

## Absorption

Substances administered to an animal, unless injected directly into the bloodstream, must be absorbed before they enter the circulating blood. Absorption is the transfer of molecules from the site of administration, across the barrier membranes and into the circulation. The most common route of administration is orally via the gastrointestinal (G.I.) tract (enteral administration).

Factors that can affect G.I. absorption are many (Riegelman and Rowland, 1973; Wagner, 1975; Welling, 1977; Houston and Wood, 1980). However, some of the more important factors that alter the rate and extent of absorption are the particle size of the foreign compound, $pK_a$, lipid solubility, the presence of other substances, motility of the G.I. tract, gut microflora and splanchnic blood flow.

Absorption from the alimentary tract can take place from the mouth, from the stomach, the intestine, the colon or from the rectum. The mechanisms for absorption are those for the transfer of compounds across membranes (Schanker et al, 1957; 1958; Hogben et al, 1959; Schanker, 1959; Kakemi et al, 1965). For the absorption from the G.I. tract, the main barrier membrane is the gastrointestinal epithelium.

Absorption from the mouth and the lower sections of the rectum differs from that from other sections of the alimentary tract in that compounds absorbed via these routes are not transported directly to the liver, nor are they exposed to the gastric juices before absorption. Both these factors can significantly

affect the amount of a foreign compound that enters the circulation (Adamson et al, 1966; 1970a; Rowland, 1977; Schwartz, 1966).

Absorption occurs from the G.I. tract by mechanisms other than simple diffusion of small non-ionised molecules. For example, quaternary ammonium compounds are absorbed from the intestine to an appreciable extent although they are completely ionized at the pH of the intestinal lumen (Levine, 1966; and Waldeck, 1969). Macromolecules, such as diphtheria, tetanus and botulinus toxins have also been shown to be absorbed enterally (Lammanna, 1960). Examples of active intestinal transport are the absorption of foreign sugars structurally similar to glucose (Wilson and Landau, 1960), and the anti-tumour agents such as 5-fluorouracil and 5-bromouracil by the process which transports the natural pyrimidines (Schanker and Jeffrey, 1962; Schanker and Tocco, 1960; 1962).

Parenteral absorption. Absorption from sites in the body such as the lung (Enna and Schanker, 1969) and muscle(Renkin, 1952; Pappenheimer, 1953) also involves transfer from the site of administration across barrier membranes and into the bloodstream. Apart from absorption through the skin this process is quite efficient and absorption is limited mainly by the supply of blood to the site of administration. Again simple diffusion and filtration are the most common mechanisms for absorption of substances from these parenteral sites.

Distribution

Once a foreign compound has entered the circulation, it will be carried to all regions of the body. The time taken to reach equilibrium between solute concentrations in the blood and tissues depends principally on the rate of supply of blood to the tissue. Other factors that will influence the distribution of a foreign compound are the ease of membrane transfer and interactions with constituents of the body, e.g. tissue and plasma proteins, nucleic acids and melanin.

For convenience, the tissues in the body can be grouped into three main categories. The first group (or compartment) usually consists of the highly perfused tissues e.g. the liver, the kidney and the lung. The second compartment normally comprises the less well perfused tissues, e.g. fat, muscle and skin. Those tissues with a negligible supply of blood, e.g. bone, teeth, hair, normally form the third compartment. In time, foreign compounds will fully distribute throughout the available space until a distribution equilibrium is obtained. The amount of drug in the different compartments at equilibrium will depend on the relative sizes of the intercompartmental transfer rate constants defined on pages 26 and 27.

It is assumed that the transfer rate constants for most drugs between tissues within a compartment are much greater than those between tissues in different compartments and that the former can be ignored. This approach simplifies the mathematical description of distribution and requires only blood (plasma or serum) concentration-time data, because at equilibrium it can be assumed that the drug concentration in the blood is proportional to that in the tissues (Gibaldi and Perrier, 1975). Blood is relatively easy to sample, and measurement of drug concentrations in blood provides a convenient method for studying the distribution of compounds in the body. By contrast, tissue samples are not usually available.

The apparent volume of distribution is defined as that volume in which the total administered dose would be uniformly distributed into, to give the observed concentration in the blood (or plasma), equation 1:1.

$$V_d = \frac{Dose}{C_o} \quad ......(1:1)$$

where $V_d$ is the apparent volume of distribution, and $C_o$ is the intercept at zero time of the whole blood (or plasma) concentration-time curve after intravenous injection of the compound.

The value of $V_d$ will depend on the degree of extravascular distribution. In man, therefore, it can vary from as little as 3 litres for a compound that is restricted almost entirely to the circulating plasma

e.g. Evans Blue (Goldstein et al, 1974) to a value of the order of 1000 times the volume of the body, e.g. quinacrine (Butler, 1971).

Membrane transfer. The blood capillary wall is relatively permeable and provides little resistance to the transfer of small molecules. Most compounds do, therefore, gain access to the extra-cellular interstitial space of tissues in a rather short time. The cellular membranes provide more resistance to penetration by foreign compounds than the capillary wall and usually control the rate of distribution into the tissue. The rate at which a molecule enters the tissue is dependent on the physical properties of the molecule. An exception is the brain which is protected from the harmful effects of many substances by a sheath of connective-tissue cells, called glial cells, around the cerebral capillaries (Rall, 1971). Only those molecules with the desired physical properties to enter the cellular membranes readily enter the extracellular fluid of the brain.

Disposition in fat. Compounds that are highly lipid soluble, e.g. organochlorine pesticides, have been shown to deposit in the fatty areas of the body (Brodie et al, 1950; 1952; Hayes et al, 1956; Chenoweth et al, 1962; Eger and Shargel, 1963; Radomski et al, 1968). Accumulation of a compound in the fat results in lower circulating levels and therefore a reduced effective concentration. For example, it has been suggested that the localization of the short acting barbiturate thiopentone in fat, and not its elimination, determines the duration of action of this compound in the body (Goldstein and Aronow, 1960).

Protein binding. Compounds usually vary in the extent to which they bind to proteins such as albumin and $\alpha_1$-acid glycoprotein. As only free (unbound) molecules transfer readily across membranes, so the extent of protein binding of a molecule can affect its distribution. The volume of distribution equation 1:1 can be modified to account for differences in the degree of protein binding equation 1:2

$$V_d = V_p + V_T \left(\frac{\alpha}{\alpha_T}\right) \quad .......(1:2)$$

where $V_p$ is the plasma volume, $V_T$ is the volume outside the plasma into which the compound distributes and $\alpha$ and $\alpha_T$ are the fractions unbound in the plasma and outside the plasma respectively (Wilkinson and Shand, 1975; Gibaldi and McNamara, 1978).

A number of compounds have been shown to interact with nucleic acids, e.g. quinacrine and chloroquine, and therefore be localized within cells (Parker and Irvin, 1952; Kurnick and Radcliffe, 1962; Hahn et al, 1966). Chloroquine has also been shown to interact with melanin as do phenothiazines and many other basic compounds (Lindquist, 1973). Accumulation of compounds such as chloroquine and phenothiazines in cells and melanin containing tissue has the effect of increasing the apparent volume of distribution of these compounds, although in true physiological terms their distribution may be rather small.

Elimination

There are many routes that a foreign compound can take to leave the body. The most common is via the kidney into the urine (renal excretion). Other routes of elimination include biliary excretion, excretion in the expired air, via sweat, tears and saliva and by secretion into the stomach and other parts of the gastrointestinal tract.

Renal excretion. The kidney is a member of the highly perfused group of tissues. The high blood flow through the kidney (in man about 1300 ml/minute, Goldstein et al, 1974) ensures that a large proportion of the circulating molecules pass through the kidney every minute. The mechanism by which molecules pass from the blood into the kidney nephron are, glomerular filtration, passive tubular transfer and active tubular transport.

In a normal man, approximately 130 ml of plasma is filtered each minute through the glomerular membranes (Goldstein et al, 1974). These

membranes appear to offer little resistance to the transfer of solute molecules. Only those molecules that are bound to plasma proteins, or of similar size as protein molecules, are not capable of entering the renal tubule. Reabsorption of the glomerular filtrate takes place across the tubular epithelium. The principles that control the reabsorption into the bloodstream are the same as for the transfer of molecules across other membranes. Highly lipid soluble molecules will therefore be reabsorbed but polar compounds and ions will be excreted, unless actively reabsorbed.

Passive tubular transfer of non-ionized molecules takes place along the entire length of the renal tubule. Solute molecules can pass in either direction (from the renal tubule into the body or from the body into the renal tubule) at a rate that is dependent on the lipid solubility of the molecule (Weiner et al, 1960). As only the non-ionized molecule passes through the tubular epithelium by this mechanism, variations in urinary pH will affect the rate of transfer in both directions (Milne et al, 1958).

The epithelial cells of the proximal tubules are particularly well adapted for active transfer. This mechanism is responsible for the reabsorption of many polar and ionic molecules, e.g. glucose, amino acids, sodium ions and water. Both acids and bases are actively secreted into the distal tubules by carrier mechanisms provided by the epithelial cells (Cafruny, 1971).

Biliary excretion

Because the membrane wall of the hepatic parenchymal cells is highly permeable, foreign compounds that have entered the liver can leave the blood and enter these cells. The foreign compound (or its metabolite) is then either returned to the blood, or excreted in the bile.

The mechanism by which some compounds are directed to the bile and others to the urine is as yet unclear. However, it is apparent that highly polar compounds, such as the bile salts and certain dyes, and conjugates of foreign compounds, which are ionic and which have a

molecular weight greater than 300, are secreted in the bile in higher concentrations than they are present in the plasma, presumably by active secretion (Brauer, 1959; Sperber, 1965). For example, sulphobromophthalein, a dye used to test hepatic function (Rosenthal and White, 1925) is secreted into bile in concentrations far exceeding those present in plasma (Brauer, 1959).

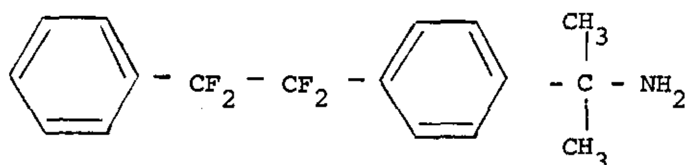
Cations such as quaternary ammonium compounds are probably secreted into the bile by active processes that are different from those for anions (Schanker and Solomon, 1963; Schanker, 1965). Indeed, different mechanisms may exist for the biliary secretion of different types of anions and cations. Furthermore, the biliary excretion of non-charged substances may sometimes be affected by presence of organic anions. Thus it would appear that pathways of biliary excretion are indeed quite complex and they may not be entirely independent of each other (for a review see Levine, 1978).

Biliary excretion is an important route of elimination of certain anions and cations that are largely ionized at intestinal pH and are therefore poorly reabsorbed from the intestine. This subject has been reviewed by Smith (1973).

Foreign compounds excreted into bile may be converted into a form that is capable of reabsorption. For example, enzymes in the bile or more often those in the intestine, produced either by intestinal secretions or gut flora (Smith, 1973) are capable of hydrolysing conjugates (e.g. glucuronides, sulphates) of foreign compounds and the free compound may be reabsorbed. Those compounds that are reabsorbed will be transported back to the liver where they may be conjugated and as conjugates again secreted into bile. The cyclic process thus set up is known as enterohepatic circulation. In this cycle, the amount of the foreign compound in the body is nevertheless gradually decreasing due to other biotransformations and urinary and faecal excretion.

There are notable species variations in the extent of biliary excretions of some substances. In a review of this subject Smith (1973) and later Hirom et al (1976) indicate that molecular weight is an

important factor. A minimum molecular weight of approximately 325 is required for significant biliary excretion in the rat, while 400 and 475 are the corresponding approximate figures in the guinea pig and rabbit, respectively (Millburn et al, 1967; Aziz et al, 1971). A molecular weight above 500-600 is probably required in man; for examples see Klaasen et al (1981). Thus, biliary excretion is the major route of elimination of the orally active anti-arrhythmic drug α,α-dimethyl-4-α,α,β,β-tetrafluorophenethyl benzylamine (after conjugation) in the rat and dog, but this compound is excreted mainly in the urine in the rhesus monkey, the baboon and man (Zacchei et al, 1976).




α,α-dimethyl-4-α,α,β,β-tetrafluorophenethyl benzylamine.

These molecular weight criteria were established mainly using organic anions. However, Hughes et al (1973) have indicated that organic cations of molecular weight as low as 200 are excreted in the bile of the rat and species differences have not been observed.

Clearance

At low concentrations biotransformation and excretion are normally assumed to proceed by first order processes, (i.e. the rate is proportional to the concentration of the solute at the site of elimination). These processes are described by the elimination rate constant $K_{el}$ (see page 27). Clearance (defined by equation 1:3) describes elimination in physiological terms.

$$Cl_{organ} = Q_{organ} \frac{C_{in} - C_{out}}{C_{in}} = Q_{organ} E_{organ} \quad \ldots\ldots\ (1:3)$$

where $Cl_{organ}$ is the clearance of a compound by an eliminating organ, $C_{in}$ and $C_{out}$ are the concentrations of the solute in the blood entering and leaving the organ respectively, $Q_{organ}$ is the organ blood flow and $E_{organ}$ is the organ extraction ratio (Rowlands et al, 1973; Wilkinson and Shand, 1975). The total clearance of a compound from the body is the sum of the individual organ clearance (Rowland, 1972; Rowland et al, 1973).

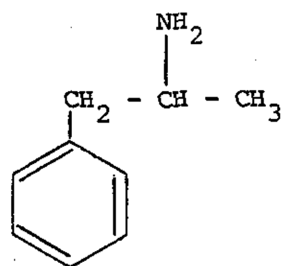
Biotransformation

Elimination of a foreign compound from an organism is assisted by biotransformation usually to increase its water solubility and so facilitate elimination from the body (Williams, 1959).

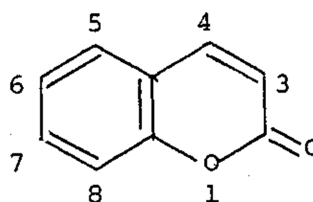
The common routes of metabolism include oxidation, reduction and hydrolysis (phase I reactions) and conjugation (phase II reactions). These reactions may take place in parallel and they may also occur sequentially. Several different metabolites may, therefore, be formed from the same compound. Metabolism usually occurs in the liver although enzymes contained in other tissues, e.g. kidney, lung and adrenal glands and the gut flora, are also catalysts in metabolic reactions (Gram, 1980).

Phase I and phase II reactions are elimination processes, but because of the possible formation of metabolites that are more toxic than the parent compound (for examples see Shuster, 1964), it is important to select a species with a pattern of metabolism which is similar to that in man when evaluating the toxicity of a foreign compound. Unfortunately, the sometimes unpredictable nature of species differences in metabolism makes the selection of a suitable species difficult.

The same general biphasic pattern of metabolism occurs in all animal species, but there are species variations in the metabolism of the many compounds. An examination of the metabolism of amphetamine and coumarin in different animal species exemplifies species differences in phase I metabolism. Amphetamine is metabolized in the rat by hydroxylation at the para position on the aromatic ring. In man and the non-human primate deamination of amphetamine occurs, the main product of metabolism being hippuric acid (Ellison et al, 1966; Dring et al, 1970). The metabolism of coumarin also shows species variations. In man and the baboon coumarin is metabolized mainly by hydroxylation at the 7 position in the coumarin ring and about 70% of the dose is excreted in the urine as 7-hydroxycoumarin (almost completely as the glucuronic acid conjugate). In other species, including the squirrel monkey, but not including one strain of mouse (DBA/2J strain), 7-hydroxycoumarin is only a minor metabolite. In species such as the rat and rabbit, the metabolism of coumarin proceeds via a pathway leading ultimately to o-hydroxyphenylacetic acid (for review see Cohen, 1979).




Amphetamine




Coumarin

In addition to species differences in phase I metabolism there are also many reported examples of species differences in phase II metabolism (Hartiala, 1955; Robinson and Williams, 1958; Williams, 1967; Capel et al, 1962; Dutton and Burchell, 1977). Table 1:1 lists eight conjugation reactions that occur in most animal species, except for glutamine conjugation which appears to be restricted to man and the non-human primates. Table 1:2 lists a number of reported species defects in conjugation reactions. These species deficiencies in phase II metabolism do however appear to be dependent on the substrate. For example, it has recently been shown that the cat can conjugate phenolphthalein, diphenylacetic acid and hydratropic acid extensively with glucuronic acid and that the pig forms about 30% of a sulphate conjugate of α-naphthol (Williams, 1974; Dixon et al, unpublished data, cited in Williams, 1978).

There are some conjugation mechanisms that are regarded as peculiar to certain animal species, namely ornithine conjugation of aromatic acids in certain birds and reptiles, and glucose conjugation of phenols in insects (Baldwin et al, 1960; Dutton, 1966; Smith, 1968).

From comparative studies of the quantitative nature of biotransformation in several species it appears that differences in metabolism are difficult to predict. However, once a certain pathway for metabolism has been shown for a particular compound, it is probable that structurally similar compounds will follow the same route in the same species. For

Table 1:1

Common conjugation mechanisms

| Mechanism | Conjugating entity |
|---|---|
| Glucuronidation | Glucuronic acid |
| Sulphation | Sulphate |
| Glycine conjugation | Glycine |
| Glutamine conjugation* | Glutamine |
| Glutathione conjugation (mercapturic acid synthesis) | Glutathione |
| Methylation | Methyl group |
| Acetylation | Acetyl group |
| Thiocyanate synthesis | Thiosulphate |

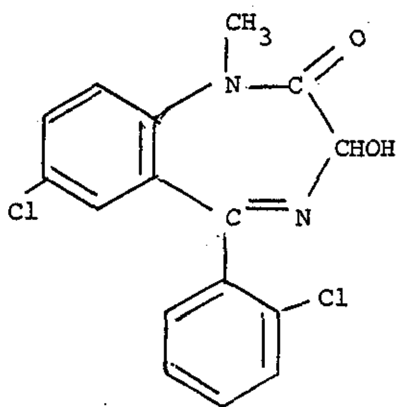
* Occurs only in man, apes and New and Old World monkeys.

Table 1:2

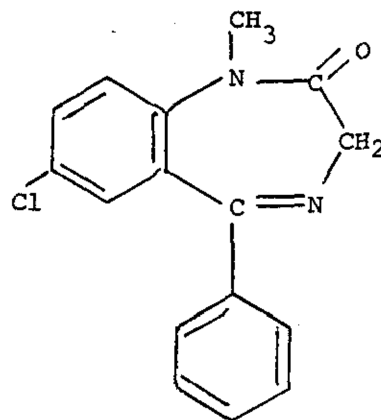
Species defects in conjugation reactions
(From Caldwell, 1980)

| Species | Defective reaction |
|---|---|
| Cat, lion, lynx, civet, genet | Glucuronidation |
| Pig | Sulphation |
| Indian fruit bat | Hippuric acid formation |
| Dog | N-Acetylation of aromatic amines and hydrazines |
| Guinea pig | N-Acetylation of S-substituted cysteines (last step of mercapturic acid formation) |
| Non-primate mammals (generally) | 1. $N_1$-glucuronidation of sulfonamides<br>2. Glutamine conjugation<br>3. O-Methylation of 4-hydroxy-3,5-diiodobenzoate |

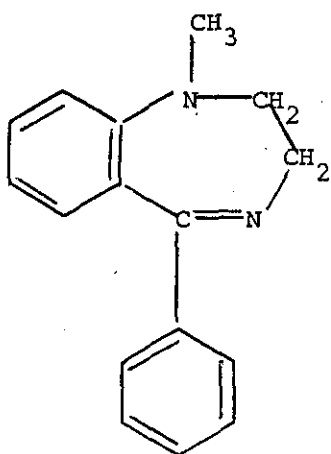
example, lormetazepam (Mayo et al, 1980; Humpel et al, 1979) has been shown to resemble other $N_1$-alkylated benzodiazepines such as medazepam (Schwartz and Carbone, 1970: Schwartz and Kolis, 1972), diazepam (Garattini et al, 1973) and pinazepam (Comi et al, 1977) in that $N_1$-dealkylation is the major phase 1 biotransformation in man and most laboratory animal species except possibly the rat.



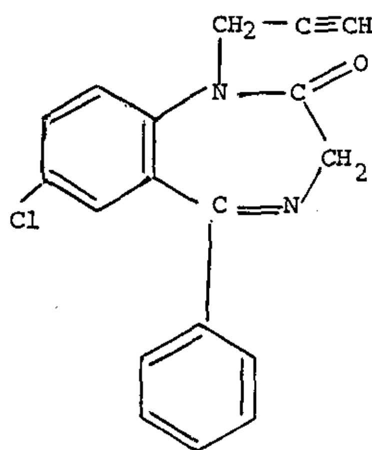

Lormetazepam




Diazepam




Medazepam




Pinazepam

There are some indications that the non-human primates, particularly the Old World monkeys, provide the best model for studying the pattern of metabolism in man. Although only a few of the available primate species have been studied and information is often scanty, most metabolic reactions that occur in man can be found in the non-human primates (Smith and Williams, 1974; Smith and Caldwell, 1977). However, differences in the relative importance of certain routes of metabolism occur. For example, simple phenols are metabolized by conjugation with sulphate and glucuronic acid in most animal species following small doses, but in man, rhesus monkey and cynomolgus monkey phenol and α-naphthol are excreted mainly as the sulphate conjugate, whereas in two species of New World monkey these phenols were excreted mainly as the glucuronide (Capel et al, unpublished data, Smith and Williams, 1974; Hirom and Mehta, unpublished data; Williams, 1978).

Of particular interest are those conjugation reactions that show some correlation with evolutionary status. For example, the formation of $N_1$-glucuronides is a major route of metabolism for certain sulphonamide drugs, e.g. sulphadimethoxine, sulphamethomidine and sulphadimethoxypyrimidine, in man, New and Old World monkeys and prosimians, but it is at a low level or even absent in non-primate species (Bridges et al, 1969a; 1969b; Adamson et al, 1970b; Walker and Williams, 1972).

The occurrence of glutamine conjugation seems to be correlated with evolutionary status since it appears to be confined to anthropoid apes and New and Old World monkeys and is not seen in prosimians or lower species. Thus several arylacetic acids are excreted as their glutamine conjugates in man and the New and Old World monkeys but as glycine conjugates in prosimians and non-primates (Smith and Caldwell, 1977; Williams, 1978).

O-Methylation has also been shown to be an important metabolic reaction of phenols in man and the non-human primates, but has not been found to occur in non-primate species (Wold et al, 1973).

Many more examples of species variations in biotransformation have been reported (e.g. for reviews see Baty 1975; 1977; 1979) but it is not the intention of this thesis to discuss these in any detail. However, species variations in pharmacokinetics will be described more fully in the relevant sections, Chapters 3 - 10.

## Pharmacokinetics

Pharmacokinetics as described by Wagner (see page 2) is the study of the relationship between the physiological disposition of a drug in the body and its pharmacological effect.

The mathematical expressions of pharmacokinetics are based on models that represent the animal body as a series of compartments and it is presumed that the foreign substance and/or its metabolite(s) are distributed in several tissues (Rescigno and Segre, 1966; Riggs, 1972). Any group of tissues which acts as if it is kinetically homogeneous is termed a 'compartment'. These compartments are separate and diffusion of compound between them is usually assumed to proceed by a first order process.

Although some complex models have been used to define the kinetics of absorption, distribution, biotransformation and excretion (Riegelman et al, 1968; Gibaldi et al, 1971; Levy and Gibaldi, 1972) and to account for certain metabolite plasma concentration-time profiles following drug administration (Rowland et al, 1970; Boxenbaun et al, 1974) the simple one and two compartment open models suffice for most purposes (Nooney, 1966; Majensohn and Gibaldi, 1971; Westlake, 1971).

Initially, pharmacokinetic data are obtained following intravenous administration of a drug, because when administered by this route the entire dose of the drug reaches the bloodstream without losses during absorption. The pharmacokinetic data, thus obtained, may be used as the primary standard for comparison with that obtained following other routes of administration.

The most useful pharmacokinetic parameters obtained following intravenous administration are half-life, clearance, area under the drug plasma (blood) concentration-time curve, and volume of distribution. The half-life of a drug is the most frequently quoted pharmacokinetic parameter, because of its importance in designing suitable dosing regimens for use in toxicology studies and in the clinic. It must be stressed, however, that many factors can influence the half-life of a drug (e.g. environmental factors that might cause stress).

The half-life of a drug might also vary as a function of dose, due to non-linear pharmacokinetics and/or by irregularities in the rate and extent of absorption. Variation in the half-life, for any reason, will produce changes in the drug plasma (blood) steady state concentration obtained following chronic administration (see equations 2:16 and 2:17; Chapter 2).

Other pharmacokinetic parameters such as clearance and volume of distribution, both of which are related to half-life as shown in equation 1:4, might also vary as a function of dose, environmental factors, diet,

$$t_{\frac{1}{2}} = \frac{\ln 2.\ V_d}{Cl} \qquad \text{.........( 1:4 )}$$

the presence of other drugs, etc., and variations in these pharmacokinetic parameters also could influence the amount of benefit or undesirable side effects obtained from the drug following any dosing regimen. It would be advantageous, therefore, to determine the pharmacokinetic profile of the drug at an early stage in its development. It is also desirable to demonstrate that the drug is being absorbed from a given dosing formulation and/or via the route of administration. A knowledge of the relationship between the drug's pharmacokinetic parameters and pharmacological (therapeutic/toxic) effects is then obtainable.

## Aim of the thesis

The objective of this thesis is to evaluate possible species-related pharmacokinetic differences between the rhesus monkey, the cynomolgus monkey and the baboon and to compare pharmacokinetic data obtained from these non-human primate species with those reported in the literature for man and other commonly used laboratory animal species. By this comparison, this thesis defines the pharmacokinetic suitability of the non-human primate species studied for use in pharmacological and toxicological studies of newer substances. Additionally the thesis aims to define the pharmaco-kinetic suitability of the cynomolgus monkey, and the baboon as alternative species to the more commonly used rhesus monkey which is becoming difficult to obtain.

CHAPTER 2

## EXPERIMENTAL

### Animals

Five mature females of each species of non-human primate, the rhesus monkey (*Macaca mulatta*), the cynomolgus monkey (*Macaca fascicularis*) and the baboon (*Papio anubis*) were obtained from a commercial supplier (Shamrock Farms (GB) Ltd., Henfield, Sussex, U.K.). All animals had been quarantined for 12 weeks and subjected to veterinary examination before and after arrival at the Huntingdon Research Centre.

### Accommodation

The animals were housed individually in cages specially designed to house small non-human primates. Each cage measured 73 cm wide, 68.5 cm deep and 91.5 cm high and was constructed of aluminium and electropolished stainless steel, incorporating squeeze back system, food hopper, bottle holder and 2.5 cm x 2.5 cm floor grid.

The cages were entirely wall-mounted, in two tiers, each tier combined with an excreta removal flushing system, and automatic drinking valves. Each animal was identified by a number tattooed on its chest and each cage was identified by a label which displayed the animal's individual identity number, study number and licencee's name.

The room in which the animals were kept was relatively isolated from external disturbances and was maintained at a temperature of 22°C ± 1°C. Although normal daylight was available to the animal rooms, artificial lighting was also used throughout the working day.

Diet

Individual animals were given free access to water and fed a controlled diet. This diet varied between the species as follows:

The rhesus monkey A daily ration of 300g per animal of mixed dry diet, containing 200g F.P.I. (Dixon and Sons Ltd., Ware, U.K.); 50g primate mazuri (Coopers Nutritional Products, Witham, Essex), and a 50g Bonio dog biscuit (Spillers Ltd., Barking, Essex) was offered, 200g in the morning and a further 100g in the afternoon.

This basic ration was supplemented on week days with one slice (35g) of wholemeal bread, and daily with fresh fruit or vegetable produce. Vitamin C, approximately 53mg (concentrated blackcurrant juice, Boots, Ltd., Nottingham) and Cytacon, a vitamin $B_{12}$ preparation containing 140μg Cyanocobalamin B.P. (Glaxo Ltd., Greenford, Middlesex), were added to the drinking water on one day in each week.

The cynomolgus monkey The daily ration per animal was 50g F.P.I. and a 50g Bonio biscuit in the morning and 50g F.P.I. and 50g Mazuri in the afternoon. This basic ration was also supplemented on week days with one slice (35g) of wholemeal bread, and daily with fresh fruit or vegetable produce and on one day a week with drink containing vitamins.

The baboon The daily ration per animal was 150g F.P.I. in the morning and 100g F.P.I., 50g Mazuri and 50g Bonio biscuit in the afternoon. Again, this basic ration was supplemented on week days with one slice (35g) of wholemeal bread, and daily with fresh fruit or vegetable produce and on one day a week with drink containing vitamins.

Dosage

Commercial preparations for intravenous injection were used when available, otherwise a suitable solution for intravenous injection was prepared. The dosage forms and manufacturers are listed in Table 2:1.

Drugs were administered at two-weekly intervals by bolus intravenous injection into a cephalic vein at levels above the normal therapeutic doses. Dose levels were chosen as those which might be selected for administration during chronic toxicity studies of these compounds (Table 2:2). The order of dosing was the same as that shown in Table 2:1.

Blood sampling

After the animals had been fed, all food was removed from the cages at 16 hours before dosing and not replaced until 7 hours afterwards, but water was provided ad libitum. The animals were dosed with the respective drugs as described in Table 2.2 and blood samples were collected at fixed time intervals for measurement of the unchanged drug in plasma. Blood (2.5 ml) was withdrawn from a femoral vein, dispensed into heparinised tubes and cooled in ice-cold water as soon as possible after mixing. Blood cells, which were discarded, were removed without delay by centrification, and the supernatant plasma was separated and stored at $-20^{o}C$ until assayed. Technical assistance was required for the dosing of the animals and in maintaining the extremely rapid bleeding schedule. For their help during this stage of the experimental work the author is grateful to colleagues in the Department of Primate Toxicology, Huntingdon Research Centre.

Table 2:1

Dosage forms and manufacturers of test compounds

| DRUG (in order of dosing) | MANUFACTURER | FORMULATION |
|---|---|---|
| Antipyrine | Sigma Chemical Company Poole, Dorset, U.K. | Solution prepared: 92 mg per ml, in dextrose 4% w/v in Saline 0.18 w/v |
| Diazepam | Roche Products Ltd., Welwyn Garden City, U.K. | Valium 10: Ampoules, 10 mg per 2 ml, for injection |
| Frusemide | Hoechst Pharmaceuticals Hounslow, Middlesex, U.K. | Lasix: Ampoules, 20 mg per ml, for injection |
| Isosorbide dinitrate | Sanol Schwarz GmbH Monheim, Germany. | Solution prepared: 2 mg per ml, in 10% ethanol: 90% water |
| Paracetamol | BDH Chemicals Ltd. Poole, Dorset, U.K. | Solution prepared: 70 mg per ml, in 15% ethanol: 85% water |
| Ibuprofen | The Boots Company Ltd. Nottingham, U.K. | Solution prepared: 15 mg per ml, in solution* |
| Pentobarbitone | May & Baker Ltd. Dagenham, Essex, U.K. | Sagatal: 60 mg in 1 ml, for injection, veterinary product |

*Solution consists of 0.1 M NaOH(13 ml), just sufficient to dissolve the ibuprofen (600 mg) plus 0.1M citric acid (1 ml) to adjust the pH of the solution to pH7 and water (26 ml) to make the value up to that required to give a concentration of 15 mg/ml.

TABLE 2:2

Therapeutic and administered dose

| Drug | Normal Daily Therapeutic Oral Dose* (mg/kg) | Administered Dose + (mg/kg) |
|---|---|---|
| Antipyrine | 4 - 9 | 100 |
| Diazepam | 0.05 - 0.5 | 0.2 and 1.0 |
| Frusemide | 0.6 - 2.3 | 3 |
| Isosorbide dinitrate | up to 1.7 | 1 |
| Paracetamol | 7 - 57 | 70 |
| Ibuprofen | 9 - 17 | 15 |
| Pentobarbitone | 1.4 - 3 | 12 |

* Data in humans obtained from Martindale (1977).

+ Dose to the rhesus monkey, bodyweight 4-6 kg.

+ Dose to the cynomolgus monkey, bodyweight 3-4 kg

+ Dose to baboon, bodyweight 4.5 - 7 kg.

## Analytical instrumentation

A wide selection of analytical instrumentation was used for this study. All instruments (listed in Table 2:3) were in the Department of Metabolism and Pharmacokinetics, Huntingdon Research Centre.

TABLE 2:3

Analytical instrumentation used in the study

| Drug | Instrument | Supplier |
|---|---|---|
| Antipyrine | SP 500 Spectrophotometer | Unicam Instruments, Cambridge, U.K. |
| Diazepam and desmethyldiazepam | High pressure liquid chromatograph | + |
| Frusemide | Aminco Bowman Spectro-photofluorimeter | American Instruments Co. Silver Spring, Maryland U.S.A. |
| Isosorbide dinitrate | Pye 104 Gas chromato-graph | Pye Unicam, Cambridge U.K. |
| Paracetamol | SP 500 Spectrophotometer | Unicam Instruments Cambridge, U.K. |
| Ibuprofen | Hewlett-Packard 5750G Gas chromatograph | Hewlett-Packard Ltd. Wokingham, Berks., U.K. |
| Pentobarbitone | Perkin-Elmer F.11 Gas chromatograph | Perkin-Elmer Ltd., Beaconsfield, Bucks., U.K. |

+ Consisting of an M 6000A pump (Waters Associates, Cheshire, U.K.), a Cecil 212 u.v. detector (Cecil Instruments, Cambridge, U.K.) and a Waters U6K injector (Waters Associates, Cheshire, U.K.).

All solvents were obtained from Fisons Scientific Apparatus Limited, Loughborough, Leicestershire, U.K. Although obtained highly pure (Distol grade), these solvents were redistilled before use. All reagents and glassware used in this study were also obtained from Fisons Scientific Apparatus Limited, whenever possible. Gas chromatographic column packings were obtained from Phase Separations Limited, Clwyd, U.K. and a high pressure liquid chromatographic column packed with $C_{18}$ μ-Bondapak was obtained from Waters Associates, Cheshire, U.K.

Determination of method parameters

The plasma calibration line for each compound was prepared by adding known amounts of drug, dissolved in a suitable solvent, to control non-human primate plasma at several different concentrations over the appropriate range and analysing the resultant plasma. Five values were obtained at each concentration and standards were analysed at the upper and lower ends of the range with at least three other points on the calibration line. The overall least squares regression line of instrument response (Y) against concentration (X) was constructed as described under Data Processing (page 24) and the accuracy of the methods for the measurement of the respective drugs in plasma was defined by the 95% confidence limits of the regression line. The precision of each assay was defined as the coefficient of variation of replicate assays of the same plasma sample containing known amounts of the appropriate drug. The sensitivity of the respective methods was defined by the limit of detection, set at twice the apparent concentration of test compound due to interfering substances present in blank predose plasma, i.e. antipyrine, frusemide and paracetamol, or by a signal to noise ratio of at least two, i.e. diazepam, desmethyldiazepam, isosorbide dinitrate, ibuprofen and pentobarbitone.

The mean recovery of each drug was determined by the repeated analysis of five standards of control non-human primate plasma containing the drug to be assayed and by comparing the instrument response from extracted plasma samples with that from known amounts of drug. When a method was used involving an internal standard, the recovery of the internal standard was determined and the recovery of the drug was calculated by comparing the gradient of the calibration line obtained from non-extracted standard with that from the calibration line obtained from extracted standards of plasma corrected for losses of the internal standard. The details of the methods used and the method parameters are reported in the Results Sections (Chapters 3 to 9).

Data processing

Optimal calibration lines were fitted by least squares regression analysis (Davies, 1961) using a Hewlett-Packard 9821A programmable calculator (Hewlett-Packard, Wokingham, U.K.) which also calculated the 95% confidence limits of the fitted line. Statistical analyses were performed with the aid of the same Hewlett-Packard 9821A programmable calculator.

Analysis of variance was performed on groups of pharmacokinetic parameters (i.e. Volumes of distribution, $V_{d(ss)}$ and $V_{d(\beta)}$ unadjusted and adjusted for bodyweight, clearance unadjusted and adjusted for bodyweight, areas under the curve, half-lives and the elimination rate constant $K_{el}$. If heterogeneity of variance was present at the 1% level of significance (Bartlett, 1937) the data was transformed using the logarithmic or square root transformation in order to stabilize the variance. If transformation of the data was unsuccessful in stabilising the variance, non-parametric methods, based on Kruskal-Wallis mean ranks (1952), were used since these are more robust than classical methods of analysis of variance. The group means were compared using the method of L.S.D.'s in conjunction with Newman-Keuls multiple comparison

procedure (1939, 1952). The Kruskal-Wallis mean ranks were compared using a non-parametric version of Newman-Kuels. Significance testing was carried out at the 5% and 1% levels.

Pharmacokinetics

The non-linear least squares curve fitting programme BLUD (kindly provided by Glaxo Group Research, Ltd., Greenford, U.K.) and adapted to suit an ICL 2903 computer (International Computers Ltd., London, U.K.) was used to fit a hypothetical model to the observed plasma concentrations. This programme not only fitted a curve to the observed data, it also calculated several pharmacokinetic parameters. In addition, it enabled an estimate of the error of these parameters to be made.

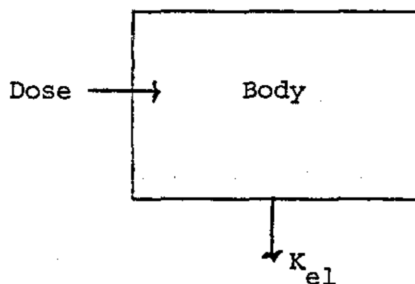
Following intravenous injection the plasma concentration time curve could be described either in terms of a one-compartment open model Equation 2:1, Scheme 2:1.

$$C = C_o e^{-K_{el} t} \quad \ldots\ldots\ldots\ldots\ldots (2:1)$$

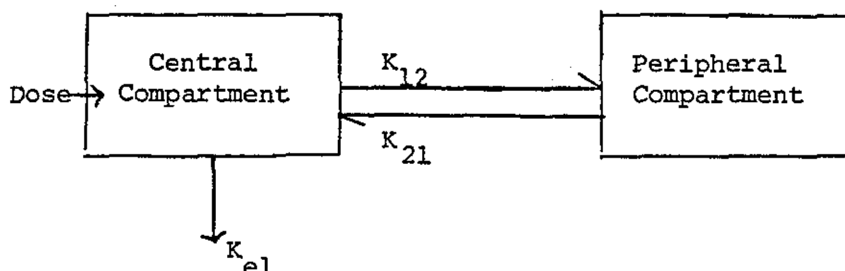
where C is the plasma concentration at any time 't' after injection, $C_o$ is the concentration immediately after injection and $K_{el}$ is the elimination constant; or as a two-compartment open model with elimination from the central compartment Equation 2:2, Scheme 2:2 .

$$C = Ae^{-\alpha t} + Be^{-\beta t} \quad \ldots\ldots\ldots\ldots (2:2)$$

where C is the plasma concentration at any time 't' after injection, A and B are the zero time intercepts and the microscopic rate constants $\alpha$ and $\beta$ are the slopes of the initial and terminal $\log_e$ - linear phases respectively.




Scheme 2:1. Schematic representation of a one-compartment open model.




Scheme 2:2 Schematic representation of a two-compartment open model with elimination from the central compartment.

The remaining pharmacokinetic parameters were calculated using the estimates of the slopes and intercepts, using standard equations 2:3 - 2:12 for the two-compartment open model (see Gibaldi and Perrier, 1975).

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta} \quad \text{......... (2:3)}$$

$$Cl = \frac{Dose}{AUC} \quad \text{......... (2:4)}$$

$$K_{21} = \frac{A\beta + B\alpha}{A+B} \quad \text{......... (2.5)}$$

$$K_{el} = \frac{\alpha\beta}{K_{21}} \quad \text{......... (2.6)}$$

$$K_{12} = \alpha + \beta - K_{21} - K_{el} \quad \text{......... (2.7)}$$

$$V_1 = \frac{Dose}{A + B} \qquad \text{.......... (2.8)}$$

$$V_2 = \frac{V_1 K_{12}}{K_{21}} \qquad \text{.......... (2.9)}$$

$$V_{d(ss)} = V_1 + V_2 \qquad \text{.......... (2.10)}$$

$$V_{d(\beta)} = Cl/\beta \qquad \text{.......... (2.11)}$$

$$t_{½} = \frac{\ln 2}{\beta} \qquad \text{.......... (2.12)}$$

where AUC is the area under the plasma concentration time curve; Cl is the clearance of the drug; $K_{12}$, $K_{21}$ are the intercompartmental transfer rate constants and $K_{el}$ is the elimination rate constant (see Scheme 2:2); $V_1$ is the apparent volume of the central compartment; $V_2$ is the apparent volume of the peripheral compartment, $V_{d(ss)}$ is the apparent total volume of distribution for a two-compartment model at steady state, and $V_{d(\beta)}$ is the volume of distribution after distribution equilibrium. For the one-compartment open model, $\alpha$ approximates to $\beta$ and $K_{el}$ is the slope of the $\log_e$-linear decline with $C_o$ as the zero time intercept. The areas under the plasma concentration time curve for a one-compartment open model were estimated using equation 2:13

$$AUC = \frac{C_o}{K_{el}} \qquad \text{.......... (2.13)}$$

the half-life of elimination was estimated using equation 2:14.

$$t_{½} = \frac{\ln 2}{K_{el}} \qquad \text{.......... (2.14)}$$

and the apparent volume of distribution and the clearance for a one-compartment model were estimated using equations 1:1 and 2:4 respectively.

Concentrations of drug in the peripheral compartment (Cp) were calculated using equation 2:15 (Gibaldi and Perrier, 1975).

$$Cp = \frac{K_{12}\,\text{Dose}}{V_{2(\alpha-\beta)}} \left(e^{-\beta t} - e^{-\alpha t}\right) \quad \text{.........} \quad (2.15)$$

The BLUD programme selected a model by comparing the relative sizes of $\alpha$ and $\beta$ but does not allow independent selection of a model. In fact, this programme tended to select a two-compartment model even though the distribution phase was quite small and a one-compartment model would be adequate (Rowland et al, 1971; Wagner, 1974 1975; Loughnan et al, 1976; Dvorchik and Vesell, 1978). In the latter case the one-compartment model was fitted to the data by normal regression techniques (Davies, 1961).

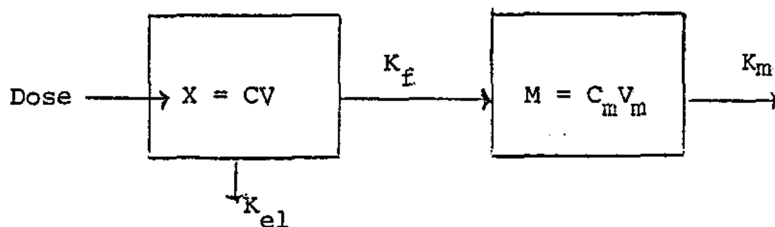
Mean steady state plasma drug concentrations ($C_{ss}$) obtained during a fixed dosing regime were predicted for a one-compartment open model and a two-compartment open model using equations 2:16 and 2:17 respectively.

$$C_{ss} = \frac{\text{Dose}}{V_d K_{el} \tau} \quad \text{.........} \quad (2.16)$$

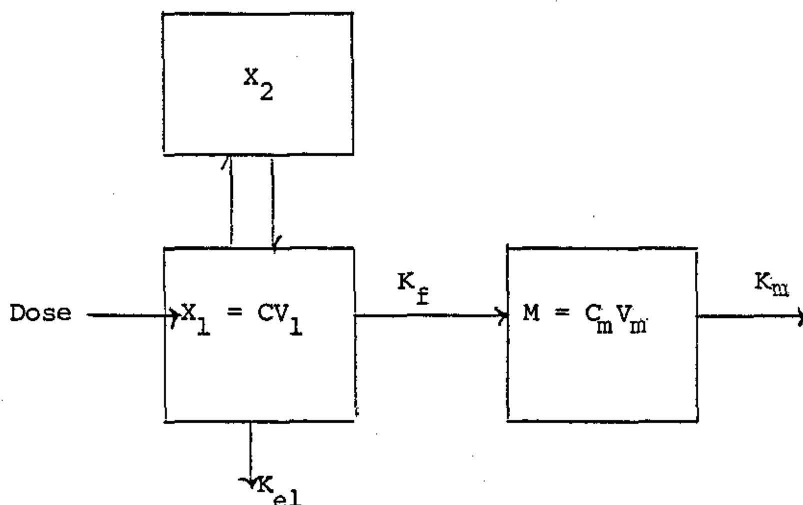
$$C_{ss} = \frac{\text{Dose}}{V_{d(\beta)} \beta \tau} \quad \text{........} \quad (2.17)$$

where $\tau$ is the dosing interval (Gibaldi and Perrier, 1975).

Metabolites. Plasma concentrations of a metabolite that obeys one-compartment kinetics following intravenous injection of a compound that distributed into the body according to either a one- or a two-compartment model (Schemes 2:3, 2:4) are described by equation 2:18 (Gibaldi and Perrier, 1975; Pang and Gillette, 1980).




Scheme 2:3: Pharmacokinetic model describing plasma concentrations of a drug and its metabolite that both distribute into the body according to one-compartment kinetics.




Scheme 2:4: Pharmacokinetic model describing plasma concentrations of a drug that obeys two-compartment kinetics and its metabolite that obeys one-compartment kinetics.

$$C_m = \frac{K_f . \text{ Dose.}}{V_m \; (K_{el} - K_m)} \left( e^{-K_m t} - e^{-K_{el} t} \right) \quad .........(2:18)$$

where X is the amount of drug in the body for a one-compartment model; $X_1$ and $X_2$ are the amounts of drug in the central and peripheral compartments, respectively, for a two-compartment model; M is the

amount of metabolite in the body, $C_m$ and $V_m$ are the metabolite concentration at any time and the apparent volume of distribution of the metabolite respectively and $K_f$ and $K_m$ are the first order rate constants of formation and elimination of the metabolite, respectively. When the rate constant for elimination of the metabolite is less than the rate of formation, the former is obtained from the terminal slope of a plot of the logarithm of metabolite levels versus time using the equation 2:19

$$\text{Slope} = \frac{-K_m}{2.303} \qquad \text{............ (2:19)}$$

and $K_f$ is estimated by the method of residuals (Gibaldi and Perrier, 1975). When $K_m > K_f$, then $K_f$ is obtained from equation 2:19 and $K_m$ is estimated by the method of residuals.

CHAPTER 3

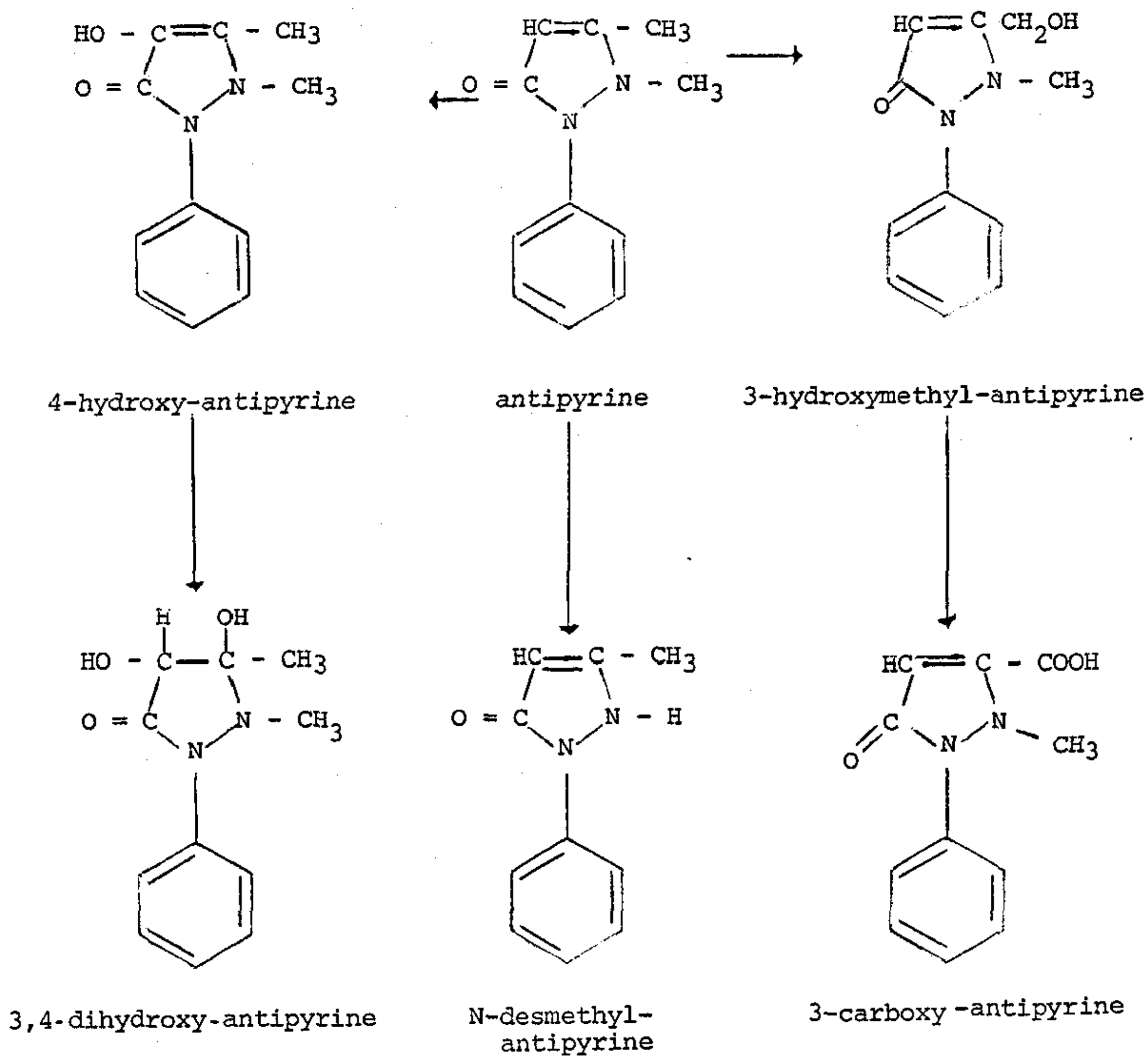
## ANTIPYRINE

### Introduction

Antipyrine (2,3-dimethyl-1-phenyl-3-pyrazoline-5-one) is a drug, possessing both analgesic and antipyretic properties, that is also used as a means of assessing oxidative enzyme induction (or inhibition) in animals (Conney, 1967) and in man (Hunter and Chasseaud, 1976; Vesell, 1979). It is a weak base ($pK_a$ 1.4) that is rapidly and completely absorbed from the gastrointestinal tract (Stevens, 1977) distributes into the total body water and is only slightly bound to plasma proteins (Brodie and Axelrod, 1950).

Antipyrine is extensively metabolised in animals and in man. The complex pattern of metabolism involves oxidation as described in scheme 3 : 1 followed by conjugation with glucuronic acid to form possibly both O- and N-glucuronic acid conjugates (Brodie and Axelrod, 1950; Yoshimura et al, 1971; Baty and Price-Evans, 1973; Huffman et al, 1974; Stafford et al, 1974; Eichelbaum et al, 1976; Horning et al, 1976; Aarbakke, 1978; Kellermann and Luyten-Kellermann, 1978; Danhof and Breimer, 1978; Danhof et al, 1978). Danhof and Breimer (1979) have shown also that the pattern of metabolism following an oral dose appears to be dose-independent.

Comparison of the rate of metabolism of antipyrine in different animal species and man, using the biological half-life as an index of oxidative metabolic rate, shows that there are large species differences in the kinetics of antipyrine metabolism (Quinn et al, 1958; Hunter and Chasseaud, 1976).




Scheme 3:1 Metabolic pathways of antipyrine.

## Measurement of concentrations of antipyrine in plasma

Plasma concentrations of antipyrine were measured using a modification of the spectrophotometric method of Welch et al (1967).

Plasma (1 ml) was diluted in the distilled water (to 1.5 ml) and made alkaline by the addition of sodium hydroxide (0.5 ml, 1M). Chloroform (15 ml) was added and the mixture was vigorously shaken for 2 minutes using a rotary mixer (Fisons Scientific Supplies Ltd., Loughborough, Leicestershire, U.K.). The organic phase was removed by filtration through phase-separation paper (Whatmans IPS Whatman Limited, Maidstone, Kent, U.K.) and evaporated to dryness under a stream of nitrogen at 50°C. The residue was dissolved in dilute sulphuric acid (6.0 ml, 0.05M) and an aliquot (3.0 ml) of this solution was transferred to a second tube. Sodium nitrite (0.1 ml of a 0.2% (w/v) solution in water) was added to the transferred sample, and the solution was left for 20 minutes at room temperature. After 20 minutes, the optical density of the 4-nitroso-antipyrine derivative was measured using the underivatised fraction as a reference.

Table 3:1

Method parameters for the measurement of concentrations of antipyrine in plasma

| | |
|---|---|
| linear range | 2-200 μg/ml |
| calibration line[a] | Y = (0.0053 ± 0.0001)X |
| accuracy | ± 14% at 200 μg/ml and ± 55% at 50 μg/ml |
| precision | ± 6% at 50 μg/ml, ± 3% at 100 μg/ml and ± 4% at 200 μg/ml |
| sensitivity | 2 μg/ml |
| recovery of antipyrine | 87% ± 1% |

[a] Least squares regression line where Y = absorbance and X = plasma concentration of antipyrine.

## Results

Plasma concentrations. After a single intravenous injection of antipyrine at a level of 92 mg/kg peak of mean concentrations of antipyrine in plasma 132 μg/ml, 137 μg/ml and 155 μg/ml in the rhesus monkey, the cynomolgus monkey and the baboon, respectively, were not reached until 5 minutes post administration (the second time of blood withdrawal) in all three species, (Table 3:2, Figures 3:1-3:3). Thereafter, mean plasma concentrations declined with an apparent half life of elimination of 1.5 - 2 hours (Table 3:3, Figures 3:1 - 3:3).

Pharmacokinetic parameters. Evaluation of the observed plasma antipyrine concentrations using the non-linear least squares curve fitting programme BLUD indicated that the data was adequately described by a bi-exponential equation (equation 2:2). The pharmacokinetic parameters shown in Table 3:3 were therefore calculated using equations 2:3 - 2:12 for a two-compartment open model with elimination from the central compartment (Scheme 2:2). However, inspection of the observed data shows that the contribution of the distribution phase $A/\alpha$ to the total area under the curve was generally less than 8% and that the two-compartment open model could collapse to a one-compartment open model described by equation 2:1. Comparison of the pharmacokinetic parameters estimated for both models (Table 3:4) shows that no notable improvements in data processing were obtained by utilising a two-compartment open model. Plasma concentrations of antipyrine predicted by both models were generally in good agreement with the observed values except at 7 hours after administration to the rhesus monkey, but this observed value was near the limit of detection of the analytical method (Figure 3:1 - 3:3).

Analysis of variance of groups of pharmacokinetic parameters showed that there was a statistically significant difference in the clearance which was lower in the cynomolgus monkey ($P < 0.05$ Newman-Keuls multiple comparison procedure) than either of the other two species. The volumes of distribution were significantly smaller in

the cynomolgus monkey ($P < 0.01$, Newman-Keuls multiple comparison procedure) than in the other species, but this difference disappeared when values were adjusted for bodyweight variations ($P > 0.05$). Although not so significant ($P > 0.05$) areas under the concentration were greater in the cynomolgus monkey than in the other two species.

The mean total volume of distribution at steady state $V_{d(ss)}$ varied between the species accounting for 88, 73 and 66% of the total bodyweight in the rhesus monkey, the cynomolgus monkey and the baboon respectively (Table 3:3). Although antipyrine is not an ideal substance for the estimation of total body water (Soberman et al, 1949) these values approximate to what would be expected as an estimate of total body water in these non-human primate species, except that it is rather large in the rhesus monkey; and are similar to those found previously in man 60% (Vesell et al, 1975; Soberman, 1949) and in the rat 76% and in other animal species 60-99% (McManus and Ilett, 1979). Adopting the two-compartment model, the compartment volume ratio (central:peripheral) for the two-compartment open model was 4.0, 3.3 and 6.2 in the rhesus monkey, the cynomolgus monkey and the baboon respectively, emphasizing the lack of importance of distribution into the peripheral compartment in the disposition of antipyrine in these non-human primates; and this fact is supported further by the similar values obtained for $V_{d(ss)}$ and for the volume of distribution estimated after attainment of pseudo-distribution equilibrium $V_{d(\beta)}$ (Table 3:3).

The measured clearance of antipyrine adjusted for bodyweight differences 5.5 ml/minute/kg, 4.4 ml/minute/kg and 5 ml/minute/kg in the rhesus monkey, the cynomolgus monkey and the baboon, respectively, are less than those found previously in the rhesus monkey (8.4 ml/minute/kg, Branch et al, 1974) in the rat (approximately 9 ml/minute/kg, Danhof et al, 1979, McManus and Ilett, 1979) and in other species (9 ml/minute/kg, McManus and Ilett, 1979) but are higher than that in man (0.9 ml/minute/kg, Vesell et al, 1975).

The mean biological half-lives of antipyrine shown in Table 3:3 are similar to those previously reported in the rhesus monkey (80-100 minutes, Hucher et al, 1972; Branch et al, 1974; Miller et al, 1978) and showed that in this respect the non-human primate species are closer to other laboratory animals such as the rat ($t_{½}$ = 144 minutes), the guinea pig ($t_{½}$ = 107 minutes) and the dog ($t_{½}$ = 110 minutes) Quinn et al (1958) than to man in which the half-life of elimination is about 12 hours (see Hunter and Chasseaud, 1976).

Adopting the two-compartment model, the ratio of $\beta/k_{el}$ of 0.76, 0.72 and 0.86 for the rhesus monkey, the cynomolgus monkey and the baboon, respectively, indicated that if a two-compartment open model is assumed, these fractions of antipyrine in the body were in the central compartment and were available for elimination once distribution was complete (Gibaldi et al, 1969).

Discussion

The plasma concentration-time course for antipyrine was thought for many years to be adequately described by a one-compartment model (Brodie and Axelrod, 1950). Swartz et al (1974) has however, suggested that a two-compartment model provides a better description of antipyrine pharmacokinetics in humans and this comment might also apply to non-human primates (Table 3:3).

In the non-human primates studied here the mean contribution from distribution phase $A/\alpha$, to the mean total area under the plasma concentration-time curve was 2%, 13% and 3% in the rhesus monkey, the cynomolgus monkey and the baboon, respectively (Table 3:3). The higher value of 13% observed for the cynomolgus monkey was mainly due to the large contribution from one animal in this group. With the exception of this one animal the contribution of the distribution phase was small, and therefore the pharmacokinetic parameters of antipyrine were estimated using a one-compartment model and found to be similar to those estimated using a two-compartment model. The systemic clearance of antipyrine mainly reflects hepatic

clearance since antipyrine is eliminated mostly by metabolism in the liver. The values for the clearance of antipyrine in non-human primates are about seven-fold lower than the liver blood flow and it would therefore be unlikely that any hepatic first-pass effects would be an important feature of antipyrine disposition in the non-human primate after oral doses particularly if some extrahepatic metabolism of antipyrine occurs.

Inspection of these relatively short half-lives of elimination of antipyrine in several species shows that during the once-daily dosage regimen, common to most chronic toxicity studies, antipyrine would not accumulate in the body in the common laboratory animal species, since each dose would have been eliminated before the next is received. In contrast, some accumulation of antipyrine would occur in humans with such a regimen and during normal therapy 250 mg antipyrine twice daily for the treatment of migraine for example; an average steady state plasma concentration of approximately 5.5 μg/ml would be achieved three days after commencement of treatment. To achieve the same steady state plasma concentration of antipyrine in non-human primates it would be necessary to administer this drug at more frequent intervals. A possible dosing regimen estimated using equation 2:16, would be an injection of antipyrine 16.6 mg intravenously every 108 minutes to the rhesus monkey, 10.4 mg every 108 minutes to the cynomolgus monkey and 14.3 mg every 90 minutes to the baboon. These considerations apply to antipyrine given intravenously. If administered orally and particularly at much larger doses other factors (such as release from the dosage form) have to be considered.

Antipyrine is an enzyme inducer in man (Breckenridge et al, 1971) and would, therefore, be eliminated more rapidly when induction occurred. However, it is unlikely that enzyme induction would be sufficient to prevent accumulation if antipyrine in man receiving a normal twice daily dose of the drug. On the basis of those studies it can be concluded that the non-human primate is not a more suitable pharmacokinetic model for man than are the other commonly used laboratory animal species.

TABLE 3:2

Mean concentrations ( ± S.D.) of antipyrine in the plasma of the rhesus monkeys, the cynomolgus monkeys and the baboons after a single blus intravenous injection of antipyrine at a level of 92 mg/kg.

Results expressed as μg/ml.

| Time | Species | | |
|---|---|---|---|
| | Rhesus | Cynomolgus | Baboons |
| 2 minute | 113 ± 33 | 104 ± 31 | 142 ± 26 |
| 5 minute | 132 ± 36 | 137 ± 8 | 155 ± 24 |
| 10 minute | 114 ± 30 | 135 ± 6 | 143 ± 28 |
| 20 minute | 100 ± 29 | 121 ± 11 | 124 ± 6 |
| 40 minute | 86 ± 21 | 102 ± 14 | 102 ± 16 |
| 1 hour | 73 ± 17 | 89 ± 9 | 87 ± 13 |
| 1.5 hour | NS* | 72 ± 7 | 74 ± 17 |
| 2 hour | 50 ± 14 | 62 ± 9 | 57 ± 12 |
| 3 hour | 36 ± 10 | 39 ± 6 | 38 ± 14 |
| 4 hour | 23 ± 8 | 29 ± 4 | 23 ± 10 |
| 5 hour | 14 ± 10 | 21 ± 4 | 16 ± 5 |
| 7 hour | 3 ± 6 | 12 ± 6 | 6 ± 3 |

*NS Indicates that no sample was taken at this time period.

Table 3:3

Mean pharmacokinetic parameters (±SD) of antipyrine determined for a two-compartment open model in the rhesus monkey, the cynomolgus monkey and the baboon after a single bolus intravenous injection of antipyrine at a level of 92 mg/kg. The data are presented as means of results from individual animals.

| Parameter[a] | Species | | |
|---|---|---|---|
| | Rhesus | Cynomolgus | Baboon |
| Bodyweight (kg) | 4.7 ± 1.1 | 3.6 ± 0.3 | 5.4 ± 0.7 |
| Dose (mg) | 434 ± 101 | 332 ± 21 | 499 ± 76 |
| Dose (mg/n) | 94 ± 2 | 94 ± 4 | 92 ± 3 |
| A (µg/n) | 30 ± 13 | 57 ± 32 | 47 ± 97 |
| B (µg/ml) | 107 ± 27 | 127 ± 35 | 136 ± 9 |
| $\alpha$ ($minute^{-1}$) | 0.130 ± 0.088 | 0.064 ± 0.054 | 0.092 ± 0.085 |
| $\beta$ ($minute^{-1}$) | 0.006 ± 0.001 | 0.006 ± 0.001 | 0.008 ± 0.002 |
| $K_{12}$ ($minute^{-1}$) | 0.030 ± 0.023 | 0.015 ± 0.015 | 0.014 ± 0.015 |
| $K_{21}$ ($minute^{-1}$) | 0.099 ± 0.069 | 0.047 ± 0.041 | 0.076 ± 0.070 |
| $K_{el}$ ($minute^{-1}$) | 0.008 ± 0.002 | 0.008 ± 0.002 | 0.009 ± 0.002 |
| AUC (µg/ml/hour) | 291 ± 84 | 357 ± 45 | 318 ± 75 |
| Cl (ml/minute) | 26 ± 5 | 16 ± 3[b] | 27 ± 8 |
| $t_{½}$ (minutes) | 111 ± 17 | 132 ± 41 | 95 ± 26 |
| $V_1$ (litres) | 3.2 ± 0.9 | 2.0 ± 0.2 | 3.1 ± 0.6 |
| $V_2$ (litres) | 0.8 ± 0.5 | 0.6 ± 0.3 | 0.5 ± 0.3 |
| $V_{d(ss)}$ (litres) | 4.1 ± 1.0 | 2.6 ± 0.4[c] | 3.6 ± 0.8 |
| $V_{d(\beta)}$ (litres) | 4.3 ± 0.8 | 2.7 ± 0.5[c] | 3.4 ± 1.0 |
| $A/\alpha$ (µg/ml/hour) | 6 ± 4 | 45 ± 70 | 8 ± 6 |
| $B/\beta$ (µg/ml/hour) | 285 ± 84 | 311 ± 60 | 310 ± 75 |

a. For details of abbreviations used see Chapter 2.

b. Significance level (analysis of variance), cynomolgus monkey compared with the other two species ($P < 0.05$).

c. Significance level (analysis of variance), cynomolgus monkey compared with the other two species ($P < 0.01$)

Table 3:4

Mean pharmacokinetic parameters of antipyrine in the rhesus monkey, the cynomolgus monkey and the baboon for a one-compartment open model compared to those for a two-compartment open model following a single intravenous injection of antipyrine (92 mg/kg). The data are presented as means of results from individual animals.

| Parameter[a] | Model and Species | | | | | |
|---|---|---|---|---|---|---|
| | One-compartment | | | Two-compartment | | |
| | Rhesus | Cyno-molgus | Baboon | Rhesus | Cyno-molgus | Baboon |
| AUC (μg/ml/hour) | 285 | 345 | 315 | 291 | 357 | 318 |
| Cl (ml/minute) | 26 | 16[b] | 28 | 26 | 16[b] | 27 |
| $t_{½}$ (minutes) | 106 | 106 | 92 | 111 | 132 [c] | 95 |
| $V_d$[d] (litres) | 4.0 | 2.5[e] | 3.6 | 4.1 | 2.6[e] | 3.6 |
| $K_{el}$ ($minute^{-1}$) | 0.007 | 0.007 | 0.008 | 0.008 | 0.008 | 0.009 |

a For details of abbreviations used see Chapter 2.

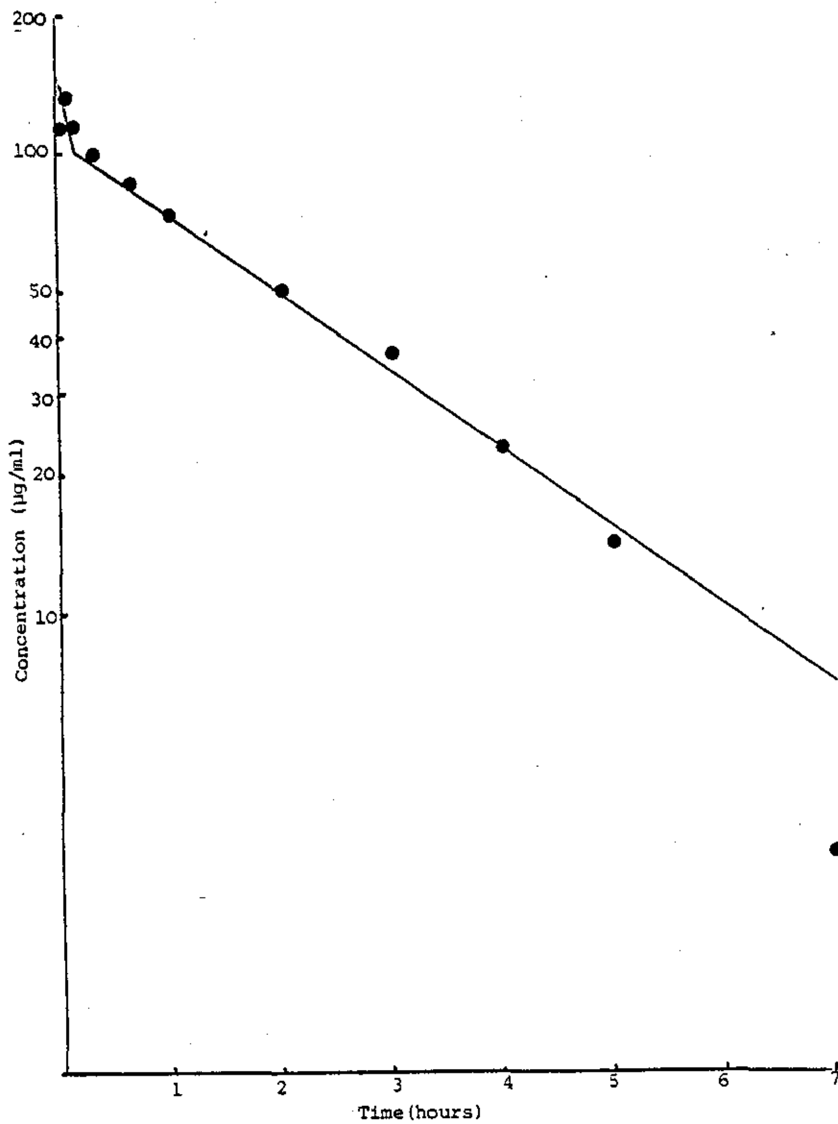
b Significance level (analysis of variance) cynomolgus monkeys compared to the other two species $P < 0.05$.

c Reduce to $t_{½}$ = 114 minutes if a high value from one animal is not included.

d $V_d$ = total volume of distribution = $V_{d(ss)}$ for the two-compartment model.

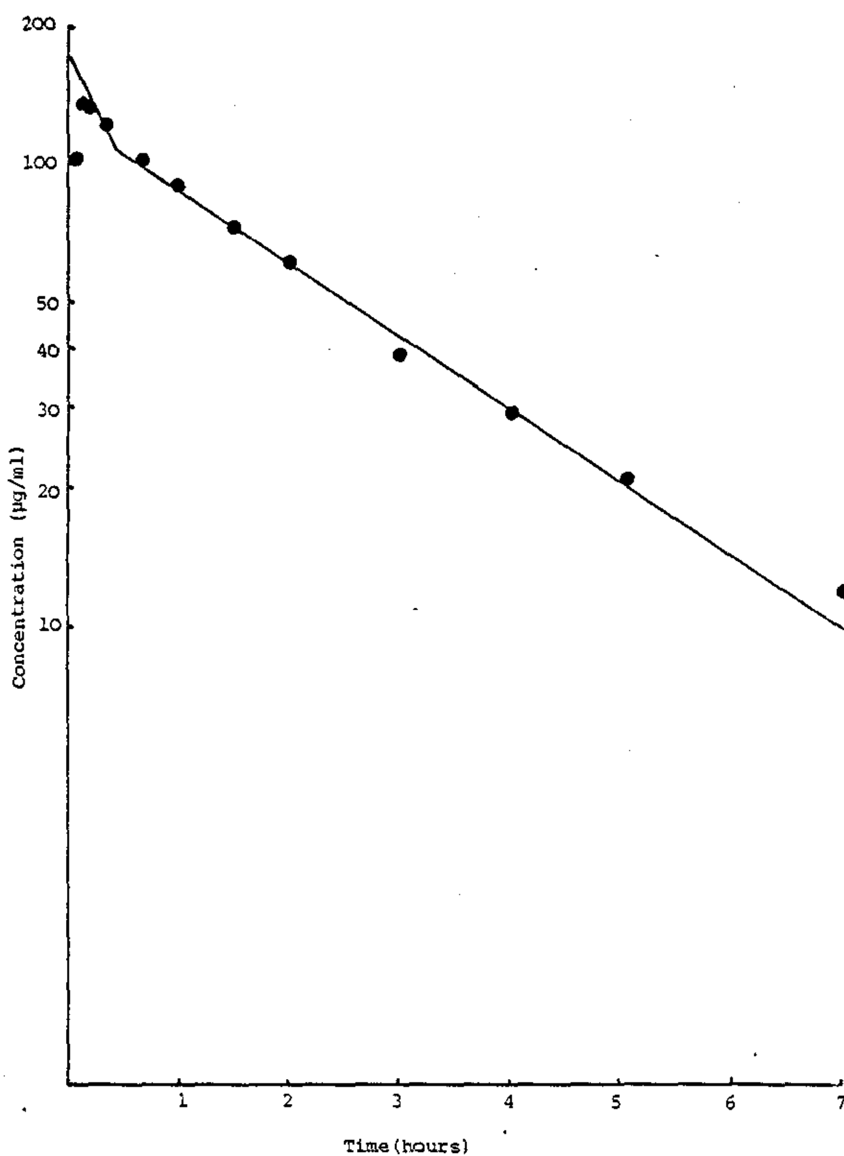
e Significance level (analysis of variance) cynomolgus monkey compared to the other two species $P < 0.01$

FIGURE 3:1



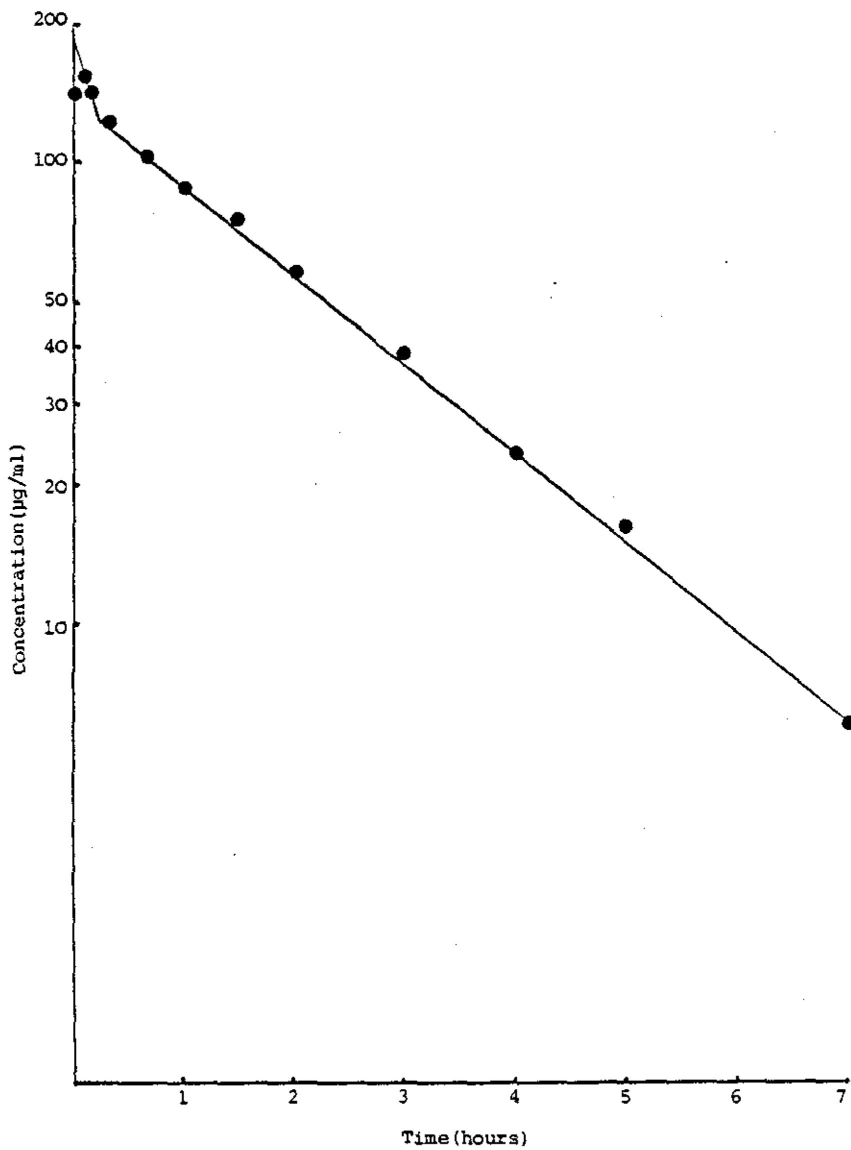

Mean concentration-time curve after a single intravenous injection of antipyrine (92 mg/kg) to rhesus monkeys. Observed concentrations ( ● ● ), estimated concentrations ( solid line).

FIGURE 3:2




Mean concentration-time curve after a single intravenous injection of antipyrine (92 mg/kg) to cynomolgus monkeys. Observed concentrations ( ● ● ), estimated concentrations (solid line).

FIGURE 3:3




Mean concentration-time curve after a single intravenous injection of antipyrine (92 mg/kg) to baboons. Observed concentrations ( ● ● ), estimated concentrations (solid line).

CHAPTER 4

# DIAZEPAM

## Introduction

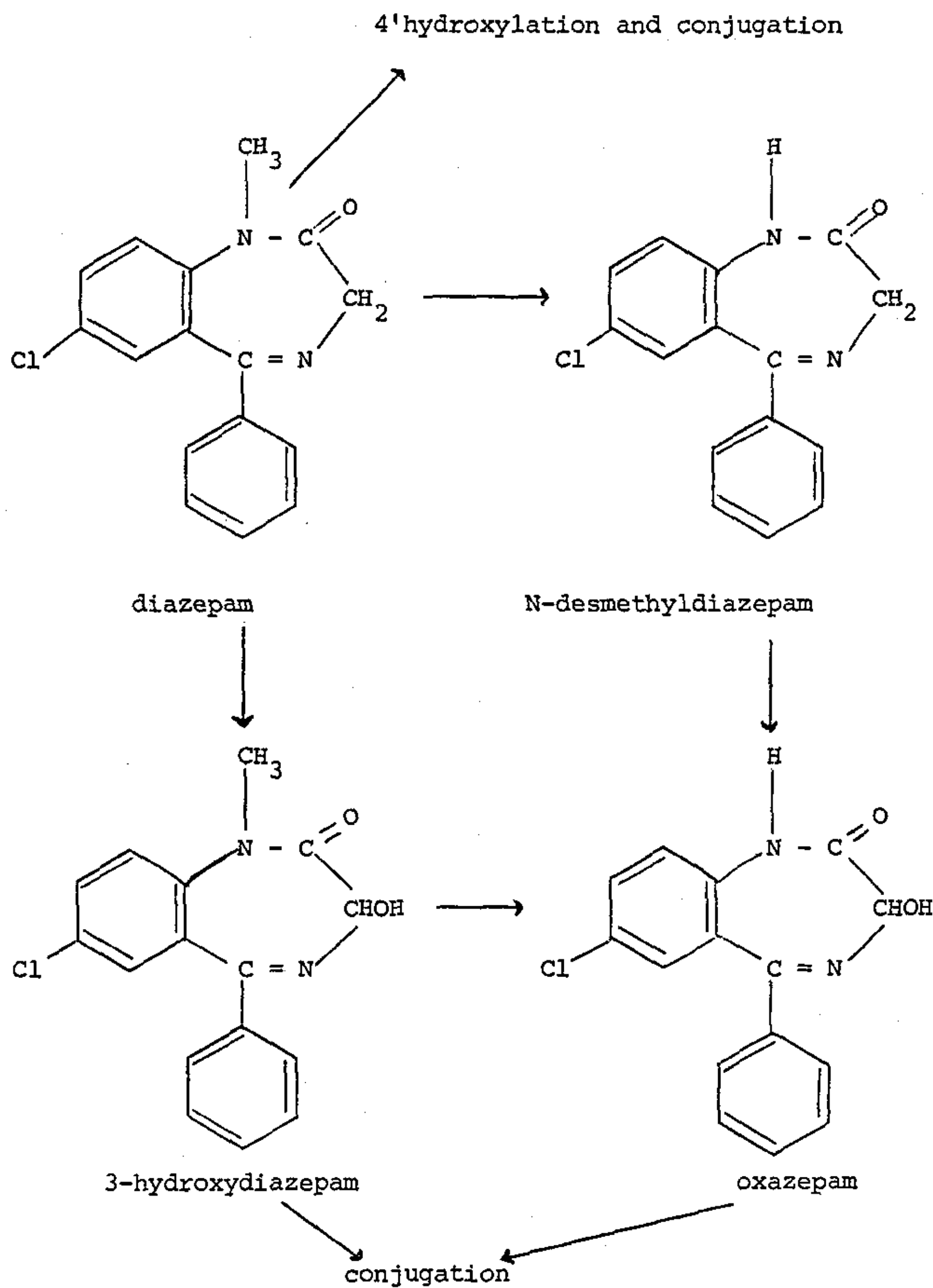
Diazepam: 7-chloro-1, 3-dihydro-1-methyl-5-phenyl 2H-1,4-benzodiazepin-2 one, is effective in the symptomatic relief of tension and anxiety states as well as for the relief of skeletal muscle spasm (Zbinden and Randell, 1967). It is a weak base ($pK_a$ 3.4), a long-acting lipophilic drug, primarily non-ionized at physiological pH, and has a high affinity for plasma proteins (Klotz et al, 1976). It has been shown to be an inducer of certain drug-metabolising enzymes in man (Heubel and Frank, 1970) and in animals (Heubel and Frank, 1970; Heubel and Patutschnick, 1972) and even to induce its own metabolism (Kanto et al, 1974; and Sellman et al, 1975).

The major metabolic pathways of diazepam have been described for several animal species including man (Ruelius et al, 1965; Schwartz et al, 1965; Coutinho et al, 1973; Garattini et al, 1973). As indicated in Scheme 4:1, the usual metabolism of diazepam is that of $N_1$-demethylation and $C_3$-hydroxylation, the two metabolites formed from these reactions undergoing further metabolism to form a common metabolite known as oxazepam (Kvetina et al, 1968; Marcucci et al, 1969, 1970; Mussini et al, 1971; Trebbi et al, 1975; Comi et al, 1977). The two hydroxylated metabolites are conjugated with glucuronic acid and excreted in the urine (Schwartz et al, 1965; Kanto et al, 1974).

In the rat, $N_1$-demethylation does not appear to be a major route of metabolism for diazepam (Marcucci et al, 1969, 1970; Comi et al, 1977) and therefore, plasma levels of desmethyldiazepam are lower in the rat than in other species. This species difference in

the metabolism of diazepam is important as the $N_1$-demethylated metabolites of diazepam have been shown to be pharmacologically active (Zbinden and Randall, 1967).

Species related differences in the proportions of metabolites excreted in the bile have also been reported (Schwartz et al, 1965) and this might reflect species differences in the rate of glucuronide formation, and/or variations in the threshold of urinary as compared to the biliary excretion of glucuronides (Smith and Williams, 1966).




Scheme 4:1 Metabolic pathways of diazepam.

Measurement of concentrations of diazepam and desmethyldiazepam in plasma

Plasma concentrations of diazepam and desmethyldiazepam were measured by a modification of the high pressure liquid chromatographic method of Brodie et al, (1978).

Plasma (1 ml) was mixed with internal standard (1-cyclo-propyl-methyl-1,3-dihydro-5-phenyl-7-chloro-2H-1,4-benzodiazepin-2-one (prazepam); 400 ng, 40 μl of a 10 μl/ml solution in spectrograde methanol) and buffered to pH 9 with borate buffer (2 ml, 1M). The mixture was extracted with freshly redistilled diethyl ether (10 ml) by shaking for 15 minutes using a mechanical shaker (Griffin and George Ltd., London, U.K.). After centrifugation, the ether phase (9 ml) was transferred to a pointed centrifuge tube and evaporated to dryness under a stream of nitrogen at 35°C. The sides of the dry centrifuge tube were washed with redistilled ether and again blown to dryness under nitrogen. The residue concentrated at the bottom of the tube was redissolved in spectrograde methanol (25 μl) and injected into the chromatograph. A stainless steel column (30 cm x 4 mm id) packed with $C_{18}$ μ-Bondapak was used and the mobile phase was spectrograde methanol : water (v/v 60/40). The water was freshly redistilled from an all-glass still and the mixture was refluxed to remove dissolved air, and cooled, before use. Varying amounts (depending on the concentration of diazepam, and desmethyldiazepam, in the residue; from 1-25 μl) were injected onto the column. Quantitation was by the internal standard technique using peak height ratios.

TABLE 4:1

Method parameters for the measurement of concentrations of diazepam and desmethyldiazepam in plasma.

| Parameter | Diazepam | Desmethyldiazepam |
|---|---|---|
| linear range | 2-500 ng/ml | 2-500 ng/ml |
| calibration line[a] | Y=(0.0091 ± 0.0001)X | Y = 0.0102X |
| accuracy | ± 4% at 200 ng/ml | ± 3% at 200 ng/ml |
| | ± 67% at 10 ng/ml | ± 66% at 10 ng/ml |
| precision | ± 1.6% at 400 ng/ml | ± 2.1% at 400 ng/ml |
| | ± 7.1% at 10 ng/ml | ± 10.8% at 10 ng/ml |
| sensitivity | 2 ng/ml | 2 ng/ml |
| recovery of IS | 99% ± 2.8 SD | - |
| recovery of test | 100 ± 2.6 SD | 98 ± 2.8 SD |

[a] Least squares regression line where Y = instrument response (peak height ratio, diazepam or desmethyldiazepam to Internal Standard) and X = plasma concentration of diazepam (or desmethyldiazepam).

I S Internal Standard

## Results

Plasma concentrations. After bolus intravenous injection of diazepam at two dose levels, 0.2 mg/kg and 1 mg/kg, peak of mean concentrations of diazepam were reached 2 minutes after injection (the first time of blood withdrawal) in all groups of animals except in the baboon after the higher dose. In this group, peak of mean plasma concentrations were not reached until 5 minutes after injection (the second time of blood withdrawal). Mean plasma concentrations of diazepam declined in a biphasic manner in all three species with with an apparent half-life of elimination of about 1 hour which was similar after both dose levels. Concentrations of diazepam in

plasma were detectable for 3-4 hours in the rhesus monkey and the cynomolgus monkey after the 0.2 mg/kg dose and up to 5 hours in these species after the 1 mg/kg dose. However, concentrations of diazepam in the baboon remained above the limit of detection (2.0 ng/ml) for 7 hours post-administration after administration of both the 0.2 mg/kg dose and the 1 mg/kg dose (Tables 4:2 and 4:3, Figures 4:1 - 4:6).

Pharmacokinetic parameters of diazepam. Observed plasma concentrations of diazepam following a single intravenous injection of diazepam at two dose levels (0.2 mg/kg and 1 mg/kg) were fitted by a biexponential equation (equation 2:2). The pharmacokinetic parameters shown in Tables 4:4 and 4:5 were, therefore, calculated using equations 2:3-2:12 for a two compartment-open model with elimination from the central compartment (Scheme 2:2). Plasma concentrations of diazepam predicted by the two-compartment model were generally in good agreement with the observed values (Figures 4:1 - 4:6).

Analysis of variance of areas under the plasma concentration-time curves after administration of the 0.2 mg/kg dose showed that the areas under the curve for the baboon were significantly higher ($P < 0.05$ non-parametric version of Newman-Keuls multiple comparison procedure) than for the other two species. However, analysis of variance of areas under the concentration time curves after administration of the 1 mg/kg dose showed that at this dose level there were no statistically significant differences between these non-human primate species ($P > 0.05$ Newman-Keuls multiple comparison procedure). Areas increased approximately in proportion to the administered dose in the rhesus monkey and the cynomolgus monkey, but there was only about a three-fold increase in the value of this pharmacokinetic parameter in the baboon.

Analysis of variance showed that the volume of distribution was significantly lower in the rhesus monkey than that in the other two species ($P < 0.05$ Newman-Keuls multiple comparison procedure) after the 1 mg/kg dose and that there was a significant increase in the volume of distribution of diazepam in the cynomolgus monkey and the baboon after the larger dose ($P < 0.005$ Newman-Keuls multiple comparison procedure).

Analysis of variance of the systemic clearance of diazepam showed that the clearance of diazepam in the baboon was significantly lower than that in the other two species ($P < 0.05$ Newman-Keuls multiple comparison procedure) after the 0.2 mg/kg dose but that there were no significant dose related differences in the clearance of diazepam ($P > 0.05$ Newman-Keuls multiple comparison procedure).

No other significant differences ($P > 0.05$) in the pharmacokinetics of diazepam in these non-human primates were observed at either dose level. The pharmacokinetics of diazepam were also found not to be greatly affected by an increase in the amount injected.

Although diazepam is strongly bound to plasma proteins, the volume of distribution of diazepam was 50, 53 and 39% of the total bodyweight in the rhesus monkey, the cynomolgus monkey and the baboon, respectively, after the 0.2 mg/kg dose and 44, 81 and 63% respectively after the 1 mg/kg dose, suggesting that the tissues also have an affinity for this compound. These values are lower than those previously reported for man (70-200% of bodyweight, Van der Kleijn et al, 1971; Kaplan et al, 1973; Klotz et al, 1976) and in other species (1277% in the dog, 714% in the rabbit, 395% in the guinea pig and 784% in the rat, Klotz et al, 1976). Thus there appears to be large interspecies variations in the degree of distribution of diazepam. These species related differences might result from differences in protein binding, but could also be an artefact resulting from differences in the method used to determine plasma concentrations (see page 66).

The mean volume of the central compartment was larger than that of the peripheral compartment in these non-human primates. The compartment volume ratio (central:peripheral) was 4.8, 3.8 and 2.1 in the rhesus monkey, the cynomolgus monkey and the baboon respectively, after the 0.2 mg/kg dose and 1.9, 2.2 and 1.7 respectively after administration of the 1 mg/kg dose, suggesting that there was relatively free transfer of diazepam between the central and the peripheral compartment. At the higher dose level there was a general increase in the apparent volume of distribution of both the central and peripheral compartments, with a slight increase in the proportion of distribution into the peripheral compartment.

The mean rate constant for the rapid distribution phase, $\alpha$, corresponded to an apparent half-life '$t_{\frac{1}{2}(\alpha)}$' of 19 minutes, 12 minutes and 15 minutes for the rhesus monkey, the cynomolgus monkey and the baboon, respectively, after the 0.2 mg/kg dose, and 11 minutes, 14 minutes and 13 minutes respectively, after the 1 mg/kg dose. These values are similar to those in other commonly used laboratory animals such as the dog ($t_{\frac{1}{2}(\alpha)}$ = 28 minutes), the rabbit ($t_{\frac{1}{2}(\alpha)}$ = 18 minutes) the guinea pig ($t_{\frac{1}{2}(\alpha)}$ = 18 minutes) and the rat ($t_{\frac{1}{2}(\alpha)}$ = 17 minutes) (Klotz et al, 1976) but are shorter than that in man ($t_{\frac{1}{2}(\alpha)}$ = 58 minutes, Kaplan, 1973; to $t_{\frac{1}{2}(\alpha)}$ = 132 minutes, Klotz et al, 1976). The value of apparent half-life of the distribution phase following intraperitoneal injection of diazepam in the rhesus monkey has been reported to be 30 minutes (Curry et al, 1977).

The ratio of $\beta/K_{el}$ of 0.4 and 0.5 for the rhesus monkey and 0.5 and 0.3 for the cynomolgus monkey and 0.6 and 0.4 for the baboon after the 0.2 mg/kg dose and the 1 mg/kg dose respectively indicates that these fractions of the diazepam in the body were in the central compartment and available for elimination at any time during the $\beta$ elimination phase (Gibaldi et al, 1969).

Simulation of concentration of diazepam in the peripheral compartment for a two-compartment open model with pharmacokinetic parameters shown in Tables 4:4 and 4:5 using equation 2:15, at both dose levels, showed that equilibrium was rapidly achieved. Peak levels of diazepam of 104 ng/ml, 127 ng/ml and 193 ng/ml in the rhesus monkey, the cynomolgus monkey and the baboon respectively, after the 0.2 mg/kg dose and 936 ng/ml, 556 ng/ml and 379 ng/ml respectively after the 1 mg/kg dose were reached 40 minutes after injection in these three non-human primate species after both administrations. Thereafter, concentrations of diazepam in the peripheral compartment were similar to those in the central compartment, except in the rhesus monkey after both dose levels and in the cynomolgus monkey after the high dose level. (Tables 4:6, 4:7; Figures 4:1 - 4:6).

TABLE 4:2

Mean concentrations ($\pm$ SD) of diazepam in the plasma of the rhesus monkey, the cynomolgus monkey and the baboon after a single bolus intravenous injection of diazepam at a level of 0.2 mg/kg. Results are expressed as ng/ml

| Time | Species | | |
|---|---|---|---|
| | Rhesus monkey | Cynomolgus monkey | Baboon |
| 2 minute | 432 ± 32 | 407 + 133 | 588 ± 180 |
| 5 minute | 386 ± 31 | 325 ± 60 | 556 ± 151 |
| 10 minute | 286 ± 73 | 264 ± 55 | 453 ± 94 |
| 20 minute | 170 ± 60 | 175 ± 42 | 371 ± 94 |
| 40 minute | 81 ± 31 | 90 ± 25 | 221 ± 63 |
| 1.0 hour | 37 ± 13 | 44 ± 7 | 142 ± 40 |
| 1.5 hour | NS | 21 ± 6 | 94 ± 31 |
| 2 hour | 10 ± 6 | 13 ± 6 | 68 ± 31 |
| 3 hour | NS | 4 ± 3 | 33 ± 26 |
| 4 hour | 2 ± 3 | ND | 17 ± 18 |
| 5 hour | ND | ND | 8 ± 11 |
| 7 hour | | | 3 ± 4 |

NS No sample taken at the time

ND Less than the limit of detection (2ng/ml)

TABLE 4:3

Mean concentrations (± SD) of diazepam in the plasma of the rhesus monkey, the cynomolgus monkey and the baboon after a single bolus intravenous injection of diazepam at a level of 1.0 mg/kg. Results are expressed as ng/ml

| Time | Species | | |
|---|---|---|---|
| | Rhesus monkey | Cynomolgus monkey | Baboon |
| 2 minute | 2984 ± 272 | 1841 ± 718 | 2172 ± 694 |
| 5 minute | 2538 ± 213 | 1692 ± 514 | 2358 ± 605 |
| 10 minute | 1892 ± 152 | 1360 ± 373 | 1888 ± 515 |
| 20 minute | 1243 ± 166 | 887 ± 274 | 1326 ± 293 |
| 40 minute | 529 ± 146 | 449 ± 77 | 662 ± 71 |
| 1.0 hour | 313 ± 84 | 254 ± 50 | 388 ± 105 |
| 1.5 hour | 147 ± 39 | 131 ± 20 | 230 ± 52 |
| 2 hour | 89 ± 28 | 72 ± 9 | 163 ± 47 |
| 3 hour | 35 ± 21 | 35 ± 9 | 89 ± 26 |
| 4 hour | 14 ± 11 | 13 ± 4 | 45 ± 13 |
| 5 hour | 3 ± 4 | 5 ± 3 | 23 ± 8 |
| 7 hour | ND | ND | 7 ± 4 |

ND Less than the limit of detection (2ng/ml)

TABLE 4:4

Mean pharmacokinetic parameters (±SD) determined for a two-compartment model after bolus intravenous injection of diazepam 0.2 mg/kg to the rhesus monkey, the cynomolgus monkey and the baboon. The data are presented as means of results from individual animals.

| Parameter[a] | | Species | | |
|---|---|---|---|---|
| | | Rhesus monkey | Cynomolgus monkey | Baboon |
| Bodyweight | (kg) | 4.6 ± 1.0 | 3.6 ± 0.3 | 5.7 ± 0.8 |
| Dose | (mg) | 0.9 ± 0.2 | 0.7 ± 0.1 | 1.1 ± 0.2 |
| Dose | (mg/kg) | 0.2 ± 0.0 | 0.2 ± 0.0 | 0.2 ± 0.0 |
| A | (ng/ml) | 440 ± 70 | 220 ± 110 | 356 ± 303 |
| B | (ng/ml) | 40 ± 20 | 160 ± 150 | 280 ± 90 |
| $\alpha$ | ($hour^{-1}$) | 2.189 ± 1.157 | 3.466 ± 3.822 | 2.773 ± 0.612 |
| $\beta$ | ($hour^{-1}$) | 0.770 ± 0.640 | 1.260 ± 0.768 | 0.693 ± 0.216 |
| $K_{12}$ | ($minute^{-1}$) | 0.003 ± 0.001 | 0.008 ± 0.061 | 0.009 ± 0.003 |
| $K_{21}$ | ($minute^{-1}$) | 0.015 ± 0.007 | 0.029 ± 0.089 | 0.019 ± 0.010 |
| $K_{el}$ | ($minute^{-1}$) | 0.031 ± 0.012 | 0.042 ± 0.008 | 0.021 ± 0.006 |
| Area | (ng/ml/hour) | 235 ± 27 | 193 ± 35 | 525 ± 176[b] |
| Cl | (ml/minute) | 66 ± 3 | 65 ± 16 | 43 ± 14[b] |
| $t_{½}$ | (minutes) | 54 ± 23 | 43 ± 17 | 60 ± 12 |
| $V_1$ | (litres) | 1.9 ± 0.6 | 1.5 ± 0.6 | 1.5 ± 1.0 |
| $V_2$ | (litres) | 0.4 ± 0.1 | 0.4 ± 0.6 | 0.7 ± 0.4 |
| $V_{(ss)}$ | (litres) | 2.3 ± 0.5 | 1.9 ± 1.1 | 2.2 ± 1.3 |
| $V_{d(\beta)}$ | (litres) | 5.1 ± 1.5 | 3.1 ± 0.8 | 3.7 ± 1.2 |
| $A/\alpha$ | (ng/ml/hour) | 91 ± 54 | 63 ± 93 | 130 ± 109 |
| $B/\beta$ | (ng/ml/hour) | 202 ± 27 | 127 ± 79 | 404 ± 149 |

a For details of abbreviations used, see Chapter 2.

b Significance level (analysis of variance) baboons compared to the other two species ($P < 0.05$).

TABLE 4:5

Mean pharmacokinetic parameters (±SD) determined for a two-compartment model after bolus intravenous injection of diazepam 1.0 mg/kg to the rhesus monkey, the cynomolgus monkey and the baboon. The data are presented as means of results from individual animals.

| Parameter[a] | | Species | | |
|---|---|---|---|---|
| | | Rhesus monkey | Cynomolgus monkey | Baboon |
| Bodyweight | (kg) | 4.5 ± 1.0 | 3.4 ± 0.3 | 5.7 ± 0.9 |
| Dose | (mg) | 4.6 ± 0.9 | 3.6 ± 0.2 | 5.7 ± 0.9 |
| Dose | (mg/kg) | 1.0 ± 0.1 | 1.1 ± 0.1 | 1.0 ± 0.0 |
| A | (ng/ml) | 2476 ± 508 | 1622 ± 589 | 2100 ± 919 |
| B | (ng/ml) | 885 ± 812 | 466 ± 329 | 620 ± 115 |
| $\alpha$ | ($hour^{-1}$) | 3.736 ± 1.165 | 3.023 ± 1.308 | 3.199 ± 0.777 |
| $\beta$ | ($hour^{-1}$) | 0.986 ± 0.716 | 0.832 ± 0.512 | 0.650 ± 0.096 |
| $K_{12}$ | ($minute^{-1}$) | 0.015 ± 0.051 | 0.010 ± 0.007 | 0.015 ± 0.005 |
| $K_{21}$ | ($minute^{-1}$) | 0.029 ± 0.089 | 0.020 ± 0.016 | 0.021 ± 0.003 |
| $K_{el}$ | ($minute^{-1}$) | 0.035 ± 0.014 | 0.033 ± 0.010 | 0.028 ± 0.009 |
| Area | (ng/ml/hr.) | 1331 ± 164 | 1035 ± 191 | 1653 ± 174 |
| Cl | (ml/minute) | 59 ± 14 | 60 ± 14 | 58 ± 14 |
| $t_{½}$ | (minute) | 45 ± 25 | 50 ± 22 | 64 ± 7 |
| $V_1$ | (litres) | 1.3 ± 0.2 | 2.0 ± 1.1 | 2.1 ± 1.0 |
| $V_2$ | (litres) | 0.7 ± 0.2 | 0.9 ± 0.3 | 1.5 ± 0.7 |
| $V_{(ss)}$ | (litres) | 2.0 ± 0.4[b] | 2.9 ± 0.9 | 3.6 ± 1.0 |
| $V_{d(\beta)}$ | (litres) | 3.6 ± 0.9[b] | 4.3 ± 1.0 | 5.4 ± 1.3 |
| $A/\alpha$ | (ng/ml/hr) | 669 ±401 | 601 ± 224 | 656 ± 177 |
| $B/\beta$ | (ng/ml/hr) | 661 ± 336 | 433 ± 242 | 954 ± 193 |

a For details of abbreviations used, see Chapter 2.

b Significance level (analysis of variance) rhesus compared to the other two species ($P < 0.05$)

TABLE 4.6

Calculated concentrations of diazepam in the peripheral compartment after administration of diazepam 0.2 mg/kg to the rhesus monkey, the cynomolgus monkey and the baboon. Results calculated from mean plasma data (Table 4.4) and expressed in ng/ml

| Time | Species | | |
|---|---|---|---|
| | Rhesus monkey | Cynomolgus monkey | Baboon |
| 2 minute | 13 | 26 | 27 |
| 5 minute | 30 | 58 | 61 |
| 10 minute | 53 | 95 | 107 |
| 20 minute | 83 | 126 | 162 |
| 40 minute | 105 | 127 | 193 |
| 1 hour | 100 | 96 | 179 |
| 1.5 hour | 79 | 55 | 138 |
| 2 hour | 57 | 30 | 100 |
| 3 hour | 28 | 9 | 51 |
| 4 hour | 13 | 2 | 26 |
| 5 hour | 6 | 1 | 13 |
| 7 hour | 1 | | 3 |

TABLE 4:7

Calculated concentrations of diazepam in the peripheral compartment after administration of diazepam 1.0 mg/kg to the rhesus monkey, the cynomolgus monkey and the baboon. Results calculated from mean plasma data (Table 4.5) and expressed in ng/ml.

| Time | Species | | |
|---|---|---|---|
| | Rhesus monkey | Cynomolgus monkey | Baboon |
| 2 minute | 183 | 75 | 57 |
| 5 minute | 407 | 182 | 130 |
| 10 minute | 669 | 327 | 222 |
| 20 minute | 930 | 472 | 330 |
| 40 minute | 936 | 533 | 379 |
| 1 hour | 751 | 466 | 344 |
| 1.5 hour | 482 | 333 | 264 |
| 2 hour | 297 | 225 | 194 |
| 3 hour | 112 | 99 | 102 |
| 4 hour | 42 | 43 | 53 |
| 5 hour | 15 | 19 | 28 |
| 7 hour | 2 | 4 | 8 |

Plasma concentrations of desmethyldiazepam. Desmethyldiazepam was detectable in the plasma at 5 minutes (the second time of blood withdrawal) and 2 minutes (the first time of blood withdrawal) after dosing with diazepam at levels of 0.2 mg/kg and 1 mg/kg respectively. Peak plasma concentrations of desmethyldiazepam of 118 ng/ml ± 39 SD, 107 ng/ml ± 19 SD and 112 ng/ml ± 13 SD in the rhesus monkey, the cynomolgus monkey and the baboon were reached 1-2 hours after administration of the 0.2 mg/kg dose. These values were similar to those in man after injection of diazepam at the same dose level (Korttila et al, 1975). After administration of diazepam at a level of 1 mg/kg peak concentrations of 813 ng/ml ± 232 SD, 551 ng/ml ± 117 SD and 516 ng/ml ± 218 SD in the rhesus monkey the cynomolgus monkey and the baboon occurred 1-2 hours after dosing. The increase in plasma concentration of the metabolite was approximately five-fold and, therefore, in proportion to the increased dose, suggesting that there is a linear relationship between the plasma concentrations of desmethyldiazepam formed after administration of different amounts of the parent drug, at least over this concentration range. After reaching peak levels, plasma concentration declined with an apparent half-life of elimination of 3-12 hours, to almost the limit of detection (2.0 ng/ml) 24-48 hours after administration of the 0.2 mg/kg dose. After administration of the 1 mg/kg dose, terminal half-lives varied from 4-18 hours and desmethyldiazepam was detectable for 72 hours after administration in all three non-human primate species (Tables 4:8, 4:9, Figures 4:1 - 4:6).

Pharmacokinetic parameters for desmethyldiazepam. A plot of the observed plasma concentrations of desmethyldiazepam against time was fitted by a biexponential curve, described by equation 2:18. The pharmacokinetic parameters shown in Tables 4:10 and 4:11 were therefore calculated for a one-compartment open model with first order input and output (Scheme 2:3). Plasma concentration of desmethyldiazepam predicted by the one-compartment model were generally in good agreement with the observed values (Figures 4:1 - 4:6).

Analysis of variance showed that the areas under the plasma concentration time curve in the baboon were significantly lower than in the other two species ($P<0.05$ non-parametric version of Newman-Keuls multiple comparison procedure) after the 0.2 mg/kg dose, and that the areas under the curve in the rhesus monkey were significantly lower ($P<0.05$ non-parametric version of Newman-Keuls multiple comparison procedure) after 1 mg/kg dose. The increased areas after the higher dose represented a four-, six- and twelve-fold increase over that after the lower dose in the rhesus monkey, the cynomolgus monkey and the baboon respectively, and were therefore approximately in proportion to the increased dose except in the baboon. The lower values obtained after the 0.2 mg/kg dose to the baboon and 1 mg/kg dose to the rhesus monkey were associated with shorter elimination half-lives of desmethyldiazepam in these species at those dose levels.

The rate constant for formation of desmethyldiazepam 'K' was estimated by the method of residuals (Gibaldi and Perrier, 1975). Mean values were similar in all three species, corresponding to an apparent half-life of formation of about 20 minutes. After the higher dose, the rate of formation of the metabolite decreased slightly and was slowest in the baboon in which the mean apparent half-life of formation was estimated to be 33 minutes. Generally, the values of $K_f$ were similar to those for the rate constant for elimination of diazepam from the central compartment '$K_{el}$' for diazepam (Tables 4:4 and 4:5). This similarity and also that no other metabolite of diazepam were detectable in the plasma of these species suggests that diazepam is eliminated by metabolism principally via the desmethyl metabolite pathway.

Assuming that demethylation of diazepam is the primary stage in the elimiantion of diazepam from the body, the volume of distribution and total systemic clearance of desmethyldiazepam can be estimated using equations 1:1 and 2:4. Volumes of distribution in the baboon after

the 1 mg/kg dose were higher than those in the other two species, but this data was variable and there were no significant differences between the species with respect to this parameter after either dose of diazepam. Analysis of variance of estimated clearances (Tables 4:10 and 4:11) shows that the total systemic clearance of desmethyldiazepam is significantly higher ($P<0.01$, Newman-Keuls multiple comparison procedure) in the baboon than in the cynomolgus monkey after administration of the 0.2 mg/kg dose. Clearances were lower in the rhesus monkey than in the baboon, but this difference was not significant ($P>0.05$). After administration of diazepam at a level of 1 mg/kg the systemic clearance was again lower in the cynomolgus monkey than in the other two species but this difference was not statistically significant ($P>0.05$). When adjusted for bodyweight differences the adjusted clearance in the baboon (3.8 ml/minute/kg) remained significantly higher than that in the rhesus monkey (2.4 ml/kinute/kg) and that in the cynomolgus monkey (2.4 ml/minute/kg) ($P<0.05$, Newman-Keuls multiple comparison procedure) after the 0.2 mg/kg dose. After the 1 mg/kg dose the adjusted clearance of desmethyldiazepam 2.8 ml/minute/kg, 2.0 ml/minute/kg and 2.6 ml/minute/kg in the rhesus monkey, the cynomolgus monkey and the baboon respectively, remained not significantly different in these non-human primate species ($P>0.05$, Newman-Keuls multiple comparison procedure).

The volume of distribution of diazepam in non-human primates was 171, 183 and 128% of bodyweight in the rhesus monkey, the cynomolgus monkey and the baboon respectively, after the 0.2 mg/kg dose and 142, 182 and 288% of bodyweight in these species respectively after the 1 mg/kg dose. These values are higher than that in man, 64% of bodyweight (Klotz et al, 1976) and in the dog approximately 100% of bodyweight (estimated from published data, Kaplan and Jack, 1980). Desmethyldiazepam clearance in the non-human primate is greater than that in man (0.2 ml/minute/kg, Klotz et al, 1977; Brodie et al, 1981; Chasseaud et al, 1980b), but much lower than that in the dog (33.4 ml/minute/kg, estimated from published data, Kaplan and Jack, 1980).

TABLE 4:8

Mean plasma concentrations (± SD) of desmethyldiazepam after bolus intravenous injection of diazepam 0.2 mg/kg to the rhesus monkey, the cynomolgus monkey and the baboon. Results are expressed as ng/ml.

| Time | Species | | |
|---|---|---|---|
| | Rhesus monkey | Cynomolgus monkey | Baboon |
| 2 minute | ND | ND | ND |
| 5 minute | 11 ± 8 | 14 ± 18 | 12 ± 20 |
| 10 minute | 34 ± 24 | 35 ± 24 | 29 ± 37 |
| 20 minute | 69 ± 20 | 59 ± 7 | 60 ± 31 |
| 40 minute | 99 ± 20 | 86 ± 15 | 87 ± 21 |
| 1 hour | 114 ± 24 | 107 ± 19 | 102 ± 10 |
| 1.5 hour | NS | 106 ± 24 | 112 ± 13 |
| 2 hour | 118 ± 39 | 101 ± 19 | 103 ± 14 |
| 3 hour | NS | 92 ± 18 | 96 ± 15 |
| 4 hour | 99 ± 33 | 85 ± 18 | 82 ± 14 |
| 5 hour | NS | 79 ± 16 | 69 ± 14 |
| 7 hour | 77 ± 23[a] | 65 ± 16 | 46 ± 15 |
| 24 hour | 20 ± 7 | 23 ± 9 | 4 ± 1 |
| 30 hour | 13 ± 5 | 15 ± 7 | ND |
| 48 hour | 2 ± 1 | 4 ± 3 | |

NS Indicating that no sample was taken at this time.

a Result refers to sample taken at 6 hours post-dose.

ND Less than the limit of detection (2ng/ml).

TABLE 4:9

Mean plasma concentrations (± SD) of desmethyldiazepam after bolus intravenous injection of diazepam 1.0 mg/kg to the rhesus monkey, the cynomolgus monkey and the baboon. Results are expressed as ng/ml.

| Time | Species | | |
|---|---|---|---|
| | Rhesus monkey | Cynomolgus monkey | Baboon |
| 2 minute | 4 ± - | 3 ± - | ND |
| 5 minute | 138 ± 111 | 29 ± 49 | 28 ± 31 |
| 10 minute | 335 ± 120 | 145 ± 59 | 82 ± 42 |
| 20 minute | 567 ± 122 | 275 ± 173 | 225 ± 42 |
| 40 minute | 813 ± 232 | 466 ± 129 | 417 ± 122 |
| 1 hour | 789 ± 238 | 526 ± 106 | 490 ± 212 |
| 1.5 hour | 742 ± 202 | 551 ± 117 | 516 ± 218 |
| 2 hour | 684 ± 177 | 549 ± 99 | 514 ± 181 |
| 3 hour | 614 ± 99 | 517 ± 87 | 501 ± 172 |
| 4 hour | 523 ± 96 | 480 ± 36 | 480 ± 224 |
| 5 hour | 449 ± 86 | 467 ± 51 | 434 ± 245 |
| 7 hour | 363 ± 75[a] | 404 ± 72 | 383 ± 255 |
| 24 hour | 66 ± 56 | 153 ± 67 | 180 ± 106 |
| 30 hour | 45 ± 51 | 120 ± 61 | 112 ± 82 |
| 48 hour | 10 ± 19 | 39 ± 29 | 47 ± 36 |
| 72 hour | 2 ± - | 12 ± 11 | 14 ± 12 |

a Result refers to sample taken at 6 hour post-dose.

ND Less than the limit of detection (2ng/ml).

TABLE 4:10

Mean pharmacokinetic parameters (± SD) determined for desmethyldiazepam after bolus intravenous injection of diazepam (0.2 mg/ml) into rhesus monkeys, cynomolgus monkeys and baboons. The data are presented as means of results from individual animals.

| Parameter[a] | | Species | | |
|---|---|---|---|---|
| | | Rhesus monkey | Cynomolgus monkey | Baboon |
| Lag time | (minutes) | 1.2 ± 1.6 | 2.9 ± 1.0 | 5.1 ± 4.5 |
| Peak concentration | (ng/ml) | 112 ± 22 | 104 ± 17 | 111 ± 10.5 |
| Time to peak concentration | (minutes) | 102 ± 56 | 82 ± 28 | 83 ± 33 |
| $t_{½}$ | (hours) | 8.5 ± 0.6 | 9.9 ± 2.5 | 4.2 ± 0.9 |
| AUC | (ng/ml/hour) | 1632 ± 766 | 1527 ± 459 | 887 ± 199[b] |
| Cl[d] | (ml/minute) | 10.6 ± 6.0 | 8.1 ± 2.8 | 20.8 ± 5.6[c] |
| $V_d$[d] | (litres) | 7.9 ± 4.4 | 6.6 ± 2.3 | 7.3 ± 2.0 |
| $K_f$ | ($hour^{-1}$) | 2.747 ± 1.822 | 2.067 ± 0.033 | 2.0586 ± 0.769 |
| $K_m$ | ($hour^{-1}$) | 0.081 ± 0.007 | 0.074 ± 0.021 | 0.1728 ± 0.049 |

a For details of abbreviations see Chapter 2.

b Significance level (analysis of variance) baboon compared to the other two species ($P < 0.05$)

c Significance level (analysis of variance) baboon compared to the cynomolgus monkey ($P < 0.01$).

d Estimated assuming that all administered diazepam is primarily metabolised to desmethyldiazepam before elimination.

TABLE 4:11

Mean pharmacokinetic parameters (± SD) determined for desmethyldiazepam after bolus intravenous injection of diazepam (1.0 mg/kg) into rhesus monkeys, cynomolgus monkeys and baboons. The data are presented as means of results from individual animals.

| Parameter[a] | | Species | | |
|---|---|---|---|---|
| | | Rhesus monkey | Cynomolgus monkey | Baboon |
| Lag time | (minutes) | 2.4 ± 0.7 | 3.5 ± 2.0 | 3.1 ± 2.0 |
| Peak concentration | (ng/ml) | 800 ± 218 | 562 ± 88 | 546 ± 223 |
| Time to peak concentration | (minutes) | 55 ± 16 | 99 ± 26 | 125 ± 47 |
| $t_{\frac{1}{2}}$ | (hours) | 6.8 ± 3.4 | 11.9 ± 4.1 | 14.7 ± 2.5 |
| AUC | (ng/ml/hour) | 6636 ± 2591[b] | 9318 ± 3261 | 10171 ± 6674 |
| Cl[c] | (ml/minute) | 12.6 ± 4.3 | 6.7 ± 2.4 | 13.1 ± 9.3 |
| $V_d$[c] | (litres) | 6.4 ± 2.2 | 6.2 ± 2.2 | 16.4 ± 11.6 |
| $K_f$ | ($hour^{-1}$) | 4.304 ± 1.311 | 2.259 ± 0.910 | 1.949 ± 0.983 |
| $K_m$ | ($hour^{-1}$) | 0.119 ± 0.042 | 0.065 ± 0.025 | 0.048 ± 0.009 |

a *For details of abbreviations see Chapter 2.*

b Significance level (analysis of variance) rhesus monkey compared with the other two species ($P < 0.05$).

c Estimated assuming that all administered diazepam is primarily metabolised to desmethyldiazepam before elimination.

Discussion

The plasma concentration-time course for diazepam has been described for many species, including man, in terms of a two-compartment open model (Klotz et al, 1976, Van den Kleijn et al, 1971; Vree et al, 1979; Kaplan et al, 1973; Mandelli et al, 1978; Curry et al, 1977). In these non-human primates also, the pharmacokinetic profile of diazepam appears to fit the two-compartment model after intravenous injection at a therapeutic dose level (0.2 mg/kg) and at a level that might be selected for administration during a chronic toxicity study of diazepam (1 mg/kg). The distribution phase, $A/\alpha$, contributed 39, 33 and 25% to the total area under the curve in the rhesus monkey, the cynomolgus monkey and the baboon respectively after the 0.2 mg/kg dose and 50, 58 and 40% respectively after the 1 mg/kg dose, increased after the higher dose.

As diazepam is eliminated almost exclusively by metabolism, the total systemic clearance reflects the metabolic clearance. The values for the clearance of diazepam in these non-human primates was about four-fold lower than the hepatic blood flow and, therefore, if diazepam metabolism occurs predominatly in the liver it is possible that an hepatic first pass effect could be a factor in diazepam disposition in the non-human primate after oral administration of this compound. When adjusted for bodyweight differences, the adjusted systemic clearance of diazepam 14.3 ml/minute/kg (13.1 ml/minute/kg), 18.1 ml/minute/kg (17.6 ml/minute/kg) and 7.5 ml/minute/kg (10.2 ml/minute/kg) in the rhesus monkey, the cynomolgus monkey and the baboon respectively after the lower dose (and higher dose) respectively, were similar to or less than those values found in the dog (18.9 ml/minute/kg), the rabbit (30.5 ml/minute/kg), the guinea pig (18.9 ml/minute/kg) and the rat (81.6 ml/minute/kg) but higher than that in man (0.35 ml/minute/kg) (Klotz et al, 1976, 1977; Kaplan et al, 1973).

The values of the apparent half-life of elimination of diazepam observed in this study with non-human primates are similar to those values previously reported for the rat (60 minutes, Klotz et al, 1976) and for the dog (80 minutes, Vree et al, 1979) but shorter than those in the guinea pig (144 minutes, Klotz et al, 1976), the dog (456 minutes Klotz et al, 1976) and for man (24-48 hours, Klotz et al, 1976, 1977; Kaplan et al, 1973; Van der Kleijn et al, 1971, Vree et al, 1979; Mandelli et al, 1978)

Curry et al (1977) reported a biexponential decline of plasma concentrations in the rhesus monkey following an intraperitoneal injection of diazepam with an $\alpha$-phase corresponding to an apparent half-life of 30 minutes and a terminal half-life of 9.7 hours. However, these workers used a gas chromatographic procedure (De Silva and Puglisi, 1970) and the terminal half-life was not observed until plasma concentrations were very low (< 40 ng/ml). No such terminal phase was observed in this study in which a high pressure liquid chromatographic method was employed. It is possible, therefore, that the longer half-life reported by Curry et al was an artefact resulting from the gas chromatographic procedure. Another example of a considerable difference in the value of the biological half-life of diazepam using these two different analytical techniques can be found in the literature. Vree et al (1979) quote a value of 80 minutes for the biological half-life of diazepam in the dog, using high pressure liquid chromatography to measure plasma concentrations, whereas Klotz et al (1976) found the biological half-life in the dog to be 7.6 hours using a gas chromatographic method (Klotz et al, 1975).

It has been reported that diazepam is an inducer of its own metabolism (Kanto et al, 1974; Sellman et al, 1975) resulting in higher plasma concentrations of desmethyldiazepam after continuous therapy. Korttila et al (1975) explained the increased plasma concentrations of desmethyldiazepam after continuous therapy resulting from the saturation of tissues with this metabolite. In this study with non-human primates, plasma concentrations of both the parent compound and the desmethyl metabolite increased approximately in proportion to the administered dose and the pharmacokinetic parameters

were similar at both dose levels. However, there was a two week interval between dosing sessions and it would, therefore, be unlikely that there was any induction effect resulting from the initial dose at 0.2 mg/kg influencing the kinetics after the 1 mg/kg dose.

The values of the half-lives of elimination of desmethyldiazepam observed in these non-human primates were shorter than those reported for man (30 - 200 hours, Van der Kleijn, 1971; Klotz et al, 1977; Kanto et al, 1975; Carrigan and Bantenback, 1979; Brodie et al, 1980; Chasseaud et al, 1980b) but similar to those previously reported for the rhesus monkey (8.2 hours, Curry et al, 1977) and for the dog (10 hours, Vree et al, 1979). Desmethyldiazepam is an unimportant metabolic product of diazepam in the rat.

Inspection of the half-lives of elimination of diazepam and desmethyldiazepam in several species shows that during the once-daily dosage regimen, common to most chronic toxicity studies, diazepam would not accumulate in the body of the commonly used laboratory animals, since each dose would have been eliminated before the next is received. However, in humans receiving 30 mg diazepam once daily for the treatment of anxiety and tension, a plasma steady state concentration of diazepam of approximately 750 ng/ml would be achieved one to two weeks after commencement of therapy. To achieve this same steady state plasma concentration in non-human primates a possible dosing regimen would be to inject diazepam intravenously 1.9 mg every 45 minutes, 2.5 mg every 50 minutes and 2.9 mg every 64 minutes into the rhesus monkey, the cynomolgus monkey and the baboon respectively.

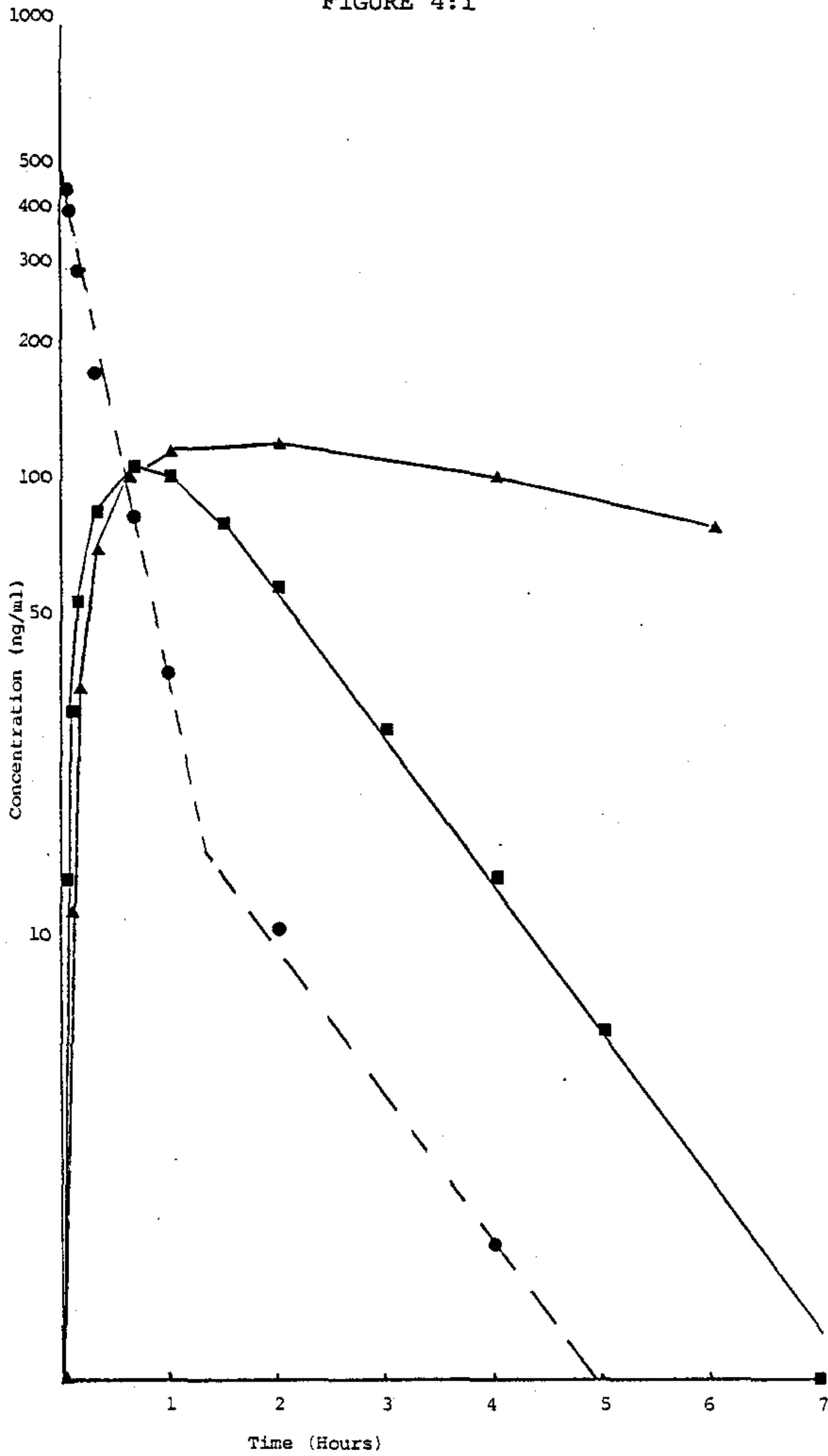
In practice, it would be more appropriate to monitor maximum and/or minimum diazepam plasma (blood) concentrations during a chronic dose study in which the drug would normally be administered orally. This is particularly relevant for diazepam, as this drug is thought to induce its own metabolism and therefore it would probably be necessary to adjust

the dosing regimen as described by Gibaldi and Perrier (1975) to obtain suitable steady state drug plasma (blood) concentration. There was no indication from this study at two dose levels in non-human primates that diazepam pharmacokinetics were dose dependent.

The accumulation of the desmethyl metabolite of diazepam in humans with the above dosing regimens can be estimated from published pharmacokinetic parameters (Brodie et al, 1980; Chasseaud et al, 1980b) if it is assumed that all diazepam is demethylated as the primary stage of elimination (see page 59). In humans, therefore, a steady state plasma concentration of desmethyldiazepam of approximately 1.8 μg/ml would be achieved about 26 days after commencement of a dosing regimen of 30 mg of diazepam once daily. The steady state plasma concentrations of desmethyldiazepam in the rhesus monkey, the cynomolgus monkey and the baboon would be approximately 3.2 μg/ml, 7.1 μg/ml and 3.3 μg/ml respectively, if diazepam was administered intravenously as described above. There is, therefore, relatively more accumulation of desmethyldiazepam in non-human primates than in humans with a dosing regimen designed to give the same steady state plasma concentration of diazepam.

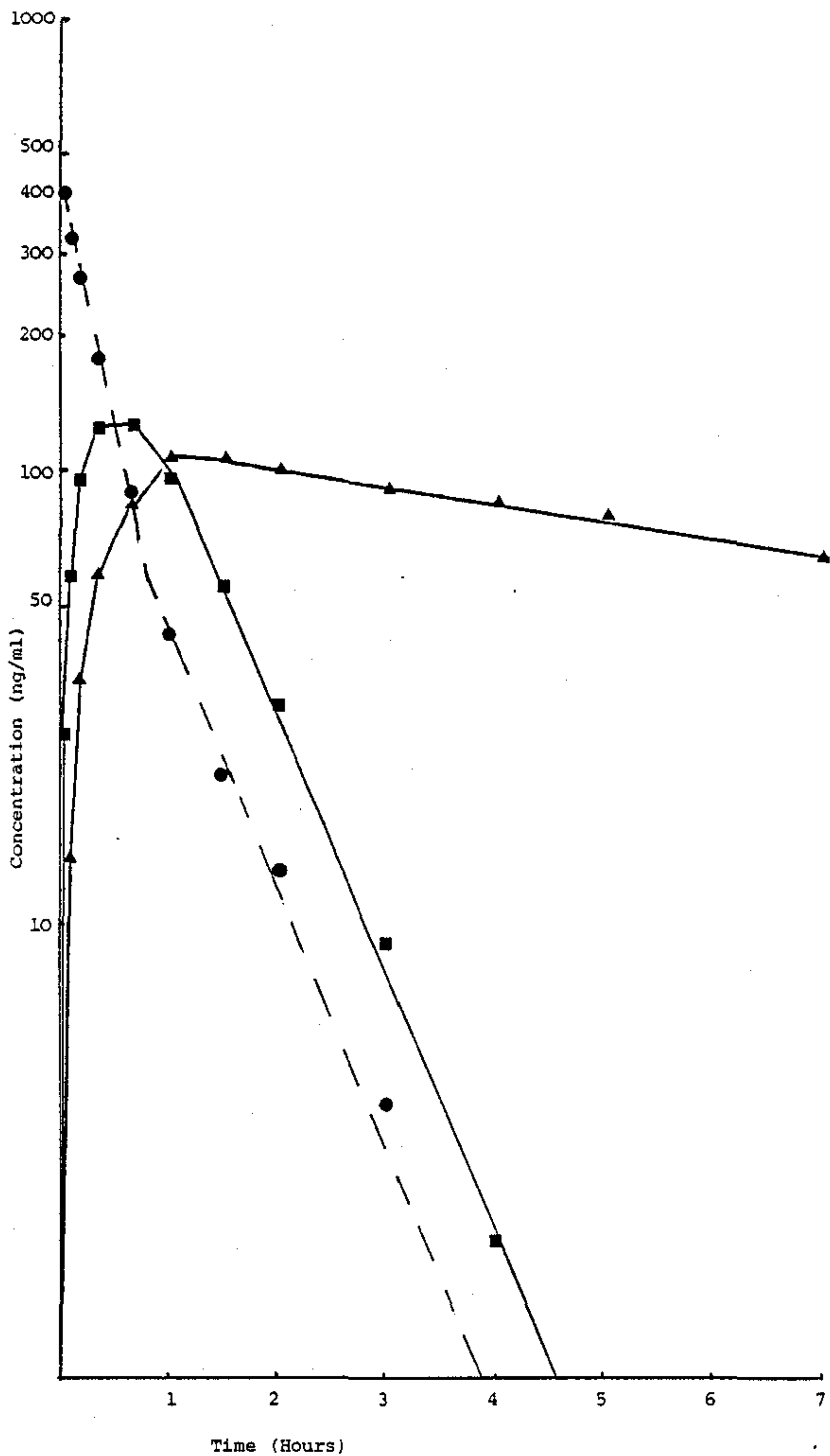
Using pharmacokinetic criteria, therefore, it can be concluded that the non-human primate is not a more suitable model for man than are the other commonly used laboratory animal species. More important factors in the choice of an animal model for man to study the fate of diazepam in vivo, would be species-related differences in metabolism, route of excretion, tissue distribution and protein binding.

FIGURE 4:1



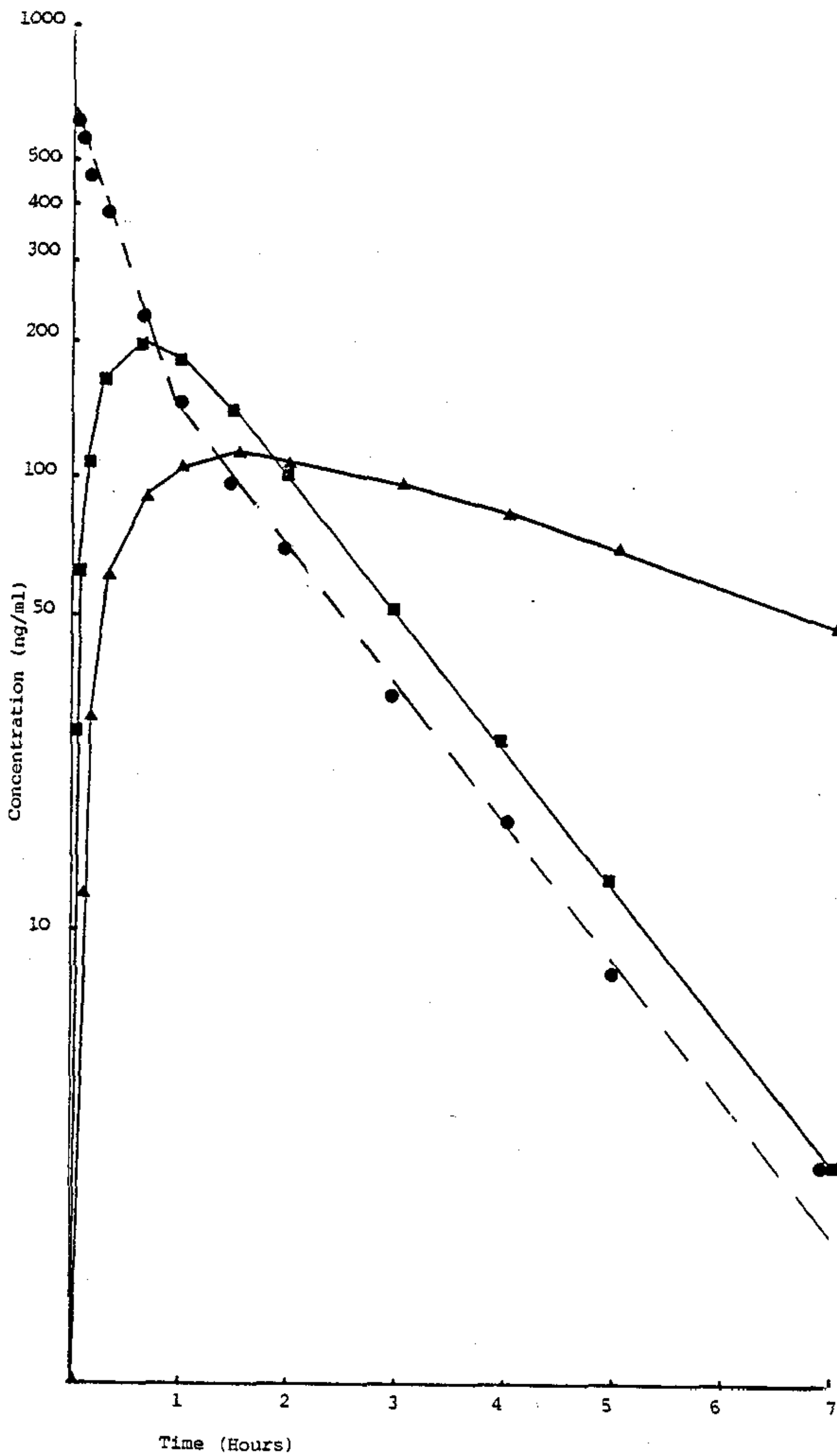

Mean concentration-time curves after a single intravenous injection of diazepam 0.2 mg/kg to the rhesus monkey. Observed concentration of diazepam ( ● ● ) and desmethyldiazepam ( ▲ ▲ ) in the central compartment. Predicted concentration for diazepam (dashed lines) and desmethyldiazepam (solid lines) in the central compartment, and for diazepam in the peripheral compartment ( ■ ■ ).

FIGURE 4:2



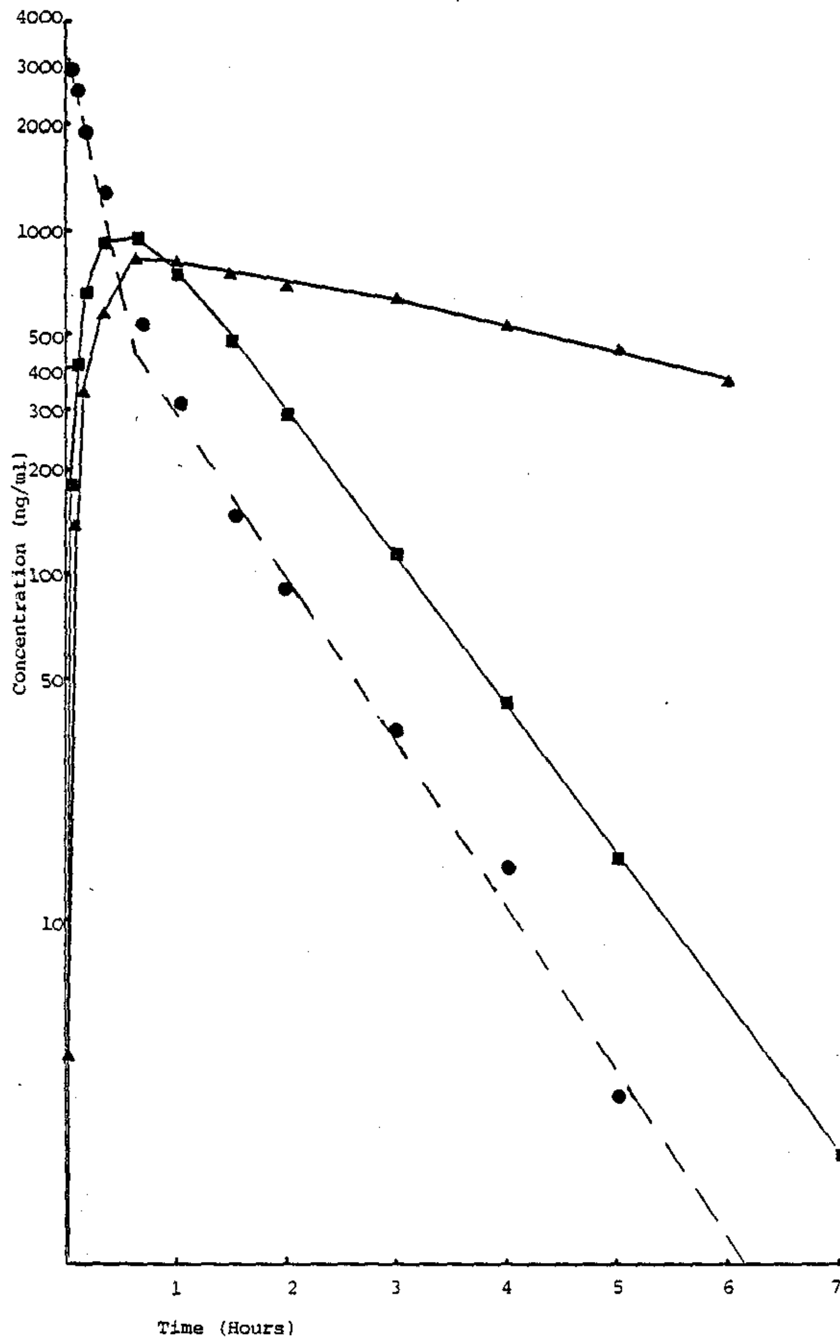

Mean concentration-time curves after a single intravenous injection of diazepam 0.2 mg/kg to the cynomolgus monkey. Observed concentrations of diazepam ( ● ● ) and desmethyldiazepam ( ▲ ▲ ) in the central compartment. Predicted concentration for diazepam (dashed lines) and desmethyldiazepam (solid lines) in the central compartment and for diazepam in the peripheral compartment ( ■ ■ )

FIGURE 4:3



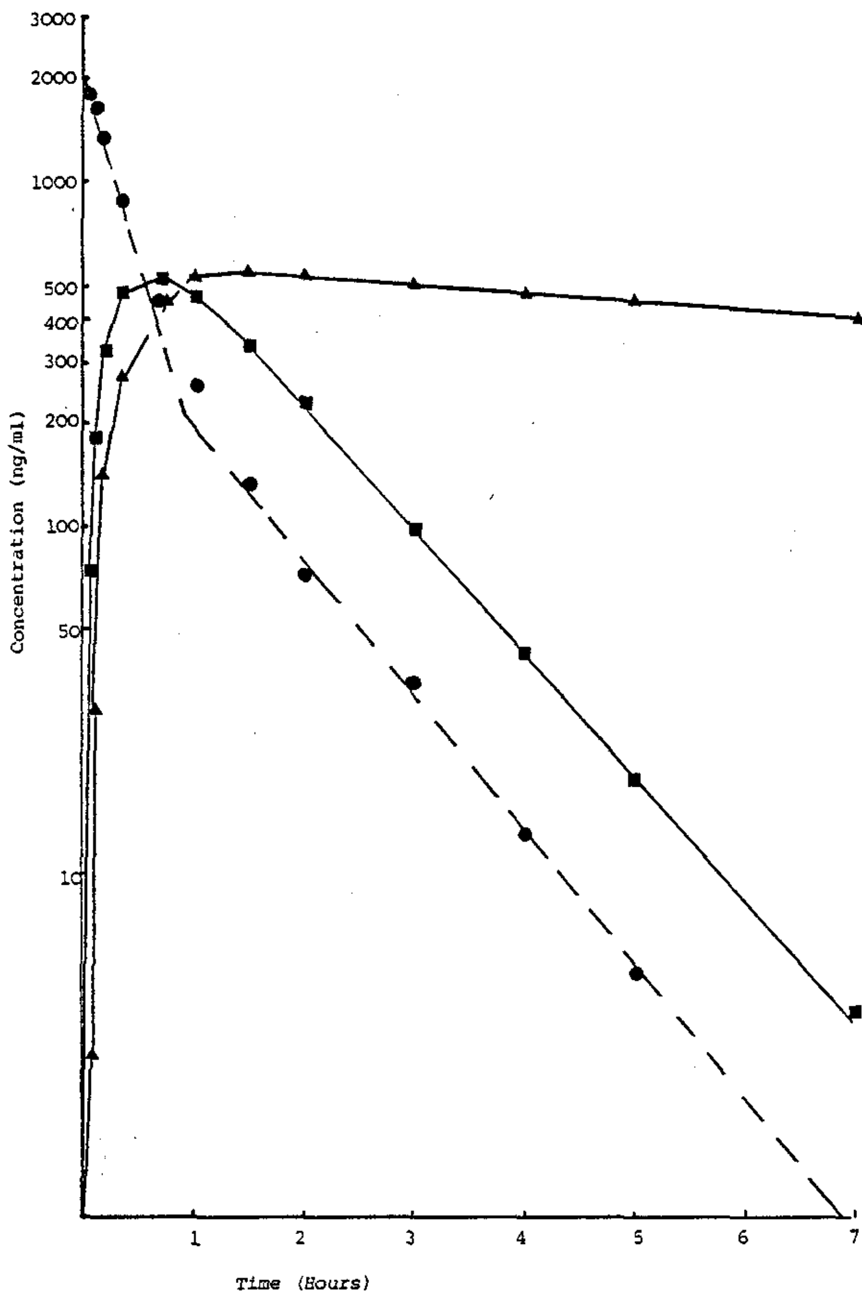

Mean concentration-time curves after a single intravenous injection of diazepam 0.2 mg/kg to the baboon. Observed concentrations of diazepam ( ● ● ) and desmethyldiazepam ( ▲ ▲ ) in the central compartment. Predicted concentrations for diazepam (dashed lines) and desmethyldiazepam (solid lines) in the central compartment and for diazepam in the peripheral compartment ( ■ ■ )

FIGURE 4:4



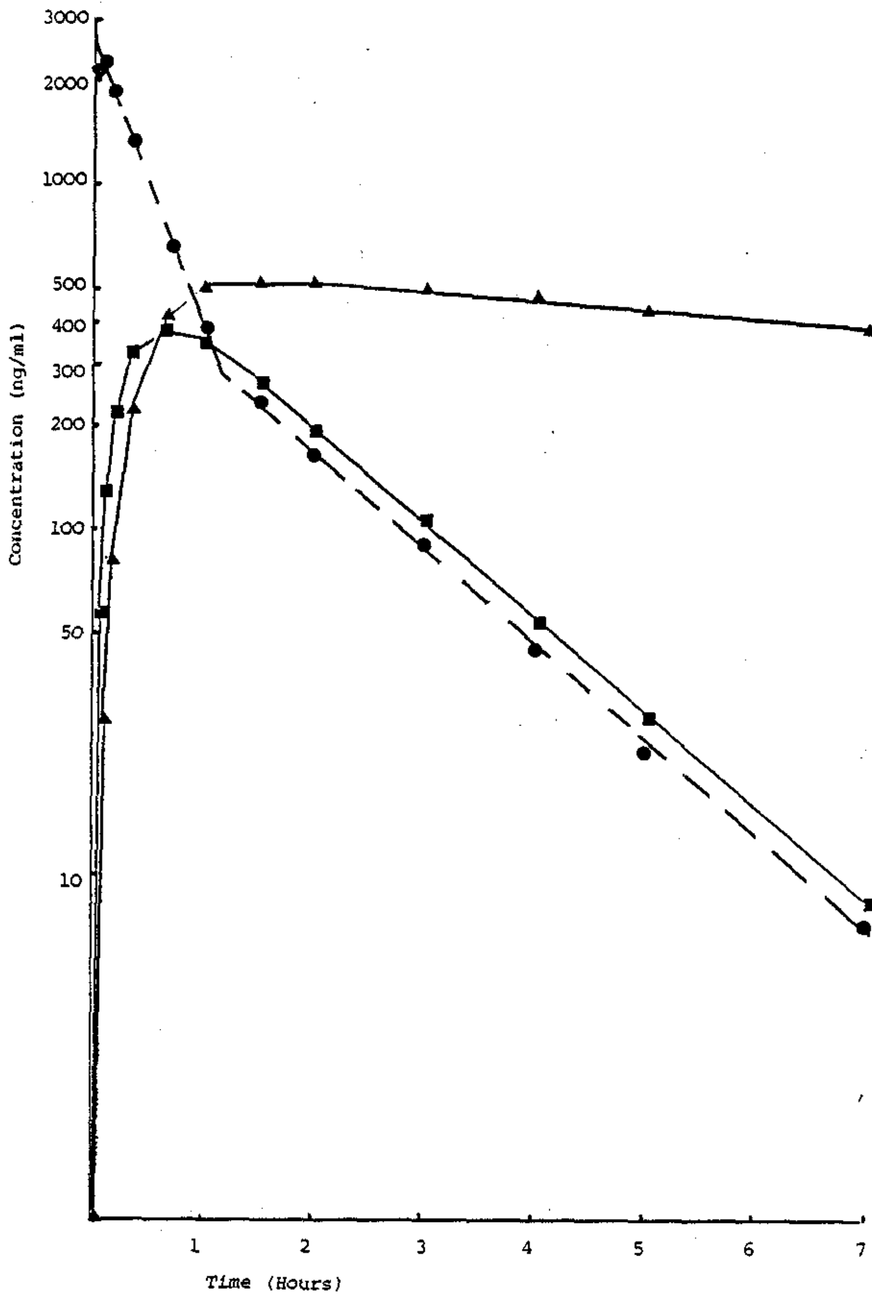

Mean concentration-time curves after a single intravenous injection of diazepam 1.0 mg/kg to the rhesus monkey. Observed concentrations of diazepam ( ● ● ) and desmethyldiazepam ( ▲ ▲ ) in the central compartment. Predicted concentrations for diazepam (dashed lines) and desmethyldiazepam (solid lines) in the central compartment and for diazepam in the peripheral compartment ( ■ ■ )

FIGURE 4:5




Mean concentration-time curves after a single intravenous injection of diazepam 1.0 mg/kg to the cynomolgus monkey. Observed concentrations of diazepam ( ● ● ) and desmethyldiazepam ( ▲ ▲ ) in the *central compartment*. Predicted concentrations for diazepam (dashed lines) and desmethyldiazepam (solid lines) in the central compartment and for diazepam in the peripheral compartment ( ■ ■ )

FIGURE 4:6




Mean concentration-time curves after a single intravenous injection of diazepam 1.0 mg/kg to the baboon. Observed concentration of diazepam ( ● ● ) and desmethyldiazepam ( ▲ ▲ ) in the central compartment. Predicted concentrations of diazepam (dashed lines) and desmethyldiazepam (solid lines) in the central compartment and diazepam in the peripheral compartment ( ■ ■ )

CHAPTER 5

## FRUSEMIDE

### Introduction

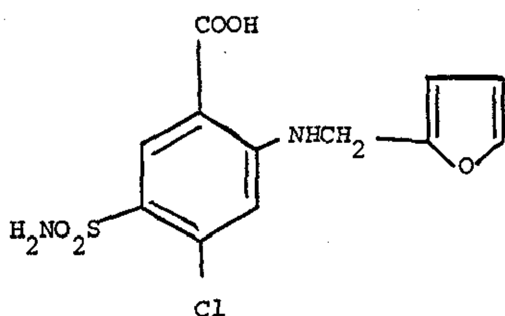
Frusemide(4-chloro-N-furfuryl-5-sulphamoyl-anthranilic acid,$pK_a$ 3.9) is a potent, short acting diuretic (Kirkendall and Stein, 1968; Kim et al, 1971). The mechanism of its effect is poorly understood, but frusemide has been shown to act at the lumen surface of the diluting segment of the loop of Henle, inhibiting active reabsorption of chloride and so preventing the reabsorption of sodium (for a review see Benet, 1979). Although incompletely absorbed from oral preparations (approximately 60% bioavailability in man, Benet, 1979, and in dogs Yakatan et al, 1976), the total diuretic response has been shown to be similar after both oral and intravenous administration of the same amount (80 mg) frusemide (Kelly et al, 1974; Branch et al, 1976).

After intravenous injection approximately 80% of the administered dose is excreted in the urine in the first 24 hours, apparently aided by active secretion (Gayer, 1965; Sorgel, 1978), and some is excreted in the faeces both in humans (Calesnick et al, 1966; Cutler et al, 1974; Beermann et al, 1975; Perez et al, 1979), and animals (Wallin et al, 1976; Yakatan et al, 1976; Sorgel et al, 1978). Of the excreted dose of up to 100 mg/kg, approximately 80% is unchanged frusemide and its glucuronide (Haussler and Hajdu, 1964; Seno et al, 1969; Yakatan et al, 1976; Perez et al, 1979) but there have been conflicting reports about other metabolites. Some investigators have been unable to isolate any metabolites other than the glucuronide (Calesnick et al, 1966; Beermann et al, 1975; Branch et al, 1977) but others have indicated that some of the administered frusemide is metabolised to the defurfuryl metabolite, (4-chloro-5-sulphamoyl anthranilic acid; saluramine); for a review see Benet (1979).

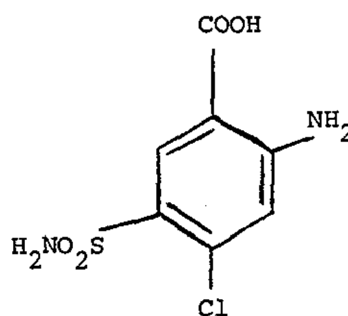
After administration of doses in excess of 100 mg/kg frusemide has been shown to produce hepatic and also possibly renal necrosis (see Mitchell and Jollow, 1975). The most likely cause of these toxic symptoms being

the possible formation of a metabolite by epoxidation of the furan ring. However, this possible route of metabolism has not been found at lower doses and therefore frusemide is thought to be a safe drug at normal doses of less than 1 mg/kg.

The apparent half-life of elimination of frusemide has been shown to be about 1 hour in normal humans (Benet, 1979) and in animals (Wallin et al, 1976; Data et al, 1978). In subjects with impaired renal function the half-life has been shown to increase and half-lives of up to 14 hours have been observed (Rupp et al, 1971; Tilstone and Fine, 1978).




Frusemide




Saluramine

Blood sampling

In addition to the normal blood sampling procedure, blood samples (1 ml) were withdrawn also from a femoral vein at predose 6.5 hours and 24 hours and dispensed into non-heparinised tubes. These samples were assayed for sodium and potassium levels by flame photometry.

Measurement of concentrations of frusemide in plasma

Plasma concentrations of frusemide were assayed by the spectrophotofluorimetric method of Hajdu and Haussler (1964). Plasma (1 ml) was acidified with concentrated hydrochloric acid (100 µl) and shaken with redistilled diethyl ether (6 ml) for 5 minutes using a mechanical shaker (Griffin and George, London, UK). After centrifugation, the

ether phase (4 ml) was transferred to a pointed centrifuge tube and evaporated to dryness under a stream of nitrogen at 35°C. The residue was redissolved in hydrochloric acid (2 ml, 0.02M) and the fluorescence of the acid solution was measured in an Aminco Bowman spectrophotofluorimeter at excitation and emission wave-lengths of 275 nm and 415 nm respectively. The fluorimeter was calibrated using a standard solution of frusemide 40 μg/ml in 0.02M hydrochloric acid and reference standards of frusemide in control non-human primate plasma in the range 2-60 μg/ml were taken through the analysis procedure with each group of 'test' plasma samples. Quantitation of plasma levels of frusemide was achieved by comparison of 'test' plasma samples to a calibration curve constructed from the reference plasma standards.

TABLE 5:1

Method parameters for the measurement of concentrations of frusemide in plasma.

| | |
|---|---|
| Linear range | 2-60 μg/ml |
| Calibration line[a] | Y = 1.5400X + 3.3204 |
| Accuracy | ± 6% at 60 μg/ml and ± 52% at 5μg/ml |
| Precision | ± 8.8% at 20 μg/ml |
| | ± 11.2% at 5 μg/ml |
| Recovery | 95% ± 2.7 SD |
| Sensitivity | 2.0 μg/ml |

a Least squares regression line where Y = % fluorescence and X = concentration of frusemide in plasma.

Measurement of concentrations of sodium and potassium in serum.

Using an IL 343 Flame Photometer. Serum concentrations of sodium and potassium in the rhesus monkeys were measured using an IL 343 flame photometer (Instrumentation Labs., Altrincham, Cheshire, UK). Serum was diluted automatically (1: 200 v/v) with distilled water containing lithium (150 mEq/litre) as internal standard. The aspiration rate of the diluted sample was 1.5 ml/minute and the instrument was calibrated using standard solutions containing 140 mEq/litre of sodium and 5.0 mEq/litre of potassium.

Using a Technicon SMA 12/60. Concentrations of sodium and potassium in serum of the cynomolgus monkey and the baboon were determined using a Technicon SMA 12/60 (Technicon Instruments Co. Ltd., Basingstoke,UK). Serum was analysed on this instrument also by flame photometry using lithium (150 mEq/litre in water) as internal standard. The sample size was 350 µl for a full profile of 10 tests, two of which were for sodium and potassium. The instrument was calibrated using serum (Wellcome Reagent Ltd., Beckenham, Kent) of known sodium and potassium concentrations.

The author is grateful to the members of the department of Clinical Pathology for the use of their equipment and for analysing the serum sodium and potassium concentrations.

Results

Plasma concentrations. After a single intravenous injection of frusemide at a level of 3.0 mg/kg,peak mean plasma concentrations of frusemide (31.6 µg/ml ± 3.2 SD, 33.6 µg/ml ± 1.1 SD and 43.6 µg/ml ± 5.5 SD in the rhesus monkey, the cynomolgus monkey and the baboon respectively) were reached at 2 minutes post administration (the first time of blood withdrawal). After an apparent rapid distribution phase, mean plasma concentrations declined with an apparent half-life of elimination of

approximately 20 minutes, to almost the limit of detection (2μg/ml) at 1-1.5 hours after administration (Table 5:3, Figures 5:1 - 5:3). Results of sodium and potassium analysis (Table 5:2) at 6.5 hours and 24 hours post-administration showed that with respect to these ions, serum levels were similar at those times after administration to those before dosing.

Pharmacokinetic parameters. Observed plasma concentrations of frusemide following a single intravenous injection of frusemide at a level of 3.0 mg/kg were fitted by a biexponential equation (Equation 2:2) but the distribution 'α' phase was very short (<10 minutes) in all three species, and in the rhesus monkey only the first sample taken after dosing did not fit a log-linear regression of the data. However, the non-linear least squares curve fitting programme BLUD does not allow the independent selection of a pharmacokinetic model and so the parameters shown in Table 5:4 were calculated using equations 2:3 - 2:12 for a two-compartment open model with elimination from the central compartment (Scheme 2:2). Plasma concentrations of frusemide predicted by the two-compartment model were generally in good agreement with the observed values (Figures 5:1 - 5:3). However, the distribution phase, if any, was so rapid, pharmacokinetic parameters were also estimated assuming a one-compartment open model (Table 5:5). Nevertheless, data for the two-compartment open model are presented also, for comparison with that published in the literature for man and many animal species. Comparison of Tables 5:4 and 5:5 shows that there were no notable differences in the parameters calculated assuming either of the two models except perhaps in '$K_{el}$' the elimination rate constant.

The mean areas under the plasma concentration-time curves were 13 μg/ml/hour ± 4 SD, 18 μg/ml/hour ± 5 SD and 14 μg/ml/hour ± 2 SD for the rhesus monkey, the cynomolgous monkey and the baboon respectively. Analysis of variance showed that the areas under the curves for the cynomolgous monkey were significantly higher than in the other two species (P<0.05 Newman Keuls multiple comparison procedure). There was no significant difference (P< 0.05) between the rhesus monkey and the baboon.

The mean total volume of distribution at steady state was similar for the rhesus monkey and the cynomolgus monkey accounting for 10.6% and 11.4% of the total bodyweight respectively. However, although the mean total volume of distribution in the baboon (8.7% of the total bodyweight) was not significantly different from that for the rhesus monkey ($P > 0.05$ Newman-Keuls multiple comparison procedure) it was significantly different from that for the cynomolgus monkey ($P < 0.05$). It is doubtful that this difference has any physiological importance as the volume difference is so small. The mean apparent volume of distribution of frusemide in these non-human primates is equivalent to 2-3 times the plasma volume and is similar to data from man (6.9-21% of bodyweight, Benet, 1979), the rat (16% of bodyweight, Wallin et al, 1977) and the dog (11% of bodyweight, Data et al, 1978). This restricted distribution volume is probably related to a high degree of plasma-protein binding, 96-98% in man, Beerman and Groschinsky-Grind, 1980.

Adopting a two-compartment model, the mean volume of the central compartment was of the same order of magnitude as that of the peripheral compartment in each species (Table 5:4). The compartment volume ratio (central:peripheral)was 1.5, 1.0 and 1.5 in the rhesus monkey, the cynomolgus monkey and the baboon respectively indicating that there was some, but not extensive distribution into the peripheral compartment. This is also implied by the similarity of the volume of distribution estimated after attainment of pseudo-distribution equilibrium $V_{d(\beta)}$ to that of the volume of distribution at steady state $V_{d(ss)}$ (Table 5:4).

Analysis of variance showed that the clearance of frusemide in the cynomolgus monkey was significantly lower than that from the other two species ($P < 0.01$ Newman-Keuls multiple comparison procedure). When adjusted for differences in bodyweight the adjusted clearance of frusemide was 4.0 ml/minute/kg, 2.9 ml/minute/kg and 3.7 ml/minute/kg in the rhesus monkey, the cynomolgus monkey and the baboon, respectively.

Analysis of variance of adjusted clearances showed that the cynomolgus monkey remained significantly different from the rhesus monkey ($P < 0.05$ Newman-Keuls multiple comparison procedure) but was not significantly different from that in the baboon ($P > 0.05$).with respect to this parameter.

The mean rate constant for the rapid distributional phase (Table 5:4) for a two-compartment model corresponded to an apparent half-life of 1.5 minutes, 1.7 minutes and 3.0 minutes for the rhesus monkey, the cynomolgus monkey and the baboon, respectively. The mean biological half-life of frusemide in the baboon 15 minutes $\pm$ 15 SD was significantly shorter($P < 0.01$ Newman Keuls multiple comparison procedure) than in the cynomolgus monkey but not from that in the rhesus monkey ($P. > 0.05$).

Adopting a two-compartment open model the mean ratio $\beta/K_{el}$ of 0.45,0.45 and 0.53 for the rhesus monkey, the cynomolgus monkey and thebaboon respectively, indicates that these fractions of frusemide in the body were in the central compartment and were available for elimination at any time after distribution equilibrium (Gibaldi et al, 1969).

Simulation of the plasma concentration time profile of frusemide in the peripheral compartment for a two-compartment open model with pharmacokinetic parameters shown in Table 5:4, showed that following intravenous administration, equilibrium between the central and peripheral compartments was rapidly achieved, peak concentrations of 23.9 µg/ml, 20 µg/ml and 18.3 µg/ml for the rhesus monkey, the cynomolgus monkey and the baboon occurring 5-10 minutes after injection (Table 5:5, Figures 5:1 - 5:3). Concentrations of frusemide in the central and peripheral compartments were similar at all times after distribution was complete.

Discussion

For man the decline of plasma concentrations of frusemide has been described by a tri-exponential equation (Rupp and Hajdu, 1970) but more generally frusemide kinetics appear to be described in terms of two-compartment kinetics (Kelly et al, 1974; Beermann et al, 1975; Andreasen et al, 1978; Andreasen and Mikkelsen, 1977; Tilstone and Fine, 1978) and occasionally in terms of a single-compartment-open model (Cutler et al, 1976). In animals also the disposition of frusemide has been described in terms of single and multicompartment models (Wallin et al, 1976; Data et al, 1978; Yakatan et al, 1979).

In non-human primates the distribution phase, if any, was short and pharmacokinetic parameters estimated for a one-compartment open model were not notably different from those estimated for a two-compartment open model, except, perhaps in '$K_{el}$'. Yakatan et al (1979) assumed one-compartment kinetics to describe the disposition of frusemide in the rhesus monkey after oral administration. In this study, the distributional phase $A/\alpha$, contributed 15, 28 and 25% to the total area under the plasma concentration-time curve in the rhesus monkey, the cynomolgus monkey and the baboon respectively, indicating that distribution into the tissues is a feature of frusemide disposition. However, these values are possibly biased by rather high predictions of the zero time intercept of the $\alpha$ phase.

Values of the systemic clearance of frusemide in animals reported in the literature are variable. Yakatan et al (1979) reported a value of 43 ml/minute for the dog and 38 ml/minute for the rhesus monkey, but Data et al (1978) reported a very high value of 246 ml/minute. The systemic clearance of frusemide in the rat is quoted as 1.74 ml/minute (Wallin et al, 1976) and in man frusemide clearance has been reported to range from 112 ml/minute (Tilstone and Fine, 1978) to 268 ml/minute Homeida et al, 1977) with an average value of 165 ml/minute. With respect to the systemic clearance of frusemide, therefore, the non-human primate is closer to man (Cl = 2.4 ml/minute/kg bodyweight) than are the other commonly used laboratory animal species.

In general, the apparent half-life of elimination of frusemide from plasma appears to be shorter when estimated by spectrofluorimetric procedures than when assayed by procedures involving chromatography. For man, average values are 44 minutes and 58 minutes when estimated by these procedures respectively, (Benet, 1979) and the range of values is represented by a half-life value of 26 minutes using a spectrofluorimetric assay (Kelly et al, 1974) and a half-life value of 72 minutes using a spectrofluorimetric method following a thin layer chromatographic separation (Andreason and Mikkelsen, 1977). The half-life of frusemide in the dog and the rat, 22 minutes using high pressure liquid chromatography and 25 minutes using $^{35}$S-frusemide respectively (Data et al, 1978; Wallin et al, 1976) are closer to those in the non-human primate species than to that in humans.

Yakatan et al (1979) using $^{35}$S-frusemide observed a slow elimination phase with a half-life of approximately 7 hours in the dog, but this value was obtained by fitting a three exponential equation to the plasma concentration time curve and the terminal phase was not observed until plasma concentrations were less than 0.2 µg/ml. The major portion of the disposition curve had a half-life of approximately 30 minutes, similar to that found by Data et al, (1978). In the rhesus monkey, Yakatan et al (1979) reported a half-life of 11 hours for frusemide after an oral dose of 5 mg/kg of $^{35}$S-frusemide. However, this half-life value might not be typical as the data was obtained from a group of animals and a complete concentration-time profile was not obtained in any one animal.

Distribution studies of $^{35}$S-frusemide in animals (Seno et al, 1969; Yakatan et al, 1979) and in humans (Calesnick et al, 1966; Beermann et al, 1975) indicate that frusemide might be sequestered somewhere in the body and accumulate during the once-daily dosing regime common to most chronic toxicity studies. However, the major portion of the dose has a short half-life in plasma and approximately 80% of the administered dose is excreted in the first 24 hours.

There is no evidence, from this study, to suggest that frusemide would accumulate in the body of the non-human primate during a once-daily dosing regimen. In man, also, receiving a normal daily dose of frusemide (80 mg once daily) for the treatment of oedema in congestive heart failure, for example, it is unlikely that frusemide would accumulate in the body. However, an average steady state plasma concentration of frusemide of approximately 0.4 μg/ml would be maintained with such a regimen. To establish this same steady state plasma concentration of frusemide in non-human primate species it would require the administration of frusemide 0.2 mg intravenously every 26 minutes to rhesus monkeys, 0.1 mg intravenously every 28 minutes to cynomolgus monkeys and 0.1 mg intravenously every 14 minutes to baboons, or alternatively, the intravenous administration of a total of 11.1 mg, 5.1 mg and 10.3 mg daily to the respective species, given in equal amounts at intervals of approximately seven half-lives.

Comparison of the relatively small extent of metabolism of frusemide in different animal species would not provide the best criteria for the selection of a suitable species for chronic toxicity studies of this drug, but rather a comparison of the drug's pharmacokinetics would be more appropriate. However, when selecting a species for study, due allowance should be made for the reported hepatotoxicity and nephrotoxicity at high dose levels in rodents (Mitchell and Jollow, 1975). Using pharmacokinetic criteria therefore, the fate of frusemide in humans (Benet et al, 1979) is more closely reflected by that in the cynomolgus monkey after intravenous injection than by that in the rhesus monkey, the baboon or other laboratory animal species (Wallin et al, 1976; Data et al, 1978), however, this might not be true after oral administration (Yakatan et al, 1979). As frusemide is known to be incompletely absorbed from the G.I. tract it would be advisable to monitor changes in frusemide pharmacokinetics that might be produced by changes in the rate and extent of absorption.

TABLE 5:2

Mean serum sodium and potassium levels (± SD) in rhesus monkeys, cynomolgus monkeys and baboons before and after injection of frusemide 3.0 mg/kg.

| | Species | | |
|---|---|---|---|
| | Rhesus monkey[a] | Cynomolgus monkey[b] | Baboon[b] |
| Sodium<br>Time | | | |
| Predose | 145 ± 1.3 | 146 ± 2.6 | 146 ± 3.4 |
| 6.5 hour | 139 ± 2.9 | 143 ± 1.9 | 143 ± 3.9 |
| 24 hour | 145 ± 2.7 | 144 ± 1.9 | 145 ± 1.9 |
| Potassium<br>Time | | | |
| Predose | 4.0 ± 0.3 | 4.9 ± 0.3 | 5.4 ± 0.6 |
| 6.5 hour | 4.0 ± 0.1 | 5.0 ± 0.9 | 4.9 ± 0.3 |
| 24 hour | 4.0 ± 0.3 | 5.1 ± 0.9 | 5.2 ± 0.2 |

a Normal range sodium 136 - 159 mEq/litre
potassium 3.4 - 5.4 mEq/litre

b Normal range sodium 137-155 mEq/litre
potassium 3.7 - 5.5 mEq/litre

TABLE 5:3

Mean concentrations (± SD) of frusemide in the plasma of the rhesus monkey, the cynomolgus monkey and the baboon after a single bolus intravenous injection of frusemide at a level of 3.0 mg/kg. Results are expressed in μg/ml.

| Time | | Species | | |
|---|---|---|---|---|
| | | Rhesus monkey | Cynomolgus monkey | Baboon |
| 2 | minute | 31.6 ± 3.2 | 33.6 ± 1.1 | 43.6 ± 5.5 |
| 5 | minute | 21.8 ± 5.3 | 26.4 ± 3.0 | 29.6 ± 1.2 |
| 10 | minute | 17.4 ± 4.0 | 19.4 ± 3.6 | 21.6 ± 2.1 |
| 20 | minute | 11.4 ± 3.0 | 16.2 ± 2.8 | 13.4 ± 2.1 |
| 40 | minute | 5.2 ± 2.7 | 9.2 ± 3.4 | 4.0 ± 1.0 |
| 1 | hour | 3.4 ± 1.1 | 5.0 ± 2.2 | ND - |
| 1.5 | hour | ND - | 2.6 ± 0.9 | ND - |
| 2 | hour | ND - | ND - | |

ND Less than the limit of detection (2.0 μg/ml)

TABLE 5:4

Mean pharmacokinetic parameters (± SD) determined for a two-compartment open model, after bolus intravenous injection of frusemide 3.0 mg/kg in the rhesus monkey, the cynomolgus monkey and the baboon. The data are presented as means of results from individual animals.

| Parameter[a] | | Species | | |
|---|---|---|---|---|
| | | Rhesus monkey | Cynomolgus monkey | Baboon |
| Bodyweight | (kg) | 4.7 ± 0.8 | 3.5 ± 0.4 | 5.7 ± 0.9 |
| Dose | (mg) | 14.0 ± 2.7 | 10.4 ± 0.9 | 17.1 ± 2.7 |
| Dose | (mg/kg) | 3.0 ± 0.1 | 3.0 ± 0.1 | 3.0 ± 0.0 |
| A | (μg/ml) | 35 ± 14 | 31 ± 17 | 48 ± 24 |
| B | (μg/ml) | 22 ± 4 | 24 ± 4 | 31 ± 6 |
| $\alpha$ | ($hour^{-1}$) | 26.978 ± 18.701 | 24.320 ± 20.953 | 13.991 ± 13.263 |
| $\beta$ | ($hour^{-1}$) | 2.090 ± 0.607 | 1.436 ± 0.642 | 2.753 ± 0.565 |
| $K_{12}$ | ($minute^{-1}$) | 0.213 ± 0.187 | 0.191 ± 0.209 | 0.075 ± 0.114 |
| $K_{21}$ | ($minute^{-1}$) | 0.194 ± 0.120 | 0.185 ± 0.136 | 0.110 ± 0.100 |
| $K_{el}$ | ($minute^{-1}$) | 0.078 ± 0.028 | 0.053 ± 0.034 | 0.085 ± 0.046 |
| AUC | (μg/ml/hour) | 13 ± 4 | 18 ± 5[b] | 14 ± 2 |
| Cl | (ml/minute) | 19 ± 4 | 10 ± 4[b] | 21 ± 3 |
| $t_{½}$ | (minutes) | 22 ± 8 | 29 ± 9[c] | 15 ± 4 |
| $V_1$ | (litres) | 0.3 ± 0.1 | 0.2 ± 0.1 | 0.3 ± 0.1 |
| $V_2$ | (litres) | 0.2 ± 0.0 | 0.2 ± 0.1 | 0.2 ± 0.2 |
| $V_{d(ss)}$ | (litres) | 0.5 ± 0.1 | 0.4 ± 0.1 | 0.5 ± 0.2 |
| $V_{d(\beta)}$ | (litres) | 0.6 ± 0.1 | 0.4 ± 0.2 | 0.5 ± 0.1 |
| $A/\alpha$ | (μg/ml/hour) | 2 ± 2 | 5 ± 8 | 4 ± 5 |
| $B/\beta$ | (μg/ml/hour) | 11 ± 3 | 16 ± 3 | 11 ± 2 |

[a] For details of abbreviations, see Chapter 2.

[b] Significance level (analysis of variance) cynomolgus monkey compared to the other two species ($P < 0.05$).

[c] Significance level (analysis of variance) cynomolgus monkey compared to the baboon ($P < 0.01$).

TABLE 5:5

Pharmacokinetic parameters of frusemide in the rhesus monkey, the cynomolgus monkey and the baboon for a one-compartment open model compared to those for a two-compartment open model following a single intravenous injection of frusemide (3.0mg/kg). The data are presented as means of results from individual animals.

| Parameter[a] | Model and Species | | | | | |
|---|---|---|---|---|---|---|
| | One-compartment | | | Two-compartment | | |
| | Rhesus monkey | Cynomolgus monkey | Baboon | Rhesus monkey | Cynomolgus monkey | Baboon |
| AUC(μg/ml/hour) | 14.1 | 18[b] | 12.4 | 13 | 18[b] | 14 |
| Cl (ml/minute) | 18.5 | 10[b] | 23.5 | 19 | 10[b] | 21 |
| $t_{½}$ (minutes) | 26 | 28[c] | 14 | 22 | 29[c] | 15 |
| $V_d$ (litres) | 0.7 | 0.4 | 0.5 | 0.5 | 0.4 | 0.5 |
| $K_{el}$ ($minute^{-1}$) | 0.026 | 0.026 | 0.050 | 0.078 | 0.053 | 0.085 |

a For details of abbreviations, see Chapter 2.

b Significance lecel (analysis of variance) cynomolgus monkey compared to the other two species ($P < 0.05$).

c Significance level (analysis of variance) cynomolgus monkey compared to the baboon ($P < 0.01$).

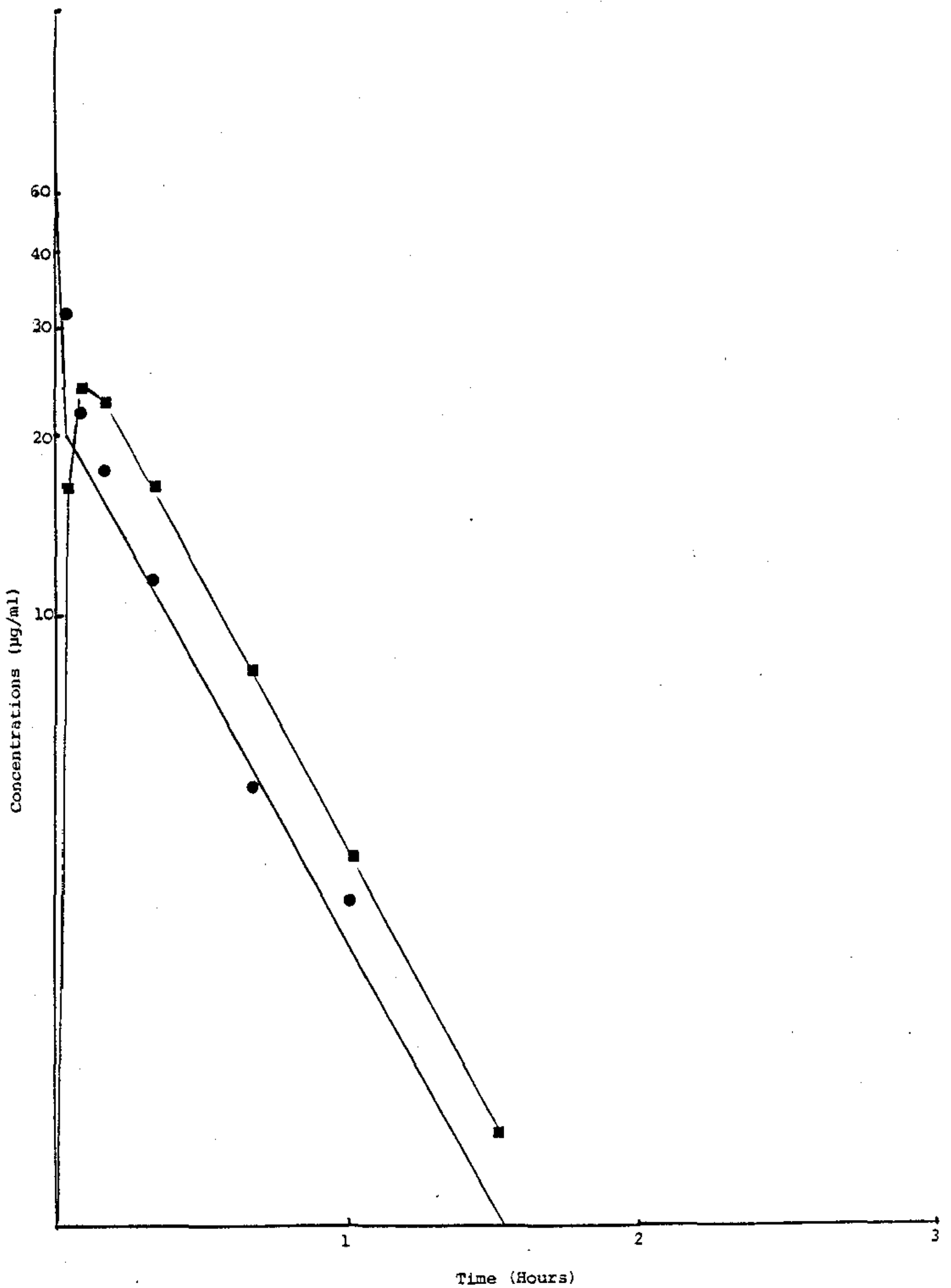
$V_d$ Total volume of distributes $= V_{d(ss)}$ for the two-compartment model.

TABLE 5:6

Calculated concentrations of frusemide in the peripheral compartment after bolus intravenous injection of frusemide 3.0 mg/kg to non-human primates. Results calculated from mean data and expressed as μg/ml.

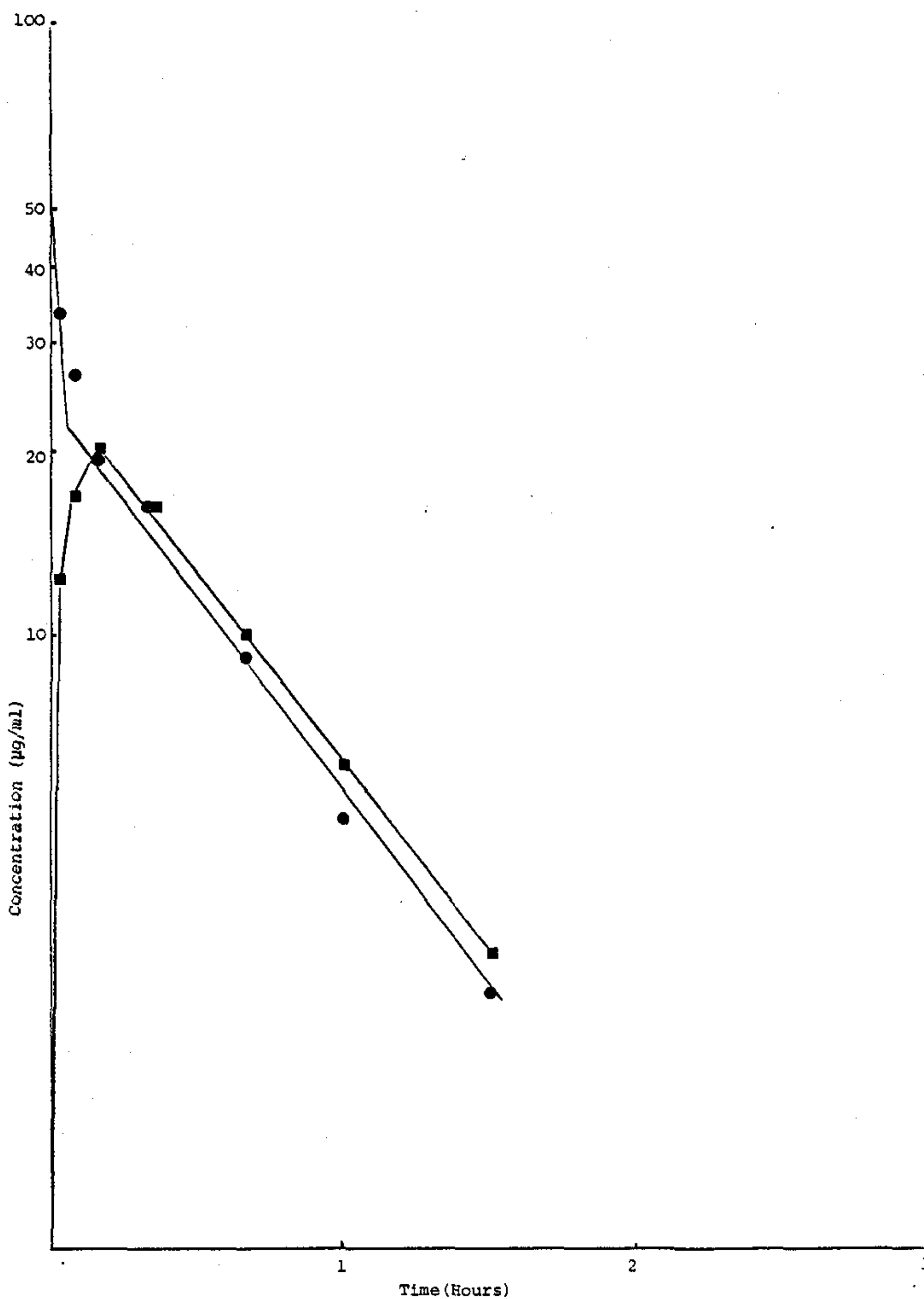
| Time | Species | | |
|---|---|---|---|
| | Rhesus monkey | Cynomolgus monkey | Baboon |
| 2 minute | 16.2 | 12.4 | 9.0 |
| 5 minute | 23.9 | 16.9 | 16.3 |
| 10 minute | 22.6 | 20.0 | 18.3 |
| 20 minute | 16.4 | 16.2 | 13.6 |
| 40 minute | 8.1 | 10.1 | 5.5 |
| 60 minute | 4.0 | 6.2 | 2.3 |
| 90 minute | 1.4 | 3.0 | |

FIGURE 5:1



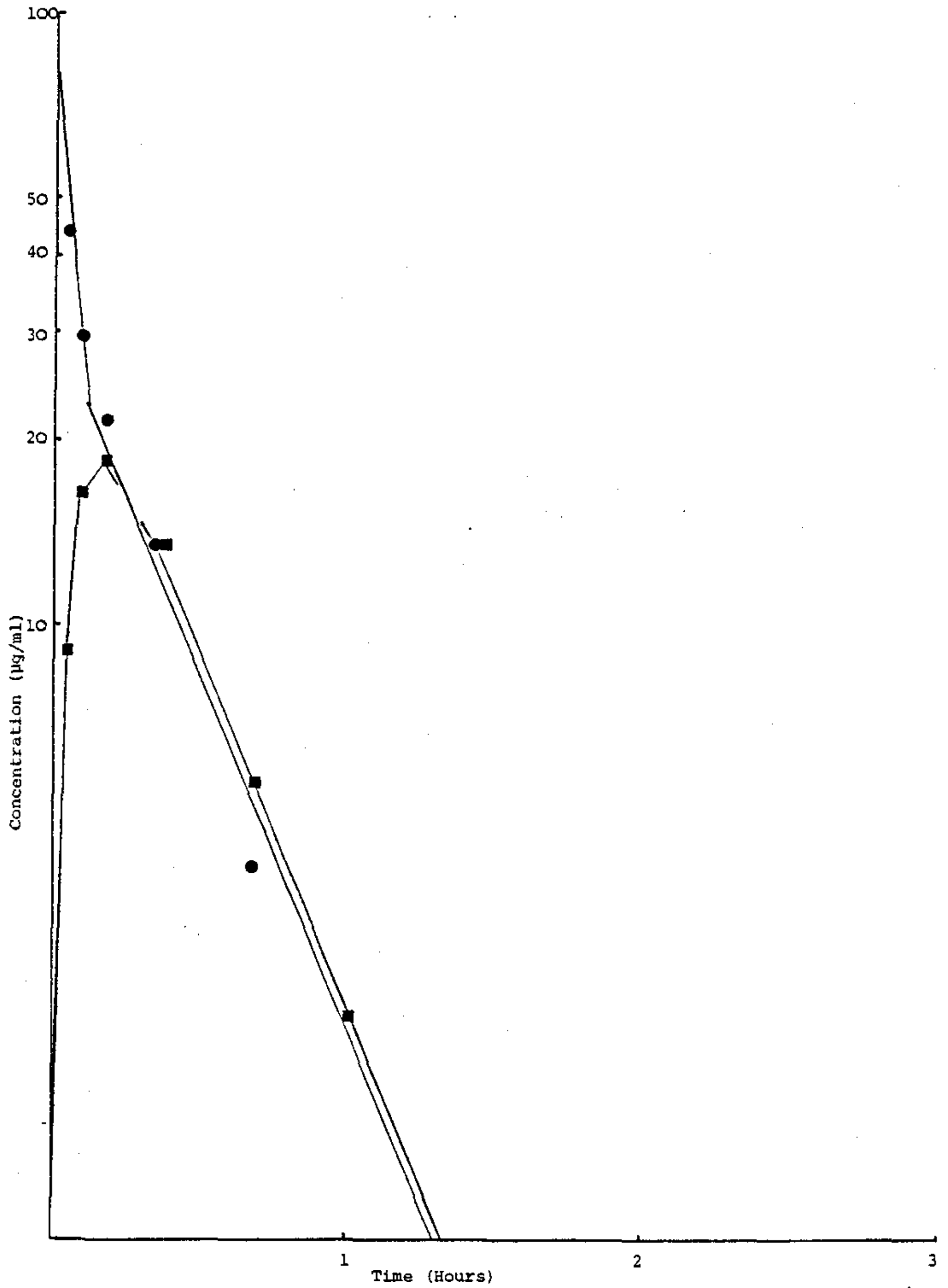

Mean concentration-time curves after a single intravenous injection of frusemide (3.0 mg/kg) to rhesus monkeys. Observed concentrations in the central compartment ( ● ● ), estimated concentrations in the central compartment (solid line) and in the peripheral compartment ( ■ ■ ).

FIGURE 5:2




Mean concentration-time curves after a single intravenous injection of frusemide (3.0 mg/kg) to cynomolgus monkeys. Observed concentrations in the central compartment ( ● ● ), estimated concentrations in the central compartment (solid line) and in the peripheral compartment ( ■ ■ ).

FIGURE 5:3




Mean concentration-time curves after a single intravenous injection of frusemide (3.0 mg/kg) to baboons. Observed concentrations in the central compartment ( ● ● ), estimated concentrations in the central compartment (solid line) and in the peripheral compartment ( ■ ■ ).

CHAPTER 6

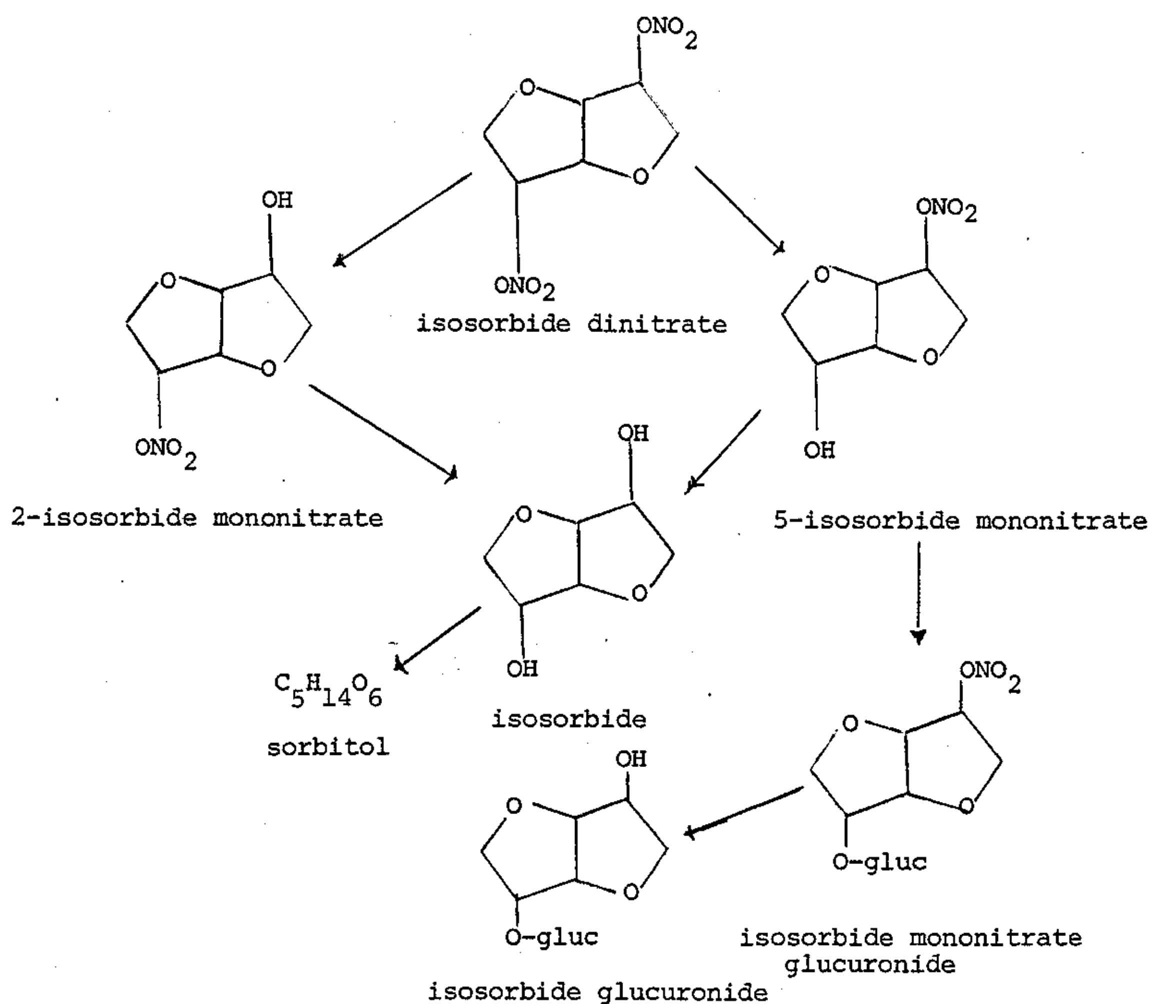
# ISOSORBIDE DINITRATE

## Introduction

Isosorbide dinitrate (1,4:3,6-dianhydrosorbitol-2,5-dinitrate) is an organic nitrate antianginal agent (for reviews see Needleman, 1975; Rudolph and Siegenthaler, 1976; Rudolph and Schrey, 1980) with a high hepatic extraction ratio (Needleman et al, 1972).

This lipophilic drug is almost completely eliminated by biotransformation being rapidly denitrated (Johnson et al, 1972; Needleman et al, 1972) by glutathione-S-transferases (Keen et al, 1976). The urinary metabolites of isosorbide dinitrate in animals (Dietz, 1967; Reed et al, 1971; Sisenwine and Ruelius, 1971; Rosseel and Bogeart, 1973) and humans (Down et al, 1974; Down and Chasseaud, 1980) are the corresponding isomeric mononitrates (1,4:3,6-dianhydrosorbitol-2-hydroxy-5-nitrate and 1,4:3,6 - dianhydrosorbitol-5-hydroxy-2-nitrate) and isosorbide which are excreted free and probably partly conjugated with glucuronic acid. Sorbitol has also been shown to be a urinary metabolite of isosorbide dinitrate in humans (Down et al, 1974).

There has been little reported regarding the pharmacokinetics of the drug, probably due to the difficulty in measuring low concentrations in plasma, and most of the early data has been obtained using $^{14}C$-isosorbide dinitrate. The recent development of adequately sensitive analytical methods has now permitted studies of the pharmacokinetics of isosorbide dinitrate in humans (e.g. Assinder et al, 1977; Chasseaud and Taylor, 1980; Taylor et al, 1980).




Scheme 6:1 Possible biotransformation of isosorbide dinitrate in humans (gluc represents glucuronic acid).

Measurement of concentrations of isosorbide dinitrate in plasma

Concentrations of isosorbide dinitrate in plasma were measured by means of a gas chromatographic method using electron capture detection (Doyle et al, 1980 ). Samples of plasma were assayed according to one of three different procedures depending on concentration of isosorbide dinitrate. These procedures covered the concentration ranges 1 - 10 ng/ml, 10-250 ng/ml and 100-1000 ng/ml.

Range 1-10 ng/ml. Plasma (1 ml) was mixed with internal standard (glyceryl trinitrate 5 ng, 5 µl of a 1 µg/ml solution in ethyl acetate) and extracted with freshly redistilled petroleum spirit (B.P. <48°C) (10 ml) by shaking the mixture using a mechanical shaker (Griffin and George, London U.K.) for 15 minutes. After centrifugation, the organic phase (9 ml) was transferred to a pointed centrifuge tube and evaporated to a volume of approximately 50µl under a stream of nitrogen at 35°C. Portions of the residual solution (1-2 µl) were injected into the gas chromatograph.

Range 10-250 ng/ml. Plasma (0.2 ml) was mixed with internal standard (glyceryl trinitrate 25 ng, 5µl of a 5µg/ml solution in ethyl acetate) and extracted with petroleum spirit (5 ml) by shaking the mixture mechanically for 15 minutes. After centrifugation the organic phase (4 ml) was transferred to a pointed centrifuge tube and evaporated to an approximate volume of 200µl under a stream of nitrogen, at 35°C. Portions of the residual solution (1-2 µl) were injected into the gas chromatograph.

Range 100-1000ng/ml. Plasma (0.1 ml) was mixed with internal standard (glyceryl trinitrate, 50 ng, 10µl of a 5µg/ml solution in ethyl acetate) and extracted with petroleum spirit (2 ml) by shaking on a rotary shaker (Fisons Scientific Apparatus Ltd., Loughborough Leicestershire, U.K.) for 2 minutes. After centrifugation, the organic phase 1.5 ml was transferred to a pointed centrifuge tube and evaporated to an approximate volume of 0.4 ml. Portions of the residual solution (1-2 µl) were injected into the gas chromatograph.

After extraction, all residues were kept at -20°C to avoid the formation of large unidentified peaks that interfered with the assay.

The gas chromatographic column was glass 1.83 m x 2 mm i.d. packed in the 3.5% QF 1 on gas chrom Q 60-80 mesh (Phase Separations Limited, Queensferry, Clwyd, U.K.). The injection port temperature was 150°C, the detector temperature was 180°C and the oven temperature was 120°C. The carrier gas was oxygen free nitrogen at a flow rate of 60 ml/minute.

TABLE 6:1

Method parameters for the measurement concentrations of isosorbide dinitrate in plasma.

| Parameter/Range | 1-10 mg/ml | 10-250 mg/ml | 100-1000 mg/ml |
|---|---|---|---|
| Calibration line[a] | Y=(0.0790±0.0011)X | Y=0.0031X-0.0275 | Y=0.0006X-0.0652 |
| Accuracy | ± 100% at 1ng/ml<br>± 10% at 10 ng/ml | ± 6% at 250ng/ml | ± 18% at 1000ng/ml |
| Precision | ± 8% at 10 ng/ml | ± 5% at 250 ng/ml | ±2.4% at 1000ng/ml |
| Sensitivity | 0.5 ng/ml | - | - |
| Recovery of glyceryl tri-nitrate (± SD) | 90%±4 at 5ng/ml | 91%±3 at 125ng/ml | 91%±3 at 500ng/ml |
| Recovery of isosorbide di-nitrate (± SD) | 90%±2 | 90%±4 | 83%±4 |

[a] Least squares regression time where Y = instrument response (peak height ratio $\frac{\text{isosorbide dinitrate}}{\text{glyceryl trinitrate}}$) and X = plasma concentration of isosorbide dinitrate.

Results

Plasma concentrations. After a single intravenous injection of isosorbide dinitrate at a level of 1.0 mg/kg mean plasma concentrations at the first time of blood withdrawal (2 minutes after injection) were similar for the rhesus monkey and the cynomolgus monkey, 565 ng/ml $\pm$ 66SD and 586 ng/ml $\pm$ 43SD respectively. In the baboon at the first time of blood withdrawal (2 minutes after injection) mean plasma concentrations of isosorbide dinitrate (1572 ng/ml $\pm$ 253SD) were approximately three-fold higher than in the other two species. In the cynomolgus monkey and the baboon, after an initial short distribution phase, mean plasma concentrations declined with an apparent half-life of elimination of about 25 minutes (Table 6:2, Figures 6:1 - 6:3) to almost the limit of detection ( $<$ 1.0 ng/ml) at 4 hours after administration. In the rhesus monkey the distribution phase was slightly longer than in the other two species and the apparent half-life of elimination was also longer (about 60 minutes). Isosorbide dinitrate was still detectable in the plasma of the rhesus monkey (10ng/ml) 4.5 hours after injection (the last time of blood withdrawal).

Pharmacokinetic parameters. Observed plasma concentrations of isosorbide dinitrate were adequately described by a biexponential equation (equation 2:2). The pharmacokinetic parameters shown in Table 6:3 were, therefore, calculated using equations 2:3 - 2.12 for a two-compartment open model with elimination from the central compartment (scheme 2:2). Plasma concentrations of isosorbide dinitrate predicted by the two-compartment model were generally in good agreement with the observed values (Figures 6:1 - 6:3). However, since the distribution phase was short, the pharmacokinetic parameters were also calculated using a one-compartment open model (equation 2:1). There were no notable differences in parameters calculated assuming a one-compartment model (equations 1:1, 2:4, 2:13, 2:14) from those for the two-compartment model except in $K_{el}$ the elimination rate constant (Table 6:4). However, $K_{el}$ obtained for a one-compartment open model is almost identical to $\beta$ calculated for a two-compartment open model.

Analysis of variance of areas under the plasma concentration time curves showed that in respect of this parameter the cynomolgus monkey was significantly different from that for the other two species ($P < 0.01$ Newman-Keuls multiple comparison procedure) and that the rhesus monkey was significantly different from that for the baboon ($P < 0.05$ Newman-Keuls multiple comparison procedure).

Analysis of variance of volumes of distribution and of apparent half-lives of elimination showed, also, that the volume of distribution was significantly larger, and that the half-life was significantly longer in the rhesus monkey than in the other two species ($P < 0.001$ Newman-Keuls multiple comparison procedure). However, analysis of variance of clearances of isosorbide dinitrate showed that in this respect these species of non-human primate were similar ($P > 0.05$ Newman-Keuls multiple comparison procedure).

The mean total volume of distribution varied between species and was equivalent to 273, 168 and 84% of the total bodyweight in the rhesus monkey, the cynomolgus monkey and the baboon respectively. The largervalues found in the rhesus monkey and the cynomolgus monkey are surprising as isosorbide dinitrate does not strongly bind to proteins. However, these observations could be explained by a rapid uptake of the drug into tissue. In the baboon the volume of distribution was not too dissimilar from the probable body water volume. Adopting the two-compartment model, the mean volume of the central compartment was larger than that of the peripheral compartment. In these non-human primates the compartment volume ratio (central:peripheral) was 1.2, 3.1 and 1.6 for the rhesus monkey, the cynomolgus monkey and the baboon respectively, indicating that there was relatively free transfer of isosorbide dinitrate between the two compartments.

The total volume of distribution,estimated after attainment of a pseudo-distribution equilibrium multiplied by the plasma concentration, indicates that only 53, 41 and 59% of the administered drug remained in the body of the rhesus monkey, the cynomolgus monkey and the baboon respectively at 45 minutes, 30 minutes and 15 minutes respectively.

The mean ratio $\beta/K_{el}$ of 0.43, 0.63 and 0.51 for the rhesus monkey, the cynomolgus monkey and the baboon respectively, indicates that these fractions of isosorbide dinitrate in the body were in the central compartment and were available for elimination during the terminal linear phase of the disposition curve (Gibaldi et al, 1969).

Simulation of the plasma concentration-time profile of isosorbide dinitrate in the peripheral compartment of a two-compartment open model with pharmacokinetic parameters shown in Table 6:3 showed that equilibrium between the central and peripheral compartments was rapidly achieved. Peak concentrations of isosorbide dinitrate in the peripheral compartment (258 ng/ml, 394 ng/ml and 727 ng/ml) in the rhesus monkey, the cynomolgus monkey and the baboon respectively, occurred 10-30 minutes after injection. Thereafter concentrations of isosorbide dinitrate were similar in both compartments (Table 6:5, Figures 6:1 - 6:3).

Discussion

There has been little reported regarding the pharmacokinetics of isosorbide dinitrate in animals. This is probably due, in part, to the lack of a suitable analytical method for the assay of this drug. However, the decline of isosorbide dinitrate concentrations in the plasma of the dog (Sisenwine and Ruelius,1971) and man (Assinder et al, 1977; Chasseaud and Taylor, 1980a; Taylor et al, 1980) appears to fit a one-compartment open model but in the rat (Needleman et al, 1972) a two-compartment model appears to be more appropriate. In the non-human primate, although a two-compartment open model seemed suitable, a one-compartment model could also be applied without introducing any important differences in the parameter estimates, except possibly in $K_{el}$. In each species there was at least three mean values (n = 5) describing the distribution phase and the contribution of this phase '$A/\alpha$' to the total area under the plasma concentration time curve was 14, 15 and 18% in the rhesus monkey, the cynomolgus monkey and the baboon respectively. This indicates that the distribution phase of isosorbide dinitrate, albeit short, should be considered in a pharmacokinetic analysis of the drug.

Although clearance of isosorbide dinitrate by enzymes in the intestinal mucosa might take place after oral administration, after intravenous administration the total systemic clearance can probably be regarded as representing hepatic clearance. In rhesus monkeys, the systemic clearance of isosorbide dinitrate was similar to but less than that of the reported hepatic blood flow of 50 ml/minute/kg in this species (Branch et al, 1973) as might be expected for a drug of high hepatic extraction ratio such as isosorbide dinitrate (Needleman et al, 1972). By contrast the systemic clearance of isosorbide dinitrate in humans appears to be about ten-fold lower than hepatic blood flow (Taylor et al, 1980). Despite this species difference in clearance, the drug is subjected to an extensive first-pass effect in both non-human primates and man (unpublished data, cited in Doyle and Chasseaud, 1981; Taylor et al, 1980) as it is in rats also (Needleman et al, 1972).

Adopting a two-compartment model, the mean rate constant for the rapid distribution phase corresponded to an apparent half-life of 5.8 minutes, 4.1 minutes, and 3.2 minutes in the rhesus monkey, the cynomolgus monkey and the baboon respectively. These values are similar to those values previously reported for the elimination half-life of isosorbide dinitrate in man (9 minutes; Taylor et al, 1980), in the dog (7 minutes; Sisenwine and Ruelius, 1971) and in the rat ( <1 minute; Needleman et al, 1972). The latter value for the rat has been disputed by Reed et al (1977) who claimed that it only represented a tissue distribution phase. The mean terminal phase of the $\log_e$- linear plot of concentrations of isosorbide dinitrate in plasma against time, for the rhesus monkey corresponded to an apparent half-life of elimination of 62 minutes and was longer than that for the other two species (23 minutes and 24 minutes in the cynomolgus monkey and the baboon respectively). These latter values are similar to the plasma terminal half-life previously found after oral administration to man (Assinder et al, 1977a; 1977b; Chasseaud et al, 1980c) and now shown to be associated with the absorption of the drug, a "flip-flop mechanism" (Taylor et al, 1980). The longer half-lives observed in these non-human primates might be explained by the deposition of some of the administered dose into a deep

(poorly perfused) compartment from which it is reabsorbed back into the central compartment. Indeed there have been previous reports of isosorbide dinitrate and a related compound, glyceryl trinitrate, apparently diffusing back into the blood from the peripheral compartment (Johnson et al, 1972; Bogeart et al, 1970). However, in humans receiving a four-times daily oral dose of 10 mg of isosorbide dinitrate for prophylactic treatment of angina, isosorbide dinitrate might accumulate due to a slow rate of absorption of the drug. To maintain a steady state plasma concentration of isosorbide dinitrate in non-human primate species of approximately 1 ng/ml it would be necessary to administer this drug intravenously 11.6 μg every 62 minutes to the rhesus monkey, 4.6 μg every 23 minutes to the cynomolgus monkey and 4.1 μg every 24 minutes to the baboon.

These considerations apply to isosorbide dinitrate given intravenously. If given orally, it would be necessary to compensate for losses due to first pass metabolism (approximately 2% bioavailability; Doyle and Chasseaud, 1981; Taylor et al, 1980). In addition, since the drug plasma terminal half-life is associated with absorption (see page 100) this factor would also have to be considered when designing a dosing regimen.

Since similar metabolites of isosorbide dinitrate are produced by animals and humans, a comparison of the relatively straightforward biotransformation of isosorbide dinitrate in different animal species would probably not provide the best criteria for the selection of a suitable species for chronic toxicity studies of the drug, rather a comparison of the drug's pharmacokinetics would be more appropriate. Using pharmacokinetic criteria therefore, the fate of isosorbide dinitrate in humans (Taylor et al, 1980) appears to be more closely reflected by that in baboons (Tables 6:3 and 6:4) than that in either of the other two non-human primate species studied.

TABLE 6:2

Mean concentrations (± SD) of isosorbide dinitrate in the plasma of the rhesus monkey, the cynomolgus monkey and the baboon after a single bolus intravenous injection of isosorbide dinitrate at a level of 1.0 mg/kg. Results are expressed as ng/ml

| Time | Species | | |
|---|---|---|---|
| | Rhesus monkey | Cynomolgus monkey | Baboon |
| 2 minute | 565 ± 66 | 586 ± 43 | 1572 ± 253 |
| 5 minute | 457 ± 120 | 581 ± 88 | 1178 ± 258 |
| 10 minute | 394 ± 70 | 447 ± 71 | 750 ± 34 |
| 15 minute | 305 ± 26 | 374 ± 67 | 599 ± 50 |
| 30 minute | 206 ± 28 | 209 ± 32 | 364 ± 14 |
| 45 minute | 153 ± 6 | 109 ± 28 | 214 ± 44 |
| 1 hour | 110 ± 14 | 65 ± 16 | 145 ± 19 |
| 1.5 hour | 82 ± 15 | 28 ± 8 | 63 ± 5 |
| 2 hour | 51 ± 7 | 11 ± 4 | 27 ± 3 |
| 3 hour | 28 ± 8 | 3 ± 1 | 6 ± 2 |
| 4 hour | 10 ± 5 + | 1 - | 1 - |

+Indicates that this sample was taken at 4.5 hours.

TABLE 6:3

Mean pharmacokinetic parameters (± SD) determined for a two-compartment open model in the rhesus monkey, the cynomolgus monkey and the baboon after a single intravenous injection of isosorbide dinitrate at a level of 1.0 mg/kg. The data are presented as means of the results from individual animals.

| Parameter[a] | | Species | | |
|---|---|---|---|---|
| | | Rhesus monkey | Cynomolgus monkey | Baboon |
| Bodyweight | (kg) | 4.8 ± 0.8 | 3.4 ± 0.4 | 5.8 ± 0.9 |
| Dose | (mg) | 4.6 ± 0.9 | 3.4 ± 0.4 | 5.8 ± 0.9 |
| Dose | (mg/kg) | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.0 ± 0.0 |
| A | (μg/ml | 435 ± 113 | 384 ± 222 | 1139 ± 557 |
| B | (μg/ml) | 220 ± 74 | 427 ± 120 | 843 ± 183 |
| $\alpha$ | ($hour^{-1}$) | 7.123 ± 7.658 | 10.061 ± 7.430 | 12.933 ± 4.500 |
| $\beta$ | ($hour^{-1}$) | 0.703 ± 0.168 | 1.788 ± 0.157 | 1.772 ± 0.256 |
| $K_{12}$ | ($minute^{-1}$) | 0.051 ± 0.066 | 0.040 ± 0.042 | 0.071 ± 0.029 |
| $K_{21}$ | ($minute^{-1}$) | 0.053 ± 0.059 | 0.110 ± 0.084 | 0.117 ± 0.055 |
| $K_{el}$ | (minute) | 0.027 ± 0.006 | 0.047 ± 0.012 | 0.058 ± 0.011 |
| AUC | (μg/ml/hour) | 409 ± 41[c] | 292 ± 47[b] | 575 ± 37 |
| Cl | (ml/minute) | 188 ± 29 | 200 ± 37 | 169 ± 30 |
| $t_{1/2}$ | (minutes) | 62 ± 13[d] | 23 ± 2 | 24 ± 3 |
| $V_1$ | (litres) | 7.1 ± 1.3 | 4.3 ± 0.8 | 3.0 ± 0.7 |
| $V_2$ | (litres) | 6.0 ± 2.4 | 1.4 ± 0.7 | 1.9 ± 0.3 |
| $V_{d(ss)}$ | (litres) | 13.2 ± 2.9[d] | 5.7 ± 0.8 | 4.9 ± 0.8 |
| $V_{d(\beta)}$ | (litres) | 16.0 ± 2.5[d] | 6.7 ± 1.2 | 5.7 ± 1.0 |
| $A/\alpha$ | (μg/ml/hour) | 61 ± 51 | 56 ± 40 | 101 ± 71 |
| $B/\beta$ | (μg/ml/hour) | 318 ± 39 | 237 ± 61 | 474 ± 74 |

a For details of abbreviations see Chapter 2.

b Significance level (analysis of variance) cynomolgus monkey compared with the other two species ($P < 0.01$).

c Significance level (analysis of variance) rhesus monkey compared with the baboon ($P < 0.05$).

d Significance level (analysis of variance) rhesus monkey compared with the other two species ($P < 0.01$).

TABLE 6:4

Mean pharmacokinetic parameters of isosorbide dinitrate in the rhesus monkey, the cynomolgus monkey and the baboon for a one-compartment open model compared to those for a two-compartment open model following a single intravenous injection of isosorbide dinitrate (1 mg/kg). The data are presented as means of results from individual animals.

| Parameter[a] | Model and Species | | | | | |
|---|---|---|---|---|---|---|
| | One-compartment | | | Two-compartment | | |
| | Rhesus monkey | Cynomolgus monkey | Baboon | Rhesus monkey | Cynomolgus monkey | Baboon |
| AUC (μg/ml/hour) | 404[c] | 281[b] | 538 | 409[c] | 292[b] | 575 |
| Cl (ml/minute) | 189 | 203 | 173 | 188 | 200 | 169 |
| $t_{½}$ (minutes) | 47[d] | 29 | 29 | 62[d] | 23 | 24 |
| $V_d$[e] (litres) | 12.2[d] | 8.5 | 7.2 | 13.2[d] | 5.7 | 4.9 |
| $K_{el}$ ($minute^{-1}$) | 0.015 | 0.024 | 0.024 | 0.027 | 0.047 | 0.058 |

a For details of abbreviations, see Chapter 2.

b Significance level (analysis of variance) cynomolgus monkey compared to the other two species ($P < 0.01$).

c Significance level (analysis of variance) rhesus monkey, compared to the baboon ($P < 0.05$).

d Significance level (analysis of variance) rhesus monkey, compared to the other two species ($P < 0.01$).

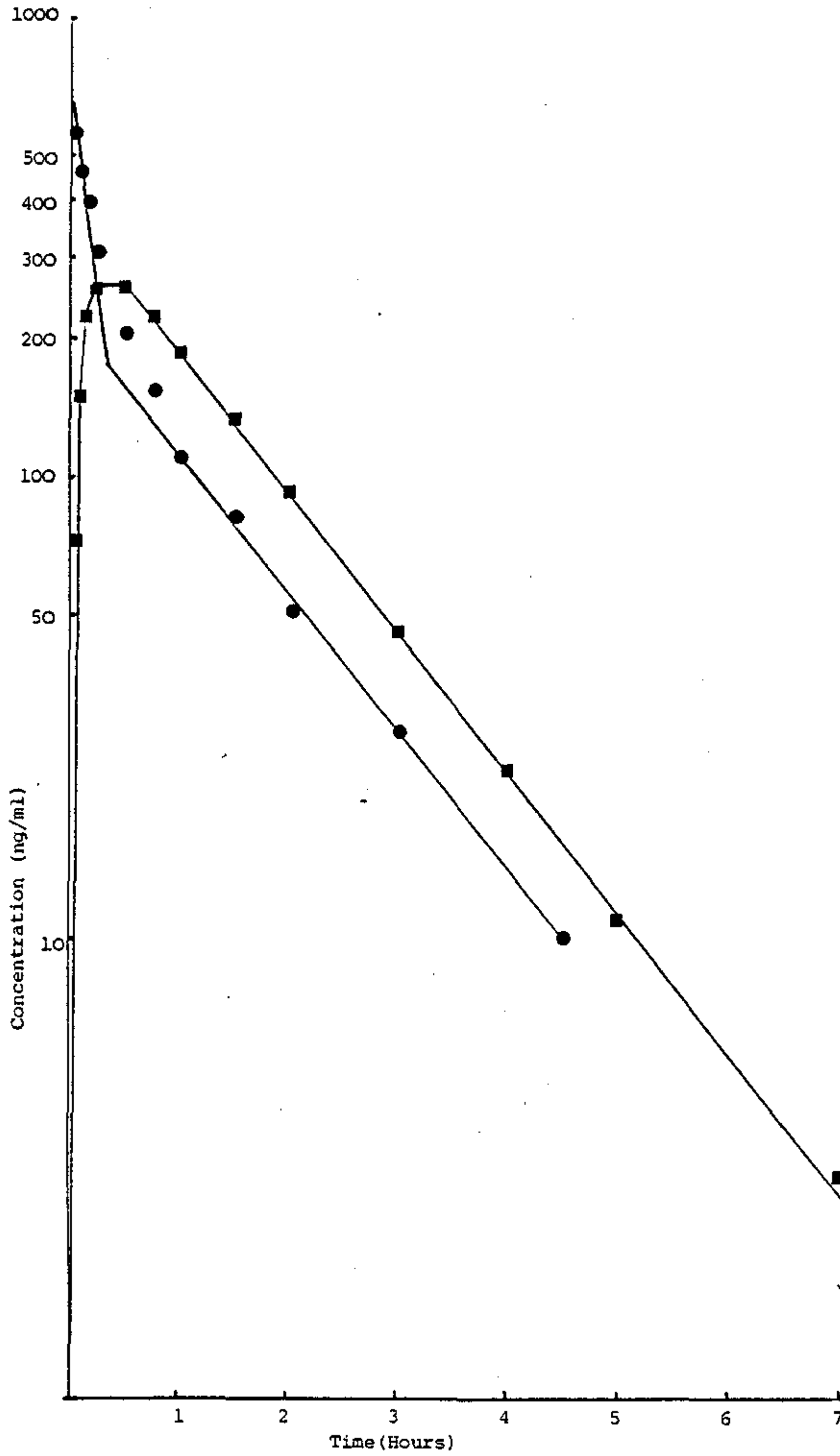
e $V_d$ = Total volume of distribution = $V_{d(ss)}$ for the two-compartment open model.

TABLE 6:5

Calculated concentrations of isosorbide dinitrate in the peripheral compartment after bolus intravenous injection of isosorbide dinitrate 1.0 mg/kg to non-human primates. Results calculated from mean data and expressed as ng/ml.

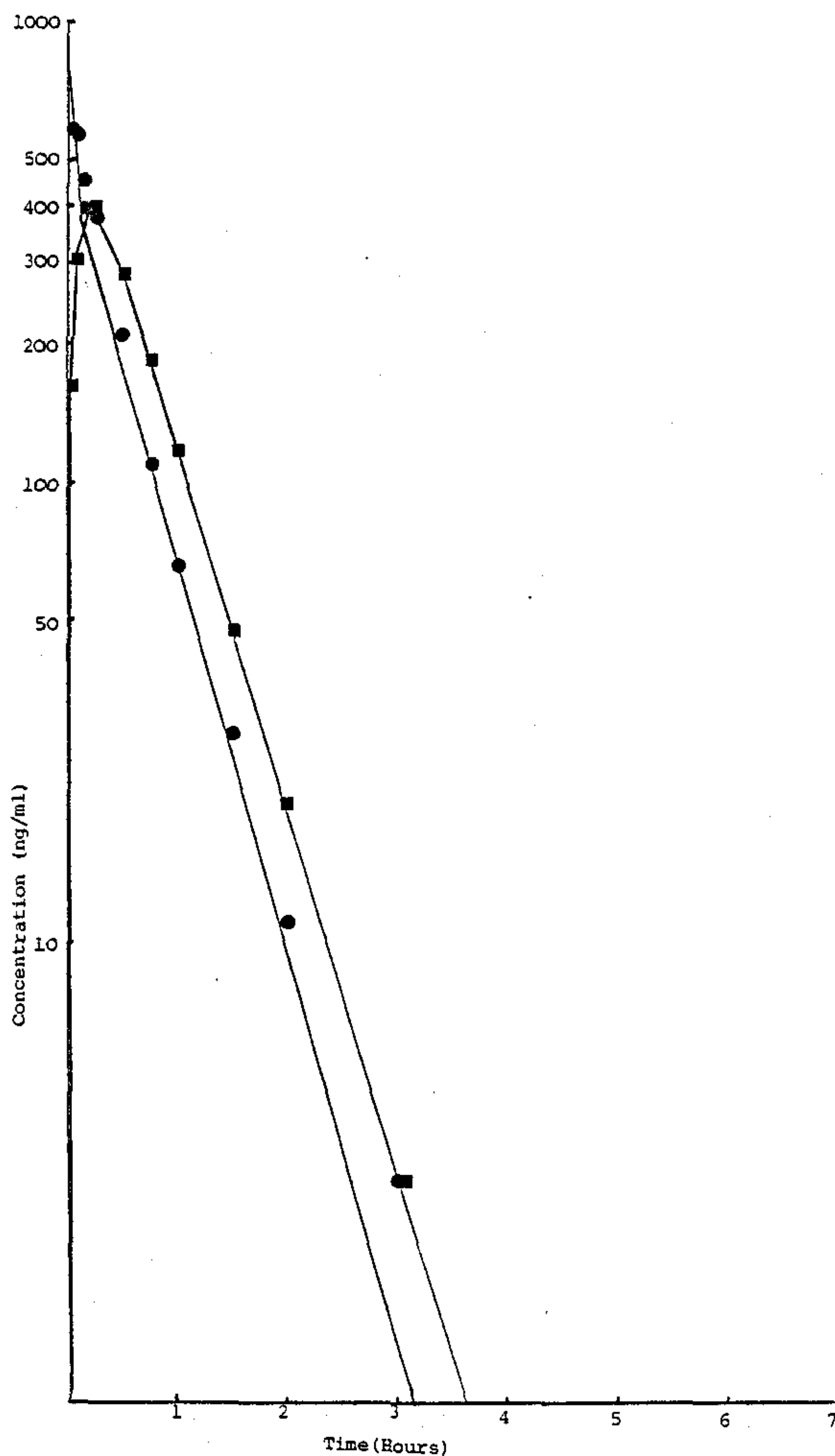
| Time | Species | | |
|---|---|---|---|
| | Rhesus monkey | Cynomolgus monkey | Baboon |
| 2 Minute | 72 | 160 | 339 |
| 5 minute | 149 | 304 | 605 |
| 10 minute | 223 | 392 | 727 |
| 15 minute | 256 | 394 | 698 |
| 30 minute | 258 | 284 | 474 |
| 45 minute | 223 | 184 | 307 |
| 1 hour | 188 | 118 | 197 |
| 1.5 hour | 133 | 48 | 81 |
| 2 hour | 93 | 20 | 33 |
| 3 hour | 46 | 3 | 6 |
| 4 hour | 23 | | |
| 5 hour | 11 | | |
| 7 hour | 3 | | |

FIGURE 6:1



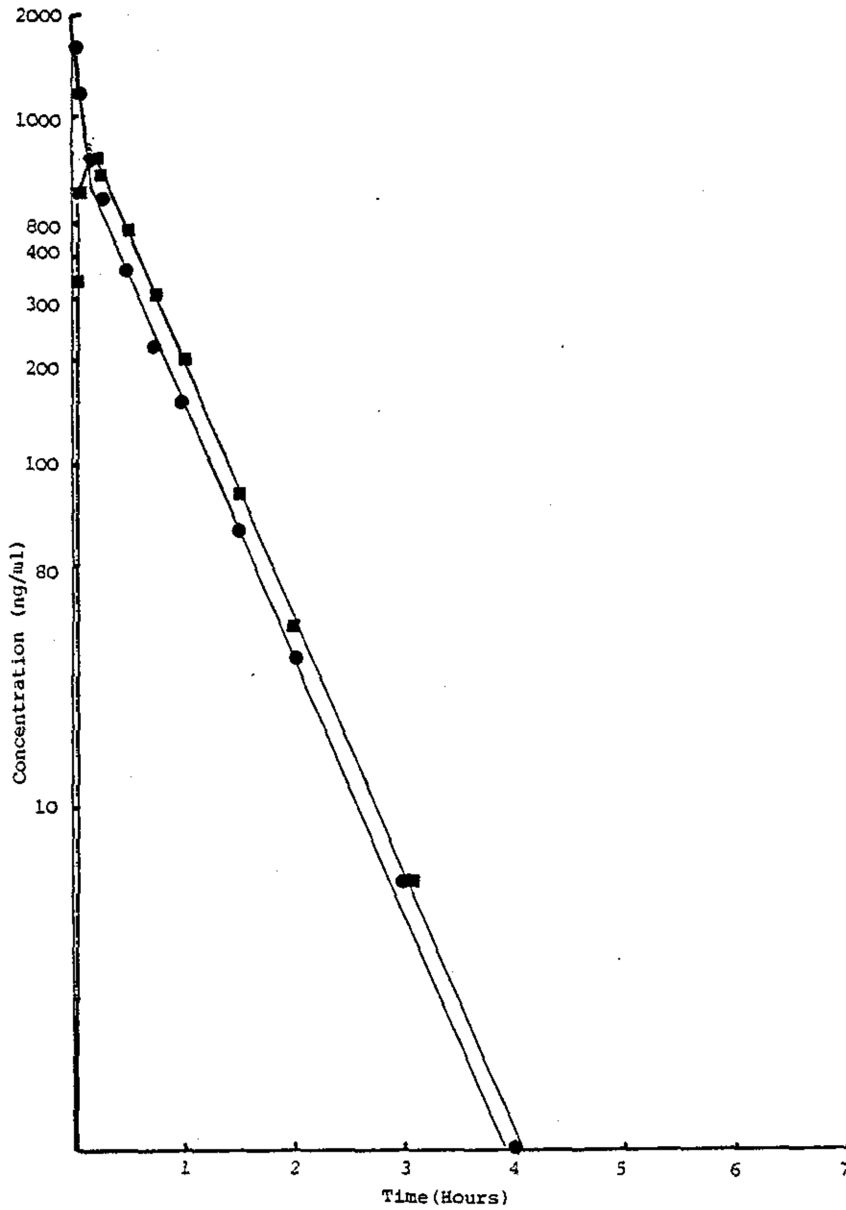

Mean concentration-time curves after a single intravenous injection of isosorbide dinitrate (1.0 mg/kg) to rhesus monkeys. Observed concentrations in the central compartment ( ● ● ). Predicted concentrations in the central compartment ( solid line) and in the peripheral compartment ( ■ ■ ).

FIGURE 6:2




Mean concentration-time curves after a single intravenous injection of isosorbide dinitrate (1.0 mg/kg) to cynomolgus monkeys. Observed concentrations in the central compartment ( ● ● ). Predicted concentrations in the central compartment ( solid line) and in the peripheral compartment ( ■ ■ ).

FIGURE 6:3




Mean concentration-time curves after single intravenous injection of isosorbide dinitrate (1.0 mg/kg) to baboons. Observed concentrations in the central compartment ( ● ● ). Predicted concentrations in the central compartment ( solid line ) and in the peripheral compartment ( ■ ■ ).

CHAPTER 7

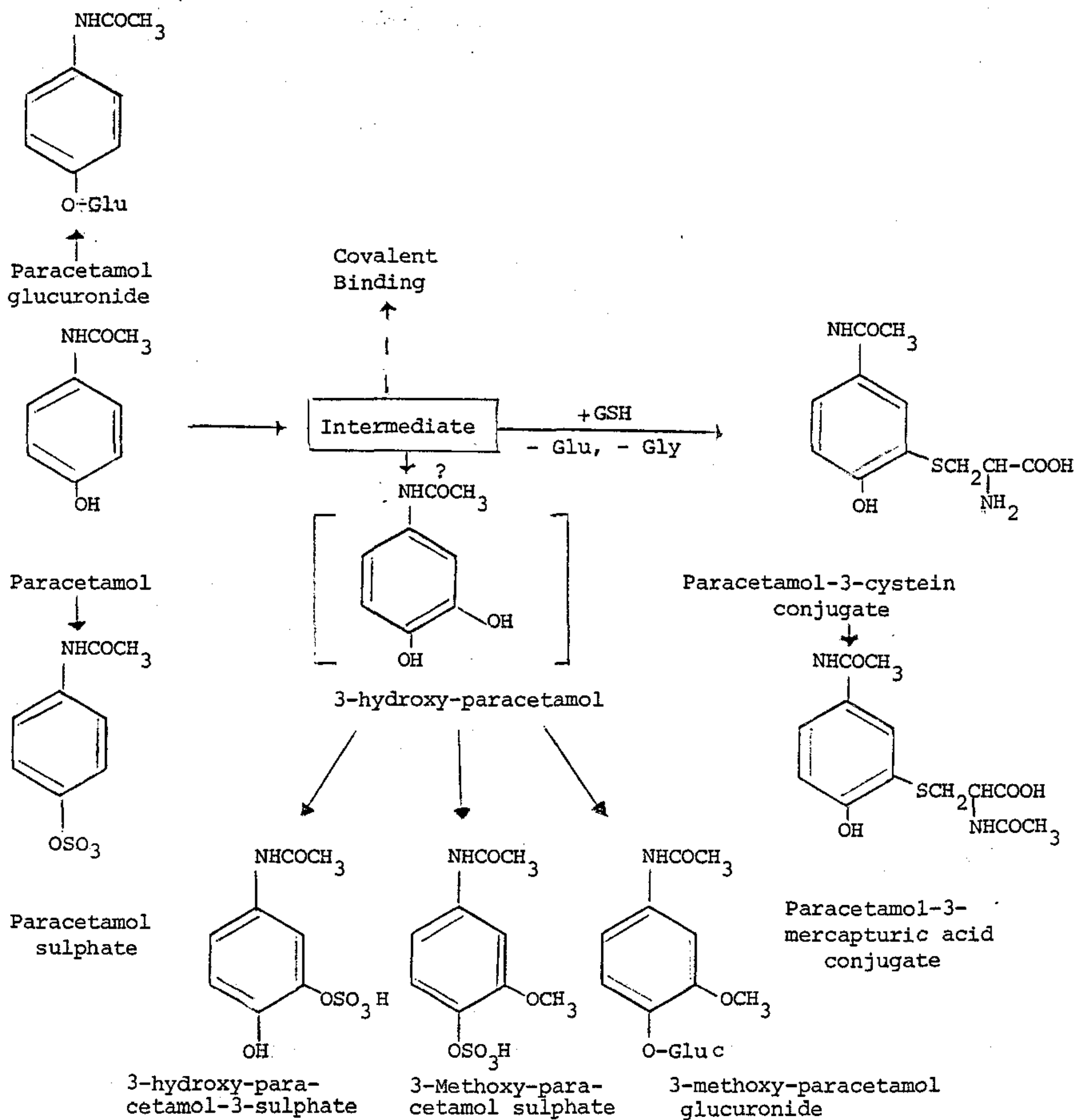
## PARACETAMOL

### Introduction

Paracetamol (N-acetyl-p-aminophenol) is a commonly used analgesic with antipyretic effect (Flinn and Brodie, 1948). It is a weak base ($pK_a$ 9.5), mainly undissociated at physiological pH and only slightly bound to plasma proteins (about 10% bound, Duggin and Mudge, 1975; Laventhal et al, 1976).

The pattern of metabolism of paracetamol is similar in man (Greenberg and Lester, 1946; Brodie and Axelrod, 1948a, 1949; Jagenberg and Toczko, 1964; Cummings et al, 1967; Jagenberg et al, 1968; Levy and Yamanda, 1971; Mitchell et al, 1974; Mrochek et al, 1974; Andrews et al, 1976) and in laboratory animal species (Shibusaki et al, 1968, 1971; Focella et al, 1972; Wong et al, 1976). Approximately 80% of the administered dose is excreted as the glucuronic acid and sulphate conjugates, the remaining fraction consists of unchanged paracetamol (less than 5%) and products of oxidative metabolism, i.e. catechol derivatives and sulphur containing conjugates (Scheme 7:1).

The hepatotoxicity of paracetamol in man following overdose was first reported by Davidson and Eastham (1966) and Thomson and Prescott (1966) and has since been confirmed in laboratory animals (Boyd and Bereczky, 1966; Dixon et al, 1971, 1975; Jollow et al, 1973). Paracetamol is partly converted by microsomal cytochrome P-450 mixed function oxidase to a reactive metabolite which may be a quinone (Scheme 7:1), although its exact structure is still uncertain (Hinson et al, 1980). This reactive metabolite is detoxified by conjugation with glutathione within hepatocytes and probably other tissue cells (Boyland and Chasseaud, 1969; Jollow et al, 1974). At high doses of paracetamol, the supply of glutathione is insufficient to prevent covalent binding of notable amounts of the reactive metabolite with hepatocyte proteins, resulting in liver damage (Mitchell et al, 1973; Davis et al, 1975).




Scheme 7:1 Metabolic pathways of paracetamol.
(GSH represents glutathione; Glu represents glutamate;
Gly represents glycine). Although 3-hydroxy-paracetamol has
been isolated and identified as a metabolite of paracetamol
(Hinson et al, 1980) its mechanism of formation is unclear.

In man, the normal half-life of elimination of paracetamol is about 2 hours but this value increases to more than 4 hours in cases of liver damage produced by paracetamol over-dosage (Prescott et al, 1971; Prescott and Wright, 1973; Peterson and Rumack, 1977). The half-life of elimination of paracetamol also increases in the rat and the mouse after high dose levels, but this increase is not thought to result from liver damage (Siegers et al, 1978). A sensitive test for the degree of damage to the liver is provided by serum alanine and aspartate transaminase (SGPT and SGOT) and hydroxybutyrate and lactate dehydrogenase (HBD and LDH) levels (Prescott et al, 1971).

Measurement of concentrations of paracetamol in plasma

Paracetamol was measured in plasma by means of the colorimetric method of Brodie and Axelrod (1948b).

Plasma (1 ml) was saturated with sodium chloride (approximately 2.5 g) and distilled water (4 ml) was added. The diluted plasma sample was extracted with purified* organic solvent [50 ml of a mixture of washed, redistilled diethyl ether (100 ml) and isoamyl alcohol (1.5 ml)] by shaking the mixture mechanically for 15 minutes. After cetrifugation, the organic phase (40 ml) was transferred to a 50 ml centrifuge tube containing sodium hydroxide (5 ml 0.1M) and the mixture was shaken mechanically for 5 minutes. After centrifugation the organic phase was discarded and the alkaline solution (4 ml) was transferred to another tube containing concentrated hydrochloric acid (1 ml). This tube was loosely stoppered and the mixture was incubated at 100°C for 30 minutes in an oven, occasionally checking that the stoppers had not blown out of the tube. The acid solution was cooled and 4 ml was transferred to another tube. Sodium nitrite

* Footnote to method:
The ether was washed with sodium hydroxide (200 ml/litre, 1M) and with hydrochloric acid (200 ml/litre, 1M) followed by 3 successive washes with distilled water (200 ml/litre) before distillation.

(0.5 ml of a 0.2% solution in water) was added and the solution was left for 20 minutes. After 20 minutes, ammonium sulphamate (0.5 ml of a 1% solution in water) was added and the solution was left for a further 3 minutes. After 3 minutes, α-naphthol (0.1 ml of a 12% solution of resublimed α-naphthol in ethanol) and sodium hydroxide (2ml, 5.5M) were added. This solution was immersed immediately in iced water and left for 5 minutes. After 5 minutes the optical density of the solution of diazo dye was measured at a wavelength of 510 nm. A reagent blank was run through the above procedure and was used for the zero setting.

Measurement of concentrations of serum enzymes using the Union Carbide Centrifichem

Serum alanine and aspartate transaminate (SGPT and SGOT) and hydroxybutyrate and lactate dehydrogenase (HBDB and LDH) levels were determined using a Union Carbide Centrifichem using Roche Diagnostic test kits (both obtained from Roche Products Ltd., Welwyn Garden City, U.K.). The author is grateful to the members of the Department of Clinical Pathology for the use of their equipment and for analysing the serum enzyme concentrations.

Results

Plasma concentrations . After a single intravenous injection of paracetamol at a level of 70 mg/kg, the peak of mean plasma concentrations of the unchanged drug, 105 μg/ml ± 8SD and 87 μg/ml ± 15 SD occurred 2 minutes after injection (the first time of blood withdrawal) in the rhesus monkey and baboon respectively. In the cynomolgus monkey, a peak of mean concentrations (91 μg/ml ± 30 SD) was not reached until 5 minutes after dosing (the second time of blood withdrawal). The apparent delay in reaching peak mean concentrations in the species was contributed by the data from two animals in which this effect was observed. Plasma concentrations of paracetamol declined from peak levels in a biexponential manner with an apparent half-life of elimination of about 1.2 hours, to almost the limit of detection (1 μg/ml) at 7 hours after administration (Table 7:3; Figures 7:1 - 7:3).

TABLE 7:1

Method parameters for the measurement of concentrations of paracetamol in plasma.

| | |
|---|---|
| Linear range | 1-100 μg/ml |
| Calibration line[a] | Y = (0.0192 ± 0.0005)X |
| Accuracy | ± 138% at 5μg/ml, ± 35% at 20 μg/ml and ± 15% at 50 μg/ml |
| Precision | ± 13% at 5μg/ml, ± 10% at 25 μg/ml and ± 7% at 50 μg/ml |
| Sensitivity | 1 μg/ml |
| Recovery of paracetamol | 77% ± 4.1 SD |

a Least squares regression line where Y = absorbance and X = paracetamol concentration in plasma.

Analysis of serum enzymes one week after dosing showed that the administered paracetamol had no prolonged adverse effects on these animals (Table 7:2). The cynomolgus monkey was affected more than the other two species and one animal of this species had a high SGPT level, but this enzyme level returned to normal before the next dosing session.

Pharmacokinetic parameters. Observed plasma concentrations of paracetamol following a single intravenous injection of paracetamol 70 mg/kg, were described by a biexponential equation (equation 2:2). The pharmacokinetic parameters shown in Table 7:4 were, therefore, calculated using equations 2:3 - 2:12 for a two-compartment open model with elimination from the central compartment (Scheme 2:2). Concentrations of paracetamol in plasma predicted by the two-compartment model were generally in good agreement with the observed values (Figures 7:1 - 7:3)

Analysis of variance of pharmacokinetic parameters showed that there was only a statistically significant difference in paracetamol clearance when these parameters were adjusted for bodyweight; clearance was higher in the cynomolgus monkey than in the other two species ($P<0.05$), Newman-Keuls multiple comparison procedure. However, mean areas under the plasma concentration time curves and volumes of distribution were lower and half-lives of elimination were shorter in the cynomolgus monkey than in the other two species, but the differences were not statistically significant.

The mean volume of distribution was equivalent to 82, 106 and 98% of the total bodyweight in the rhesus moneky, the cynomolgus monkey and the baboon respectively. These values are of the same order of magnitude as those previously reported for man (85%, unpublished results, in Prescott and Wright, 1973; 95%, Rawlins et al, 1977) but higher than those values calculated from data published for the rat (35%, Pang and Gillette, 1978) and dog (60% Kampffmayer, 1974).

The mean volume of the central compartment was larger than that of the peripheral compartment. The compartment volume ratio (central: peripheral) was 3:4, 2:1 and 4:4 in the rhesus monkey, the cynomolgus monkey and baboon respectively. These values indicate that there is relatively free transfer of paracetamol between the two compartments and that in this respect the non-human primate is similar to man (Rawlins et al, 1977).

The total volume of distribution $V_{d(\beta)}$, estimated after attainment of pseudo-distribution equilibrium multiplied by the plasma concentration at 1 hour indicates that 49, 46 and 59% of the administered drug remained in the body at 1 hour after dosing in the rhesus monkey, the cynomolgus monkey and the baboon respectively.

The mean ratio $\beta/K_{el}$ of 0.64, 0.59 and 0.77 for the rhesus moneky, the cynomolgus monkey and the baboon respectively indicates that these fractions of paracetamol in the body were in the central compartment and were available for elimination at any time during the terminal linear phase of the disposition curve (Gibaldi et al, 1969).

Simulation of the plasma concentration-time profile of paracetamol in the peripheral compartment for a two-compartment open model with pharmacokinetic parameters shown in Table 7:4, showed that equilibirium between the central and peripheral compartments was achieved 1-2 hours after administration. Peak concentrations of paracetamol in the peripheral compartment (52.8 µg/ml, 34.4 µg/ml and 47.8 µg/ml in the rhesus monkey, the cynomolgus monkey and the baboon respectively) occurred at 40 minutes after injection (Table 7:5, Figures 7:1 - 7:3). Concentrations of paracetamol in the central and peripheral compartments were similar at all times after the distribution phase was complete, reflecting the ease of transfer between the two compartments. These results agree with findings in the dog, for which concentrations of paracetamol in the liver, kidney, heart, spleen, lungs, brain and muscle were similar to those in the plasma,

although concentrations of paracetamol in the fat were lower (Gwilt et al, 1963).

The mean total systemic clearance of paracetamol of 46 ml/minute, 49 ml/minute and 51 ml /minute in the rhesus monkey, the cynomolgus monkey and the baboon respectively, shows a remarkably similar capability of elimination of paracetamol in these non-human primate species. When adjusted for differences in bodyweight the clearance of paracetamol 9.2 ml/minute/kg, 14.0 ml/minute/kg and 8.5 ml/minute/kg in the rhesus monkey, the cynomolgus monkey and the baboon respectively was higher than that in man (5 ml/minute/kg, Rawlins et al, 1977) but lower than that in the rat (34 ml/minute/kg, Pang and Gillette, 1978) and than that estimated from data published for the dog (18 ml/minute/kg, Kampffmayer, 1974).

The mean rate constants for the rapid distribution '$\alpha$' phase (Table. 7:3) correspond to an apparent half-life of 16 minutes, 12 minutes and 14 minutes for the rhesus monkey, the cynomolgus monkey and the baboon respectively and are similar to those values found previously for man 16 minutes - 44 minutes (Rawlins et al, 1977; Albert et al, 1974). The mean biological half-lives of 72 minutes, 59 minutes and 75 minutes for the rhesus monkey, the cynomolgus monkey and the baboon respectively, are shorter than that in man (about 2 hours) after therapeutic administration (Nelson and Morioka, 1963: Prescott et al, 1971; Prescott and Wright, 1973; Albert et al 1974; Rawlins et al, 1974; Laventhal et al, 1976). The half-life of elimination of paracetamol after intravenous injection to the rat (37 minutes, Pang and Gillette, 1978) and to the dog (32 minutes, Kampffmayer, 1973) are shorter than that in the non-human primates. However, the half-life of paracetamol has been shown to be dose dependent, increasing from 1.8 hours after an intravenous injection at a level of 100 mg/kg to 3 hours after injection of 400 mg/kg in the rat, and in the mouse also after oral dosage (0.5 hours after a dose of 200 mg/kg and 0.9 hours after a dose of 1g/kg. The increased half-life of elimination is thought to be due to saturation of the

of the metabolic pathways as no associated liver damage occurred (Siegers et al, 1978).

Discussion

Plasma concentrations of paracetamol decline in a biexponential manner in man after both oral and intravenous administration of the drug (Levy and Yamada, 1971; Albert et al, 1974; Rawlins et al, 1977) and after intravenous administration to the rat (Pang and Gillette, 1978) but paracetamol elimination appears to obey one-compartment kinetics after intravenous administration to the dog (Kampffmeyer, 1973).

In non-human primates, plasma concentrations of paracetamol after intravenous administration of the drug decline in a biphasic manner. The contribution of the distribution phase '$A/\alpha$' to the total area under the plasma concentration time curve was 16 and 19.6% in the rhesus monkey and cynomolgus monkey respectively but the rapid distribution phase contributed only 12% to the total area under the plasma concentration time curve in the baboon. Nevertheless, the distribution phase was clearly defined in all three species and distribution into the peripheral compartment does appear to be a feature of paracetamol disposition in the non-human primate.

Paracetamol is eliminated almost exclusively by biotransformation, so much so that it has been used as a model substrate for examining conjugation mechanisms (Shively and Vesell, 1975; Triggs et al, 1975). The total systemic clearance, therefore, approximates to the metabolic clearance which after intravenous administration presumably occurs predominantly in the liver. In the non-human primate clearance values were about fourfold lower than the hepatic blood flow, suggesting that the clearance of paracetamol might be dependent on both hepatic blood flow and on the hepatic extraction ratio. The dependence of paracetamol clearance on the hepatic blood flow suggests that first-pass metabolism would be an important factor in paracetamol disposition after oral administration to non-human primates. Paracetamol has already been shown to exhibit a significant first pass effect in man due to biotransformation in the liver and gut wall (Rawlins et al, 1977).

Inspection of the half-lives of elimination of paracetamol shows that during the once-daily dosage regimen common to most chronic toxicity studies paracetamol would be unlikely to accumulate in the body of the commonly used laboratory animal species since each dose would have been eliminated before the next was received. In man, the half-life was longer, approximately 2 hours, but paracetamol would still not accumulate during a once-daily dosage regimen. However, when administered three times daily to patients in pain, a dose of 1 g of paracetamol would accumulate in the body particularly if the elimination half-life of the drug increased as a result of liver damage caused by this treatment. In normal subjects with an elimination half-life of 2 hours a plasma steady state concentration of 6 μg/ml would be achieved after the first day with a 1 g three times daily dosing regimen. To achieve this same steady state plasma concentration in non-human primates, paracetamol would have to be injected intravenously at a level of about 19.9 mg, 17.3 mg and 23.0 mg at intervals of 72 minutes, 59 minutes and 75 minutes to the rhesus monkey, the cynomolgus monkey and the baboon respectively, or a similar dosing regimen adopted. These considerations apply to paracetamol administered intravenously. If administered orally, and particularly at much higher dosages, other factors (such as release from the dosage form) have to be assessed.

Although the half-life might increase at higher dose levels due to saturation of metabolic pathways with or without liver damage, it appears that the non-human primate is closer to man than are the other commonly used laboratory animals. Using pharmacokinetic criteria, therefore, the fate of paracetamol in humans (Albert et al, 1974; Rawlins et al, 1977) appears to be more closely reflected by that in the non-human primate, except perhaps the cynomolgus monkey, than in the other commonly used laboratory animals.

TABLE 7:2

Mean serum alanine and aspartate transaminase and hydroxybutyrate and lactate dehydrogenase levels (± SD) in the rhesus monkey, cynomolgus monkey and baboon after injection of paracetamol 70 mg/kg.

| Enzyme | Species | | |
|---|---|---|---|
| | Rhesus monkey | Cynomolgus monkey | Baboon |
| SGPT[a] | 41.8 ± 16.8 | 84.4 ± 76.9[e] | 31.2 ± 5.6 |
| SGOT[b] | 35.8 ± 7.8 | 39.6 ± 20.2 | 32.2 ± 4.8 |
| HBD[c] | 210.8 ± 43.9 | 422.0 ± 40.9 | 205.2 ± 37.9 |
| LDH[d] | 428.2 ± 84.0 | 215.6 ± 14.8 | 474.4 ± 86.1 |

a Alanine transaminase, upper limit of normal 50 mu/ml.

b Aspartate transaminase, upper limit of normal 60 mu/ml.

c Hydroxybutyrate dehydrogenase, upper limit of normal 1000 mu/ml.

d Lactate dehydrogenase, upper limit of normal 1000 mu/ml.

e High value in one animal was observed one week after dosing but the serum enzyme level returned to normal before the next dosing session.

TABLE 7:3

Mean observed concentrations of paracetamol (± SD) in the plasma of the rhesus monkey, the cynomolgus monkey and the baboon after a single bolus intravenous injection of paracetamol at a level of 70 mg/kg. Results are expressed as μg/ml.

| Time | Species | | |
|---|---|---|---|
| | Rhesus monkey | Cynomolgus monkey | Baboon |
| 2 minute | 105 ± 8 | 77 ± 6 | 87 ± 15 |
| 5 minute | 91 ± 10 | 91 ± 30 | 78 ± 6 |
| 10 minute | 84 ± 10 | 72 ± 25 | 81 ± 9 |
| 20 minute | 70 ± 8 | 55 ± 23 | 70 ± 8 |
| 40 minute | 53 ± 8 | 39 ± 12 | 54 ± 3 |
| 1 hour | 36 ± 5 | 29 ± 10 | 40 ± 8 |
| 1.5 hour | 26 ± 3 | 17 ± 4 | 31 ± 8 |
| 2 hour | 17 ± 3 | 12 ± 7 | 25 ± 8 |
| 3 hour | 11 ± 1 | 6 ± 2 | 14 ± 6 |
| 4 hour | 7 ± 1 | 4 ± 2 | 9 ± 5 |
| 5 hour | 3 ± 1 | 2 ± 1 | 5 ± 4 |
| 7 hour | ND - | 1 - | 2 ± 2 |

ND Less than the limit of detection (1 μg/ml).

TABLE 7:4

Mean pharmacokinetic parameters (± SD) determined for a two-compartment open model for the rhesus monkey, the cynomolgus monkey and the baboon after single intravenous injection of paracetamol at a level of 70 mg/kg. The data are presented as means of results from individual animals.

| Parameter[a] | | Species | | |
|---|---|---|---|---|
| | | Rhesus monkey | Cynomolgus monkey | Baboon |
| Bodyweight | (kg) | 5.0 ± 0.7 | 3.5 ± 0.3 | 6.0 ± 0.9 |
| Dose | (mg) | 350 ± 55 | 245 ± 25 | 410 ± 63 |
| Dose | (mg/kg) | 70 ± 2 | 70 ± 3 | 69 ± 1 |
| A | (μg/ml) | 48 ± 33 | 55 ± 12 | 28 ± 91 |
| B | (μg/ml) | 64 ± 31 | 51 ± 8 | 65 ± 12 |
| $\alpha$ | ($hour^{-1}$) | 2.601 ± 1.175 | 3.464 ± 2.423 | 2.881 ± 0.908 |
| $\beta$ | ($hour^{-1}$) | 0.578 ± 0.270 | 0.747 ± 0.180 | 0.507 ± 0.098 |
| $K_{12}$ | ($minute^{-1}$) | 0.009 ± 0.003 | 0.015 ± 0.013 | 0.009 ± 0.009 |
| $K_{21}$ | ($minute^{-1}$) | 0.029 ± 0.045 | 0.034 ± 0.013 | 0.032 ± 0.041 |
| $K_{el}$ | ($minute^{-1}$) | 0.015 ± 0.004 | 0.021 ± 0.006 | 0.011 ± 0.007 |
| AUC | (μg/ml/hour) | 129 ± 25 | 90 ± 27 | 141 ± 34 |
| Cl | (ml/minute) | 46 ± 12 | 49 ± 15 | 51 ± 18 |
| $t_{½}$ | (minutes) | 72 ± 24 | 59 ± 15 | 75 ± 30 |
| $V_1$ | (litres) | 3.1 ± 0.4 | 2.5 ± 0.2 | 4.8 ± 2.3 |
| $V_2$ | (litres) | 0.9 ± 0.7 | 1.2 ± 1.3 | 1.1 ± 1.6 |
| $V_{d(ss)}$ | (litres) | 4.1 ± 0.7 | 3.7 ± 1.3 | 5.9 ± 1.3 |
| $V_{d(\beta)}$ | (litres) | 4.8 ± 1.3 | 3.9 ± 1.2 | 6.0 ± 2.1 |
| $A/\alpha$ | (μg/ml/hour) | 20 ± 25 | 28 ± 27 | 17 ± 36 |
| $B/\beta$ | (μg/ml/hour) | 109 ± 13 | 71 ± 28 | 129 ± 12 |

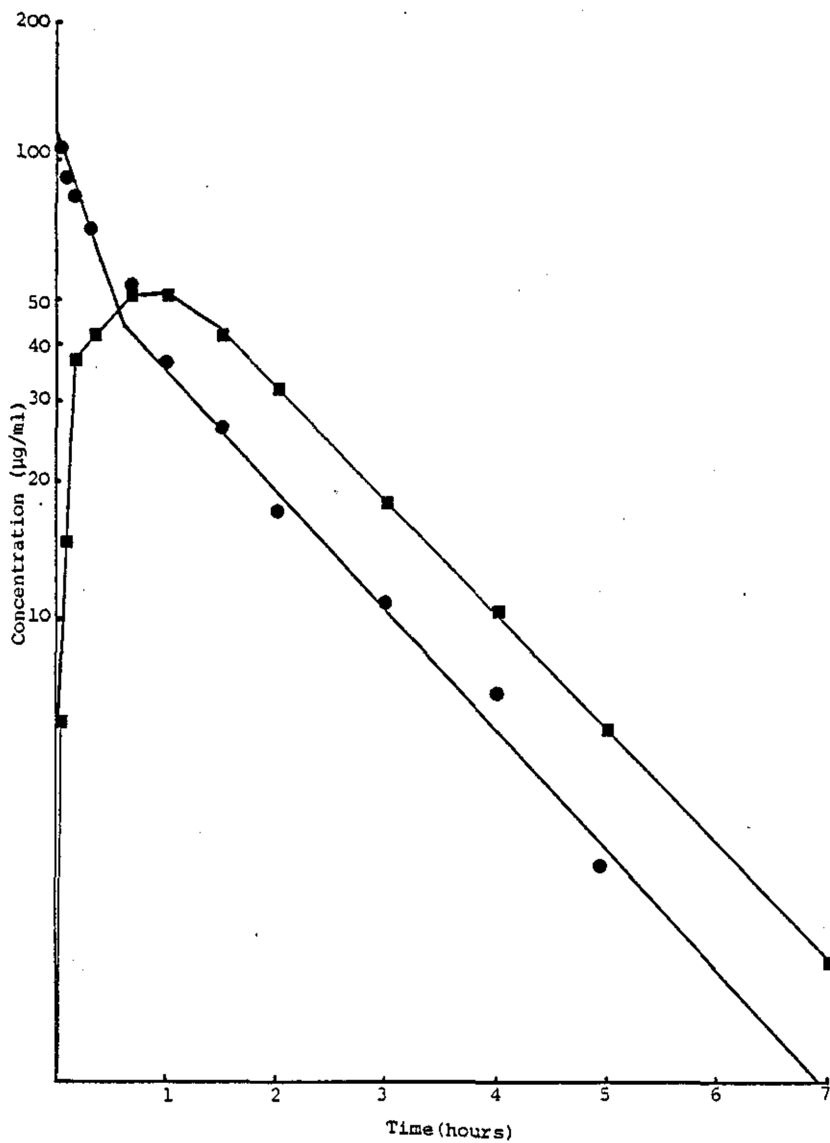
[a] For details of abbreviations used see Chapter 2.

TABLE 7:5

Calculated concentrations of paracetamol in the peripheral compartment after bolus intravenous injection of paracetamol 70 mg/kg to non-human primates. Results calculated from mean data and expressed as μg/ml.

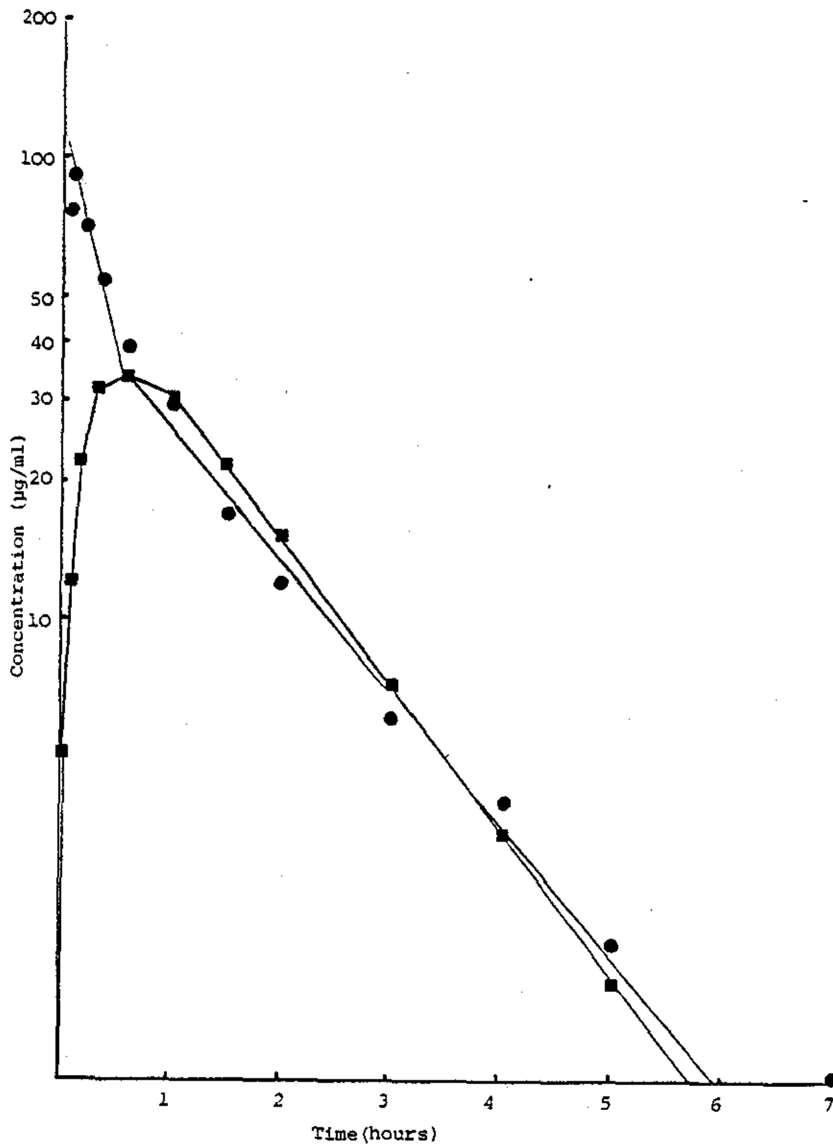
| Time | Species | | |
|---|---|---|---|
| | Rhesus monkey | Cynomolgus monkey | Baboon |
| 2 minute | 6.0 | 5.2 | 6.3 |
| 5 minute | 14.9 | 12.4 | 23.0 |
| 10 minute | 37.7 | 22.0 | 25.3 |
| 20 minute | 41.8 | 31.3 | 39.0 |
| 40 minute | 52.8 | 34.4 | 47.8 |
| 1 hour | 50.6 | 30.0 | 46.1 |
| 1.5 hour | 41.5 | 21.6 | 38.3 |
| 2 hour | 32.1 | 15.1 | 30.6 |
| 3 hour | 18.4 | 7.2 | 18.4 |
| 4 hour | 10.3 | 3.4 | 11.1 |
| 5 hour | 5.8 | 1.6 | 6.7 |
| 7 hour | 1.8 | ND | 2.4 |

FIGURE 7:1



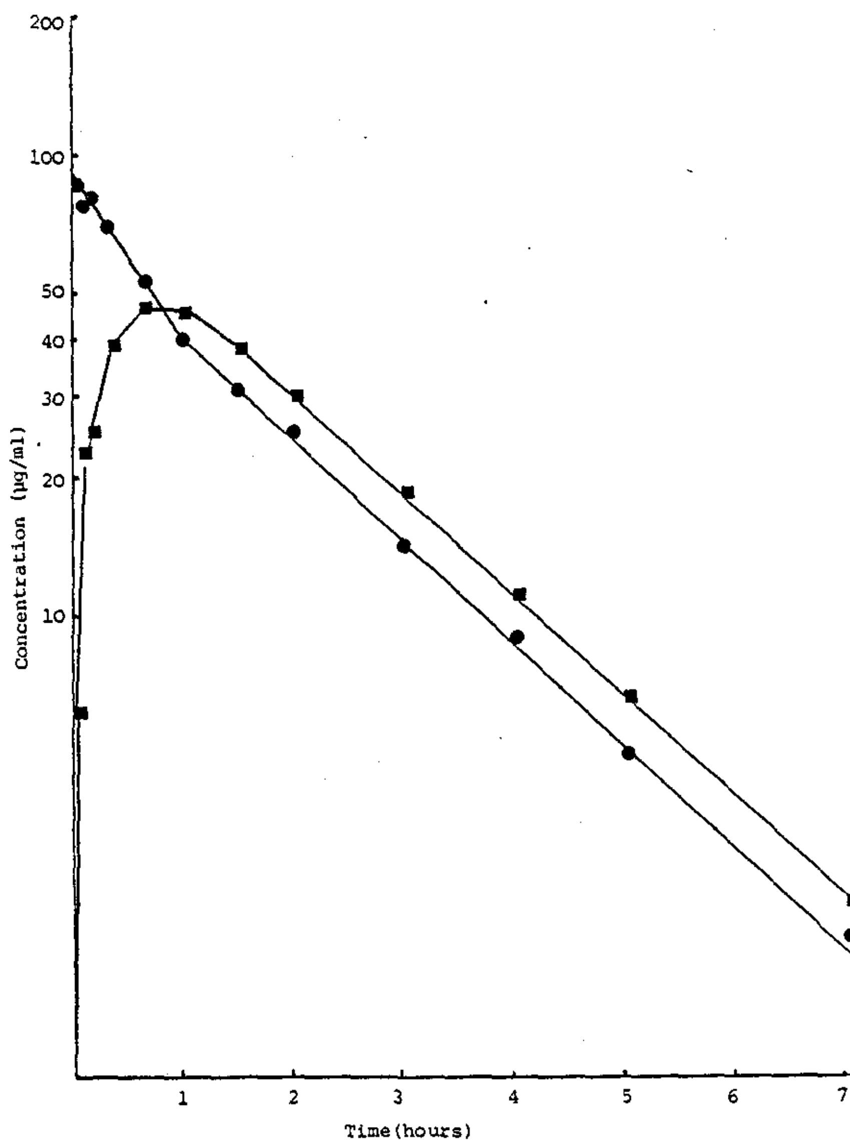

Mean concentration-time curves after a single intravenous injection of paracetamol (7 0 mg/kg) into rhesus monkeys. Observed concentrations in the central compartment ( ● ● ); estimated concentrations in the central compartment (solid line) and in the peripheral compartment ( ■ ■ ).

FIGURE 7:2




Mean concentration-time curves after a single intravenous injection of paracetamol (70 mg/kg) into cynomolgus monkeys. Observed concentrations in the central compartment ( ● ● ); estimated concentrations in the central compartment (solid line) and in the peripheral compartment ( ■ ■ ).

FIGURE 7:3




Mean concentration-time curves after a single intravenous injection of paracetamol (70 mg/kg) into baboons. Observed concentrations in the central compartment ( ● ● ); estimated concentrations in the central compartment (solid line) and in the peripheral compartment ( ■ ■ ).

CHAPTER 8

## IBUPROFEN

### Introduction

Ibuprofen 2-[4-isobutylphenyl] propionic acid is a non-steroid with proven anti-inflammatory properties in animals (Adams et al, 1967, 1969a, 1969b, 1970; Masumato et al, 1972) and in man (Brooks et al 1970, 1973; Davies and Avery, 1971). It is administered as a racemic mixture, is a weak acid ($pK_a$ 5.2), only slightly soluble in water and strongly bound to plasma proteins.

In man and the commonly used laboratory animal species there are four metabolites of ibuprofen that are excreted into urine. These are formed by oxidation and hydroxylation of the isobutyl side chain (Scheme 8:1). In addition a fifth metabolite, 2-(4-carboxyphenyl) propionic acid has been isolated from dialysis fluid samples taken from nephrectomized patients (Pettersen et al, 1978). The major urinary metabolites are the dextrorotatory isomers of 2-[4-(2-hydroxymethyl-2-methylpropyl)phenyl] propionic acid and 2-[4-(2-hydroxy-2-methylpropyl)phenyl] propionic acid and these are excreted both unchanged and conjugated (Adams et al, 1967, 1969a; Brooks and Gilbert, 1974) but there are marked species variations in the proportions of these metabolites and the extent of conjugation (Mills et al, 1973). The conjugates of ibuprofen and its metabolites have not been identified.

The observation that only the dextrorotatory isomers are excreted into urine has been explained by Wechter et al (1974a) and Kaiser et al (1976). These workers demonstrated that ibuprofen undergoes a facile epimerization of the laevorotatory isomer prior to metabolism.

Biliary excretion has been shown to be an important route of elimination of ibuprofen for the rat and dog. Approximately 25% of the administered dose of $^{14}C$-ibuprofen was eliminated into bile (both

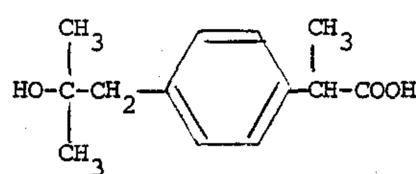
free and conjugated) of these species in 3 hours (Mills et al, 1973). These same workers also demonstrated that there was negligible enterohepatic circulation of ibuprofen in the rat and dog (Mills et al, 1973).

After oral administration, ibuprofen appears mainly unchanged in the plasma of humans and the common laboratory animal species, although the two major metabolites are detectable in plasma, except in the dog (Adams et al, 1969; Mills et al, 1973). The half-life of elimination of ibuprofen in man (about 2 hours, Kaiser and Van Giessen, 1974; Kaiser and Martin, 1977) is similar to that in the rat ($t_{\frac{1}{2}}$ = 1.4 hours, Kaiser and Glenn, 1974) and other laboratory animal species ($t_{\frac{1}{2}}$ = about 3 hours, Adams et al, 1969a; Mills et al, 1973).

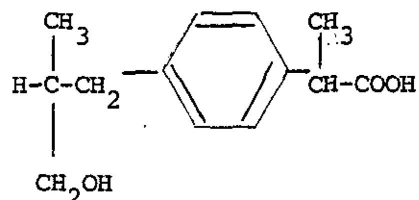
## Measurement of concentrations of ibuprofen in plasma

Concentrations of ibuprofen in plasma were measured by a gas chromatographic procedure developed and supplied by the Boots Company Limited (Glass and Hind, 1974).

Plasma (0.5 ml) was mixed with internal standard (4-n-butyl phenylacetic acid, 1 ml of a 10 µg/ml solution in water containing sufficient 1M sodium hydroxide solution to dissolve the acid) and hydrochloric acid (250 µl, 1M) in a 10 ml graduated tube. The mixture was transferred to a glass column 10.0cm x 4 mm i.d. containing washed Amberlite XAD-2 resin (25-30 mesh, approximately 7.0 cm, obtained from British Drug Houses Chemicals Ltd., Poole, U.K.). The tube was rinsed with distilled water (2 x 2 ml) and the washings were added to the column. The aqueous eluate was discarded. The test-tube was rinsed with methanol (Distol, 4 ml, obtained from Fisons Scientific Apparatus Ltd., Loughborough, U.K.), and the washings were transferred to the column. The eluate, a mixture of water and methanol, was collected in a conical centrifuge tube and concentrated by evaporating the methanol at 40°C under a strong current of nitrogen. The remaining solution was extracted by vigorous mixing with dichloromethane (5 ml). After centrifugation, the bottom organic layer was transferred to a pointed centrifuge tube and evaporated to dryness at 40°C under a stream of nitrogen. The residue was redissolved in hexane (200 µl) and



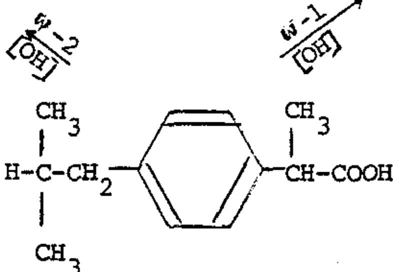

2 [4 -(2-hydroxy-2-methylpropyl) phenyl] propionic acid




2-[4 -(2-hydroxymethyl-2-methylpropyl) phenyl] propionic acid

ω-2 [OH]

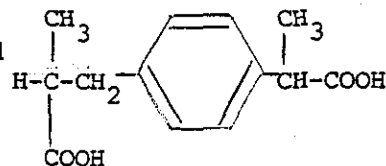
ω-1 [OH]




Ibuprofen

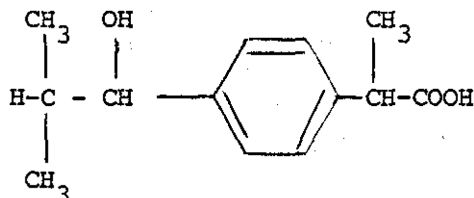
2-(4-isobutylphenyl) propionic acid

[O]



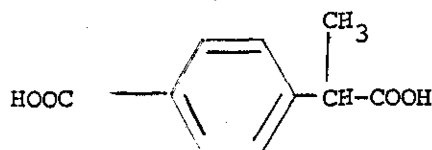

2-[4-(2-hydroxy-2-methylpropyl)phenyl] propionic acid

ω-3 [OH]




2-[4-(1-hydroxy-2-methylpropyl)phenyl] propionic acid

β[O]




2-( 4'-carboxyphenyl ) propionic acid

Scheme 8:1 Metabolic pathways of ibuprofen.

methylated by bubbling diazomethane through the solution until the solution was permanently coloured yellow. The solution was allowed to stand for 5 minutes and then carefully evaporated to dryness at room temperature, under nitrogen. The residues were dissolved in methyl acetate (20 µl) and an aliquot (1-3 µl) injected onto the gas chromatographic column. Quantitation was by peak height ratio using the internal standard technique.

A Hewlett-Packard Model 5750G gas chromatograph fitted with a flame ionisation detector was used to measure ibuprofen in the extracts of plasma. The column was 6 ft. long x 1/8th inch i.d. stainless steel silanised and packed with 5% carbowax 20M on 100/120 mesh Gas Chrom Q support (Phase Separations Limited, Queensferry, Clwyd, U.K.) conditioned for 16 hours at 210°C. The carrier was nitrogen (oxygen free and dried) at a flow rate of 40 ml/minute. The column temperature was 150°C increased at a rate of 4°C/minute until the internal standard had eluted (at about 172°C), and then increased to 210°C and kept at this temperature for 4 minutes. The detector temperature was 295°C and the injector temperature was 255°C. Gas supply to the flame-ionization detector was optimized to give maximum sensitivity (hydrogen about 25 ml/minute and air about 300 ml/minute). Under these conditions the retention times of ibuprofen and the internal standard were 4.2 and 5.6 minutes respectively.

TABLE 8:1

Method parameters for the measurement of concentrations of ibuprofen in plasma.

| Parameter | Value |
|---|---|
| Linear range[a] | 0.4-80 µg/ml |
| Calibration line[b] | Y=(0.0554 ± 0.0013)X |
| Accuracy | ± 14.4% at 80µg/ml,<br>± 26.4% at 41.5 µg/ml and<br>± 109% at 10 µg/ml |
| Precision | ± 9% at 80 µg/ml,<br>± 10% at 50 µg/ml and<br>± 9% at 10 µg/ml |
| Sensitivity | 0.4 µg/ml |
| Recovery of ibuprofen | 46% ± 10 SD |
| Recovery of internal standard | 46% |

a Samples containing ibuprofen at levels greater than 80 µg/ml were assayed using a smaller volume of plasma (0.25ml) diluted with control plasma to a volume of 0.5 ml.

b Least squares regressions where Y= instrument response(peak height ratio, ibuprofen to internal standard) and X = plama concentration of ibuprofen.

## Results

Plasma concentrations. After a single bolus intravenous injection of ibuprofen at a level of 15 mg/kg, the peak of mean plasma concentrations in the rhesus monkey and baboon of 171 μg/ml ± 43SD and 179 μg/ml ± 23 SD respectively, were reached at 2 minutes after injection (the first time of blood withdrawal). However, the peak of mean plasma concentrations of ibuprofen in the cynomolgus monkey (235 μg/ml ± 62 SD) was not reached until 5 minutes post-administration (the second time of blood withdrawal). The delayed time to peak concentrations in the cynomolgus monkey was due to data from two animals in which this effect was observed. In all three species, plasma concentrations declined in a biphasic manner, with an apparent half-life of elimination of about 1 hour and were at or near the limit of detection (0.4 μg/ml) at 7 hours post-administration (Table 8:2, Figures 8:1 ; 8:3).

Pharmacokinetic parameters. Observed plasma concentrations of ibuprofen following a single intravenous injection of ibuprofen at a level of 15 mg/kg were described by a biexponential equation (Equation 2:2). The pharmacokinetic parameters shown in Table 8:3 were, therefore, calculated using equations 2:3 - 2:12 for a two compartment open model with elimination from the central compartment (Scheme 2:2). Plasma concentrations of ibuprofen predicted by the two-compartment model were generally in good agreement with the observed values. That a two-compartment model is appropriate to describe ibuprofen kinetics is also indicated by the contribution of the distribution phase ($A/\alpha$) to the total area under the plasma concentration-time curve which was 59, 62 and 28% for the rhesus monkey, cynomolgus monkey and baboon respectively.

Analysis of variance showed that the areas under the plasma concentration-time curve for the rhesus monkey was significantly lower than that for the other two species ($P < 0.01$ Newman-Keuls

multiple comparison procedure) and that the total volume of distribution and the total systemic clearance in the cynomolgus monkey were significantly lower than in the other two species ($P < 0.01$). When adjusted for differences in bodyweight, there were no significant differences in the adjusted volumes of distribution ($P > 0.05$) but the clearance in the rhesus monkey (mean 3.1 µg/ml/kg) was significantly higher ($P < 0.05$ Newman-Keuls multiple comparison procedure) than in the cynomolgus monkey (mean 2.0 µg/ml/kg) and the baboon (mean 2.1 µg/ml/kg).

In common with other anti-inflammatories (for review see Hucher et al, 1980), the mean total volume of distribution $V_{d(ss)}$ of ibuprofen was small, accounting for 14, 8 and 10% of the total bodyweight in the rhesus monkey, the cynomolgus monkey and the baboon respectively, presumably reflecting the high degree of plasma protein binding of this compound. The mean compartment volume ratio (central:peripheral) 0.8, 2.0 and 2.0 for the rhesus monkey, the cynomolgus monkey and the baboon respectively, indicates that ibuprofen is able to transfer freely between the two compartments in the cynomolgus monkey and the baboon but may be released more slowly from the peripheral compartment in the rhesus monkey. In these non-human primate species, the volume of the central compartment is approximately 7% of the total bodyweight and therefore approximates to the blood volume.

The mean rate constants for the rapid distribution phase, calculated for the rhesus monkey, the cynomolgus monkey and the baboon corresponds to an apparent half-life for the $\alpha$ phase of 12 minutes, 16 minutes and 12 minutes respectively.

The mean terminal half-lives of ibuprofen in these non-human primates, 91 minutes ± 57 SD, 70 minutes ± 40 SD and 49 minutes ± 129 SD for the rhesus monkey, the cynomolgus monkey and the baboon respectively showed large inter-animal variations in the value of this parameter. These mean values are shorter than those in man (about

2 hours, unpublished data Davies and Avery, 1971; Kaiser and Van Giesson, 1974; Collier et al, 1978) and that estimated for the dog (2.9 hours, calculated from data published by Mills et al, 1973) but are similar to those reported for the rat (1.4 hours Kaiser and Glenn, 1974).

The total volume of distribution $V_{d(\beta)}$ estimated after attainment of pseudo-distribution equilibrium multiplied by the plasma concentration at 1.5, 2 and 1.5 hours after dosing in the rhesus monkey, cynomolgus monkey and the baboon respectively, indicates that 25, 15 and 21% respectively of the administered dose remained in the body at these times.

The mean ratio $\beta/\bar{k}_{el}$ of 0.2, 0.3 and 0.5 indicates that these fractions of the drug in the body were in the central compartment and available for elimination at any time during the β elimination phase.

Simulation of the plasma concentration-time profile of ibuprofen in the peripheral compartment for a two-compartment open model with pharmacokinetic parameters shown in Table 8:3, showed that following intravenous injection, equilibrium was rapidly achieved. Peak concentrations of 39 μg/ml, 81 μg/ml and 112 μg/ml in the rhesus monkey, the cynomolgus monkey and the baboon respectively, were reached at 40 minutes post administration (Table 8:4, Figures 8:1 - 8:3). Estimated concentrations of ibuprofen in the peripheral compartment of these species were higher than those in the central compartment. In dogs, total radioactivity measured in most tissues after administration of $^{14}C$-ibuprofen were lower than that in plasma, although total radioactivity in the bile was twenty to forty five-fold greater than that in plasma (Adams et al, 1969a). In rats, however, these same workers found that total radioactivity measured in several tissues, including well perfused tissues such as the liver and kidney, were higher than that in plasma.

## Discussion

Ibuprofen is normally administered orally and only limited information concerning the pharmacokinetics of this compound following intravenous administration has been reported in the literature. Mills et al (1973) have, however, published total radioactivity measurements of $^{14}C$ in plasma after intravenous administration of $^{14}C$ ibuprofen to dogs and these data can be used to estimate the half-life of ibuprofen because no metabolites are detectable in the plasma of this species. There is not sufficient information in the report by Mills et al. to estimate other pharmacokinetic parameters for ibuprofen in the dog.

In the dog after intravenous dosing, (Mills et al, 1973) and in man after oral doses (Collier et al, 1978), ibuprofen obeys two compartment kinetics, although a mono-exponential decline to the concentration-time curve was observed for some arthritic human volunteers (Collier et al, 1978). Using the computerised non-linear least squares curve fitting programme BLUD, the plasma concentration-time curve for ibuprofen following intravenous injection of this compound to non-human primates was described by a biexponential equation.

Although concentrations of ibuprofen in the tissue compartment in the rat and the non-human primates are higher than those in the central compartment, the reverse is true in the dog. Nevertheless, there is no evidence to suggest that the fraction of administered ibuprofen that diffuses into the tissue compartment is not free to diffuse back into the plasma of these species after an intravenous injection.

Inspection of the values for the apparent half-life of elimination of ibuprofen in the non-human primate (reported in Table 8:3), other laboratory animal species and man following oral administration (Mills et al, 1973; Collier et al, 1978), shows that ibuprofen would not accumulate during the once-daily dosing regimen common to most chronic toxicity studies. However, ibuprofen would

accumulate in man if given 400 mg three times daily, for the treatment of osteo-arthrosis, for example. To maintain steady state concentrations of ibuprofen in the plasma of non-human primates, equal to the limit of detection of the analytical method (0.4 μg/ml), a possible dosing regimen would be the injection of doses of 0.6 mg, 0.2 mg and 0.3 mg intravenously into the rhesus monkey, cynomolgus monkey and the baboon respectively at intervals of 91 minutes, 70 minutes and 49 minutes respectively. Such a dosing regimen would be impractical during a chronic toxicity study of this compound. An alternative dosing regimen would be the injection of doses of 9.5 mg, 4.1 mg and 10.5 mg of ibuprofen to the rhesus monkey, the cynomolgus monkey and the baboon respectively, once daily . These doses would not harm the animals as they are similar to those given in this study, however, with such a dosing regimen ibuprofen would have been completely eliminated from the body after 7 to 12 hours in these non-human primate species. Ideally, a twice daily, or preferably a three times daily, oral dose designed to achieve a suitable steady state plasma drug level would be more suitable, as such a regimen would ensure that some ibuprofen remained in the body of the non-human primate during the whole day.

Using pharmacokinetic criteria it can be concluded that the non-human primate would not be a more suitable model for man than other commonly used laboratory animals. Other factors that need to be considered in the choice of an animal model for man to study the fate of ibuprofen in vivo would be species-related differences in metabolism, routes of excretion, tissue distribution and protein binding.

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TABLE 8:2

Mean observed concentrations of ibuprofen (± SD) in the plasma of the rhesus monkey, the cynomolgus monkey and the baboon after a single bolus intravenous injection of ibuprofen at a level of 15 mg/kg. Results are expressed as µg/ml.

| Time | | Species | | |
|---|---|---|---|---|
| | | Rhesus monkey | Cynomolgus monkey | Baboon |
| 2 | minute | 171 ± 43 | 221 ± 30 | 179 ± 23 |
| 5 | minute | 159 ± 43 | 235 ± 62 | 158 ± 15 |
| 10 | minute | 114 ± 30 | 160 ± 18 | 129 ± 10 |
| 20 | minute | 65 ± 12 | 107 ± 16 | 89 ± 9 |
| 40 | minute | 31 ± 6 | 58 ± 17 | 57 ± 15 |
| 1 | hour | 17 ± 5 | 36 ± 7 | 39 ± 21 |
| 1.5 | hour | 9 ± 3 | 21 ± 7 | 21 ± 8 |
| 2 | hour | 7 ± 2 | 10 ± 5 | 12 ± 10 |
| 3 | hour | 4 ± 2 | 6 ± 4 | 4 ± 3 |
| 4 | hour | 3 ± 1 | 4 ± 2 | 2 ± 2 |
| 5 | hour | 2 ± 1 | 2 ± 1 | 1 ± 1 |
| 7 | hour | 1 - | 1 ± 1 | ND |

ND Less than the limit of detection (0.4 µg/ml).

TABLE 8:3

Mean pharmacokinetic parameters (± SD) determined for a two-compartment model in the rhesus monkey, the cynomolgus monkey and the baboon, after a single intravenous injection of ibuprofen at a level of 15 mg/kg. The data are presented as means of results of individual animals.

| Parameter[a] | | Species | | |
|---|---|---|---|---|
| | | Rhesus monkey | Cynomolgus monkey | Baboon |
| Bodyweight (kg) | | 4.8 ± 0.8 | 3.5 ± 0.3 | 6.1 ± 0.9 |
| Dose | (mg) | 73.2 ± 12.4 | 53.6 ± 3.6 | 90.0 ± 14.2 |
| Dose | (mg/kg) | 15.0 ± 0.4 | 15.2 ± 0.6 | 14.9 ± 0.4 |
| A | (µg/ml | 174 ± 83 | 205 ± 82 | 114 ± 73 |
| B | (µg/ml | 17 ± 9 | 33 ± 22 | 70 ± 39 |
| $\alpha$ | ($hour^{-1}$) | 3.460 ± 1.061 | 2.529 ± 1.212 | 3.457 ± 0.615 |
| $\beta$ | ($hour^{-1}$) | 0.467 ± 0.244 | 0.555 ± 0.224 | 0.850 ± 0.422 |
| $K_{12}$ | ($minute^{-1}$) | 0.017 ± 0.008 | 0.010 ± 0.006 | 0.023 ± 0.009 |
| $K_{21}$ | ($minute^{-1}$) | 0.012 ± 0.004 | 0.014 ± 0.006 | 0.066 ± 0.042 |
| $K_{el}$ | ($minute^{-1}$) | 0.036 ± 0.006 | 0.029 ± 0.003 | 0.028 ± 0.008 |
| AUC | (µg/ml/hour) | 85 ± 23[b] | 127 ± 6 | 123 ± 23 |
| Cl | (ml/minute) | 15 ± 3 | 7 ± 1[c] | 13 ± 2 |
| $t_{½}$ | (minutes) | 91 ± 40 | 70 ± 33 | 49 ± 19 |
| $V_1$ | (litres) | 0.3 ± 0.2 | 0.2 ± 0.1 | 0.4 ± 0.1 |
| $V_2$ | (litres) | 0.4 ± 0.3 | 0.1 ± 0.1 | 0.2 ± 0.5 |
| $V_{d(ss)}$ | (litres) | 0.7 ± 0.4 | 0.3[c] ± 0.1 | 0.6 ± 0.1 |
| $V_{d(\beta)}$ | (litres) | 2.0 ± 0.4[b] | 0.8 ± 0.1 | 0.9 ± 0.2 |
| A/$\alpha$ | (µg/ml/hour) | 50 ± 18 | 79 ± 3.1 | 34 ± 23 |
| B/$\beta$ | (µg/ml/hour) | 38 ± 14 | 63 ± 47 | 74 ± 32 |

a For details of abbreviations used see Chapter 2.

b Significance level (analysis of variance) rhesus monkeys compared with the other two species ($P < 0.01$).

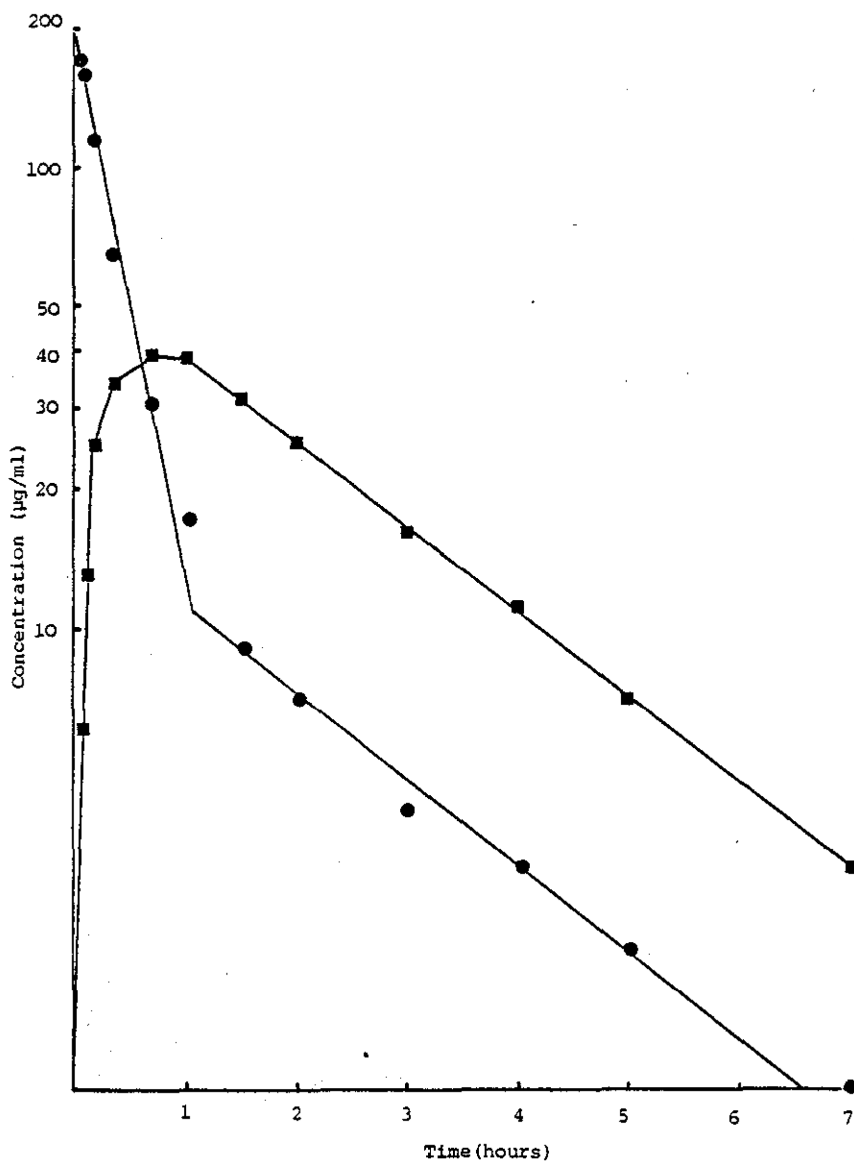
c Significance level (analysis of variance), cynomolgus monkeys compared with the other two species ($P < 0.01$).

TABLE 8:4

Calculated concentrations of ibuprofen in the peripheral compartment after bolus intravenous injection of ibuprofen 15 mg/kg to non-human primates. Results calculated from mean data and expressed as μg/ml.

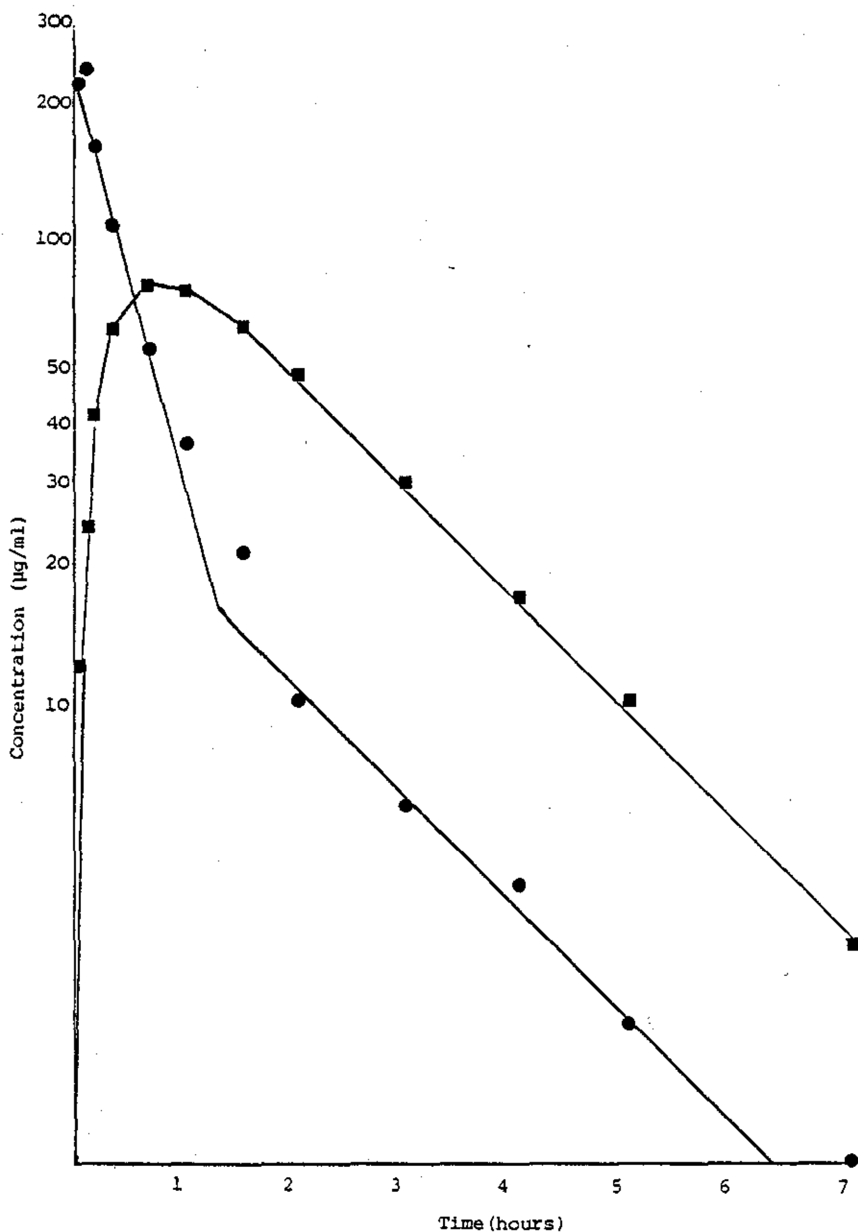
| Time | | Species | | |
|---|---|---|---|---|
| | | Rhesus monkey | Cynomolgus monkey | Baboon |
| 2 | minute | 6 | 12 | 18 |
| 5 | minute | 13 | 24 | 42 |
| 10 | minute | 25 | 42 | 74 |
| 20 | minute | 34 | 65 | 105 |
| 40 | minute | 39 | 81 | 112 |
| 1 | hour | 38 | 79 | 94 |
| 1.5 | hour | 31 | 65 | 65 |
| 2 | hour | 25 | 51 | 44 |
| 3 | hour | 16 | 30 | 19 |
| 4 | hour | 11 | 17 | 8 |
| 5 | hour | 7 | 10 | 3 |
| 7 | hour | 3 | 3 | 1 |

FIGURE 8:1



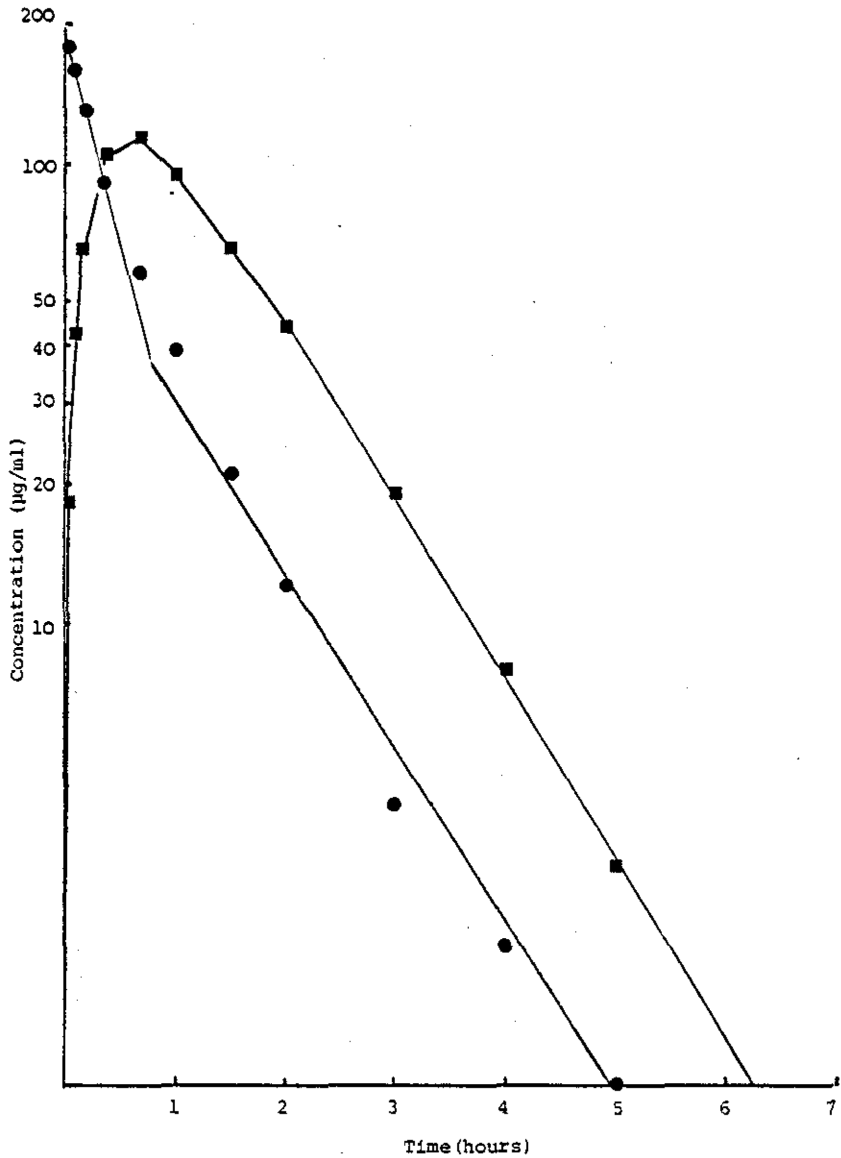

Mean concentration-time curves after single intravenous injection of ibuprofen 15 mg/kg to rhesus monkeys. Observed concentrations in the central compartment ( ● ● ), estimated concentrations in the central compartment (solid line) and in the peripheral compartment ( ■ ■ ).

FIGURE 8:2




Mean concentration-time curves after single intravenous injection of ibuprofen 15 mg/kg to cynomolgus monkeys. Observed concentrations in the central compartment ( ● ● ), estimated concentrations in the central compartment (solid line) and in the peripheral compartment ( ■ ■ ).

FIGURE 8:3




Mean concentration-time curves after single intravenous injection of ibuprofen 15 mg/kg to baboons. Observed concentrations in the central compartment ( ● ● ), estimated concentrations in the central compartment (solid line) and in the peripheral compartment ( ■ ■ ).

CHAPTER 9

PENTOBARBITONE

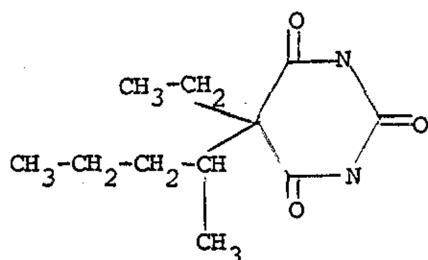
Introduction

Pentobarbitone (5-ethyl-5-(1′-methylbutyl) barbituric acid) is a short acting barbiturate with hypnotic and sedative properties (Goodman and Gilman, 1975). It is a weak acid ($pK_a$ 8.1), lipid soluble and only slightly bound to plasma proteins (Brodie et al, 1953; Ehrnebo, 1974).

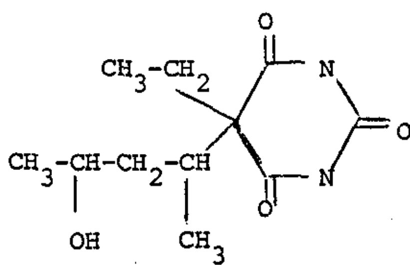
Pentobarbitone is metabolized by hydroxylation and oxidation, presumably in the liver, in both humans and the commonly used laboratory animal species. Only trace amounts of the administered pentobarbitone are excreted unchanged (Brundage and Gruber, 1937; Herwick, 1933; Van Dyke et al, 1947); the major fraction (about 75%) is found in urine as the diastereoisomeric forms of 5-ethyl-5-(3′-hydroxy-1′-methylbutyl) barbituric acid and their glucuronic acid conjugates (Maynert and Dawson, 1952; Brodie et al, 1953; Titus and Weiss, 1955; Tang et al, 1977). Approximately twice as much of the laevorotatory isomer appears in the urine unchanged as does the dextrorotarory isomer, and this has been explained by the preferential conversion of the dextrorotatory isomer to the glucuronide (Titus and Weiss, 1955). Of the remaining dose, Maynert and Van Dyke (1950) suggested that small amounts are converted to urea; Algeri and McBay (1952) and Titus and Weiss (1955) isolated 5-ethyl-5-(1′-methyl-3′-carboxypropyl) barbituric acid.

Measurement of concentrations of pentobarbitone in plasma.

Concentrations of pentobarbitone in plasma were measured by means of a modification of the gas chromatographic procedure of Inaba and Kalow (1972).

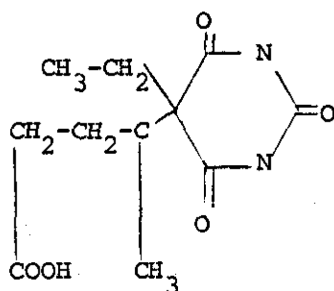



5-ethyl-5-(-1′-methylbutyl)barbituric acid
(pentobarbitone)




(+)5-ethyl-5-(-3′-hydroxy-1′-methylbutyl) barbituric acid

glucuronic acid conjugates




5-ethyl-5-(-3′-carboxy-1′-methylpropyl) barbituric acid

Scheme 9:1 Metabolic pathways of pentobarbitone.

Plasma (1 ml) was mixed with internal standard (0.5 ml of a solution of methyl stearate 1μg/ml in chloroform) and extracted with freshly redistilled diethyl ether by shaking on a rotary mixer (Fisons Scientific Apparatus Limited, Loughborough, Leicestershire, U.K.) for 30 seconds. After centrifugation the organic layer was transferred to a 50 ml centrifuge tube. The plasma was extracted twice again with a mixture of redistilled diethyl ether and redistilled chloroform (3 ml, 80:20 v/v) and the extracts were combined. The combined extracts were washed with hydrochloric acid (0.5 ml; M) before the organic phase was transferred to a 10 ml pointed centrifuge tube and evaporated to dryness under a stream of nitrogen at 40°C. The residue was dissolved in 0.5 ml of the solvent mixture, washing the sides of the tube, and blown carefully to dryness once again. The concentrated sample was dissolved in chloroform (10 ml) and a fraction of the final solution (about 1 μl) was injected into the chromatograph. Quantitation was by peak height ratio using the internal standard technique. A Perkin-Elmer F11 gas chromatograph (Perkin-Elmer Limited, Beaconsfield, U.K.) fitted with a flame ionization detector was used. The column was glass, 5 ft. x 2 mm id packed with 3% SE-30 on 60-80 mesh, AW-DMCS Chromosorb W support (Phase Separations Limited, Queensferry, Clwyd, U.K.) conditioned for 16 hours at 280°. The carrier was helium at a flow rate of 60 ml/minute. The column temperature was 200°C and the detection and injection temperatures were maintained at about 240°C and about 280°C respectively. The supply of air and hydrogen to the detector were optimized for maximum sensitivity at about 25 ml/minute for hydrogen and 300 ml/minute for air.

To avoid interference from high boiling materials extracted from plasma, the column temperature was increased to 280°C after every 3 or 4 injections and maintained at the elevated temperature until the long running peaks had been eluted (about 20 minutes). Under these conditions pentobarbitone and the internal standard were eluted as symmetrical peaks with retention times of 1.2 minutes and 4 minutes respectively.

TABLE 9:1

Method parameters for the measurement of concentrations of pentobarbitone in plasma.

| | |
|---|---|
| Linear range | 0.3-2.0 µg/ml |
| Calibration line[a] | Y=(0.1434± 0.0035) X |
| Accuracy | ± 14.7% at 15 µg/ml, ± 24.0% at 8.8 µg/ml and 68.9% at 3µg/ml. |
| Precision | ± 5% at 15 µg/ml, ± 6% at 9µg/ml and 5.3% at 3 µg/ml. |
| Sensitivity | 0.3 µg/ml |
| Recovery of pentobarbitone | 97% ± 2.3 SD |
| Recovery of internal standard | 97% ± 2.8 SD at a level of 2 µg/ml |

a Least squares regression line where Y = instrument response peak height ratio, pentobarbitone (to internal standard) and X = plasma concentrations of pentobarbitone.

Results

Plasma concentrations. After a single intravenous injection of pentobarbitone at a level of 12 mg/kg, peak of mean concentrations of pentobarbitone in plasma occurred, as would be expected, at the first time of blood withdrawal (2 minutes after injection), in the cynomolgous monkey and the baboon. However, the peak of mean concentrations of pentobarbitone in the plasma of the rhesus monkey was not reached until 5 minutes after injection (the second time of blood withdrawal). After an initial rapid distribution phase, mean plasma concentrations declined with an apparent half-life of elimination about 2 hours and were still detectable at 7 hours post-administration (Table 9:2; Figures 9:1 - 9:3)

Pharmacokinetic parameters. Observed plasma concentrations of pentobarbitone following a single intravenous injection of pentobarbitone at a level of 12 mg/kg were fitted by a biexponential equation (Equation 2:2). The pharmacokinetic parameters shown in Table 9:3 were, therefore, calculated using equations 2:3 - 2:13 for a two-compartment open model with elimination from the central compartment (Scheme 2:2). Plasma concentrations of pentobarbitone predicted by the two-compartment model were generally in good agreement with the observed values (Figures 9:1 - 9:3). These parameters were compared to those estimated by fitting a one-compartment open model to the data (Equation 1:1, 2:4, 2:13, 2:14) and the parameters estimated by both models were found to be similar except for the half-life values for the rhesus monkey and the cynomolgus monkey (Table 9:4).

The contribution of the distribution phase, $A/\alpha$, to the total area under the plasma concentration-time curve was only small (8, 7 and 6% in the rhesus monkey, the cynomolgus monkey and the baboon respectively) and does not therefore make an important contribution to the total disposition of pentobarbitone. However, inspection of the data shows that the one-compartment model provides a poor fit to the observed concentrations at early times after injection.

Analysis of variance showed that areas under the plasma pentobarbitone concentration time curves for the baboon were significantly lower ($P < 0.05$, non-parametric version of Newman-Keuls multiple comparison procedure) and that the clearance of pentobarbitone in the baboon was significantly higher ($P < 0.05$, Newman-Keuls multiple comparison procedure) than in the other two species. When adjusted for bodyweight differences, the adjusted clearance in the baboon (mean 6.0 μg/ml/kg) was not significantly different from that in the other two species (mean 4.6 μg/ml/kg) in both the rhesus monkey and the cynomolgus monkey, ($P > 0.05$ Newman-Keuls multiple comparison procedure). No other significant species related differences were observed in the pharmacokinetics of pentobarbitone in these non-human primate species.

The mean total volume of distribution of pentobarbitone was equivalent to 82, 96 and 86% of the total bodyweight in the rhesus monkey, the cynomolgus monkey and the baboon respectively, suggesting that pentobarbitone does distribute beyond the body water and into the tissues. In this respect the non-human primate is similar to man (99-170% of total bodyweight, Ehrnebo, 1974; Wagner et al, 1977), the rat (90-120% of total bodyweight, Ossenberg et al, 1975; Buice, 1978) and the rabbit (63-83% of total bodyweight, Kozlowski et al, 1977). In the dog, also, concentrations of pentobarbitone were similar in the plasma, plasma water, cerebrospinal fluid, red cells, liver, brain, muscle, kidney, heart, lung, spleen and lumbodorsal fat, suggesting extensive distribution (Brodie et al, 1953).

Adopting the two-compartment model the compartmental volume ratio (central:peripheral) was 2.3, 1.9 and 2.6 in the rhesus monkey, cynomolgus monkey and baboon respectively, suggesting that there was relatively free transfer of pentobarbitone between the two compartments.

In man, the compartment volume ratio is 0.6 - 0.8 (Ehrnebo, 1974; Ossenberg et al, 1975) and it can be seen from equation 2:9 (page 27) that this ratio implies that in man the rate of transfer of pentobarbitone from the central compartment to the peripheral compartment is greater than that from the reverse transfer process. Thus, if the site of action of this drug (the brain) is in the central compartment and if the fat is in the peripheral compartment, these findings would then

support the view that deposition into fat and not elimination from the body might be responsible for the short duration of action of this type of barbiturate in man as suggested by Goldstein and Aronow (1960).

Pentobarbitone distributes rapidly in the non-human primate, the mean rate constants for the rapid distribution phase corresponding to an apparent half-life of 12 minutes, 9 minutes and 8 minutes in the rhesus monkey, cynomolgus monkey and baboon respectively. These values are similar to those in man (10 minutes, Ehrnebo, 1974) but are three to fourfold longer than those in the rat (3 minutes, Ossenberg et al, 1975).

The mean elimination half-lives of pentobarbitone of 132 minutes, 153 minutes and 106 minutes in the rhesus monkey, the cynomolgus monkey and the baboon, respectively were shorter than those in man ($>$ 22 hours, Brodie et al, 1953; Ehrnebo, 1974; Misra et al, 1971; Smith et al, 1973 and Wagner et al, 1977), but were similar to those in the rat (2 hours, Ossenberg et al, 1975; Buice, 1978) and other laboratory animals (0.6 - 3 hours, Brodie et al, 1953; Kazlowski et al, 1977).

The total volume of distribution estimated after attainment of a pseudo-distribution equilibrium multiplied by the plasma concentration indicates that only 65, 77 and 77% of the administered drug remained in the body of the rhesus monkey, cynomolgus monkey and baboon respectively at 1, 1.0 and 0.67 hours respectively.

The mean ratio of $\beta/K_{el}$ of 0.72, 0.62 and 0.64 for the rhesus monkey, cynomolgus monkey and baboon respectively indicates that if a two-compartment open model is assumed these fractions of the pentobarbitone in the body were in the central compartment and available for elimination at any time during the terminal linear phase of the disposition curve (Gibaldi et al, 1969).

Concentrations of pentobarbitone in the peripheral compartment simulated for a two-compartment open model with pharmacokinetic parameters shown in Table 9:3,using equation 2:16,showed that equilibrium between the central and peripheral compartments was rapidly achieved. Peak concentrations of pentobarbitone of 11.4 μg/ml, 11.3 μg/ml and 12.6 μg/ml in the rhesus monkey, cynomolgus monkey and the baboon, respectively were reached at 40 minutes post-administration in all three species of non-human primates. Thereafter, plasma concentrations of pentobarbitone in the central and peripheral compartments were similar (Table 9:5, Figures 9:1 - 9:3).

## Discussion

In man (Brodie et al, 1953; Ehrnebo, 1974; Smith et al, 1973) and the rat (Ossenberg et al, 1975) pentobarbitone has been shown to distribute according to a two-compartment open model. In non-human primates, the distribution phase does not contribute more than 8% to the total area under the plasma concentration-time curve, suggesting that pentobarbitone kinetics could be described by a one-compartment open model in the non-human primate species without introducing notable errors in the estimation of the pharmacokinetic parameters (Gibaldi and Perrier, 1975). However, inspection of the data shows that the rapid distribution phase is clearly defined and that distribution into the peripheral compartment does appear to be a feature of pentobarbitone disposition in the non-human primate.

The total systemic clearance of pentobarbitone, adjusted for bodyweight differences, in the non-human primate is approximately tenfold higher than in man (0.5 μg/ml/kg, Ehrnebo, 1974; Wagner et al, 1977) but was similar to that in the rat (5 μg/ml/kg - 9 μg/ml/kg, Ossenberg et al, 1975; Bruice, 1978) and in the rabbit (18.6 μg/ml/kg, Kozlowski et al, 1977). Since pentobarbitone is eliminated almost exclusively by metabolism, the total systemic clearance presumably reflects metabolic clearance. If, after intravenous administration biotransformation occurs predominantly in the liver, the values for the clearance of pentobarbitone reflect hepatic clearance in the non-human primate species, therefore clearance values of sevenfold lower than in the liver blood flow imply that it is unlikely that an hepatic first-pass effect would be an important

feature of pentobarbitone disposition in the non-human primate after an oral dose and this would be true also if there were extra-hepatic sites of biotransformation except for biotransformation in the gut wall.

Inspection of the half-lives of elimination of pentobarbitone in the non-human primate, the rat and the dog, shows that during the once-daily dosage regimen common to most chronic toxicity studies, pentobarbitone would not accumulate in the body of these commonly used laboratory animals, since each dose would have been eliminated before the next is received. In contrast, some accumulation of this drug would occur in man when administered once-daily for the treatment of insomnia. For a normal human subject receiving 200 mg of pentobarbitone once-daily a steady state plasma concentration of approximately 4.0 μg/ml would be achieved one week after commencement of treatment. To achieve this same steady state plasma concentration of pentobarbitone in the non-human primate it would be necessary to inject pentobarbitone 13.3 mg, 9.8 mg and 15.2 mg intravenously to the rhesus monkey, cynomolgus monkey and baboon respectively at intervals of 124 minutes, 126 minutes and 100 minutes, respectively or to adopt a similar dosing regimen. Alternatively, a more practical dosing regimen would be the administration of 154.5 mg, 112.0 mg and 218.9 mg orally, to the rhesus monkey, cynomolgus monkey and baboon respectively, once daily, assuming complete availability of pentobarbitone from the G.I. tract. However, even when completely absorbed, the total dose would have been eliminated from the body of the non-human primate after 12 to 15 hours, if given once-daily. It would, therefore be necessary to administer this compound at intervals of about 12 hours to maintain concentrations of pentobarbitone in the plasma of non-human primates throughout the day.

Using pharmacokinetic criteria, therefore, it can be concluded that the non-human primate is not a more suitable model to use to study the fate of pentobarbitone in man than are the other commonly used laboratory animal species.

TABLE 9:2

Mean observed concentrations of pentobarbitone (± SD) in the plasma of the rhesus monkey, the cynomolgus monkey and the baboon after a single bolus intravenous injection of pentobarbitone at a level of 12 mg/kg. Results are expressed as μg/ml.

| Time | | Species | | |
|---|---|---|---|---|
| | | Rhesus monkey | Cynomolgus monkey | Baboon |
| 2 | minute | 13.3 ± 2.5 | 18.3 ± 3.8 | 19.8 ± 7.2 |
| 5 | minute | 17.8 ± 1.8 | 17.0 ± 1.1 | 19.0 ± 5.3 |
| 10 | minute | 17.1 ± 1.6 | 15.0 ± 1.6 | 15.9 ± 4.0 |
| 20 | minute | 13.9 ± 1.8 | 12.8 ± 1.1 | 13.4 ± 2.8 |
| 40 | minute | 11.8 ± 2.4 | 11.0 ± 1.2 | 10.4 ± 1.2 |
| 1 | hour | 9.7 ± 2.6 | 9.3 ± 1.1 | 8.8 ± 1.2 |
| 1.5 | hour | 7.9 ± 2.2 | 7.4 ± 0.9 | 7.2 ± 1.1 |
| 2 | hour | 6.6 ± 2.2 | 6.2 ± 0.7 | 5.8 ± 1.1 |
| 3 | hour | 4.6 ± 2.0 | 5.0 ± 0.8 | 4.0 ± 1.0 |
| 4 | hour | 3.6 ± 2.1 | 4.0 ± 0.8 | 2.4 ± 0.6 |
| 5 | hour | 2.7 ± 1.7 | 2.9 ± 0.7 | 1.6 ± 0.8 |
| 7 | hour | 1.8 ± 1.3 | 1.6 ± 0.4 | 1.1 ± 0.5 |

TABLE 9:3

Mean pharmacokinetic parameters (± SD) determined for a two-compartment model after a single intravenous injection of pentobarbitone (12 mg/kg) into the rhesus monkey, the cynomolgus monkey and the baboon. The data are presented as means of results from individual animals.

| Parameter[a] | | Species | | |
|---|---|---|---|---|
| | | Rhesus monkey | Cynomolgus monkey | Baboon |
| Bodyweight (kg) | | 5.0 ± 0.8 | 3.5 ± 0.3 | 6.1 ± 0.9 |
| Dose | (mg) | 60.0 ± 11.2 | 41.4 ± 4.5 | 72.0 ± 85 |
| Dose | (mg/kg) | 12.1 ± 0.4 | 12.1 ± 2.2 | 12.0 ± 0.6 |
| A | (μg/ml) | 5.9 ± 3.6 | 8.1 ± 3.3 | 8.7 ± 7.5 |
| B | (μg/ml) | 12.9 ± 3.6 | 11.5 ± 2.1 | 13.2 ± 2.4 |
| $\alpha$ | ($hour^{-1}$) | 3.530 ± 3.579 | 4.5 ± 4.308 | 5.390 ± 3.706 |
| $\beta$ | ($hour^{-1}$) | 0.375 ± 0.242 | 0.283 ± 0.060 | 0.409 ± 0.089 |
| $K_{12}$ | ($minute^{-1}$) | 0.013 ± 0.015 | 0.026 ± 0.026 | 0.028 ± 0.026 |
| $K_{21}$ | ($minute^{-1}$) | 0.045 ± 0.046 | 0.046 ± 0.045 | 0.058 ± 0.037 |
| $K_{el}$ | ($minute^{-1}$) | 0.008 ± 0.004 | 0.008 ± 0.002 | 0.011 ± 0.004 |
| AUC | (μg/ml/hour) | 48 ± 24 | 44 ± 7 | 35 ± 6[b] |
| Cl | (ml/minute) | 23 ± 10 | 16 ± 3 | 36 ± 9[b] |
| $t_{½}$ | (minutes) | 132 ± 63 | 153 ± 35 | 106 ± 24 |
| $V_1$ | (litres) | 3.2 ± 0.6 | 2.1 ± 0.2 | 3.7 ± 1.6 |
| $V_2$ | (litres) | 0.9 ± 0.6 | 1.1 ± 0.5 | 1.4 ± 0.8 |
| $V_{d(ss)}$ | (litres) | 4.1 ± 1.2 | 3.3 ± 0.4 | 5.2 ± 1.8 |
| $V_{d(\beta)}$ | (litres) | 4.0 ± 1.7 | 3.4 ± 0.1 | 5.3 ± 1.3 |
| A/$\alpha$ | (μg/ml/hour) | 4 ± 4 | 3 ± 1 | 2 ± 2 |
| B/$\beta$ | (μg/ml/hour) | 45 ± 21 | 41 ± 6 | 32 ± 6 |

a For details of abbreviations used see Chapter 2.

b Significance level (analysis of variance) baboons compared to the other two species ($P < 0.05$).

TABLE 9:4

Mean pharmacokinetic parameters of pentobarbitone in the rhesus monkey, the cynomolgus monkey and the baboon for a one-compartment open model compared to those for a two-compartment open model following a single intravenous injection of pentobarbitone (12 ng/kg). The data are presented as means of results from individual animals.

| Parameter[a] | | Species | | | | | |
|---|---|---|---|---|---|---|---|
| | | One-compartment | | | Two-compartment | | |
| | | Rhesus monkey | Cynomolgus monkey | Baboon | Rhesus monkey | Cynomolgus monkey | Baboon |
| AUC | (μg/ml/hour) | 45 | 44 | 36[b] | 48 | 44 | 35[b] |
| Cl | (ml/minute) | 25 | 16 | 34[b] | 23 | 16 | 36[b] |
| $t_{1/2}$ | (minutes) | 124 | 126 | 100 | 132 | 153 | 106 |
| $V_d$ | (litres)[c] | 4.1 | 2.8 | 4.9 | 4.1 | 3.3 | 5.2 |
| $K_{el}$ | (minute$^{-1}$) | 0.006 | 0.006 | 0.007 | 0.008 | 0.008 | 0.011 |

a For details of abbreviations used see Chapter 2.

b Significance level (analysis of variance), baboons compared to the other two species ($P < 0.05$).

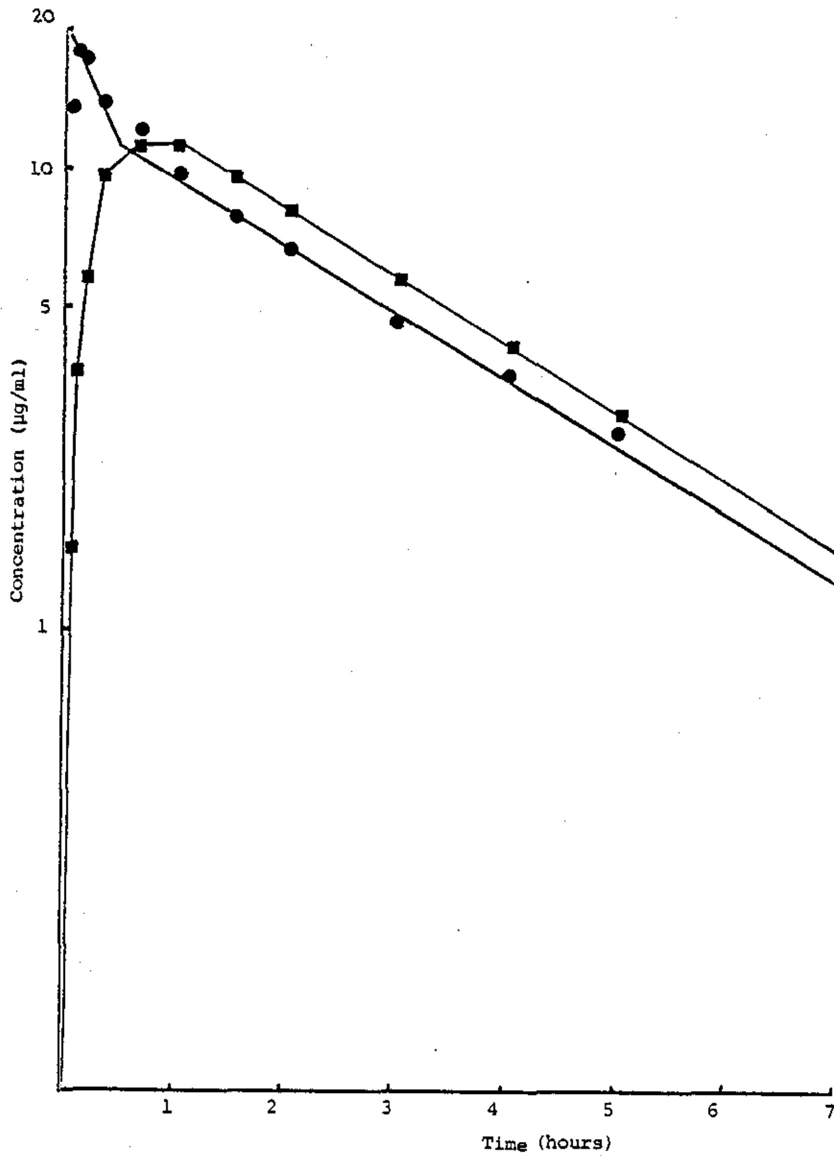
c $V_d$ = total volume of distribution = $V_{d(ss)}$ for the two-compartment model.

TABLE 9:5

Calculated concentrations of pentobarbitone in the peripheral compartment after bolus intravenous injection of pentobarbitone 12 µg/ml to non-human primates. Results calculated from mean data and expressed as µg/ml.

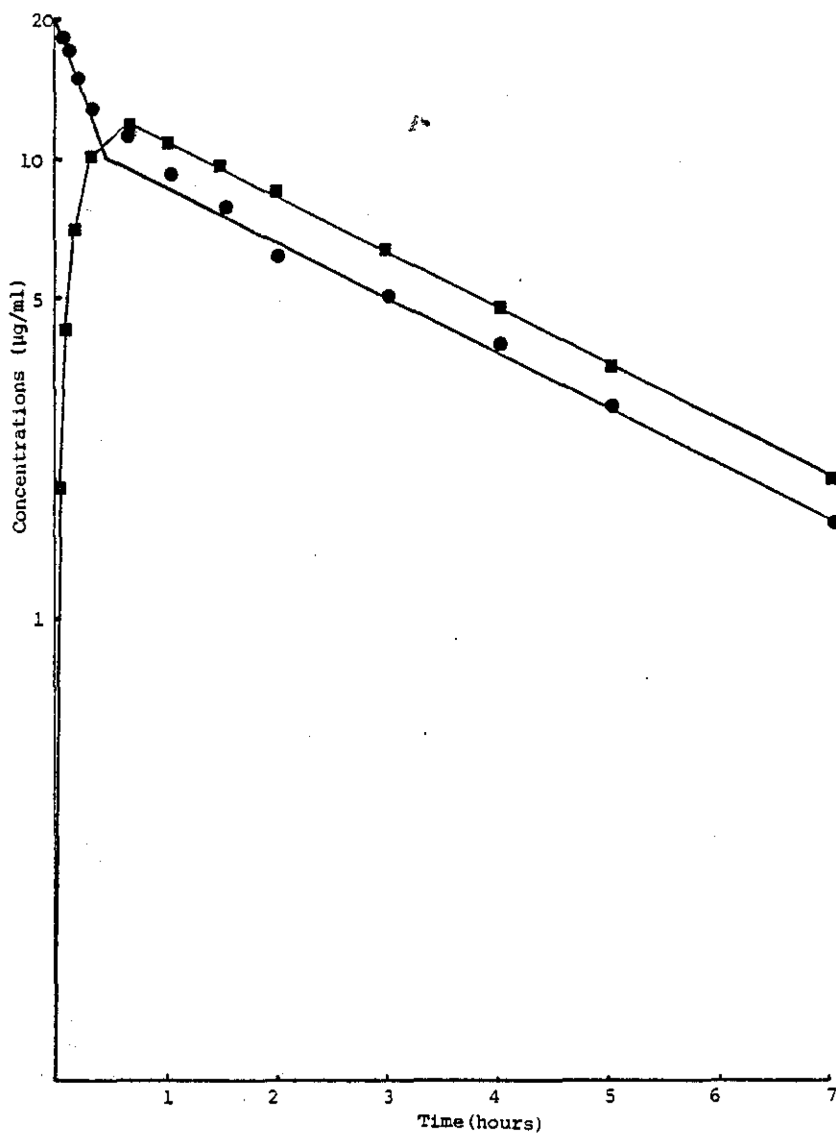
| Time | | Species | | |
|---|---|---|---|---|
| | | Rhesus monkey | Cynomolgus monkey | Baboon |
| 2 | minute | 1.5 | 1.9 | 1.6 |
| 5 | minute | 3.6 | 4.2 | 3.7 |
| 10 | minute | 5.8 | 7.0 | 6.3 |
| 20 | minute | 9.5 | 10.0 | 9.5 |
| 40 | minute | 11.4 | 11.3 | 12.6 |
| 1 | hour | 11.1 | 10.8 | 10.9 |
| 1.5 | hour | 9.6 | 9.5 | 9.4 |
| 2 | hour | 8.2 | 8.3 | 7.9 |
| 3 | hour | 5.8 | 6.2 | 5.5 |
| 4 | hour | 4.1 | 4.7 | 3.9 |
| 5 | hour | 2.9 | 3.5 | 2.7 |
| 7 | hour | 1.4 | 2.0 | 1.3 |

FIGURE 9:1



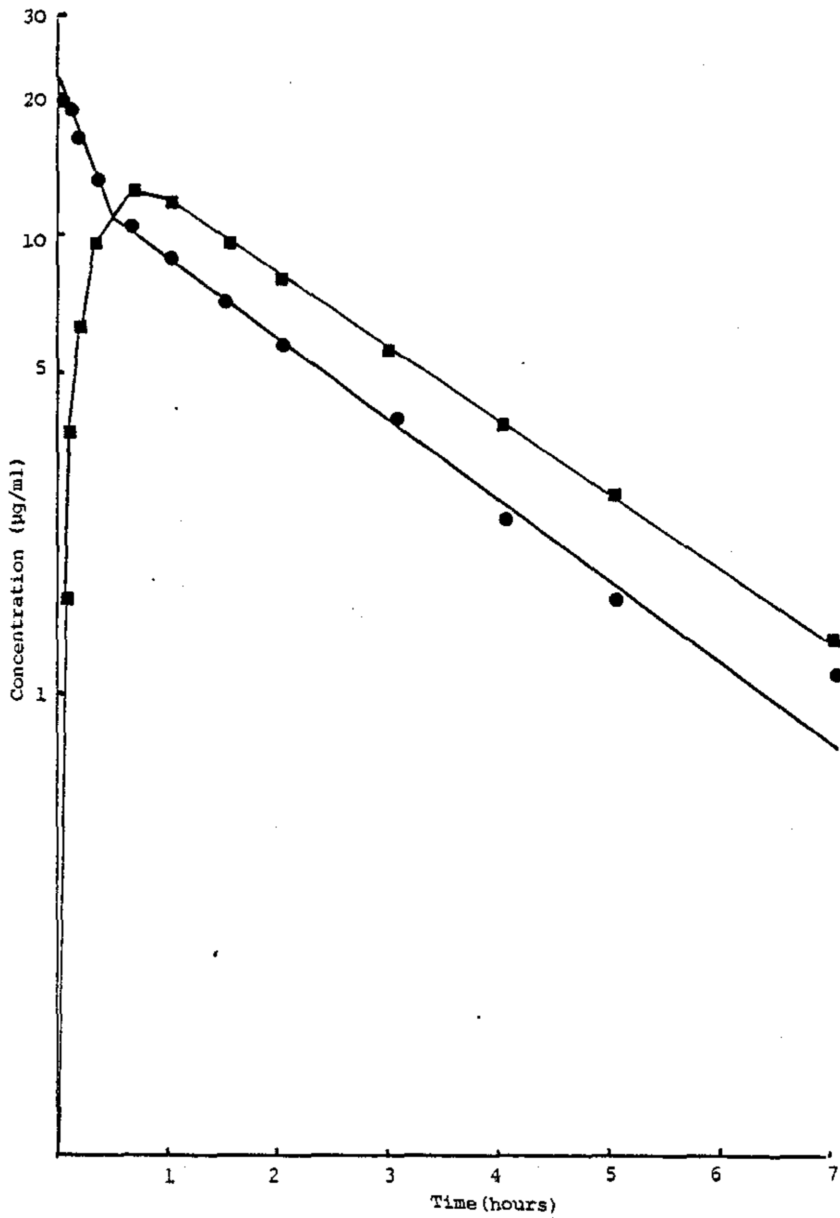

Mean concentration-time curves after single intravenous injection of pentobarbitone 12 mg/kg to rhesus monkeys. Observed concentrations in the central compartment ( ● ● ), and predicted concentrations in the central compartment (solid line) and in the peripheral compartment( ■ ■ )

FIGURE 9:2




Mean concentration-time curves after single intravenous injection of pentobarbitone 12 mg/kg to cynomolgus monkeys. Observed concentration in the central compartment (● ●), and predicted concentration in the central compartment (solid line) and in the peripheral compartment ( ■ ■ ).

FIGURE 9:3




Mean concentration-time curves after single intravenous injection of pentobarbitone 12 mg/kg to baboons. Observed concentrations ( ● ● ), and predicted concentrations in the central compartment (solid lines) and in the peripheral compartment ( ■ ■ ).

CHAPTER 10

## GENERAL DISCUSSION

After single intravenous injection of antipyrine, diazepam, frusemide, ibuprofen, isosorbide dinitrate, paracetamol and pentobarbitone, plasma drug concentration-time curves were fitted by a biexponential equation in the rhesus monkey, the cynomolgus monkey and the baboon suggesting that the pharmacokinetics of these compounds could be described by a two-compartment open model. However, the non-linear least squares curve fitting programme used, BLUD, described these curves by a biexponential equation even though the initial distribution phase was short. For those compounds, i.e. antipyrine, frusemide, isosorbide dinitrate and pentobarbitone, for which an inspection of the plasma concentration-time curve suggested that a one-compartment might be more appropriate, the data was also fitted by normal regression techniques and the parameters predicted by both models were compared. Whichever model was appropriate, either a one-compartment open model or a two-compartment open model there were no species differences in the kinetic model that was most suitable for these compounds. A review of the literature of the pharmacokinetics of the compounds studied, in man and the commonly used laboratory animal species, reveals that in general, the same kinetic model applies in non-human primate species and other species.

Analysis of variance of pharmacokinetic parameters showed that there were few significant pharmacokinetic differences between the rhesus monkey, the cynomolgus monkey and the baboon. Table 10:1 shows the apparent total volume of distribution expressed as a percentage of bodyweight for the non-human primate, man, the rat and other commonly used laboratory animals such as the dog, rabbit and guinea pig. From Table 10:1 it can be seen that the volume of distribution per kg bodyweight is similar in the non-human primate species and in man, the rat and other species, for each compound except diazepam and isosorbide

TABLE 10:1

Volumes of distribution ($V_{d(ss)}$) expressed as a percentage of bodyweight. A summary of individual results reported in Chapters 3-9.

| Compound | Species | | | | | |
|---|---|---|---|---|---|---|
| | Rhesus monkey | Cynomolgus monkey | Baboon | Rat[a] | Man[a] | Others[a] |
| Antipyrine | 88 | 73 | 66 | 76 | 40-60 | 60-99 |
| Diazepam (0.2 mg/kg) | 50 | 53 | 39 | 784 | 70-200 | 400-1300 |
| Desmethyldiazepam (0.2 mg/kg)[b] | 171 | 183 | 128 | NA | 64 | 100 |
| Diazepam (1.0 mg/kg) | 44[c] | 81 | 63 | - | - | - |
| Desmethyldiazepam (1 mg/kg)[b] | 142 | 182 | 288 | - | - | - |
| Frusemide | 11 | 11 | 9 | 16 | 7-21 | 11 |
| Isosorbide dinitrate | 273[d] | 168 | 84 | NA | 3-6 | NA |
| Paracetamol | 82 | 106 | 98 | 85 | 85-95 | 58 |
| Ibuprofen | 14 | 8 | 10 | NA | NA | NA |
| Pentobarbitone | 82 | 96 | 86 | 90-120 | 99-170 | 63-83 |

a For references see Chapters 3 - 9.

b Estimated, assuming that all the administered diazepam is primarily metabolised to desmethyldiazepam before elimination.

c Significant difference (analysis of variance) rhesus monkey compared with the cynomolgus monkey and baboon ($P < 0.05$).

d Significant difference (analysis of variance) rhesus monkey compared with the cynomolgus monkey and baboon ($P < 0.01$).

NA Value not available.

dinitrate. The relatively low values obtained for diazepam in the non-human primate are possibly attributable to the method of analysis of plasma concentrations, which was by high pressure liquid chromatography. This technique is presumably more accurate at low concentrations than the previously used gas chromatographic methods. For fuller discussion see Chapter 4.

The large differences in the apparent volume of distribution per kg bodyweight of isosorbide dinitrate in the non-human primate and man are difficult to explain. However, it does appear that in the non-human primate following intravenous injection at a high dose level (1 mg/kg), that isosorbide dinitrate might be sequestered into a deep peripheral compartment and this does not seem to be detected in man after infusion of this compound (12 mg in 2.5 hours; Taylor et al, 1980).

Clearance is dependent on the organ extraction efficiency (extraction ratio) and on the supply of compound (i.e. blood flow) to the eliminating organs. For those compounds with clearance rates less than 45 ml/min, antipyrine, N-desmethyldiazepam, frusemide, ibuprofen and pentobarbitone, the clearance in the lighter cynomolgus monkey is lower than in the other two species (Table 10:2) and presumably reflects the corresponding activities of the drug metabolising enzymes responsible for the biotransformation of these compounds. Those compounds with higher clearance rates, diazepam, isosorbide dinitrate and paracetamol, were cleared at a similar rate in all three species although it might have been expected that the lighter cynomolgus monkey would have a lower rate of clearance because of its smaller organ size; the sizes of the organs being approximately proportional to (bodyweight) $^{2/3}$ (Dedrick, 1972).

As antipyrine is eliminated almost exclusively by oxidative metabolism, this compound has been used as a model compound for the measurement of oxidative enzyme activity (Conney, 1967; Hunter and Chasseaud, 1976; Vesell, 1979). Using antipyrine clearance as an indicator of oxidative enzyme activity, Table 10:2 implies that the cynomolgus monkey has a significantly lower enzyme activity than the

TABLE 10:2

Total systemic clearance values observed in the rhesus monkey, the cynomolgus monkey and the baboon. A summary of individual results reported in Chapters 3 - 9.

| Compound | Species | | |
|---|---|---|---|
| | Rhesus monkey (ml/minute) | Cynomolgus monkey (ml/minute) | Baboon (ml/minute) |
| Antipyrine | 26 | 16[b] | 28 |
| Diazepam (0.2 mg/kg) | 66 | 65 | 43[d] |
| Desmethyldiazepam (0.2mg/kg[a]) | 11.2 | 8.5 | 21.9[e] |
| Diazepam (1.0mg/kg) | 59 | 60 | 58 |
| Desmethyldiazepam (1mg/kg[a]) | 13.3 | 7.1 | 13.8 |
| Frusemide | 18 | 10[b] | 23 |
| Isosorbide dinitrate | 188 | 200 | 169 |
| Paracetamol | 46 | 49 | 51 |
| Ibuprofen | 15 | 7[c] | 13 |
| Pentobarbitone | 23 | 16 | 36[d] |

a Estimated assuming that all the administered diazepam is primarily metabolised to desmethyldiazepam before elimination.

b Significant difference (analysis of variance) cynomolgus monkey compared to the other two species ($P < 0.05$).

c Significant difference (analysis of variance) cynomolgus monkey compared to the other two species ($P < 0.01$).

d Significant difference (analysis of variance) baboon compared to the other two species ($P < 0.05$).

e Significant difference (analysis of variance) baboons compared to the other two species ($P < 0.01$).

other two species. However, when adjusted for bodyweight differences, the clearance of antipyrine in the cynomolgus monkey was only slightly less than in the other two species (Table 10:3), and this difference was not statistically significant ($P<0.05$). The oxidative enzyme activity per kg bodyweight, therefore, would appear to be similar in these three species of non-human primate.

Isosorbide dinitrate has been shown to have a high hepatic extraction ratio (Needleman et al, 1972) and, therefore, the clearance of this compound approximates to hepatic blood flow. If isosorbide dinitrate clearance is taken as a measure of hepatic blood flow this suggests that the liver blood flow is similar in the rhesus monkey, cynomolgus monkey and baboon and is approximately 30 ml/minute/kg (Table 10:2). Isosorbide dinitrate clearance is, however, only an approximate measure of the hepatic clearance which is approximately 50 ml/minute/kg (see page 100).

When adjusted for bodyweight, clearances tended to be different in the cynomolgus monkey compared with the other two species of non-human primates (Table 10:3), but in general adjusted clearances were similar in the non-human primate species to those in other commonly used laboratory animal species except for the rat. However, with the exception of frusemide and possibly ibuprofen, clearances were higher in animal species than in humans (Table 10:3).

The biological half-life is frequently used to compare the rate of elimination of foreign compounds by different animal species. Inspection of Table 10:4 shows that with respect to this parameter, the non-human primate species are closer to the rat and to other commonly used laboratory animal species, than to man. In particular, antipyrine, diazepam, desmethyldiazepam and pentobarbitone which have biological half-lives greater than 12 hours in man, have got considerably shorter half-lives in the commonly used laboratory animal species. A similar comparison of other compounds reported in the literature shows the same trend, whereby, with the possible exception of indomethacin, isoniazid and

TABLE 10:3

Total systemic clearance adjusted for differences in bodyweight. A summary of individual results reported in Chapters 3-9.

| Compound | Species | | | | | |
|---|---|---|---|---|---|---|
| | Rhesus monkey (ml/minute /kg) | Cynomolgus monkey (ml/minute /kg) | Baboon (ml/minute /kg) | Rat[a] (ml/minute /kg) | Man[a] (ml/minute /kg) | Others[a] (ml/minute/kg) |
| Antipyrine | 5.5 | 4.4 | 5.0 | 9.0 | 0.9 | 9.0 |
| Diazepam (0.2 mg/kg) | 14.3 | 18.1[c] | 7.5 | 81.6 | 0.4 | 19-31 |
| Desmethyldiazepam (0.2mg/kg[b]) | 2.4 | 2.4 | 3.8[h] | NA | 0.2 | 33.4 |
| Diazepam (1.0 mg/kg) | 13.1 | 17.6[d,e] | 10.2 | - | - | - |
| Desmethyldiazepam (1mg/kg[b]) | 2.8 | 2.0 | 2.6 | - | - | - |
| Frusemide | 4.0 | 2.9[d] | 3.7 | 7.0 | 2.4 | 4.3 |
| Isosorbide dinitrate | 39 | 59[f] | 29 | NA | 0.2-0.5 | NA |
| Paracetamol | 9.2 | 14.0 | 8.5 | 34 | 5 | 18 |
| Ibuprofen | 3.1[g] | 2.0 | 2.1 | NA | NA | NA |
| Pentobarbitone | 4.6 | 4.6 | 6.0 | 5-9 | 0.5 | 6-18 |

NA Value not available.

a For references see Chapters 3 - 9.

b Estimated, assuming that all administered diazepam is primarily metabolised to desmethyldiazepam before elimination.

c Significant difference (analysis of variance) cynomolgus monkey compared with the other two non-human primate species ($P<0.01$).

d Significant difference (analysis of variance) cynomolgus monkey compared with the rhesus monkey ($P<0.05$).

e Significant difference (analysis of variance) cynomolgus monkey compared with the baboon ($P<0.01$).

f Significant difference (analysis of variance) cynomolgus monkey compared with the baboon ($P<0.05$).

g Significant difference (analysis of variance) rhesus monkey compared with the other two non-human primate species ($P<0.05$).

h Significant difference (analysis of variance) baboon compared with the other two non-human primate species ($P<0.05$).

TABLE 10:4

Half-lives of elimination in non-human primates, the rat, man and other commonly used laboratory animal species. A summary of individual results reported in Chapters 3-9.

| Compound | Species | | | | | |
|---|---|---|---|---|---|---|
| | Rhesus monkey (minutes) | Cynomolgus monkey (minutes) | Baboon (minutes) | Rat[a] (minutes) | Man[a] (minutes) | Others[a] (minutes) |
| Antipyrine[b] | 108 | 108 | 90 | 144 | 12[c] | 110 |
| Diazepam (0.2 mg/kg) | 54 | 43 | 60 | 60 | 24-48[c] | 80-456 |
| Diazepam (1.0 mg/kg) | 45 | 50 | 64 | - | - | - |
| Desmethyldiazepam(0.2mg/kg) | 7.9[c] | 9.3[c] | 4.5[c] | - | 30-200[c] | 10[c] |
| Desmethyldiazepam(1.0mg/kg) | 5.8[c] | 10.5[c] | 10.6[c] | - | - | - |
| Frusemide[b] | 26 | 28 | 14 | 22 | 26-72 | 25 |
| Isosorbide dinitrate[d] | 62[e] | 23 | 24 | <1 | 9 | 7 |
| Paracetamol | 72 | 59 | 75 | 37 | 2-3[c] | 32 |
| Ibuprofen | 91 | 70 | 49 | 84 | 2[c] | 3.5[c] |
| Pentobarbitone | 132 | 153 | 106 | 123 | >22[c] | 36-180 |

a For references, see Chapters 3-9.
b Half-lives estimated for a one-compartment open model.
c Half-lives are expressed in hours.
d Half-lives shown are possibly associated with slow release from a deep peripheral compartment (see Chapter 6).
e Significant difference (analysis of variance) rhesus monkey compared to the other two non-human primate species (P<0.01).

caffeine, the plasma biological half-lives are shorter in the common laboratory animals than in man (Table 10:5). In particular, there does not appear to be a suitable kinetic model for man amongst these species for those compounds with half-lives greater than 30 hours in man.

The relatively short half-lives found in the non-human primate species and other commonly used laboratory animal species indicate that during the once-daily dosing regimen common to most chronic toxicity studies, most compounds would not accumulate in the body of the laboratory animal species. This is because each dose would have been eliminated before the next is received ; thus the toxic effects of the compound may be under-estimated relative to human use. In contrast some accumulation of foreign compounds with half-lives longer than 4 hours would occur in humans during a once-daily dosing regimen. In clinical practice, however, drugs are administered at intervals throughout the day that are too short to allow complete elimination of the administered dose before the next is received. It is possible, therefore, with such a dosing regimen, that drug would always be present in the body to produce advantageous pharmacological effects, but also possibly undesirable side effects. Equations 2:16 and 2:17 can be used to estimate the steady state plasma concentrations of drug that would be achieved during normal therapy in humans; and also to define a dosing regimen that needs to be followed during the administration of compounds to non-human primates to achieve the same steady state plasma concentrations as those observed in man.

Table 10:6 shows the intravenous doses required, if administered at intervals of one elimination half-life to achieve the normal steady state drug concentrations in the rhesus monkey, the cynomolgus moneky and the baboon, for the compounds described in this thesis. From Table 10:6 it can be seen that it would be necessary to administer

TABLE 10:5

Plasma half-lives in the rhesus monkey, rat, man and dog (from Smith and Caldwell, 1977).

| Compound | Species | | | |
|---|---|---|---|---|
| | Rhesus monkey (hours) | Rat (hours) | Man (hours) | Dog (hours) |
| Indomethacin | 1.5 | 4 | 2 | 1.5 |
| Isoniazide | 5 | 10 | 5 | 5 |
| Caffeine | 2.4 | 2 | 3.5 | 5 |
| Pethidine | 1.2 | - | 5.5 | 0.9 |
| Halofenate | 16 | 26 | 24 | 24 |
| Diazoxide | 19 | - | 29 | - |
| Myalex | 3 | 29 | 31 | 30 |
| Oxisuran | 21 | 10 | 55 | 12 |
| Phenylbutazone | 8 | - | 72 | 6 |
| Oxyphenylbutazone | 8 | - | 72 | 0.5 |

TABLE 10:6

Possible dosing regimes to achieve normal steady state concentrations of drug in the plasma of rhesus monkeys, cynomolgus monkeys and baboons.

| Drug | Species | | | | | | | | Predicted mean steady state plasma concentration |
|---|---|---|---|---|---|---|---|---|---|
| | Rhesus monkey | | Cynomolgus monkey | | Baboon | | Human | | |
| | Dose (mg) | $\tau$ (minutes) | Dose (mg) | $\tau$ (minutes) | Dose (mg) | $\tau$ (minutes) | Dose (mg) | $\tau$ (hours) | |
| Antipyrine | 16.6 | 108 | 10.4 | 108 | 14.3 | 90 | 250 | 12 | 5500 |
| Diazepam | 1.9 | 45 | 2.5 | 50 | 2.9 | 64 | 30 | 24 | 750 |
| Frusemide | 0.2 | 26 | 0.1 | 28 | 0.1 | 14 | 80 | 24 | 400 |
| Isosorbide dinitrate | 11.6[a] | 62 | 4.6[a] | 23 | 4.1[a] | 24 | 10 | 6 | 1 |
| Paracetamol | 19.9 | 75 | 17.3 | 59 | 23.0 | 75 | 1000 | 8 | 6000 |
| Ibuprofen | 0.6 | 91 | 0.2 | 70 | 0.3 | 49 | 400 | 8 | 400 |
| Pentobarbitone | 13.3 | 134 | 9.8 | 153 | 15.2 | 106 | 200 | 24 | 4000 |

Dose — Amount of drug injected intravenously.

$\tau$ — Dosing interval.

a — Dose administered in µg quantities.

relatively more drug per day to non-human primates than to man to maintain the same steady state plasma concentrations of drug in both non-human primates and humans and it would be necessary to administer the drug at frequent intervals to ensure that some drug remains in the body of the non-human primate throughout the day. For example, it would be necessary to administer 61 mg (1.9 mg every 45 minutes) of diazepam to the rhesus monkey to maintain the same steady state plasma concentration of diazepam observed in man following a once-daily administration of 30 mg diazepam.

In most pharmacology and toxicology studies, however, the drug is given orally. The amount of drug that will reach the systemic circulation will therefore partly depend on the dose matrix and would be impossible to predict. Table 10:6 is, therefore, intended to be a primary standard dosing regimen to obtain the steady state concentrations aimed for.

Using pharmacokinetic criteria, therefore, it can be concluded that the cynomolgus monkey and the baboon are possible replacement species for the rhesus monkey for use in pharmacological and toxicological studies, but that the non-human primate is not a more suitable model for man than are the other commonly used laboratory animal species. However, it is possible to design a dosing regimen to compensate for species differences in the rate of elimination if the pharmacokinetic parameters are known. Other factors in the choice of animal model for man for use in safety testing of foreign compounds are differences in the pattern and nature of biotransformation, distribution of drug into target tissue and protein binding. It is not possible to compensate for species differences with respect to these latter factors, but it has been suggested that with respect to biotransformation of foreign compounds, the non-human primate is a more suitable model for man than are other commonly used laboratory animal species (Smith and Caldwell, 1977).

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# APPENDIX

Concentrations of antipyrine in the plasma of rhesus monkeys after intravenous injection of antipyrine 92 mg/kg. Results are expressed as µg/ml.

| TIME | ANIMAL | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| 2 minute | 157 | 79 | 86 | 136 | 107 |
| 5 minute | 186 | 129 | 93 | 143 | 107 |
| 10 minute | 157 | 100 | 79 | 129 | 107 |
| 20 minute | 136 | 86 | 64 | 121 | 93 |
| 40 minute | 107 | 79 | 57 | 107 | 79 |
| 1 hour | 93 | 71 | 50 | 86 | 64 |
| 2 hour | 71 | 43 | 36 | 57 | 43 |
| 3 hour | 50 | 36 | 21 | 36 | 36 |
| 4 hour | 36 | 21 | 14 | 21 | 21 |
| 5 hour | 29 | 14 | ND | 14 | 14 |
| 7 hour | 14 | ND | ND | ND | ND |

ND: Less than the limit of detection (2µg/ml).

Concentrations of antipyrine in the plasma of cynomolgus monkeys after intravenous injection of antipyrine 92 mg/kg. Results are expressed as µg/ml.

| TIME | ANIMAL | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| 2 minute | 137 | 137 | 98 | 67 | 82 |
| 5 minute | 141 | 149 | 131 | 133 | 133 |
| 10 minute | 137 | 143 | 131 | 133 | 129 |
| 20 minute | 125 | 137 | 118 | 114 | 110 |
| 40 minute | 108 | 116 | 112 | 84 | 90 |
| 1 hour | 94 | 98 | 92 | 86 | 75 |
| 1.5 hour | 75 | 80 | 75 | 69 | 63 |
| 2 hour | 65 | 71 | 67 | 57 | 49 |
| 3 hour | 41 | 45 | 43 | 31 | 35 |
| 4 hour | 29 | 33 | 31 | 24 | 27 |
| 5 hour | 24 | 22 | 24 | 18 | 16 |
| 7 hour | 14 | 18 | 16 | 4 | 8 |

Concentrations of antipyrine in the plasma of baboons after intravenous injection of antipyrine 92 mg/kg. Results are expressed as μg/ml.

| TIME | ANIMAL | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| 2 minute | 108 | 167 | 123 | 144 | 167 |
| 5 minute | 148 | 179 | 121 | 150 | 175 |
| 10 minute | 129 | 121 | 144 | 131 | 190 |
| 20 minute | 117 | 121 | 131 | 121 | 129 |
| 40 minute | 102 | 76 | 106 | 115 | 112 |
| 1 hour | 79 | 76 | 79 | 104 | 97 |
| 1.5 hour | 69 | 50 | 79 | 77 | 96 |
| 2 hour | 40 | 49 | 62 | 67 | 67 |
| 3 hour | 29 | 19 | 55 | 39 | 46 |
| 4 hour | 14 | 12 | 30 | 22 | 35 |
| 5 hour | 10 | 8 | 16 | 17 | 21 |
| 7 hour | 4 | 4 | 4 | 7 | 10 |

Concentrations of diazepam in plasma after bolus intravenous injection of diazepam 0.2 mg/kg to rhesus monkeys. Results are expressed as ng/ml.

| TIME | ANIMALS | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| 2 minute | 450 | 399 | 414 | 417 | 478 |
| 5 minute | 401 | 419 | 368 | 343 | 401 |
| 10 minute | 346 | 300 | 310 | 160 | 315 |
| 20 minute | 213 | 201 | 173 | 66 | 196 |
| 40 minute | 91 | 79 | 84 | 33 | 117 |
| 1 hour | 43 | 43 | 38 | 15 | 48 |
| 1.5 hour | NS | NS | NS | NS | NS |
| 2 hour | 15 | 5 | 5 | 5 | 18 |
| 4 hour | 2 | ND | ND | ND | 7.5 |
| 6 hour | ND | ND | ND | ND | 3 |

ND: Less than the limit of detection (2 ng/ml).

NS: No sample taken at this time.

Concentrations of diazepam in plasma after intravenous injection of diazepam 0.2 mg/kg to cynomolgus monkeys. Results are expressed as ng/ml.

| TIME | ANIMAL | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| 2 minute | 428 | 439 | 266 | 602 | 298 |
| 5 minute | 395 | 323 | 279 | 375 | 254 |
| 10 minute | 267 | 231 | 249 | 356 | 215 |
| 20 minute | 198 | 190 | 157 | 220 | 112 |
| 40 minute | 115 | 82 | 85 | 113 | 56 |
| 1 hour | 54 | 46 | 41 | 46 | 34 |
| 1.5 hour | 30 | 15 | 18 | 21 | 21 |
| 2 hour | 20 | 3 | 16 | 13 | 11 |
| 3 hour | 3 | ND | 8 | 5 | 3 |
| 4 hour | ND | ND | 3 | 2 | ND |
| 5 hour | ND | ND | ND | ND | ND |

ND: Less than the limit of detection (2 ng/ml).

Concentrations of diazepam in plasma after bolus intravenous injection of diazepam 0.2 mg/kg to baboons. Results are expressed as ng/ml.

| TIME | ANIMAL | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| 2 minute | 884 | 630 | 450 | 520 | 458 |
| 5 minute | 740 | 700 | 460 | 440 | 440 |
| 10 minute | 572 | 530 | 400 | 416 | 348 |
| 20 minute | 488 | 458 | 304 | 304 | 300 |
| 40 minute | 200 | 331 | 194 | 207 | 173 |
| 1 hour | 108 | 210 | 124 | 128 | 138 |
| 1.5 hour | 72 | 148 | 80 | 90 | 82 |
| 2 hour | 48 | 116 | 48 | 82 | 44 |
| 3 hour | 22 | 76 | 20 | 36 | 10 |
| 4 hour | 11 | 46 | 8 | 18 | ND |
| 5 hour | 5 | 26 | ND | 9 | ND |
| 7 hour | ND | 10 | ND | 3 | ND |

ND Less than the limit of detection (2 ng/ml).

Concentrations of diazepam in plasma after bolus intravenous injection of diazepam 1.0 mg/kg to rhesus monkeys. Results are expressed as ng/ml.

| TIME | ANIMAL | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| 2 minute | 3071 | 2879 | 3338 | 2599 | 3092 |
| 5 minute | 2274 | 2696 | 2355 | 2759 | 2604 |
| 10 minute | 1677 | 2066 | 1966 | 1946 | 1804 |
| 20 minute | 1326 | 1390 | 1105 | 1369 | 1026 |
| 40 minute | 556 | 605 | 310 | 696 | 478 |
| 1 hour | 356 | 340 | 226 | 417 | 226 |
| 1.5 hour | 191 | 124 | 102 | 183 | 135 |
| 2 hour | 130 | 86 | 51 | 94 | 86 |
| 3 hour | 66 | 44 | 15 | 16 | 32 |
| 4 hour | 30 | 16 | 2 | 4 | 16 |
| 5 hour | 8 | 5 | ND | ND | ND |
| 7 hour | ND | ND | ND | ND | ND |

ND Less than the limit of detection (2 ng/ml).

Concentrations of diazepam in plasma after intravenous injection of diazepam 1.0 mg/kg to cynomolgus monkeys. Results are expressed as ng/ml.

| TIME | ANIMAL | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| 2 minute | 2705 | 779 | 1918 | 2197 | 1607 |
| 5 minute | 2148 | 902 | 1853 | 2082 | 1475 |
| 10 minute | 1721 | 787 | 1541 | 1557 | 1197 |
| 20 minute | 1016 | 533 | 1098 | 1131 | 656 |
| 40 minute | 475 | 344 | 426 | 557 | 443 |
| 1 hour | 269 | 180 | 295 | 298 | 230 |
| 1.5 hour | 131 | 98 | 148 | 131 | 148 |
| 2 hour | 66 | 66 | 82 | 66 | 82 |
| 3 hour | 33 | 33 | 35 | 25 | 49 |
| 4 hour | 12 | 16 | 16 | 7 | 16 |
| 5 hour | 5 | 7 | 5 | ND | 7 |
| 7 hour | ND | ND | ND | ND | ND |

ND Less than the limit of detection (2 ng/ml)

Concentrations of diazepam in plasma after bolus intravenous injection of diazepam 1.0 mg/kg to baboons. Results are expressed as ng/ml.

| TIME | | ANIMAL | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 2 | minute | 3300 | 2320 | 1950 | 1530 | 1760 |
| 5 | minute | 3360 | 2500 | 1920 | 1980 | 2030 |
| 10 | minute | 2700 | 2000 | 1750 | 1320 | 1670 |
| 20 | minute | 1100 | 1000 | 1440 | 1350 | 1740 |
| 40 | minute | 630 | 600 | 780 | 628 | 670 |
| 1 | hour | 286 | 262 | 476 | 444 | 472 |
| 1.5 | hour | 220 | 145 | 250 | 279 | 255 |
| 2 | hour | 186 | 86 | 194 | 200 | 150 |
| 3 | hour | 76 | 55 | 108 | 120 | 86 |
| 4 | hour | 42 | 24 | 50 | 56 | 55 |
| 5 | hour | 24 | 10 | 25 | 25 | 32 |
| 7 | hour | 7 | 2 | 7 | 7 | 12 |

Plasma concentrations of diazepam metabolite after bolus intravenous injection of diazepam 0.2 mg/kg to rhesus monkeys. Results are expressed as ng/ml

| TIME | | ANIMAL | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 2 | minute | ND | ND | ND | ND | ND |
| 5 | minute | 23 | 13 | 0 | 9 | 10 |
| 10 | minute | 14 | 76 | 30 | 23 | 28 |
| 20 | minute | 42 | 94 | 58 | 78 | 71 |
| 40 | minute | 89 | 127 | 73 | 106 | 99 |
| 1 | hour | 94 | 156 | 105 | 112 | 105 |
| 2 | hour | 80 | 142 | 94 | 101 | 175 |
| 4 | hour | 69 | 121 | 80 | 80 | 146 |
| 6 | hour | 56 | 92 | 63 | 62 | 110 |
| 24 | hour | 10 | 25 | 20 | 18 | 28 |
| 30 | hour | 7 | 17 | 12 | 11 | 20 |
| 48 | hour | ND | 3 | 3 | 3 | 3 |
| 72 | hour | ND | ND | ND | ND | ND |

ND: Less than the limit of detection(2 ng/ml)

Plasma concentrations of diazepam metabolite after bolus intravenous injection of diazepam 0.2 mg/kg to cynomolgus monkeys. Results are expressed as ng/ml.

| TIME | | ANIMAL | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 2 | minute | ND | ND | ND | ND | ND |
| 5 | minute | ND | 41 | ND | 24 | 7 |
| 10 | minute | 50 | 70 | 17 | 13 | 27 |
| 20 | minute | 53 | 67 | 57 | 53 | 67 |
| 40 | minute | 100 | 69 | 71 | 99 | 90 |
| 1 | hour | 121 | 107 | 74 | 121 | 113 |
| 1.5 | hour | 147 | 93 | 87 | 97 | 104 |
| 2 | hour | 131 | 79 | 101 | 94 | 101 |
| 3 | hour | 119 | 69 | 91 | 86 | 97 |
| 4 | hour | 106 | 61 | 84 | 75 | 99 |
| 5 | hour | 96 | 57 | 80 | 69 | 93 |
| 7 | hour | 81 | 40 | 65 | 63 | 75 |
| 24 | hour | 26 | 7 | 29 | 21 | 30 |
| 30 | hour | 16 | 4 | 21 | 14 | 21 |
| 48 | hour | 4 | ND | 7 | 3 | 7 |
| 72 | hour | ND | ND | ND | ND | ND |

ND: Less than the limit of detection (2 ng/ml).

Plasma concentrations of diazepam metabolite after bolus intravenous injection of diazepam 0.2 mg/kg to baboons. Results are expressed as ng/ml.

| TIME | | ANIMAL | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 2 | minute | ND | ND | ND | 4 | ND |
| 5 | minute | ND | ND | 45 | 14 | ND |
| 10 | minute | 5 | 4 | 80 | 58 | ND |
| 20 | minute | 32 | 93 | 79 | 72 | 22 |
| 40 | minute | 76 | 100 | 114 | 84 | 60 |
| 1 | hour | 100 | 106 | 110 | 107 | 86 |
| 1.5 | hour | 94 | 116 | 120 | 104 | 126 |
| 2 | hour | 89 | 118 | 100 | 90 | 117 |
| 3 | hour | 80 | 106 | 100 | 80 | 112 |
| 4 | hour | 76 | 90 | 84 | 62 | 100 |
| 5 | hour | 66 | 80 | 70 | 47 | 84 |
| 7 | hour | 48 | 55 | 46 | 22 | 60 |
| 24 | hour | 4 | 4 | 3 | 3 | 5 |
| 30 | hour | ND | ND | ND | ND | ND |

ND: Less than the limit of detection (2 ng/ml).

Plasma concentrations of diazepam metabolite after bolus intravenous injection of diazepam 1.0 mg/kg to rhesus monkeys. Results are expressed as ng/ml.

| TIME | | ANIMALS | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 2 | minute | ND | 22 | ND | ND | ND |
| 5 | minute | 179 | 310 | 22 | 89 | 89 |
| 10 | minute | 465 | 410 | 177 | 244 | 377 |
| 20 | minute | 466 | 731 | 465 | 509 | 665 |
| 40 | minute | 565 | 1174 | 709 | 731 | 886 |
| 1 | hour | 520 | 1152 | 665 | 864 | 742 |
| 1.5 | hour | 487 | 1041 | 675 | 797 | 709 |
| 2 | hour | 465 | 953 | 642 | 720 | 642 |
| 3 | hour | 499 | 753 | 554 | 665 | 598 |
| 4 | hour | 444 | 665 | 443 | 576 | 487 |
| 5 | hour | 430 | 563 | 400 | 457 | 396 |
| 7 | hour | 422 | 443 | 310 | 377 | 265 |
| 24 | hour | 160 | 66 | 44 | 44 | 15 |
| 30 | hour | 133 | 44 | 22 | 22 | 5 |
| 48 | hour | 44 | 4 | 2 | ND | ND |
| 72 | hour | 10 | ND | ND | ND | ND |

ND: Less than the limit of detection (2 ng/ml).

Plasma concentrations of diazepam metabolite after bolus intravenous injection of diazepam 1.0 mg/kg to cynomolgus monkeys. Results are expressed as ng/ml.

| TIME | ANIMALS | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| 2 minute | 14 | ND | ND | ND | ND |
| 5 minute | 114 | ND | 27 | ND | 6 |
| 10 minute | 229 | 143 | 171 | 103 | 80 |
| 20 minute | 343 | 157 | 429 | 414 | 33 |
| 40 minute | 400 | 357 | 429 | 686 | 457 |
| 1 hour | 471 | 429 | 543 | 700 | 486 |
| 1.5 hour | 471 | 529 | 514 | 757 | 486 |
| 2 hour | 486 | 471 | 600 | 700 | 486 |
| 3 hour | 443 | 457 | 571 | 643 | 471 |
| 4 hour | 471 | 457 | 457 | 543 | 471 |
| 5 hour | 457 | 443 | 407 | 543 | 486 |
| 7 hour | 414 | 314 | 350 | 457 | 486 |
| 24 hour | 214 | 61 | 106 | 186 | 200 |
| 30 hour | 171 | 39 | 71 | 150 | 170 |
| 48 hour | 71 | 4 | 14 | 49 | 57 |
| 72 hour | 25 | ND | 2 | 19 | 14 |

Plasma concentrations of diazepam metabolite after bolus intravenous injection of diazepam 1.0 mg/kg to baboons. Results are expressed as ng/ml.

| TIME | | ANIMAL | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 2 | minute | ND | ND | ND | 4 | ND |
| 5 | minute | 32 | 10 | 20 | 79 | ND |
| 10 | minute | 128 | 66 | 86 | 110 | 20 |
| 20 | minute | 277 | 222 | 258 | 180 | 190 |
| 40 | minute | 330 | 434 | 500 | 260 | 560 |
| 1 | hour | 336 | 403 | 760 | 282 | 670 |
| 1.5 | hour | 324 | 456 | 780 | 312 | 710 |
| 2 | hour | 366 | 426 | 720 | 360 | 700 |
| 3 | hour | 344 | 430 | 700 | 360 | 670 |
| 4 | hour | 258 | 350 | 760 | 350 | 680 |
| 5 | hour | 162 | 330 | 770 | 310 | 600 |
| 7 | hour | 96 | 282 | 750 | 268 | 520 |
| 24 | hour | 48 | 200 | 340 | 136 | 177 |
| 30 | hour | 35 | 66 | 248 | 106 | 106 |
| 48 | hour | 13 | 24 | 104 | 60 | 32 |
| 72 | hour | 4 | 5 | 28 | 26 | 6 |

ND: Less than the limit of detection (2 ng/ml).

Concentrations of frusemide in plasma after bolus intravenous injection of frusemide 3.0 mg/kg to rhesus monkeys. Results are expressed as μg/ml.

| TIME | ANIMALS | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| 2 minute | 33 | 33 | 32 | 34 | 26 |
| 5 minute | 29 | 22 | 22 | 22 | 14 |
| 10 minute | 23 | 17 | 16 | 19 | 12 |
| 20 minute | 16 | 10 | 11 | 12 | 8 |
| 40 minute | 9 | 3 | 4 | 7 | 3 |
| 1 hour | 5 | 3 | 3 | 4 | 2 |
| 1.5 hour | 3 | 2 | ND | 2 | ND |
| 2 hour | 2 | ND | ND | ND | ND |
| 3 hour | ND | ND | ND | ND | ND |

ND: Less than the limit of detection (2 μg/ml).

Concentrations of frusemide in plasma after bolus intravenous injection of frusemide 3.0 mg/kg to cynomolgus monkeys. Results are expressed as µg/ml.

| TIME | | ANIMAL | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 2 | minute | 34 | 35 | 33 | 32 | 34 |
| 5 | minute | 28 | 30 | 25 | 27 | 22 |
| 10 | minute | 18 | 25 | 21 | 16 | 17 |
| 20 | minute | 15 | 21 | 16 | 15 | 14 |
| 40 | minute | 11 | 14 | 8 | 8 | 5 |
| 1 | hour | 6 | 8 | 5 | 4 | 2 |
| 1.5 | hour | 2 | 4 | 3 | 2 | 2 |
| 2 | hour | 2 | 3 | 2 | ND | ND |
| 3 | hour | ND | ND | ND | ND | ND |

ND: Less than the limit of detection (2 µg/ml).

Concentration of frusemide in plasma after bolus intravenous injection of frusemide 3.0 mg/kg to baboons. Results are expressed as μg/ml.

| TIME | ANIMALS | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| 2 minute | 52 | 46 | 38 | 42 | 40 |
| 5 minute | 28 | 29 | 30 | 29 | 32 |
| 10 minute | 18 | 22 | 23 | 23 | 22 |
| 20 minute | 10 | 13 | 15 | 14 | 15 |
| 40 minute | 3 | 4 | 5 | 3 | 5 |
| 1 hour | ND | ND | 2 | 2 | 2 |
| 1.5 hour | ND | ND | ND | ND | ND |

ND: Less than the limit of detection (2 μg/ml).

Concentration of isosorbide dinitrate in the plasma of the rhesus monkey after a single bolus intravenous injection of isosorbide dinitrate at a level of 1.0 mg/kg. Results are expressed as ng/ml.

| TIME | | ANIMAL | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 2 | minute | 658 | 514 | 530 | 506 | 557 |
| 5 | minute | 648 | 428 | 317 | 434 | 457 |
| 10 | minute | 481 | 394 | 288 | 386 | 420 |
| 15 | minute | 285 | 300 | 280 | 317 | 343 |
| 30 | minute | 232 | 157 | 215 | 211 | 214 |
| 45 | minute | 149 | 146 | 151 | 160 | 157 |
| 1 | hour | 99 | 106 | 126 | 123 | 94 |
| 1.5 | hour | 71 | 86 | 80 | 106 | 69 |
| 2 | hour | 56 | 43 | 55 | 57 | 43 |
| 3 | hour | 40 | 20 | 28 | 31 | 20 |
| 4.5 | hour | 17 | 7 | 7 | 11 | 6 |

Concentrations of isosorbide dinitrate in the plasma of the cynomolgus monkey after a single bolus intravenous injection of isosorbide dinitrate at a level of 1.0 mg/kg. Results are expressed as ng/ml.

| TIME | | ANIMAL | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 2 | minute | 630 | 527 | 608 | 608 | 556 |
| 5 | minute | 660 | 673 | 478 | 586 | 508 |
| 10 | minute | 530 | 421 | 350 | 430 | 502 |
| 15 | minute | 420 | 332 | 299 | 354 | 463 |
| 30 | minute | 234 | 179 | 251 | 181 | 201 |
| 45 | minute | 150 | 75 | 98 | 102 | 119 |
| 1 | hour | 90 | 50 | 52 | 63 | 68 |
| 1.5 | hour | 39 | 22 | 20 | 33 | 25 |
| 2 | hour | 18 | 9 | 8 | 10 | 10 |
| 3 | hour | 5 | 2 | 2 | 3 | 2 |
| 4 | hour | 3 | ND | ND | 1 | ND |
| 5 | hour | ND | ND | ND | ND | ND |

ND: Less than the limit of detection (1 ng/ml).

Concentrations of isosorbide dinitrate in the plasma of the baboon after a single bolus intravenous injection of isosorbide dinitrate at a level of 1.0 mg/kg. Results are expressed as ng/ml.

| TIME | | ANIMALS | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 2 | minute | 1475 | 1478 | 1344 | 2003 | 1561 |
| 5 | minute | 831 | 1134 | 1127 | 1548 | 1249 |
| 10 | minute | 771 | 694 | 742 | 771 | 774 |
| 15 | minute | 599 | 659 | 570 | 532 | 633 |
| 30 | minute | 368 | 353 | 378 | 347 | 376 |
| 45 | minute | 188 | 195 | 290 | 185 | 213 |
| 1 | hour | 167 | 125 | 163 | 128 | 143 |
| 1.5 | hour | 67 | 67 | 67 | 55 | 61 |
| 2 | hour | 29 | 29 | 29 | 23 | 26 |
| 3 | hour | 6 | 7 | 7 | 3 | 6 |
| 4 | hour | 1 | 1 | 1 | ND | 1 |
| 5 | hour | ND | ND | ND | ND | ND |

ND: Less than the limit of detection (1 ng/ml).

Concentrations of paracetamol in the plasma of the rhesus monkey after a single bolus intravenous injection of paracetamol at a level of 70 mg/kg. Results are expressed as µg/ml.

| TIME | | ANIMAL | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 2 | minute | 112 | 100 | 116 | 100 | 98 |
| 5 | minute | 100 | 90 | 102 | 86 | 77 |
| 10 | minute | 89 | 84 | 93 | 84 | 68 |
| 20 | minute | 72 | 68 | 75 | 77 | 58 |
| 40 | minute | 53 | 54 | 64 | 53 | 42 |
| 1 | hour | 34 | 36 | 42 | 40 | 29 |
| 1.5 | hour | 26 | 31 | 27 | 24 | 22 |
| 2 | hour | 14 | 22 | 18 | 15 | 14 |
| 3 | hour | 10 | 13 | 12 | 10 | 12 |
| 4 | hour | 7 | 8 | 8 | 6 | 7 |
| 5 | hour | 4 | 3 | 4 | 4 | 2 |
| 7 | hour | 2 | ND | ND | ND | ND |

ND: Less than the limit of detection (1 µg/ml).

Concentrations of paracetamol in the plasma of the cynomolgus monkey after a single bolus intravenous injection of paracetamol at a level of 70 mg/kg. Results are expressed as µg/ml.

| TIME | | ANIMAL | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 2 | minute | 85 | 76 | 67 | 80 | 79 |
| 5 | minute | 123 | 95 | 116 | 61 | 60 |
| 10 | minute | 97 | 74 | 95 | 55 | 39 |
| 20 | minute | 85 | 44 | 74 | 36 | 37 |
| 40 | minute | 49 | 41 | 52 | 23 | 32 |
| 1 | hour | 42 | 28 | 32 | 13 | 28 |
| 1.5 | hour | 19 | 18 | 18 | 10 | 22 |
| 2 | hour | 15 | 10 | 11 | 6 | 18 |
| 3 | hour | 8 | 4 | 10 | 3 | 7 |
| 4 | hour | 5 | 4 | 5 | 1 | 4 |
| 5 | hour | 4 | 2 | 3 | ND | 2 |
| 7 | hour | 1 | ND | 2 | ND | ND |

ND: Less than the limit of detection (1 µg/ml).

Concentrations of paracetamol in the plasma of the baboon after a single bolus intravenous injection of paracetamol at a level of 70 mg/kg. Results are expressed as µg/ml.

| TIME | | ANIMAL | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 2 | minute | 67 | 90 | 84 | 87 | 109 |
| 5 | minute | 80 | 87 | 77 | 71 | 77 |
| 10 | minute | 79 | 87 | 80 | 68 | 91 |
| 20 | minute | 77 | 75 | 59 | 65 | 75 |
| 40 | minute | 58 | 53 | 52 | 55 | 52 |
| 1 | hour | 38 | 28 | 48 | 45 | 41 |
| 1.5 | hour | 32 | 17 | 32 | 39 | 34 |
| 2 | hour | 28 | 10 | 27 | 31 | 28 |
| 3 | hour | 14 | 3 | 16 | 20 | 18 |
| 4 | hour | 10 | ND | 8 | 13 | 12 |
| 5 | hour | 4 | ND | 5 | 9 | 8 |
| 7 | hour | ND | ND | 2 | 4 | 3 |

ND: Less than the limit of detection (1 µg/ml).

Concentrations of ibuprofen in the plasma of the rhesus monkey after a single bolus intravenous injection of ibuprofen at a level of 15 mg/kg. Results are expressed as µg/ml.

| TIME | | ANIMAL | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 2 | minute | 242 | 157 | 164 | 169 | 124 |
| 5 | minute | 191 | 129 | 134 | 218 | 122 |
| 10 | minute | 161 | 90 | 88 | 125 | 105 |
| 20 | minute | 83 | 67 | 50 | 65 | 61 |
| 40 | minute | 34 | 27 | 24 | 35 | 37 |
| 1 | hour | 17 | 14 | 14 | 24 | 17 |
| 1.5 | hour | 13 | 8 | 6 | 10 | 9 |
| 2 | hour | 10 | 6 | 4 | 7 | 6 |
| 3 | hour | 6 | 4 | 3 | 5 | 2 |
| 4 | hour | 4 | 2 | 2 | 4 | 2 |
| 5 | hour | 2 | 2 | 2 | 2 | 1 |
| 7 | hour | 1 | 1 | 1 | 1 | 1 |

Concentrations of ibuprofen in the plasma of the cynomolgus monkey after a single bolus intravenous injection of ibuprofen at a level of 15 mg/kg. Results are expressed as µg/ml.

| TIME | | ANIMAL | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 2 | minute | 202 | 230 | 181 | 230 | 260 |
| 5 | minute | 203 | 344 | 191 | 211 | 228 |
| 10 | minute | 148 | 187 | 154 | 142 | 170 |
| 20 | minute | 90 | 95 | 116 | 102 | 130 |
| 40 | minute | 51 | 33 | 69 | 61 | 77 |
| 1 | hour | 36 | 24 | 40 | 39 | 43 |
| 1.5 | hour | 19 | 21 | 18 | 14 | 32 |
| 2 | hour | 10 | 7 | 6 | 7 | 19 |
| 3 | hour | 6 | 3 | 4 | 4 | 12 |
| 4 | hour | 5 | 1 | 3 | 3 | 7 |
| 5 | hour | 2 | 1 | 2 | 1 | 2 |
| 7 | hour | 2 | ND | ND | ND | 2 |

ND: Less than the limit of detection (0.4 µg/ml).

Concentrations of ibuprofen in the plasma of the baboon after a single bolus intravenous injection of ibuprofen at a level of 15 mg/kg. Results are expressed as µg/ml.

| TIME | | ANIMAL | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 2 | minute | 200 | 200 | 151 | 186 | 159 |
| 5 | minute | 167 | 167 | 133 | 170 | 154 |
| 10 | minute | 143 | 134 | 119 | 129 | 119 |
| 20 | minute | 80 | 80 | 94 | 100 | 91 |
| 40 | minute | 35 | 48 | 68 | 70 | 64 |
| 1 | hour | 10 | 23 | 55 | 58 | 49 |
| 1.5 | hour | 8 | 17 | 28 | 22 | 28 |
| 2 | hour | 4 | 7 | 28 | 14 | 8 |
| 3 | hour | 1 | 2 | 7 | 4 | 6 |
| 4 | hour | ND | 1 | 5 | 3 | 2 |
| 5 | hour | ND | ND | 2 | 2 | 1 |
| 7 | hour | ND | ND | 1 | 1 | ND |

ND: Less than the limit of detection (0.4 µg/ml).

Concentrations of pentobarbitone in the plasma of the rhesus monkey after a single bolus intravenous injection of pentobarbitone at a level of 12 mg/kg. Results are expressed as µg/ml.

| TIME | ANIMAL | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| 2 minute | 13.8 | 11.6 | 11.1 | 17.4 | 12.4 |
| 5 minute | 20.0 | 16.3 | 16.3 | 19.4 | 17.1 |
| 10 minute | 18.2 | 16.9 | 14.5 | 18.7 | 17.1 |
| 20 minute | 13.1 | 13.7 | 11.8 | 16.6 | 14.4 |
| 40 minute | 9.7 | 11.1 | 10.6 | 15.8 | 11.6 |
| 1 hour | 7.9 | 9.0 | 7.3 | 13.8 | 10.3 |
| 1.5 hour | 7.5 | 5.5 | 6.6 | 11.1 | 9.0 |
| 2 hour | 6.9 | 4.4 | 4.4 | 9.7 | 7.4 |
| 3 hour | 5.9 | 2.5 | 2.4 | 6.9 | 5.1 |
| 4 hour | 5.2 | 1.5 | 1.4 | 5.9 | 4.1 |
| 5 hour | 4.5 | 1.0 | 0.8 | 4.1 | 3.0 |
| 7 hour | 3.3 | 0.5 | 0.4 | 2.2 | 2.4 |

Concentrations of pentobarbitone in the plasma of the cynomolgus monkey after a single bolus intravenous injection of pentobarbitone at a level of 12 mg/kg. Results are expressed as μg/ml.

| TIME | ANIMAL | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| 2 minute | 17.9 | 22.4 | 14.5 | 15.9 | 20.7 |
| 5 minute | 17.9 | 17.9 | 16.6 | 15.2 | 17.3 |
| 10 minute | 14.2 | 17.3 | 15.2 | 13.1 | 15.2 |
| 20 minute | 11.3 | 13.1 | 13.6 | 12.1 | 14.1 |
| 40 minute | 9.0 | 11.7 | 11.9 | 10.6 | 11.7 |
| 1 hour | 7.9 | 9.1 | 10.4 | 8.6 | 10.4 |
| 1.5 hour | 6.9 | 6.5 | 8.6 | 6.9 | 8.3 |
| 2 hour | 5.2 | 5.9 | 6.9 | 5.9 | 6.9 |
| 3 hour | 3.7 | 5.5 | 5.5 | 4.8 | 5.4 |
| 4 hour | 2.8 | 4.8 | 4.1 | 4.0 | 4.5 |
| 5 hour | 1.9 | 3.9 | 2.8 | 2.8 | 3.1 |
| 7 hour | 1.2 | 1.9 | 1.2 | 2.0 | 1.7 |

Concentrations of pentobarbitone in the plasma of the baboon after a single bolus intravenous injection of pentobarbitone at a level of 12 mg/kg. Results are expressed as μg/ml.

| TIME | | ANIMAL | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 2 | minute | 31.2 | 20.4 | 19.9 | 14.2 | 13.2 |
| 5 | minute | 26.9 | 17.0 | 21.0 | 17.3 | 12.7 |
| 10 | minute | 22.6 | 15.0 | 14.6 | 14.4 | 12.0 |
| 20 | minute | 17.8 | 12.2 | 13.5 | 13.4 | 10.1 |
| 40 | minute | 10.1 | 11.3 | 11.0 | 11.1 | 8.4 |
| 1 | hour | 8.9 | 9.3 | 9.9 | 9.2 | 6.7 |
| 1.5 | hour | 7.1 | 6.9 | 8.3 | 8.0 | 5.5 |
| 2 | hour | 5.2 | 5.8 | 7.7 | 5.2 | 5.2 |
| 3 | hour | 3.0 | 4.5 | 5.6 | 3.6 | 3.5 |
| 4 | hour | 1.9 | 2.3 | 3.5 | 2.0 | 2.4 |
| 5 | hour | 1.2 | 1.1 | 3.0 | 1.3 | 1.6 |
| 7 | hour | 0.7 | 1.8 | 1.6 | 0.8 | 0.8 |