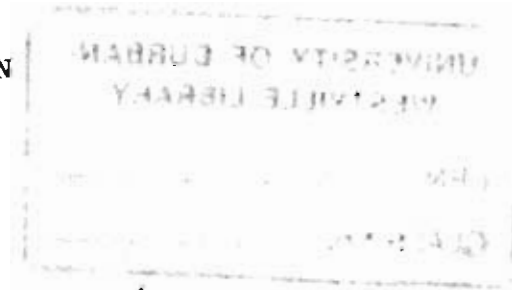# THE PHARMACOKINETICS OF PHENOBARBITONE IN FASTING AND NON-FASTING DOGS

by

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To mom and dad
for their support and
faith in my convictions

ACKNOWLEDGEMENTS

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GLOSSARY OF IMPORTANT SYMBOLS AND ABBREVIATIONS

$^{\circ}$C	-	degrees Celsius
GABA	-	gamma-aminobutyric acid
EEG	-	electroencephalogram
μM	-	micro Mole
μg.ml^{-1}	-	microgram per milliliter
hr	-	hour
CSF	-	cerebrospinal fluid
L.kg^{-1}	-	liters per kilogram
mg.kg^{-1}	-	milligram per kilogram
Vd	-	volume of distribution
PHB	-	phenobarbitone
PBOH	-	p-hydroxyphenobarbitone
LC	-	liquid chromatography
GLC	-	gas liquid chromatography
HPLC	-	high pressure liquid chromatography
GC-MS	-	gas chromatography-mass spectrometry
$t\frac{1}{2}$	-	half-life
LD	-	lethal dose

FPIA	–	fluorescence polarization immunoassay
FDR	–	fluorogenic drug reagent
f	–	bioavailability factor
S	–	salt factor
τ	–	dosing interval
Ri	–	rate of administration
Ro	–	rate of elimination
MEC	–	minimum effective concentration
MSC	–	maximum safe concentration
Cp	–	drug concentration in the plasma
Ab	–	amount of drug in the body
Cl	–	clearance
$\overline{Cp}_{ss}$	–	average steady state drug concentration
K_E	–	elimination rate constant
K_A	–	absorption rate constant
AUC	–	area under the curve
pe	–	predictive error
mpe	–	mean predictive error
mse	–	mean squared error

CONVERSION FACTOR

Throughout most of this text, serum phenobarbitone concentration measurements are reported in the locally popular $\mu g.ml^{-1}$ units and not in the SI units of $mmol.l^{-1}$. The relevant conversion factor for $\mu g.ml^{-1}$ to $mmol.l^{-1}$ is 5.55.

INTRODUCTION

Practicing clinical veterinarians in large companion animal prac-
tices are often faced with the phenomena of epileptic seizures
which occur commonly in dogs. The high incidence of non-
responsive cases is often frustrating, and the literature offers
incomplete, conflicting and often inaccurate information. The
concept of therapeutic anti-epileptic drug concentration monitor-
ing, as applied in man as an aid to treatment, appears attractive
in order to provide an improved service to the patient and
client.

An investigation into the pharmacokinetics of phenobarbitone,
particularly at steady state, became necessary in order to inter-
pret the application of drug serum concentration monitoring. The
trend of veterinarians to extrapolate human kinetics to dogs is
common and unsound. This study was an attempt to identify the
similarities and dissimilarities between the pharmacokinetics of
dogs and humans.

No literature was available, both for man or animal, on the ef-
fect of food on the absorption of phenobarbitone. As dog owners
frequently have to administer oral medication in food, this was
an important factor to examine.

The kinetics of the drug was determined in a group of epileptic
dogs in order to provide a possible base-line therapeutic regime
on commencement of treatment, and the practical application of
therapeutic drug monitoring in order to individualize and improve
response to treatment was explored.

Phenobarbitone - Pharmaceutical Chemistry, Mechanism of Action, Pharmacology, Toxicity and Pharmacokinetics.

1.1 PHARMACEUTICAL CHEMISTRY

1.1.1 Structure and Solubility
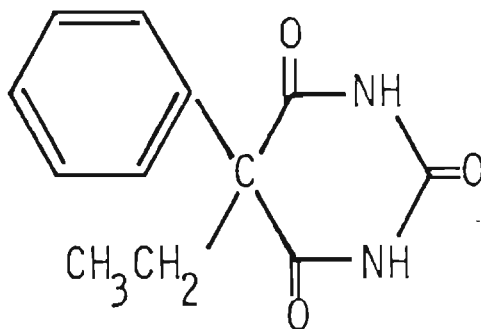
Fig.1 - Structure of 5-ethyl-5-phenyl barbituric acid (phenobarbitone)

Phenobarbitone Sodium, USP (soluble phenobarbitone, Luminal sodium), was the second barbituric acid derivative of clinical importance to be developed. It was synthesized in 1912 in Germany by HAUPTMANN and patented under the name of Luminal. It is a white crystalline material with a somewhat bitter taste. The molecular weight is 232.23, and the melting point is 176°C (*Johannessen,1989*).

The free acid is only slightly soluble in water, whereas the sodium salt is freely soluble (*Booth,1988; Schafer,1985; Johannessen,1989*). Phenobarbitone is soluble in organic solvents such as chloroform, diethyl ether, and ethanol. The partition coefficient between chloroform and water is 4.2 at pH 3.4 (*Johannessen, 1989*). The pKa is 7.3 and it is a stronger acid than other barbiturates (*Rimmer and Richens,1988; Johannessen,1989*). These acidic properties are important in relation to the distribution and excretion of the drug. The pKa of phenobarbitone is such that a change of extracellar pH without a corresponding change of intracellular pH will cause a shift of the drug from one compartment to the other.

1.2 MECHANISM OF ACTION

In humans, phenobarbitone is effective against generalized tonic-clonic seizures and partial seizures but has very little effect in absence seizures. All the different barbiturates are not equally effective as antiepileptic drugs in ambulatory patients with epilepsy. The sedative-anaesthetic barbiturates, such as pentobarbitone, produce marked sedation at anticonvulsant doses, whereas phenobarbitone is clinically useful because it has antiepileptic activity without causing excessive sedation (*Rimmer and Richens,1988*).

Although phenobarbitone has been extensively studied in humans, its basic mechanisms of selective antiepileptic action are not yet fully understood (*Prichard and Ransom,1989*). Earlier workers proposed several mechanisms that seemed plausible in light of the data then available, but the more recent advance of neurobiological knowledge has rendered most of the older theories improbable. Some are still quoted in recent literature:

* The antiepileptic effects of phenobarbitone are probably the results of at least two mechanisms. Decreased monosynaptic and polysynaptic transmission which

presumably results in reduced neuronal excitability. Also, the motor cortex's threshold for electrical stimulation is increased (*Shell, 1984*).

*   The mechanism of action of the benzodiazepines and the barbiturates is thought to be the facilitation of chloride-ion transport through postsynaptic neuronal cell membranes (*Rimmer and Richens,1988*).

*   Presynaptic action to reduce calcium entry into the neurons and block neurotransmitter release (*Rimmer and Richens,1988*).

*   Non-synaptically to reduce voltage dependent sodium and potassium conductances and block repetitive firing (*Rimmer and Richens,1988*).

Although current knowledge of epileptic pathophysiology has advanced considerably, the chain of events that occur from cellular defect to clinical seizure is still too vaguely defined and therefore, the point in this chain where the critical action of antiepileptic drugs occur remains obscure.

Recent evidence points toward modulation of the inhibitory postsynaptic actions of gamma-aminobutyric acid (GABA) and the excitatory postsynaptic actions of amino acids, such as glutamate, as key phenobarbitone-sensitive phenomena relevant to seizure control (*Prichard and Ransom,1989*).

1.2.1 Action of phenobarbitone on abnormal phenomena

In experimental animals phenobarbitone elevates electroshock seizure threshold and protects against pentylenetetrazol-induced convulsions more effectively than phenytoin does (*Prichard and Ransom,1989*). This phenomena could imply that phenobarbitone has some property not shared with phenytoin that causes elevation of threshold to electrical and chemical epileptic stimuli. This

cannot be a selective effect on abnormal nervous tissue, because elevated thresholds can be demonstrated in normal animals (*Prichard and Ransom,1989*). In work done by *Aston and Domino (1961)*, it was determined in the monkey that pentobarbitone equally raised the motor cortical and reticular formation after-discharge thresholds. However, phenobarbitone raised cortical threshold more than the reticular threshold, and phenytoin raised only the cortical threshold. These findings seem quite consistent with the relative antiepileptic and hypnotic potencies of the three drugs. The depression of consciousness, elevation of thresholds for electrical and chemical seizures, and production of EEG fast activity, all of which distinguish phenobarbitone from phenytoin, therefore could all be related to actions in the reticular formation. Further studies are required to extend their results and to move toward a cellular explanation of them (*Prichard and Ransom,1989*).

Comparisons of phenobarbitone and pentobarbitone in a different study showed phenobarbitone to be .more potent against after-discharges in isolated cortex and the interictal spikes in hippocampal slices exposed to penicillin (*Vazquez et al,1975*). This study also included phenytoin, which was substantially less potent than phenobarbitone, and diazepam, which was very slightly more potent. A study on rabbits showed that phenobarbitone inhibited spread of abnormal activity from the focus to adjacent cortex and diencephalon and suppressed the firing of the focus itself. Phenytoin was a better inhibitor of cortical spread but had no effect on the focus or on spread to the diencephalon (*Morell et al,1959*).

The above data suggests that phenobarbitone exerts an important part of its antiepileptic action on abnormal neurons, in contrast to phenytoin which may act primarily on normal neurons. This has not been proven, and the cellular basis for the difference is unknown (*Prichard and Ransom,1989*).

1.2.2 Actions of phenobarbitone on normal phenomena

Extraneural Tissues

Enzyme induction is the only well-defined barbiturate action outside the nervous tissue that has received attention as a possible antiepileptic mechanism. It has been shown in numerous studies that barbiturates cause increased synthesis and concentration of a number of enzymes in a variety of tissues. Phenobarbitone is the most effective inducer among them, but whether this is related to any property also important for its antiepileptic potency is not known. The most thoroughly studied inductions are of hepatic microsomal enzymes, several of which have been implicated in barbiturate toxicity (*Prichard and Ransom,1989; Booth,1988*). One author has proposed that some antiepileptic drugs, including phenobarbitone, act against seizures because they alter folate metabolism; the mechanisms are not completely understood but may involve hepatic enzyme induction (*Reynolds,1973*).

The relevant data as a whole does not provide strong support for this theory but does prompt reflection on the possibility that what antiepileptic drugs do in the rest of the body may influence what they do in the brain. At present, however, there is no good evidence to suggest that enzyme induction or any other extraneural action of phenobarbitone is responsible for its antiepileptic activity.

Sleep and Anesthesia

Barbiturates have been used to cause sleep more frequently than for any other purpose. The exact mechanism of their sedative action is not known, but it is known that the state they produce is not equivalent to physiological sleep. Total time spent in the rapid eye movement phase of sleep is reduced and rebounds after barbiturate use is stopped (*Prichard and Ransom,1989*). Since all barbiturates and many quite different drugs have similar effects, it seems unlikely that clues to the selective antiepileptic ac-

tion of phenobarbitone will be found in the mechanisms by which it can cause sleep.

Electroencephalogram and Evoked Potentials

Most barbiturates appear to exert quite similar actions on the EEGs of man and common experimental animals, although their potencies and durations of action vary greatly. Electroencephalogram power spectrum analysis has revealed some differences among barbiturates, but these have not been shown to correlate with antiepileptic potency (*Prichard and Ransom,1989*). These authors also report that evoked potential studies, like those on the EEG, have so far shown no selective effect of antiepileptic barbiturates. In general, barbiturates depress nonspecific and neospinothalamic sensory-evoked potentials at doses that spare or enhance other specific ones. Further, a study done on the differences between antiepileptic concentrations of phenobarbitone and phenytoin at several sites in the cat brain, showed the mesencephalic reticular formation to be most sensitive to phenobarbitone. There is no evidence at present that any aspect of barbiturate antiepileptic action depends on the mesencephalic reticular formation, but experiments likely to detect such dependency have not been done (*Prichard and Ransom,1989*).

Synaptic Transmission

The most potent neuropharmacological actions of barbiturates yet demonstrated are on synaptic transmission or phenomena related to it. Some of its synaptic actions occur at concentrations as low as 25 μM, which is well within the range of concentrations found in the serum of patients whose seizures are controlled by the drug. These actions are the possible basis of phenobarbitone's antiepileptic effectiveness (*Prichard and Ransom,1989*).

The concentration of free phenobarbitone in serum correlates better with seizure control than does total concentration. About 40% of serum phenobarbitone is protein-bound. The therapeutic range of free phenobarbitone concentration is 25 to 100 $\mu M.l^{-1}$ (6-24 $\mu g.ml^{-1}$). In human's, free phenobarbitone concentrations greater than 100 $\mu M.l^{-1}$ frequently produce sedation, and still higher concentrations produce anaesthesia. It is not known whether these clinical effects of high phenobarbitone concentrations are due to a more pronouced effect of the same mechanisms that are responsible for the drug's antiepileptic action, or to different ones (*Prichard and Ransom, 1989*).

1.3 PHARMACOKINETICS

1.3.1 Absorption

The majority of orally administered phenobarbitone is absorbed in the small intestine due to the extensive surface area and ample time for absorption. Studies in man have shown that after oral or intramuscular administration, peak plasma concentrations occur within 2 to 12 hr, and are linearly related to dose within a wide range of doses. The average time to peak plasma concentrations is 2 hr after oral dosing, but the peak may be considerably delayed in patients with diminished gastrointestinal motility or poor circulation (*Rust and Dodson,1989*).

In a study on dogs, peak serum concentrations were observed at 3.2 ± 1.0 hr (*Ravis et al,1984*).

Phenobarbitone has a high bioavailability in adults after oral or intramuscular administration. Studies in dogs and humans have shown 80% to 100% absorption of orally administered phenobarbitone (*Rust and Dodson,1989; Pedersoli et al,1987; Gallagher and Freer, 1985*)

Gastric absorption of sodium phenobarbitone is enhanced if it is dissolved prior to administration. The presence of food and neutralizing agents or rapid gastric emptying slows phenobarbitone absorption, and the presence of even small amounts of ethanol in the stomach or blood accelerates gastric absorption (*Rust and Dodson,1989*).

The role of enterohepatic circulation in phenobarbitone pharmacokinetics is incompletely characterized. Several studies have demonstrated unmetabolized phenobarbitone in bile within 1 hr of intravenous administration. Biliary phenobarbitone concentration has also been found to be twice the peak serum concentration (*Rust and Dodson,1989*).

1.3.2 Distribution

Phenobarbitone disseminates into all body tissues. Lowering serum pH increases the non-ionized portion in serum, enhancing diffusion into tissue, whereas higher serum pH has the opposite effect (*Rust and Dodson,1989*).

Phenobarbitone binding to plasma proteins ($\pm$ 45%) plays a minor role in distribution. Binding is readily reversible and independent of drug concentration, ionic dissociation, and serum calcium concentration (*Rust and Dodson,1989; Rimmer and Richens,1988*). This relatively low degree of protein binding means that the drug is less susceptible to alterations in plasma protein concentrations and is unlikely to be significantly involved in drug interactions in which there is competition for binding sites (*Rimmer and Richens,1988*).

Concentrations of phenobarbitone in CSF of adults are 43% to 60% of plasma concentrations and correlate well with the unbound phenobarbitone concentration in serum. The CSF levels in infants are similar, ranging from 48% to 83% of plasma levels (*Rust and Dodson,1989*). The phenobarbitone concentration in CSF provides a reliable index of phenobarbitone concentration in the brain.

Under nonequilibrium dosing conditions, actions of phenobarbitone correlate better with CSF levels than with either dose or the rate of drug administration. It has been shown that the loss of righting reflex in rodents is related to CSF concentrations and is independent of either the dose or the rate of administration (*Rust and Dodson,1989*).

Phenobarbitone concentrations are higher in CSF than saliva, a more acidic but similarly low-protein fluid. The concentration in saliva is, however, sensitive to pH changes and it is therefore unwise to use saliva for serum drug level monitoring for phenobarbitone(*Rimmer and Richens,1988*).

After intravenous administration, the distribution of phenobarbitone into body organs has two phases. The early phase involves distribution to highly vascular organs including liver, heart, and kidney, but not the brain, muscle, or intestine. During the late phase, phenobarbitone achieves fairly even distribution throughout the body except for relative exclusion from fat. This pattern of relatively slow entry into brain and limited distribution into fat is related to phenobarbitone's low lipid solubility, as drugs with higher lipophilicity rapidly enter brain (*Rust and Dodson,1989*).

The relative exclusion of phenobarbitone from body fat should be taken into consideration when dosages are based on weight to avoid overdosing obese patients. The slow distribution into the brain also makes phenobarbitone not an ideal drug for the treatment of status epilepticus.

1.3.3 Volume of Distribution

In dogs, the relative volume of distribution (Vd) of phenobarbitone is 0.7 ± 0.151 l.kg^{-1} after an oral dose of 5.5 mg.kg^{-1} and 0.689 ± 0.136 l.kg^{-1} after an oral dose of 15 mg.kg^{-1} (*Pedersoli et al, 1987*). In a similar study, the Vd was found to

be 0.7436 ± 0.698 l.kg^{-1} after oral administration of 2 mg.kg^{-1} daily for 5 days to dogs (*Ravis et al,1984*).

In adult humans, the relative volume of distribution ranges from 0.36 to 0.67 L.kg^{-1} after intramuscular doses and from 0.42 to 0.73 l.kg^{-1} after oral doses. The average Vd for phenobarbitone is larger in newborns, averaging approximately 1.0 l.kg^{-1}. This is probably the consequence of their relatively larger extracellular fluid volume (*Rust and Dodson,1989*).

1.3.4 Metabolism and Excretion

In spite of decades of use and the introduction of other agents, phenobarbitone has remained the most widely used antiepileptic drug in the world. Although the main pathways of phenobarbitone clearance have been identified, knowledge of its quantitative disposition is based on a series of observations rather than definitive studies. This is surprising in the light of recent advances in analytical and enzymatic techniques. Few studies have been performed in the last few years and today there remains a large discrepancy between the degree of investigation and the degree of use. For example, phenobarbitone is widely used in children but disposition in this population is virtually unknown. Definitive quantitative investigations of phenobarbitone metabolism are still warranted (*Anderson,1989*). The following metabolic pathways have been studied and reported in various publications:

Excretion of Unchanged Phenobarbitone

Phenobarbitone is partly metabolized and partly excreted unchanged in the urine (*Shell,1984*). There would appear to be considerable intersubject and intrasubject variability in the amount of phenobarbitone excreted unchanged in the urine. Studies by numerous researchers and referred to by *Anderson(1989)* report that the fraction of phenobarbitone excreted unchanged in the urine range between 9% to 48% (average of 22-25%).

The renal clearance of phenobarbitone is dependent on both urine flow and urine pH due to the lipophilicity and pKa of this drug (*Anderson,1989; Rimmer and Richens,1988; Gallagher and Freer,1985; Booth,1988*). This phenomena may explain some of the intersubject variability found in the fraction of dose excreted unchanged in the urine. After a drug is filtered by the glomerulus and possibly actively secreted into the tubule, it may be subject to passive reabsorption. Drug reabsorption takes place primarily in the distal tubule where the tubule membranes favour the transport of lipid-soluble and unionized compounds. The efficient reabsorption of water from the proximal tubule and Loop of Henle results in a large concentration gradient between drug in the distal tubule and drug in the plasma. Increasing urine flow decreases this concentration gradient, resulting in a decrease in passive reabsorption. Small changes in urine pH can cause large increases/decreases in the percentage of an unionized weak acid, like phenobarbitone, that is passively reabsorbed (*Anderson,1989; Booth,1988*). Alkaninization of the urine increases the renal excretion of unchanged phenobarbitone by reducing the non-ionic back diffusion from the distal renal tubule to the plasma. This property has been exploited in the treatment of phenobarbitone overdosage. The renal clearance can be increased from 4-6 ml.min^{-1} to 30 ml.min^{-1} by bicarbonate administration (*Rimmer and Richens,1988*).

Major Metabolic Pathways

Aromatic Hydroxylation

The major site of metabolism of phenobarbitone is in the liver. Through microsomal enzyme action, it is hydroxylated in the *para* position to form *p*-hydroxyphenobarbitone (PBOH) (*Booth,1988; Anderson,1989; Rimmer and Richens,1988; Shell,1984; Gallagher and Freer,1985*). This metabolite has weak anticonvulsant activity and does not contribute significantly to the action of phenobarbitone (*Booth,1988; Rimmer and Richens,1988*). A substantial fraction of the PBOH is then conjugated with glucuronic acid to form PBOH glucuronide. An earlier article written by *Butler(1956)* and referred to by *Gallagher and Freer(1985)* state that PBOH may also be conjugated with sulfate. This sulfate conjugate of PBOH has, however, not been substantiated experimentally and may have been due to problems in the analytical method (*Anderson,1989*).

N-Glucosidation

Recent research has proposed that 1-(β-D-glucopyranosyl) phenobarbitone is a quantitatively significant metabolite of phenobarbitone. Thin-layer chromatography of urine identified the *N*-glucoside conjugate of phenobarbitone after comparison to a synthetic standard. Glucosidation may be a significant pathway in the disposition of phenobarbitone in the neonate accounting for 50% of the drug and metabolites in the urine (*Anderson,1989*).

Other routes of Metabolism

Epoxidation and Subsequent Reactions

The hydroxylation of phenobarbitone to PBOH is presumed to be mediated by the cytochrome P-450 system. Cytochrome P-450-mediated aromatic hydroxylation is postulated to occur through epoxide intermediates. Due to the highly unstable nature of epoxides, they can undergo spontaneous rearrangement to phenols primarily in the *para* orientation. Theoretically, *meta-*

hydroxyphenobarbitone could also be formed. Meta-hydroxyphenobarbitone has been identified by gas chromatography-mass spectrometry (GC-MS) analysis as a minor metabolite in rats and guinea pigs (*Anderson,1989*).

Aliphatic Hydroxylation

Due to steric hindrance, cytochrome P-450-mediated aliphatic oxidation of the ethyl group of phenobarbitone is not a favoured reaction. However, small amounts of 5(1-hydroxyethyl)5-phenylbarbituric acid was detected by GC-MS analysis of the urine of rats and guinea pigs. This metabolite has not been found in human urine (*Anderson,1989*).

Hydrolysis

Phenobarbitone in aqueous solution is subject to spontaneous hydrolysis to a greater extent than any of the dialkylbar-biturates. The extent to which phenobarbitone is subject to hydrolysis in the human body is unknown. Studies with $2\text{-}^{14}C$ phenobarbitone in mice and rats found only trace amounts of ^{14}C-labelled CO_2 in expired air. However, since there is still a significant part of the phenobarbitone dose that is not accounted for by excretion of unchanged phenobarbitone and metabolites, this mode of elimination cannot be totally discounted (*Anderson,1989; Gallagher and Freer,1985*).

Autoinduction

Phenobarbitone is regarded as the classic inducer of hepatic microsomal metabolism. *Anderson(1989)* reports on two studies on elimination rates of phenobarbitone in humans. Based on the results the author concluded that phenobarbitone does not undergo time-dependent changes in total plasma clearance. An earlier study of three volunteers suggested that the elimination rate may actually decrease after prolonged dosing of phenobarbitone. However, *Anderson* discounts the results of this study due to

problems in analytical methodology and the small number of sub-
jects.

In contrast to the results in humans, there is evidence in both
dogs and the rat that autoinduction occurs. During prolonged
dosing in the dog, the daily elimination rate increases and it is
suggested that autoinduction occurs, resulting in a decrease in
phenobarbitone $t\frac{1}{2}_\beta$ (*Ravis et al,1984*). In a study done on the
urine of two groups of Sprague Dawley rats by *Anderson*, there was
a significantly lower recovery of total radioactivity from the
administration of ^{14}C-phenobarbitone in the induced group of rats
than the noninduced rats. The author concluded that this result
would be consistent with the effect of induction on metabolites,
but was also shown in another study in the rat where biliary
excretion of PBOH resulted in a decreased fraction of the dose
metabolized to PBOH or excreted as phenobarbitone. The pathway of
metabolism of phenobarbitone involved is at this time not fully
understood as only 40% to 50% of the phenobarbitone dose in the
rat has been isolated and identified (*Anderson,1989*).

1.3.5 Excretion

A number of factors contribute to the elimination of phenobar-
bitone. The phenobarbitone half-life is the longest among the
frequently used antiepileptic drugs. Phenobarbitone elimination
is slowed when liver metabolism or renal clearance is reduced, or
in the case when urine is rendered more acidic.

As previously discussed, phenobarbitone is eliminated from the
body both by hepatic metabolism and by renal excretion. In the
liver, phenobarbitone is parahydroxylated and subsequently con-
jugated to glucuronic acid. Both unmetabolized and parahydroxy-
lated phenobarbitone are excreted in urine. Enterohepatic cir-
culation and faecal excretion are not regarded as important con-
tributors to the net disposition under usual circumstances (*Rust
and Dodson,1989*).

Phenobarbitone elimination has first-order kinetics and thus the fraction of drug removed is independent of concentration (*Pedersoli et al.,1987; Ravis et al.,1984; Rust and Dodson,1989*). In humans, from 11% to 50% of phenobarbitone is eliminated from the body per day corresponding to a half-life range of 24 to 140 hr. Average half-lives after single doses range from 75 to 126 hr and are not influenced by route of administration. However, the rate of urine flow and urinary pH do influence the phenobarbitone elimination rate (*Rust and Dodson,1989*).

Both urinary pH and flow influence phenobarbitone reabsorption in the distal nephron. Under usual circumstances total renal clearance of phenobarbitone ranges from 0.7 to 8.8 $ml.kg^{-1}.hr^{-1}$ in adults (*Rust and Dodson,1989*). This is much less than the glomerular filtration rate, indicating extensive reabsorption in the nephron. In a study on healthy dogs following single IV or oral administration at two dosage rates, the mean total clearance was 5.60 ± 2.31 and 6.66 ± 0.78 $ml.kg^{-1}.hr^{-1}$ for doses of 5 and 15 $mg.kg^{-1}$, respectively (*Pedersoli et al., 1987*). In an earlier study, where phenobarbitone was administered orally 3 times a day to healthy mature dogs at a dose of 2 $mg.kg^{-1}$, total body clearance was found to be 10.38 ± 4.68 $ml.kg^{-1}.hr^{-1}$ (*Ravis,1984*). *Frey et al.(1985)* measured renal clearance of phenobarbitone after intravenous administration of a single dose of phenobarbitone in beagle and mongrel dogs. Beagles were found to have a clearance rate of 13.0 ± 1.7 $ml.kg^{-1}.hr^{-1}$, while that for mongrel dogs was 7.0 ± 1.3 $ml.kg^{-1}.hr^{-1}$. The differences were statistically significant and related to different pharmacokinetic models in these dogs.

The phenobarbitone half-life varies with age in humans. It is longest in premature and full-term newborns who have similar values typically ranging from 43 to 404 hr, and shortest in children ranging from 37 to 94 hr.

In the dog, phenobarbitone (2 $mg.kg^{-1}$) administered orally 3 times a day for 5 days resulted in an elimination half-life between 37 and 75 hrs ; the mean elimination half-life was 53 ± 15

hrs (*Ravis et al.,1984*). In the study done by *Pedersoli et al.*
(1987) following single IV and oral administration to dogs at 5
and 15 mg/kg, the mean elimination half-life was found to be 92.6
± 23.7 and 72.3 ± 15.5 hrs, respectively. No studies have been
done on the variation of the half-life with age in animals.
Epilepsy and therefore its control is, however, a rare occurrence
in young animals and such a study may not be justified.

1.3.6 Concentration-Effect Relationship

Although studies examining the relationship between plasma levels
of phenobarbitone and seizure control have produced conflicting
results, it would appear that plasma phenobarbitone levels
greater than 42 μmol.l^{-1} are associated with improved clinical
efficacy. With plasma levels over 172 μmol.l^{-1} the additional
therapeutic benefits decline and there is a higher incidence of
adverse effects (*Rimmer and Richens,1988*). It is well recognized
both in man and in dogs that tolerance occurs to the sedative ef-
fects of the drug so that a serum level of 20 μmol.l^{-1} produced
acutely may have a greater sedative effect than a level of
200 μmol.l^{-1} which has been maintained chronically (*Shell,1984*).
A therapeutic range of 42-170 μmol.l^{-1} has been suggested as a
guide to therapy (*Rimmer and Richens,1988*).

1.4 TOXICITY

Worldwide use of phenobarbitone for almost a century has allowed
the accumulation of considerable knowledge of its toxicity.
Phenobarbitone enjoys a reputation of safety because serious
systemic side effects are very uncommon. Neurological and
psychological toxicity is, however, frequent.

1.4.1   Neurotoxicity

With the long-term usage of phenobarbitone, even the usual dosage-producing serum concentrations of the drug in the broad therapeutic range of 42 to 170 μmol.l^{-1}, adverse changes in affect, behavior, and cognitive function are often encountered in the human patient. High serum concentrations cause neurological signs of "drunkenness", including nystagmus, dysarthria, incoordination, and ataxia. Often, the neurotoxic side effects occur together in different degrees and have been reported in both man and animals (*Mattson and Cramer,1989; Shell,1984*).

Sedation

Sedation would appear to be the major side effect of phenobarbitone. Complaints of fatigue and tiredness are difficult to quantify and are often variable and subtle. The patient and family may describe listlessness or lack of spontaneity even when excessive sleeping time is not observed. As the dosage is increased, overt sleepiness is observable and often apparent by difficulty in arousal in the morning and naps after school or work. Patients, however, complained of sedation for the first few days of treatment which clears rapidly as tolerance develops (*Mattson and Cramer,1989*).

Neurological Side Effects

Increasing the dosage of phenobarbitone eventually leads to neurological signs similar to those found with the use of other antiepileptic drugs. Dysarthria, incoordination, ataxia, dizziness, and nystagmus appear as serum levels exceed 170 μmol.l^{-1} (*Mattson and Cramer,1989*). Side effects of sedation and ataxia have also been observed in dogs on phenobarbitone therapy (*Shell,1984*).

Behaviour

Instead of the sedative effect of phenobarbitone common in adults, in children, and the elderly a paradoxical effect of the drug may produce insomnia and hyperkinetic activity. Phenobarbitone has also been reported to exacerbate aggressiveness and overactivity. The pattern of behaviour included signs of distractibilty, shortened attention span, fluctuation of mood, and aggressive outbursts. The incidence in children was higher in boys. Behavioural disturbances are more likely to become evident in children in the presence of organic brain disease or deficits. These behavioural disturbances have not been reported in animals receiving phenobarbitone therapy.

Character

Phenobarbitone therapy can produce an alteration in the character of the patient and in particular can cause depression. It is difficult to determine whether such mood changes are a reaction to the often newly diagnosed illness, the addition of another drug to treat severe seizures, or a direct neurotoxic effect of phenobarbitone. Clinical observations suggest a direct effect of phenobarbitone, because changes to carbamazepine therapy have been associated with improved mood scores (*Mattson and Cramer,1989*).

Cognition

A side effect of phenobarbitone of considerable importance, especially in children, is a possible disturbance in cognitive function. Problems with memory or compromised work and school performance may develop independent of sedation and hyperkinetic activity, although these factors may play a contributory role (*Mattson and Cramer,1989*).

Barbiturate Overdosage

Frank overdosage of phenobarbitone causing serum levels in excess
of 200 μmol.l^{-1} leads to progressive neurological dysfunction
and depression in levels of consciousness, even in patients on
long term therapy. Excessively high doses first cause ataxia,
dysarthria, nystagmus, incoordination, and uncontrolled sleepi-
ness. As the serum levels rise further, these effects progress to
stupor and coma. Ultimately, depression of cardiorespiratory
function may lead to death (*Mattson and Cramer,1989*).

In the mouse, orally administered phenobarbitone has a median
neurotoxic dose (LD$_{50}$) of 51 mg.kg^{-1}. Subcutaneous administration
increases the LD$_{50}$ to about 100mg.kg^{-1}. In the rat comparable
LD$_{50}$ values are 35 mg.kg^{-1} p.o. and 90 mg.kg^{-1} s.c. The rabbit is
identical to that for the rat, while the cat is more sensitive to
phenobarbitone, with a LD$_{50}$ of 5 mg.kg^{-1} (*Gallagher and
Freer,1988*).

Dependence, Habituation, and Withdrawal

Physical dependence on phenobarbitone occurs and abrupt discon-
tinuation after high dosage produces abstinence symptoms, includ-
ing anxiety, insomnia, tremors, confusion and seizures. These
symptoms can be reversed by reinstituting the drug. If a decision
is made to stop phenobarbitone therapy, it must be tapered off
very slowly to avoid withdrawal seizures (*Mattson and
Cramer,1989; Rimmer and Richens,1988*).

A neonatal withdrawal syndrome has been described in infants born
to epileptic mothers taking phenobarbitone. Phenobarbitone can
cross the placenta and enter the foetal system, and therefore
special care must be taken during the neonatal period of children
born to mothers who received phenobarbitone. The rate of
phenobarbitone elimination in neonates is probably slower than in
adults, possibly because neonatal liver is not fully capable of

metabolizing barbiturates until enzyme induction has occurred (*Mattson and Cramer,1989*).

Some evidence suggests that discontinuation of phenobarbitone in epileptic patients may lead to an exacerbation of seizures not only because of the underlying epilepsy but also because of an additional barbiturate withdrawal mechanism (*Mattson and Cramer,1989*).

1.4.2 Long-Term Developmental Effects

Phenobarbitone given orally and daily for 2 weeks to infant rats at 60 mg.kg^{-1} and 15 mg.kg^{-1} produces a 12 and 3% respective reduction in brain growth (*Booth,1988*).

In addition, phenobarbitone fed to rats in a diet at 0.25% results in a reduced gain in body weight. It is suggested that the lower weight gain in animals chronically exposed to phenobarbitone occurs from alterations in hepatic metabolism, however, the effect of phenobarbitone on food intake may also be a factor in growth reduction (*Booth,1988*).

Mattson and Cramer(1989) report a decreased foetal head growth associated with maternal use of phenobarbitone and this may be of concern because phenobarbitone is currently the drug of choice in pregnancy and treatment of neonates.

1.4.3 Systemic Toxicity

Haematological

Phenobarbitone is particularly benign in its likelihood to produce serious haematological changes. There are no apparent reports in the literature to indicate that blood tests in anticipation of haematological changes should be taken. Mild and clinically unimportant leukopenia (3000-5000 white blood count)

may occur following initiation of treatment, as is also observed with the use of other antiepileptic drugs (*Mattson and Cramer,1989*).

Megaloblastic Anaemia

Megaloblastic anaemia has been described during treatment with phenobarbitone alone, or more commonly, when used with other antiepileptic drugs (*Rimmer and Richens,1988; Mattson and Cramer,1989*). Anticonvulsant megaloblastic anaemia occurs in less than 1% of patients (*Mattson and Cramer,1989*). The aetiology and pathogenesis of macrocytosis and megaloblastic anaemia during antiepileptic therapy are unknown, but these conditions usually respond to folate therapy.

Folate Deficiency

Frank serum and red blood cell folate deficiency is relatively common. In a study done by *Reynolds(1974)* and referred to by *Mattson and Cramer(1989)*, a survey indicated a 27% to 91% subnormal serum folate level in 52% of patients receiving long-term therapy with phenytoin, phenobarbitone, or primidone.

Reynolds also reports an improvement in psychiatric abnormalities in patients with low serum folate concentrations that were treated with folate therapy. This was, however, a subjective observation and as such difficult to assess. No controlled trials have been done to confirm this.

Mattson and Cramer(1989), however, discount the significance of folate deficiency as being speculative. These authors state that serum phenobarbitone concentrations decreased when folic acid was given in very high doses and, therefore, it is possible that reports of seizure exacerbation resulted in part from the

decrease in drug concentration rather than from an epileptogenic activity of folate; although the mechanism of this interaction is unknown. These authors also make mention that animal studies done by *Klipstein (1964)* showed that even in severe folate deprivation, the brain maintains sufficient folate.

Vitamin K

Phenobarbitone and phenytoin can enter the liver of the foetus and compete with vitamin K to prevent production of vitamin K-dependent clotting factors. This can occur even in the presence of normal clotting factors in mothers receiving drug therapy. The neonate can suffer from intraperitoneal, interthoracic, or intracranial bleeding if vitamin K-dependent coagulation factors are deficient. Vitamin K administered to mothers prepartum will prevent this coagulation deficiency (*Mattson and Cramer,1989; Rimmer and Richens,1988*).

Bone Disorders

Antiepileptic drug therapy may affect calcium and vitamin D metabolism, leading to hypocalcaemia or, rarely, osteomalacia (*Rimmer and Richens,1988; Mattson and Cramer,1989*). Induction of liver enzymes leading to increased hydroxylation of vitamin D is a probable mechanism for altered calcium metabolism (*Mattson and Cramer,1989*).

1.4.4 Hepatic Disorders

Phenobarbitone is only a hepatotoxin in unusually susceptible individuals. Liver disease induced by the drug would appear not to be dose-dependent and has a low incidence (*Rimmer and Richens,1988*). The induction of hepatic microsomal enzymes has been discussed before, and this can lead to the enhanced metabolism of other drugs or endogenous substances. These interactions can be considered a hepatic side effect of phenobarbitone therapy.

1.4.5 Hypersensitivity Reactions

Phenobarbitone causes various types of skin reactions. These are usually mild maculopapular, morbilliform, or scarlatiniform rashes that fade rapidly when drug administration is stopped. The incidence is reported to be as low as 1% to 3% of all patients receiving barbiturates (*Mattson and Cramer,1989*).

1.4.6 Teratogenicity

There is an increased risk of foetal malformation in the offspring of mothers receiving antiepileptic therapy, but the role played by the various antiepileptic drugs in their aetiology is far from clear. Evidence for the teratogenic potential of phenobarbitone is much less than for phenytoin (*Rimmer and Richens,1988; Mattson and Cramer,1989; Gallagher and Freer,1985*).

1.5 DRUG INTERACTIONS

Most of the reported drug interactions involving phenobarbitone occur in situations where phenobarbitone has altered the kinetics of other drugs. Alteration in protein binding has not been implicated as an important factor in any reported interactions. However, phenobarbitone is a potent inducer of hepatic mixed function oxidase enzymes and thus can alter the metabolism of numerous drugs, e.g. warfarin, oral contraceptive steroids, and various endogenous substances (*Rimmer and Richens,1988; Kutt,1989*). The effect of enzyme induction on other drugs is largely unpredictable and seems to be dependent on genetic factors and previous contact with environmental inducing factors (*Kutt,1989*).

Inhibition of the metabolism of phenobarbitone by other drugs may also occur. Accumulation of phenobarbitone caused by *valproate* is clinically important since it occurs predictably in the majority of patients taking these two drugs together (*Rimmer and Richens,1988; Kutt,1989*).

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Chapter Two

The principles of applied pharmacokinetics and analytical methods
of determination of serum levels of phenobarbitone

2.1 INTRODUCTION

Advances have been made in the management of epilepsy in man over
recent years. Increased knowledge of antiepileptic drug phar-
macokinetics has been invaluable and the use of serum drug con-
centration monitoring is very useful for selected antiepileptic
drugs. Because of our greater awareness of the hazards of mul-
tiple antiepileptic therapy, in particular the risks of chronic
drug toxicity and the dangers of drug interactions, monotherapy
has become the general policy. The widespread availability of
reliable anticonvulsant drug assays have improved the prospects
for seizure control with monotherapy. Therapeutic drug monitoring
is a neglected field in veterinary medicine. The use of drug con-
centrations, pharmacokinetic principles and pharmacodynamic
criteria should be used to optimize drug therapy in refractory
animal cases.

2.1.1 Why monitor phenobarbitone levels?

The value of therapeutic drug monitoring is to maximize efficacy
of therapy and minimize side effects. It is well recognized in
human therapy that dosage of most drugs must be individualized to
achieve optimum efficacy with minimal side effects (*Frewin,1982*).
The therapeutic ranges of serum concentrations of antiepileptic
drugs have been reasonably well defined. The term "therapeutic
range" describes the relationship between the desired phar-
macologic activity and the drug concentration in serum or plasma.
Patients vary in their response to antiepileptics. Some benefit

with concentrations that are low, whereas, others may require high levels. Similarly, some patients, exquisitely sensitive to the adverse effects of antiepileptic drugs, cannot tolerate even low serum concentrations, while others can tolerate high concentrations without experiencing drug toxicity. Therefore, the therapeutic ranges for the antiepileptic drugs are useful primarily as guidelines in initiation of therapy (*Welty et al,1983; Kutt and Penry,1974*).

In a study done on dogs where the serum concentrations, drug dosages, and seizure control were monitored in 142 dogs receiving a variety of antiepileptic treatment regimens, the author found that of the dogs in which seizures were uncontrolled, a large proportion had serum phenobarbitone concentrations that appeared to be inadequate in spite of what was considered adequate dosage. Further, for dogs given phenobarbitone, there was a sixfold variation between dosage and an achieved serum concentration (*Farnbach,1984*). This underscores the need for serum concentration monitoring as an adjunct to any drug protocol in seizure control since effectiveness is correlated far better with serum concentrations than with oral dosing.

2.2 ANALYTICAL METHODS

During the past 25 years enormous advances have been made in the development of sensitive and specific methods of determination of drug and drug metabolite concentrations in biologic fluids. The improved methodologies have resulted in an almost explosive proliferation of commercial drug assay kits and instrumentation. The different methods available differ in important aspects including sensitivity, specificity, sample size needed, technical difficulty, amount of technician time required and initial equipment costs (*Hendeles and Weinberger,1981*). The following discus-

sion traces the evolution of the available phenobarbitone assay
techniques from initial tedious, technically difficult assays to
modern day automated immunoassays with extremely rapid turnaround
time.

2.2.1 UV spectrophotometric assay

Phenobarbitone was initially measured by spectrophotometry. In
this method phenobarbitone is separated from other drugs, espe-
cially phenytoin, and extracted from the serum with organic sol-
vent and the absorbance of ultraviolet light is measured in a
spectrophotometer. Since most laboratories have a
spectrophotometer, the method does not require an investment in
new and expensive equipment. However, it does require a rela-
tively large sample size. In addition, a variety of commonly used
drugs (e.g. furosemide and aspirin) interfere with this assay,
producing false results. Patients are often treated with various
drugs, and these are not always reported to the laboratory. Ac-
cordingly, there is a great risk of false results, as has been
shown in quality control schemes (*Johannessen,1989; Hendeles and
Weinberger,1981*). Dogs, however, are not commonly treated with
concomitant medication and, therefore, this problem may not be as
acute as in the case of man.

Other disadvantages include the need for more technician time and
the poor reproducibility of the results compared to other avail-
able methods. Even if spectrophotometric methods for determina-
tion of phenobarbitone can produce excellent results in the ab-
sence of interfering compounds, these techniques can hardly be
recommended today for routine monitoring of patients with
epilepsy.

2.2.2 Thin-layer chromatography

To avoid interfering substances and problems with spectrophotometric analysis of phenobarbitone, thin-layer chromatographic systems have been useful. This technique also allows analysis of multiple antiepileptic drugs. Instead of extensive solvent partition extractions, separation was achieved on the chromatographic plate. Although many of the thin-layer chromatographic methods are specific and reproducible, they are rather complicated, time consuming, with low output, and cannot be recommended today (*Johannessen,1989*).

2.2.3 Gas-liquid chromatography

The determination of phenobarbitone by gas-liquid chromatography (GLC) soon followed thin-layer chromatography and has been widely used because of its high selectivity and sensitivity. The gas chromatographic determination of phenobarbitone depends on various conditions; variables include, specific quantification of phenobarbitone alone or in multiple drug analysis, simple or complex extraction procedures, choice of internal standards, use of derivatization, type of column, isothermal or programmed temperature, and type of detector.

The first step in a GLC assay involves an extraction step to dissolve the drug into an organic solvent. The sample plus an internal standard with chemical properties similar to the drug being assayed, is then injected into the gas chromatograph. This is carried into a high temperature column by an inert gas (e.g. helium) which constitutes the mobile phase. Column temperatures of 100°C to 350°C volatilize the sample, which then comes into contact with an inert stationary phase, usually coated with a non-volatile liquid. As the mobile phase passes through the column, the analyte is separated from other sample constituents

based on affinity for the stationary phase. The mobile phase then passes from the column to a detecting device that records peaks graphically which correspond to the concentrations of the unknown substances. The internal standard produces a reference peak. Detecting devices include electron capture, mass spectrometry, or more commonly, flame ionization (*Bottorff and Stewart,1986*).

Advantages of GLC include flexibility in drug assays (by altering column length and temperature), simultaneous assay of parent drug and metabolites and the ability to use small sample volumes. The disadvantages are the time consuming procedure, high equipment cost, column deterioration and the need for a skilled analyst. Few laboratories currently use this technique (*Hendeles and Weinberger,1981; Bottorff and Stewart,1986; Johannessen,1989*).

2.2.4 High-pressure liquid chromatography

Determination of phenobarbitone by liquid chromatography (LC) is a suitable alternative to GLC. Liquid chromatography is similar to GLC except that the mobile phase is a liquid, usually a mixture of acetonitrile or methanol with water. Compared to GLC, the need for high temperatures is eliminated and columns packed with stationary phase are kept at ambient temperature. Substances are separated in LC according to their solubility in aqueous or organic solvents. Highly polar compounds will be more soluble in highly polar solvents like water, whereas, less polar drugs will dissolve better in organic solvents such as chloroform. As the mobile liquid phase and the stationary phase come into contact in a column, separation occurs in a fashion similar to other chromatographic methods.

The addition of 200 to 1000 pounds of pressure per square inch to the separation column converts liquid chromatography into high pressure liquid chromatography (HPLC), producing rapid separation and determination of drug concentration.

Reverse phase liquid chromatography uses a non-polar column packing and polar mobile phase. This technique allows for the rapid determination of phenobarbitone concentrations without the need for an organic extraction step (*Bottorff and Stewart,1986*). The reverse phase model is the one most often used, and offers distinct advantages over other LC methods (*Johannessen,1989*).

Liquid chromatography offers several advantages over gas chromatographic methods, such as the absence of derivatization, faster separation, better sample stability, smaller sample size, and low cost once the method has been established.

The disadvantages are the high initial cost of the equipment; the high degree of technician skill required and the difficulty in performing stat or small batch testing due to the equipment preparation time required. Large doses of some drugs e.g. ampicillin, cephalothin, acetazolamide and trisulfapyrimidine cause falsely elevated results under some operating conditions and represents another disadvantage of this technique (*Hendeles and Weinberger,1981; Bottorff and Stewart,1986, Johannessen,1989*).

2.2.5 Gas chromatography-mass spectrometry

The quantitation of both phenobarbitone and its metabolites using selected ion detection with a gas chromatograph-mass spectrometer-computer system operated in the chemical ionization mode has been reported in several studies. Alternatively, phenobarbitone can be quantitated using a stable isotope-labelled internal standard and chemical ionization/mass spectrometry

without prior chromatographic separation. Selected ion monitoring is the most sensitive and specific method for drug analysis. Although this method can be considered a reference procedure, the instrumentation is costly and technically difficult to operate. It is therefore limited to research laboratories (*Johannessen,1989*).

2.2.6 Radioimmunoassay

The principle of a radioimmunoassay consists of a radiolabelled ligand (usually ^{57}Co, ^3H, or ^{125}I) that binds to a specific antibody. Added unlabelled ligand competes with the label for binding sites. Measurement of the free or bound label is used for the quantitation of drug present. Specific radioimmunoassays for determination of phenobarbitone are not commercially available, but the technique has been described in several reports. Generally, radioimmunoassays have the advantage of excellent sensitivity, and it is possible to make the antisera highly specific. Furthermore, a large number of samples can be processed and because of the ability to detect concentrations in the picogram range, small sample volumes are adequate. The disadvantages are the long turnaround times; the many interfering substances; radiation hazards; inconvenience of recording and disposing of radioactive waste; short shelf life of RIA reagents and the need for daily calibration of the instruments. This method is now less popular because of these many disadvantages (*Johannessen,1989; Hendeles and Weinberger,1981; Bottorff and Stewart,1986*).

2.2.7 Homogenous enzyme immunoassay

The development of the homogenous enzyme immunoassay (EMIT[R], Syva Company) for phenobarbitone and other antiepileptic drugs has been a major advance in the rapid and accurate analysis of microsamples.

Whereas the label in radioimmunoassays is a radioactive isotope, the homogenous enzyme immunoassays employ an enzyme as a label. The assay is based on competition between drug in sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the sample can be measured spectrophotometrically in terms of enzyme activity based upon the conversion of oxidized nicotinamide-adenine dinucleotide (NAD^+) to the reduced form of NAD(NADH). No separation step is required in this assay.

The phenobarbitone assay is easily run on one of the Syva Lab Systems, including the Syva Advance Fluorescence Immunoassay System. In running phenobarbitone EMIT assay on the Advance System, rather than measuring the absorbance of NADH in the turnover of NAD^+ to NADH, the fluorescence emission of NADH is measured (*Johannessen,1989*).

The advantages of this method include the rapid turnaround time and high precision. The disadvantages are the need to frequently recalibrate the instrument and the technician time required to perform dilution steps to analyse abnormally high serum concentrations (*Bottorff and Stewart,1986*).This technique is one of the more popular methods currently available.

2.2.8 Fluorescence polarization immunoassay

Another system of therapeutic monitoring of phenobarbitone and other drugs is based on fluorescence polarization immunoassay (FPIA). An automated analyzer TDx was introduced for such measurements (Abbott Diagnostics).

This method combines competitive protein binding with fluorescence polarization to give a direct measurement without the need for a separation procedure. All competitive immunoassays for measuring therapeutic drugs are based on competition between the drug in the patient sample and a labeled drug, called the tracer. In the TDx system, the label on the tracer drug is the fluorescent dye, fluorescein. The polarization of fluorescent light emitted by fluorescein tracer increases as the tracer is bound to antibody. Polarization is measured using a sophisticated optical detection system. A calibration curve stored in the systems memory is used to automatically determine the concentrations of unknown patient samples. FPIA is a precise, sensitive measurement technique for rapid analysis of phenobarbitone in small sample volumes (*Johannessen,1989*).

The advantages are the high degree of automation; rapid turnaround times, good sensitivity (lower limit of detection is 0.5 $\mu g.ml^{-1}$ at the 95% confidence limit), good stability of reagents; good stability of calibration curves and the ability to analyse small or large batches of samples. A disadvantage is the presence of background interference inherent in some serum samples. In order to minimize this, blank readings are taken and background interferences are subtracted (*Hendeles et al,1989; Bottorff and Stewart,1986*).

2.2.9 Substrate-labelled fluorescent immunoassay

This type of test for determination of phenobarbitone utilizes a technique in which the drug in the specimen competes with a drug-labeled fluorogenic substrate for binding sites on the antibody. The drug is labeled with a derivative of the fluorogenic enzyme substrate umbelliferyl-β-D-galactoside. This fluorogenic drug reagent (FDR) is nonfluorescent under the conditions of the assay. However, hydrolysis catalyzed by β-galactosidase yields a fluorescent product. When antibody to the drug reacts with FDR, it is virtually inactive as a substrate for the β-galactosidase. Competitive binding reactions are set up with a constant amount of FDR, a limiting amount of antibody to the drug, and the clinical sample containing the drug. The drug in the sample competes with the FDR for antibody binding sites. FDR not bound to antibody is hydrolyzed by β-galactosidase to produce the fluorescent product. Hence, the fluorescence produced is proportional to the drug concentration in the sample. Each test requires only a small volume of serum or plasma and can easily be run on Ames Fluorescent Chemistry Systems (*Johannessen,1989*).

2.2.10 Nephelometric inhibition immunoassay

Phenobarbitone is also measured by a rate nephelometric inhibition immunoassay, which is a homogenous, competitive binding assay for quantitation of haptens. It utilizes a precipitation procedure that does not require the use of fluorescent, radioactive, or enzymatic tracers. The drug in each patient sample competes with a drug-protein conjugate for a fixed amount of antibody that is injected into each test reaction. Since the rate of light scattering results only from the reaction of the antibody with the drug-protein conjugate (and not the reaction with the drug in the patient sample), the nephelometric signal is inversely proportional to the amount of drug present in the test sample.

This system has been very convenient for the precise determination of phenobarbitone in patient samples (Beckman Immunochemistry Systems).

(*Johannessen,1989*)

2.2.11 Radial partition immunoassay

A radial partition immunoassay for phenobarbitone has also been developed. This assay is an integral part of the Stratus Enzyme Immunoassay System (American Dade) which is a rapid and sensitive procedure for the automated determination of therapeutic phenobarbitone levels in serum and plasma, based upon the competitive immunoassay technique. The clinical sample is premixed with alkaline phosphatase labelled phenobarbitone and spotted onto glass fiber paper containing preimmobilized anti-phenobarbitone distributed throughout the paper at the analysis site. The two antigens then compete for binding sites on the antibody molecule and any unbound labeled drug is washed out of the field of view of the fluorometric analyzer. A substrate for the enzyme label is incorporated into the wash solution and the enzyme reaction is initiated simultaneously with the wash. The reaction rate of the bound fraction is measured via front surface fluorometry. These rates are inversely proportional to the phenobarbitone concentration. This system has proven to be a rapid and sensitive procedure for determination of phenobarbitone levels.

(*Johannessen,1989*)

2.2.12 Newer techniques

2.2.12.1 Dry-phase apoenzyme reactivation immunoassay system (ARIS)

Ames Diagnostics have recently introduced the ARIS reagent strip test for determination of phenobarbitone which is performed on the Seralyzer reflectance photometer (Ames). In the assay, the drug in the sample competes with a flavine adenine dinucleotide (FAD)-drug conjugate for binding to a specific antibody. The unbound conjugate then activates apoglucose oxidase to reconstitute glucose oxidase, whose activity is kinetically monitored by a coupled chromogenic reaction.

This homogenous competitive colorimetric immunoassay is partially suitable for emergency use, for testing small batches of samples, and wherever prompt results are needed. The dry reagent strip technology is very convenient since all reagents are contained in a ready-to-use cellulose pad fixed to a plastic strip, the colour developed on the pad is kinetically monitored by a reflectance photometer, and test results are displayed directly in clinically useful units.

(Johannessen,1989)

2.2.12.2 Enzyme immunochromatography

Syntex Medical Diagnostics recently developed a noninstrumented quantitative method for therapeutic monitoring of phenobarbitone and other antiepileptic drugs using a factory-calibrated unit test format and a novel single-level approach to quality control. This method is based on the principles of immunochromatography, which provides a number of convenient protocol advantages without sacrificing assay performance or quality assurance, mainly because quantification is dependent on enzyme migration rather than

enzyme activity. Since migration height is almost solely a function of a highly stable, immobilized, dry antibody reagent, this test is extremely insensitive to environmental factors. The specificity of the assay should be comparable to that of other since the principle involved viz. use of monoclonal antibodies is the same (*Hendeles et al,1986*).

The AccuLevel test consists of three main components: a chromatographic paper strip coated with monoclonal antibodies against a specific drug, an enzyme reagent that contains horseradish peroxidase-labeled drug and the enzyme glucose oxidase, and a developing reagent that contains glucose and 4-chloro-1-naphthol. It requires only 12 µl of whole blood from a finger prick and results can be obtained within 15 min using a simple 2-incubation protocol which does not require sample dilution (*Johannessen,1989*). The ability to use whole blood rather than serum or plasma means that the time-consuming centrifugation step is eliminated, and no electronic or other instrumentation is required. This is a real advantage, and this new approach to therapeutic drug monitoring represents a step forward toward immediate laboratory information for better patient care.

2.3 BIOLOGIC FLUID PHENOBARBITONE CONCENTRATIONS

The penetration of phenobarbitone to well perfused tissue occurs fairly rapidly. The distribution of phenobarbitone is sensitive to variations in the pH of plasma because it has a pKa close to the physiological plasma pH. Acidosis causes a shift of the drug from plasma to tissues, and alkalosis results in an increased phenobarbitone concentration in the plasma (*Rimmer and Richens,1988*).

The phenobarbitone concentration in the CSF of adults is 43% to 60% of plasma concentration and correlates well with the unbound phenobarbitone concentration in serum. The CSF levels in infants are similar, ranging from 48% to 83% of plasma levels. The phenobarbitone concentration in the CSF provides a reliable index of phenobarbitone concentration in the brain. Under nonequilibrium dosing conditions, actions of phenobarbitone correlate better with CSF levels than with either dose or the rate of drug administration (*Rust and Dodson,1989*).

Phenobarbitone concentrations are higher in CSF than saliva, a more acidic but similarly low-protein fluid. Both salivary and sweat phenobarbitone concentrations vary with flow rate. Salivary phenobarbitone concentrations are also sensitive to pH changes and saliva-to-serum phenobarbitone ratios have a greater interindividual than intraindividual variation. It is therefore unwise to use saliva for serum drug level monitoring in the case of phenobarbitone (*Rust and Dodson,1989; Rimmer and Richens,1988*). Since tears have a more constant pH than saliva, some investigators have suggested that tears may be more reliable than other nonsanguinous fluids for estimating phenobarbitone concentrations (*Rust and Dodson,1989*).

Phenobarbitone readily crosses the placenta and is excreted in breast milk. Infants born to mothers have equivalent serum concentrations in the immediate postnatal period. Concentrations of phenobarbitone in breast milk were 36 ± 20% and 41 ± 16% of maternal serum concentration in two studies referred to by *Rust and Dodson*.

Due to the variability of results from nonsanguinous fluids, there is a danger in possible dosage adjustment errors. The ability of newer techniques to measure levels as little as 12 μl (i.e. sufficient from a finger or heel prick) would make the sampling and analysis of blood the preferred method.

2.4   WHEN TO DRAW SAMPLES

Since drug administration is a dynamic process, the timing of sample collection can be critical to its proper interpretation. It is important that samples for serum phenobarbitone concentration monitoring be drawn at steady state since levels measured before steady state may be misleading. Steady state usually occurs after approximately 3-4 weeks of stable dosage in man (*Gal,1986; Rimmer and Richens,1988*) and in a study done on dogs, *Ravis et al(1984)* concluded that time to steady state would be between 8 to 15.5 days. However, if computer facilities are available to perform the relevant complex calculations, then samples may be drawn prior to steady state. Blood samples should not be drawn during the absorption phase in view of the many factors that affect absorption of phenobarbitone (*Robinson and Taylor,1986*). The absorption phase in adult man is 1-6 hours after oral dosing (*Rimmer and Richens,1988; Rust and Dodson,1989*), and in dogs, maximum concentrations in plasma were reached in 4-8 hours (*Frey and Loscher,1985*). Since peak/trough fluctuations of phenobarbitone in the dog are relatively small, the time when the blood sample is taken is not crucial.

2.5 PHENOBARBITONE DOSING METHODS AND THE APPLICATION OF PHARMACOKINETIC CALCULATIONS IN THE INTERPRETATION OF SERUM CONCENTRATIONS

The pharmacokinetic properties of phenobarbitone given orally, intravenously, or intramuscularly, allows prediction of plasma levels that are therapeutically efficacious. Until the last decade, the use of phenobarbitone for the treatment of epilepsy in man was based on empiric information of clinical response rather than plasma or brain tissue concentrations attained during therapy (*Painter,1989*). The clinical response to phenobarbitone is still, however, the method of assessment of drug efficacy in the dog, and only recently has an attempt been made to investigate the practical application of therapeutic drug monitoring in the treatment of canine epilepsy (*Morton and Honhold,1988*).

Clinical pharmacokinetics concerns the use of pharmacokinetic concepts and techniques with or without drug levels to promote effective and safe therapy in patients and is based upon the concepts of drug disposition. The interpretation of serum drug concentrations requires an understanding of the basic concepts of pharmacokinetics and a background in pathophysiology and pharmacotherapeutics (*Beane,1979*). However, in the case of phenobarbitone, this is is not that significant due to the drugs low degree of protein-binding and its relative safety in respect of drug interactions. Numerous dosing methods have been developed in an attempt to improve the relationship between dose, serum phenobarbitone concentration and response. The various pharmacokinetic parameters that will affect the therapeutic objective may be considered under the following headings:

* Bioavailability
* Desired plasma concentration
* Volume of distribution and loading dose
* Clearance and steady-state
* Elimination rate constant (kd) and half-life

2.5.1   Bioavailability

Bioavailability has been defined as the *rate* and *extent* to which the drug reaches the general circulation in an unchanged form. It is defined in reference to direct intravenous administration where the extent of drug entry is considered to be 100% and the rate of entry is determined by the infusion rate. Bioavailability of a particular dosage form is expressed as the fraction of the administered dose that reaches the systemic circulation of the patient and is designated by the parameter *f*, which varies between 0 and 1; *f* = 1 refers to 100% extent of bioavailability. Although the rate of absorption can be important when a rapid onset of drug action is required, it is not usually important when a drug is administered chronically.

The bioavailability of phenobarbitone varies among the different formulations of the drug. Salts of phenobarbitone are more soluble than phenobarbitone crystal. Preparations for intravenous or intramuscular administration are from the sodium salt in slightly alkaline solutions. Tablets for oral administration are usually compounded from fine, somewhat polymorphic crystals of the sodium phenobarbitone salt (*Rust and Dodson,1989*).

Phenobarbitone has a high bioavailability in adults after oral or intramuscular administration. Studies in dogs and humans, at steady-state kinetics, have shown 80% to 100% absorption of

orally administered phenobarbitone. In one study, the
bioavailability of phenobarbitone tablets was 95% and for
phenobarbitone elixir was 100% (*Rust and Dodson,1989; Levy et
al,1986; Pedersoli et al,1987*).

To calculate the amount of drug which should reach the systemic
circulation, the administered dose is multiplied by the
bioavailability factor (f).

$$f \times DOSE = AMOUNT\ OF\ DRUG\ ABSORBED \quad \dots\dots\dots\dots\dots(1)$$

The chemical form in which the drug is administered must also be
considered. When a salt or ester of a drug is administered, the
bioavailability factor (f) should be multiplied by the fraction
of the total molecular weight which the active drug represents
(S). The salt factor is not significant in the case of phenobar-
bitone where one is working from one dose to another of the same
form.

Drugs given chronically, as in the case of phenobarbitone, are
usually administered at a constant rate. This then enables one to
calculate the administration rate (Ri) which is the average rate
at which the absorbed drug reaches the systemic circulation. This
is calculated by dividing the amount of drug absorbed by the time
over which the drug was administered, or the dosing interval (τ).

$$Ri = \frac{(S)\ (f)\ (DOSE)}{\tau} \quad \dots\dots\dots\dots\dots(2)$$

Poor patient compliance may be mistaken for decreased
bioavailability. A true decrease in bioavailability may result
from a poorly formulated dosage form that fails to disintergrate

or dissolve in the gastrointestinal fluids, interactions between other drugs in the gastrointestinal tract, metabolism of the drug in the gastrointestinal tract, and first-pass hepatic metabolism.

2.5.2   Desired plasma concentration

A therapeutic objective or endpoint must be selected when designing a therapeutic regime. The preferred endpoint would obviously be the relief of the treated condition, but this is not always feasible and therefore, the drug serum concentration may used as an intermediate endpoint. Study of drugs which show some relationship between serum concentration and the effect has served to establish "therapeutic ranges" which is defined by a Minimum Effective Concentration (MEC) and a Maximum Safe Concentration (MSC). The therapeutic range of a drug represent average values only. A beneficial response to a drug may occur below the MEC in a particular patient, and likewise, a patient may exhibit drug toxicity at levels below the average MSC or tolerate levels higher than the average MSC. Drug efficacy and drug toxicity can, therefore, only be determined in the individual patient from clinical assessment of the response to the drug. Drug levels are used in conjunction with clinical observations in order to investigate the contribution of the drug to the patient's clinical state.

The therapeutic range of phenobarbitone has been reported as 10 to 40 $\mu g.ml^{-1}$ (42-170 $\mu mol.l^{-1}$) in man(*Painter,1989; Rimmer and Richens,1988; Morton and Honhold,1988*). In a study done by Feely *et al* (1980) and referred to by *Painter*, it was found that the dose necessary to achieve a plasma concentration of more than 10 $\mu g/ml$ were 1.0 to 1.5 $mg.kg^{-1}$ for adult patients and 1.5 to 3 $mg.kg^{-1}$ for children. The therapeutic range has not been calculated for canines but is assumed to be the therapeutic range for

man (*Morton and Honhold,1988; Schwartz-Porsche et al,1984; Shell,1984*).In a study done by *Farnbach (1984)* where seizure control was monitored in dogs, the serum concentrations of phenobarbitone in the dogs with effective control ranged from 14.3 μg.ml^{-1} to 43.1 μg.ml^{-1}.

The status of the binding of drugs to plasma proteins is often of importance in the choice of the desired steady-state concentration. The drug concentration in plasma (Cp) which is reported by the laboratory represents drug that is bound to plasma protein plus drug that is unbound or free. Phenobarbitone binding to plasma protein plays a minor role in distribution. Binding is readily reversible and independent of drug concentration, ionic dissociation, and serum calcium concentration within the physiological range of pH. In a study by *Rust and Dodson (1989)*, it was concluded that since approximately 55% of phenobarbitone in serum is unbound, changes in the extent of phenobarbitone binding in serum will have little effect on the unbound phenobarbitone level. In dogs, 45% to 46% of phenobarbitone is bound to canine serum proteins, which is slightly less than the 48.5-50% determined in human serum(*Frey and Loscher,1985*).

2.5.3 Volume of distribution

The volume of distribution (Vd) is the size of the compartment necessary to account for all the drug in the body if it were present at the same concentration everywhere as in the sample measured. The volume of distribution does not necessarily refer

to any real volume but is simply a constant relating the amount of drug in the body (Ab) and the plasma concentration. The equation for the volume of distribution is expressed as follows:

$$Vd = \frac{Ab}{Cp} \quad \ldots\ldots\ldots\ldots\ldots\ldots(3)$$

where Ab = (S) (*f*) (DOSE)

Estimates of the apparent volume of distribution for phenobarbitone vary, sometimes as much as fourfold (*Rust and Dodson,1989*). This wide range of values is in part attributable to the different methods that have been employed; most recent investigators have used a single compartment kinetic model (*Ravis et al,1984; Pedersoli,1987*). In dogs, the relative volume of distribution of phenobarbitone was calculated to be 0.68 ± 0.029 l.kg⁻¹ (*Frey and Loscher,1985*). In another canine study by *Ravis et al (1984)*, a value of 0.7436 ± 0.0698 l.kg⁻¹ for Vd/*f* was obtained using non-linear least squares regression. To avoid the assumption of complete systemic absorption, the value of Vd was expressed as a function of the extent of absorption (*f*). In adults and children, the relative volume of distribution ranges from 0.36 to 0.67 l.kg⁻¹ after intramuscular doses and from 0.42 to 0.73 l.kg⁻¹ after oral doses. A wider range (0.39 to 2.25 l.kg⁻¹) has been reported for newborns than for older children or adults(*Rust and Dodson,1989*). This is probably the consequence of their relatively larger extracellular fluid volume.

Since the volume of distribution is the parameter which accounts for all of the drug in the body, it can be used to estimate the loading dose necessary to rapidly achieve a desired plasma concentration.

$$\text{LOADING DOSE} = \frac{Vd \times Cp}{(S)\ (f)} \quad \ldots\ldots\ldots\ldots\ldots\ldots(4)$$

Using a loading dose is beneficial in the case of status epilepticus or where the patient/owner is particularly anxious to rapidly bring the seizures under control. It has been recommended that in the treatment of canine seizures, a oral loading dose of 30 mg/9 kg body weight stat should be given followed by a maintenance dose of 15 mg/9 kg every 6-24 hours(*Booth,1988*). In humans, in the treatment of status epilepticus, a loading dose is given in the form of an intravenous infusion at a rate of 100 mg.min^{-1} until a dose of 10 mg.kg^{-1} is achieved. This is also used in the treatment of neonatal seizures to promptly achieve therapeutically effective but nontoxic concentrations. Loading doses of the drug are given intravenously at 15 to 20 mg.kg^{-1} to achieve a predictable plasma level based on the volume of distribution for neonates (0.81 ± 0.18 l.kg^{-1}). Plasma levels of phenobarbitone can then be maintained in the range of 20 µg.ml^{-1} with doses of 3 to 4 mg.kg^{-1} per day following loading (*Painter,1989*).

2.5.4 Clearance

The overall apparent loss of an active drug moiety from the system may be simplified and summarized by the parameter drug *clearance* (Cl). Drug clearance principles are similar to the clearance concepts in renal physiology, in which creatinine clearance is defined as the rate of elimination of the creatinine in the urine relative to the plasma creatinine concentration. Clearance of a drug is, therefore, the rate of elimination (Ro) by all routes relative to the concentration of drug in any biological fluid.

$$\text{RATE OF ELIMINATION (Ro)} = Cl \times Cp \quad \dotsb (5)$$

and, therefore

$$\text{CLEARANCE} = \frac{Ro}{Cp} \quad \dotsb (6)$$

Clearance does not indicate how much drug is being removed but rather the volume of blood or plasma which would be completely cleared of drug if it were present.

2.5.5 Steady state

In most cases of prolonged treatment, as in the case of phenobarbitone, it is desirable to maintain steady state drug concentrations within a known therapeutic range. To maintain this desired drug concentration (steady state), the drug must be replaced at a rate equal to its loss. At steady state, therefore, the rate of drug administration (Ri) and the rate of drug elimination (Ro) must be equal.

$$Ri = Ro$$

and since Ro = Cl x Cp (Equ. 5)

therefore, at steady state

$$Ri = Cl \times \overline{Cp_{ss}} \qquad \dots\dots\dots\dots\dots\dots(7)$$

$$\text{where } \overline{Cp_{ss}} = \text{average steady state concentration}$$

If the average steady state plasma concentration and rate of drug administration are known, then clearance can be calculated by rearranging equation (7)

$$Cl = \frac{Ri}{\overline{Cp_{ss}}} \qquad \dots\dots\dots\dots\dots\dots(8)$$

For intermittent dosing, as in the case of oral dosing,

$$Ri = \frac{(S)\ (f)\ (DOSE)}{\tau} \qquad \dots\dots\dots\dots\dots\dots\dots(9)$$

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therefore, if the average steady state plasma concentration can be estimated, clearance can be calculated using the modified equation (8):

$$Cl = \frac{(S)\ (f)\ (DOSE)}{\tau\ \times\ \overline{Cp}_{ss}} \qquad \dots\dots\dots\dots\dots\dots\dots\dots\dots(10)$$

If serial serum drug concentrations are measured and plotted graphically, then the area under the curve (AUC) can be calculated by the linear trapezoidal rule. The AUC is a measure of extent of drug absorption if clearance is constant. The total body clearance at steady state can be calculated in a model-independent fashion by using the following equation:

$$Cl = f \times DOSE/AUC\ (l.hr^{-1}.kg^{-1}) \qquad \dots\dots\dots\dots(11)$$

Maintenance dose

If an estimate for clearance is obtained from the literature or calculated from a steady state concentration-dose pair, the clearance formula (Equ. 10) can be rearranged to calculate the rate of administration or maintenance dose that will produce a desired average plasma concentration at steady state:

$$\text{Maintenance dose} = \frac{Cl \times \overline{Cp}_{ss} \times \tau}{S \times f} \qquad \dots\dots\dots\dots\dots\dots(12)$$

2.5.6 Elimination rate constant (K_E) and half-life

The amount of drug removed per unit time (Ro) varies proportionately with the drug concentration although the fraction of the total amount of drug present in the body (Ab) which is removed at any instant in time remains constant and independent of dose. The follow equation describes the relationship between Ro and Ab.

$$Ro = K_E \times Ab \quad \dots\dots\dots\dots\dots\dots\dots\dots(13)$$

Where K_E is the elimination rate constant. This constant (K_E) is the fraction of the total amount of drug in the body removed per unit time and is a function of clearance and volume of distribution:

$$K_E = \frac{Cl}{Vd} \quad \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots(14)$$

Half-life

The elimination rate constant is often expressed, in its reciprocal form, as the half-life ($t_{\frac{1}{2}\beta}$), which is defined as the time required for the total amount of drug in the body or the plasma drug concentration to decrease by one half. It can be calculated from the following equation:

$$t_{\frac{1}{2}\beta} = \frac{0.693 \ Vd}{Cl} \quad \dots\dots\dots\dots\dots\dots\dots\dots(15)$$

and thus, from equation (14)

$$t_{\frac{1}{2}\beta} = \frac{0.693}{K_E} \quad \dots\dots\dots\dots\dots\dots\dots\dots\dots(16)$$

The above equations combined with the knowledge of good estimates of the population pharmacokinetic parameters of a particular drug will ensure effective and safe dosing.

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Chapter Three

The pharmacokinetics of phenobarbitone in a group of healthy dogs
and the effect of food on the absorption of the drug

3.1   INTRODUCTION

Seizure disorders are amongst the most frequently seen neurologic
problems in small animal practice.   Seizures often present a
diagnostic dilemma because they are episodic, frequently not
observed by the veterinarian and often present with no other
clinical abnormality.   Epileptic seizures in dogs may be
controlled by a variety of drugs, the most common of which being
the administration of oral phenobarbitone alone or in combination
with other anticonvulsant drugs (*Shell,1984*).

The control of epileptic seizures is not as well described as is
the case in man. The dose of phenobarbitone in dogs as quoted in
the literature offers dose ranges as variable as 2 to 20 mg kg^{-1}
which may be divided into twice daily or even three times daily
dosing or given "to effect" (*Oliver,1980; Shell,1984; Parker AJ,
1987 University of Illinois, Notes prepared for small animal
neurology seminar*).   As phenobarbitone in man requires only once
daily administration because of its long half life (50-120
hours), it would therefore appear that, in dogs, the clearance of
phenobarbitone is more rapid than in man thereby necessitating
more frequent dosing. *Parker* is of the opinion that the meta-
bolism of antiepileptic drugs in dogs may be up to ten times more
rapid the human rate.   It is generally regarded by veterinarians
that only 60 to 70% of epileptic dogs are medically controllable
and in some breeds where epilepsy is considered to be hereditary,
this control may drop as low as 25% (*Parker, 1987*).

The purpose of the study was to determine the pharmacokinetics of
orally administered phenobarbitone to clinically healthy dogs
over 3 weeks on daily dosing.   The effect of food on the absorp-
tion of the drug was also investigated.

3.2 MATERIALS AND METHODS

3.2.1 Model System

Ten clinically healthy adult dogs were selected (Table 1). Ideally, for standardization, it was preferred that they should have been of the same breed, mass and sex. In this study, it was not possible to achieve this, and the dogs selected were of mixed breeds and sex (eight males and two females). All were short haired and without excess body fat, and of a tractable disposition.

The dogs were kenneled separately in standard kennels with outside runs. Adequate shade, warmth and shelter were provided. Routine daily kennel management was applied in accordance with regulated practice. Diurnal changes in the environment were recorded by means of a max/min thermometer (see Table 2 in Appendix A). The highest temperature recorded was 33 degrees Celsius and the lowest 18 degrees Celsius.

Prior to the drug study, all the test animals were evaluated for suitability and clinical soundness. This involved recording the mass of each animal, a thorough physical examination, and the collection of venous blood for haematological and biochemical screening (full blood count, plasma(P)-glucose, serum(S)-urea, serum(S)-creatinine, alanine transaminase(ALT), serum(S)-bilirubin, serum alkaline phosphatase(ALP), total serum proteins(S-TSP), serum(S)-albumin and serum(S)-globulins). On conclusion of the drug trial, these same blood parameters were again measured to determine the clinical soundness of the animals and the adverse effect,if any, of the drug on any organ system. Animals showing any deviation from the accepted norm were excluded from the test group (see Table 3 & 4 in Appendix A). The selected animals were inoculated (Rabguard TC, SmithKline Beecham A.H. and Vanguard 5/L, SmithKline A.H.) and dewormed (Vitaminthe, Kruger-Med) in accordance with standard veterinary preventative medicine and quarantined individually in kennels for a period of two weeks prior to the study. For the duration of the drug trial

TABLE 1:   CHARACTERISTICS OF THE DOGS (n=10) USED IN THE PHENOBARBITONE
PHARMACOKINETIC STUDY

N°.	BREED	SEX	AGE (yrs)	MASS (kg)	CONDITION
1	Lab. X G.S.D.	M	4	24	lean
2	Mixed	M	9	17	lean
3	Mixed	M	1	15	lean
4	Fox Terrier X	M	2	24	lean
5	Bull Terrier	M	2	23	well muscled
6	Mastiff X	M	7	23	lean
7	Bull Mastiff X	M	8	28	lean
8	Lab. X Bull Terrier	F	1	19	well muscled
9	G.S.D.	F	2	22	lean
10	Doberman	M	8	27	lean

M = Male; F = Female; X = Mixed breed.
G.S.D. = German Shepherd Dog
Lab. = Labrador

the animals were provided with water *ad lib.* and were fed dry commercial pelleted dog food at 15h00 daily. Following the quarantine period, the dogs were again clinically examined for soundness and their mass recorded.

3.2.2 Experimental procedure

Drug administration

Sodium phenobarbitone (PHB) was administered orally in tablet form (Lethyl, Lennon Lab., 30 mg per tablet) at a dosage rate of 5 mg.kg^{-1} of body mass per day given as a single dose. Where necessary, the tablets were scored and broken to provide as accurate a dose as possible. It was administered with 20 mls of tap water to all dogs on an empty stomach at 08h00 each day until the last day of the trial. In the case of Dog 7, however, the above method of administration could not be achieved due to aggression, but the dog readily swallowed the tablets in no more than a teaspoonful of minced meat.

On Day 24 the normal daily food intake was given directly after dosing in order to evaluate the effects of food on drug absorption.

Blood collection and sampling intervals

On the sampling days where multiple blood samples were required, to facilitate the collection of blood, and to reduce the discomfort and stress and possible infection from multiple needle punctures, an intravenous catheter (Bard I-Cath, 17g) was inserted in the jugular vein of each test animal. Prior to insertion of the catheter, the skin was aseptically prepared and a small bleb of local anaesthetic (Procaine) was injected subcutaneously to reduce the discomfort on insertion of the catheter. A small amount of topical antiseptic ointment (Providine-iodine) was applied to the skin surrounding the catheter. The catheter was secured by means of a nonelastic bandage and covered with adhesive plaster. Once the catheter was in position, blood could

then be comfortably and easily withdrawn into a sterile dry 3 ml syringe and deposited immediately into a capped test tube without anticoagulant (Venoject-plain). The blood was then stored under refrigeration (± 4°C) until centrifugation approximately 6 to 8 hours later. After a blood sample had been withdrawn, the catheter was flushed with heparinized physiological saline in order to prevent blood from clotting in the catheter between sampling. The catheter was then plugged with the cap provided. This technique of blood collection was used on Day 1 (the first day of drug dosing), Day 22 (empty stomach) and Day 24 (with food), when serial venous blood samples (n=9) were withdrawn over a 24 hour period following drug administration (i.e. at 0.0; 0.5; 1.0; 2.0; 4.0; 6.0; 8.0; 12.0; and 24.0 hours after dosing).

On Day 7, 14, and 21, one blood sample was withdrawn from the cephalic vein just prior to dosing (i.e. trough-level).

The volume of blood collected at any one time did not exceed 3 mls. The collected blood was allowed to clot for approximately one hour, and then centrifuged at 2500 r.p.m. for 5 min. at 20 degrees Celsius. On separation, the serum was aspirated by means of disposable plastic pipettes and placed into pre-labelled containers (see Table 5 in Appendix A) and stored at -20 °C until assayed for PHB concentration.

3.2.3 Clinical observations and side effects

The animals were monitored daily for poor health or side effects and in particular polyphagia, polydipsia, polyuria, sedation, ataxia, and hyperkinesis which have been reported in the literature(Oliver,1980; Shell,1984). These were recorded on a data collection form (see Figs. 2 and 2A-2J in Appendix A). The animals mass was recorded weekly for signs of weight gain or loss.

3.2.4 Drug serum concentration assay

The concentration of phenobarbitone in the serum samples was determined using the TDx System which uses the fluorescence polarization immunoassay (FPIA) technology and is described in Chapter Two (2.2.8).

3.2.5 Pharmacokinetic calculations

The serum concentration time pairs for each dog were fitted for single dose, steady state without food, and steady state with food using nonlinear least squares regression (*STATIS 2, Version 2.1., 1987*). A one and two compartment open model were compared for goodness of fit by the residual sum of squares and the Akaike's Information Criterion (AIC) (*Yamaoka and Nagagawa, 1978*). Estimation of the absorption rate constant (K_A) and elimination rate constant (K_E) could then be obtained.

The first-order elimination constant (K_E) was used to calculate the biological half-life using equation (16):

$$t_{\frac{1}{2}} = 0.693/K_E \qquad \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots(16)$$

The area under the curve (AUC) was calculated by the linear trapezoidal rule. The total body clearance at steady state can be calculated in a model independent fashion by using equation (11):

$$Cl = f \times DOSE/AUC \ (l.hr^{-1}.kg^{-1}) \qquad \dots\dots\dots\dots(11)$$

Since phenobarbitone was not administered intravenously it is not possible in this study to estimate the absolute bioavailability (f) of oral phenobarbitone. Clearance in this situation is therefore more correctly termed oral clearance since it includes the oral bioavailability factor (Cl/f). In humans and dogs bioavailability has been shown to be essentially complete (80 - 100%) (*Rust and Dodson, 1989; Levy et al, 1986; Pedersoli et al, 1984*). Oral clearance (Cl/f) will therefore closely approximate total body clearance (Cl).

3.2.6 Statistical evaluation

Analysis of variance was used in order to test variation between subjects and also between treatments for half-life and the AUC (P < 0.05 taken to be significant).

3.3 RESULTS

3.3.1 Drug absorption

The serum concentration time pairs for each dog were fitted for single dose (Day 1), steady state without food (Day 22), and steady state with food (Day 24), using nonlinear regression (STATIS)(Figs. 3A-3J in Appendix B). A one compartment open model was adequate to describe the data. Calculated pharmacokinetic values indicating the absorption, accumulation and excretion of PHB on Days 1, 22 and 24 are presented (Tables 6 & 7 and Fig.4). On the first day of dosing (Day 1), there was a fairly rapid absorption of PHB with average peak serum concentrations of 28.03 ± 2.84 μmol.l^{-1} occurring two hours after dosing (Fig.4 and Appendix B). Absorption rate constants (Tables 6 & 7) showed a large variation (3.0085 ± 2.7085 hr^{-1} and 0.9068 ± 0.7287 hr^{-1} for Day 1 and Day 22 respectively).

Serum levels measured on Day 22 showed a trough concentration of 52.96 ± 8.40 μmol.l^{-1}. Following drug administration, there was again a fairly rapid absorption with an average peak concentration of 80.52 ± 6.63 μmol.l^{-1} attained within 2 - 4 hours (Figs. 4 & 5 and Appendix B). Serum levels measured on Day 24 showed a similar pharmacokinetic pattern as observed on Day 22, except that the average peak concentration was slightly lower although the trough level was similar (Figs. 4 & 5 and Appendix B).

TABLE 7: PHENOBARBITONE PHARMACOKINETICS IN DOGS GIVEN ONCE DAILY ORAL DOSING

	$t\frac{1}{2}$ hr		K_A hr^{-1}		K_E hr^{-1}		AUC $\mu mol.hr.l^{-1}$		Cl/F $hr^{-1}.kg^{-1}$	$\triangle C_P$ $\mu mol.l^{-1}$
	DAY 1	DAY 22	DAY 1	DAY 22	DAY 1	DAY 22	DAY 22	DAY 24	DAY 22	DAY 22
MEAN	46.3	29.3	3.0085	0.9068	0.0156	0.0242	1656.17	1493.06	0.0133	27.60
SD	11.31	4.6	2.7085	0.7287	0.0032	0.0036	186.45	205.4	0.0016	7.49
CV %	24.4	15.7	90.02	80.35	20.5	14.9	11.3	13.8	12.0	27.18

$\triangle C_P$ = average peak - trough serum concentrations

Fig. 4: Average serum phenobarbitone concentrations in dogs
for the initial day of dosing (Day 1), 22 days after
dosing (Day 22) and after the intake of food on Day
24

CONCENTRATION (µg/ml)

TIME (hours)

DAY 0   DAY 22 (FASTING)   DAY 24 (FOOD)
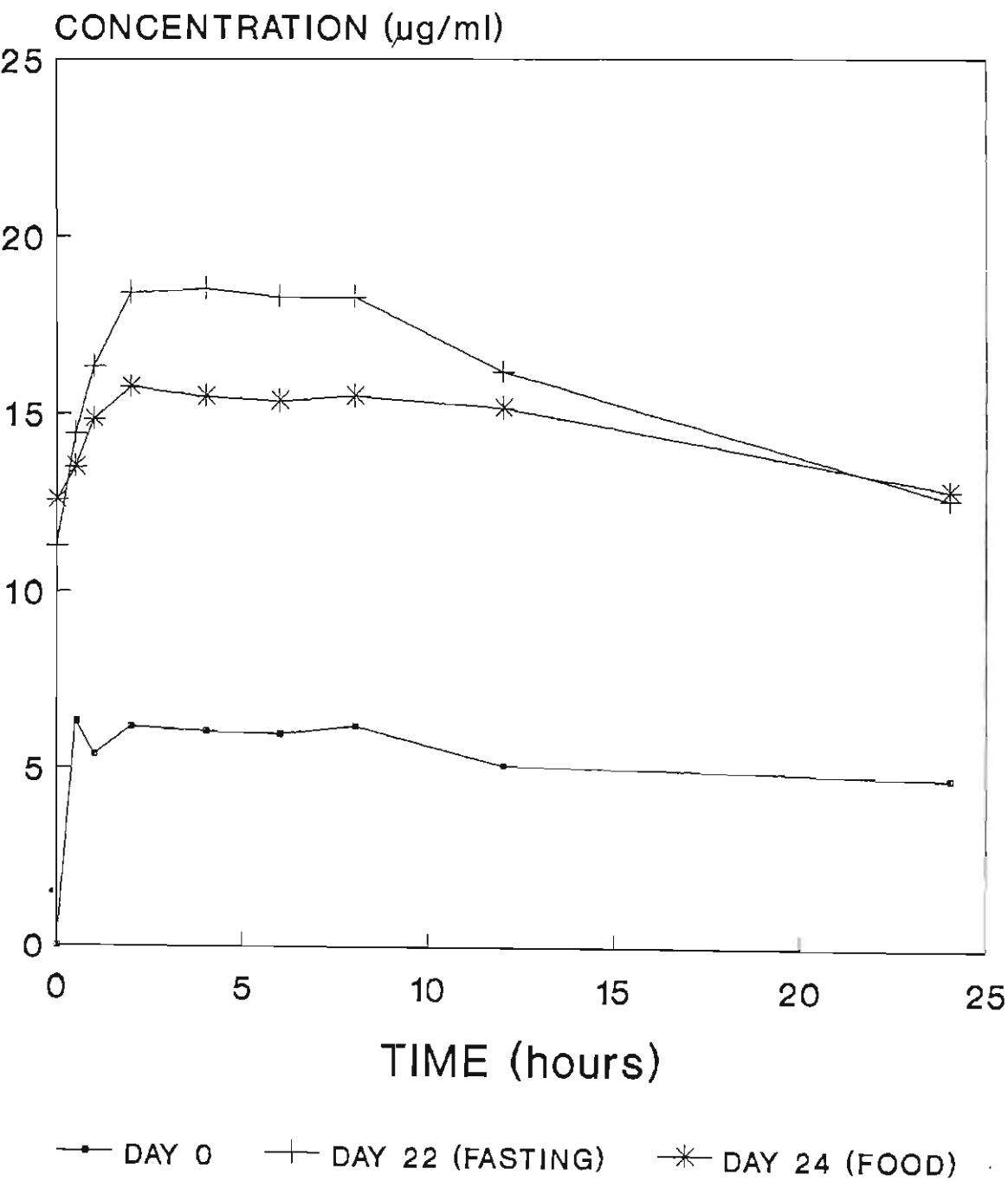
Fig. 5: Average trough serum phenobarbitone concentrations in dogs (n=10) over 24 days
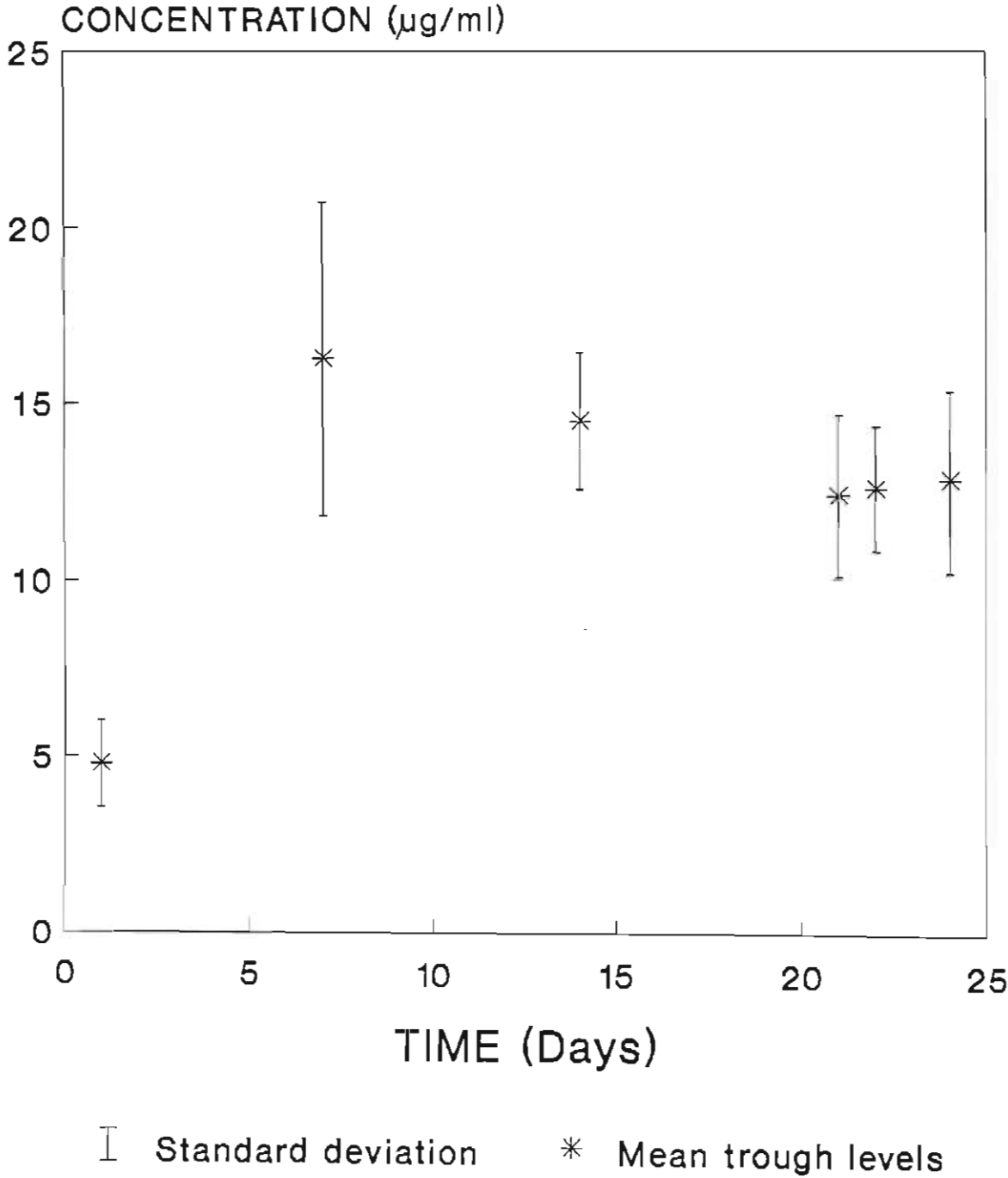
3.3.2 Trough levels

Following Day 1 there was a slow accumulation in the PHB plasma concentration followed by a decline to stable trough levels of 52.96 ± 8.18 μmol.l^{-1} which occurred approximately 22 days from commencement of initial dosing (Fig.5 and Appendix B).

3.3.3 Half-life determination

The elimination half-life (t½) was calculated for each dog on Day 1 and Day 22 from elimination constant (K_E) and using equation 16 (Chapter 2.6.5). The results are given in Table 7 & 8. The mean elimination t½ for Day 1 was 46.3 ± 11.3 hours and for Day 22 was 29.3 ± 4.6 hours. Dog 5, a bull terrier, could not be used in this comparison as there was doubt as to the dose he received on Day 1. The results were compared by two-way analysis of variance which showed that there was no significant difference between subjects (P < 0.5), but there was a significant difference between Day 1 and Day 22 (P < 0.0066).

3.3.4 Area under the curve

AUC is a measure of the extent of drug absorption if clearance is constant and is calculated by the linear trapezoidal rule. The AUC values for the dogs were compared for Day 22 and Day 24 to examine the effect of food on the extent of PHB absorption (Table 9). A difference of approximately 10% in the AUC was found when Day 22 and Day 24 were compared (P = 0.0496), suggesting that less drug had been absorbed when given with food (Table 7 & 9).

3.3.5 Clearance

The clearance was calculated from the AUC and using equation 11. The mean clearance value for the sample group on Day 22 was 0.0133 ± 0.0016 l.hr^{-1}.kg^{-1} (Table 7).

3.3.6 Clinical observations

The majority of dogs showed mild to moderate side effects to the drug. The side effects of polyphagia, polydipsia and sedation were initially observed from Day 2 in all test animals. In addition three dogs showed hindquarter ataxia. All these side effects, were, however, of short duration and were no longer evident by Day 10 (see Figs. 2A-2J in Appendix A).

3.3.7 Clinical pathology

No significant changes were recorded in the haematological or biochemical analysis of the blood, with all parameters measured and compared remaining within normal physiological limits (see Table 3 & 4 in Appendix A).

3.4 DISCUSSION

Phenobarbitone distributes into most body tissues fairly rapidly permitting the plasma pharmacokinetics to be described adequately by a one compartment open model. Absorption rate constants showed an 80 - 90 % variation but were within the ranges found in other studies (*Pedersoli et al,1987; Ravis et al,1984*). The time required to reach peak levels after oral PHB was between two to four hours on Day 1, Day 22, and Day 24. It would therefore appear that the administration of food together with the tablets has little effect on rate of absorption.

However, if one compares the extent of absorption using the AUC values, there is a statistically significant difference between administration with and without food. The lower AUC on Day 24 is unlikely to be the result of further enzyme induction and increased clearance as trough levels on Day 21, 22 and 24 are similar. Thus, it would appear that food, together with the tablets, reduces the extent of PHB absorption by approximately 10%. Continuous dosing together with food therefore, would be ex-

pected to produce serum level profiles on average 10 % lower than if dosing were given on an empty stomach. In general a difference of less than 20 % is considered to be bioequivalent (*United States Food and Drug Administration,1977*). Therefore it is unlikely that feeding and simultaneously dosing the animals will have a clinically significant effect on the control of epilepsy.

In this particular study the mean elimination half-life of PHB declined from 46.3 ± 11.6 hours calculated for Day 1 to 29.3 ± 4.6 hours after three weeks of daily oral dosing. It is assumed that this decline is due to microsomal enzyme induction (*Ravis et al.,1984; Saunders and Penry,1981*). The initial rise in trough levels from Days 1 to 7 and then the decline and stabilization by 3 weeks supports this assumption. Both the initial and final half-lives in this study were considerably shorter than those reported in a single dose study by *Pedersoli et al.(1987)* where the $t_{\frac{1}{2}}$ for oral dosing was 72.3 ± 15.5 hours. The half-life of 53.0 ± 15 hours reported in another study (*Ravis et al.,1984*) after 5 days of oral dosing is comparable to the initial half-life reported in this study. Five days is probably not long enough for enzyme induction to reach a maximum. Another possible reason for the relatively short half-lives found in the present study could be the relatively short sampling interval of only 24 hours after dosing whereby distribution may still be operative. The other studies sampled over 288 and 131 hours respectively (*Pedersoli et al.,1987; Ravis et al.,1984*). Considerable inter-subject variation in the half-life has also been reported (*Pedersoli et al.,1987; Ravis et al.,1984*) which was not as apparent in this study (coefficient of variation for Day 22 = 15.7 %) possibly because of our larger numbers.

Steady state is assumed to be close to five times the elimination $t_{\frac{1}{2}}$. Using this value to estimate the time necessary to reach steady state in this study would have given a period of nine to six days utilizing our calculated $t_{\frac{1}{2}}$ for Day 1 and Day 22 respectively. *Ravis et al.(1984)* in their study assumed steady state would be reached at approximately eleven days, However, in this trough levels only stabilized between 14 - 21 days, as indicated

in Fig.4. It is therefore apparent that approximately three weeks of daily dosing is required before enzyme induction is complete resulting in stable clearance and steady state levels.

Clearance of phenobarbitone in dogs was calculated as 0.0133 ± 0.0016 $l.hr^{-1}.kg^{-1}$ which is more than four times that for humans (0.0032 $l.hr^{-1}.kg^{-1}$) (*Gal, 1986*). Using oral clearance, the following equation can be used to calculate a dose for a desired concentration (C_P) in dogs. [Note 0.232 is the conversion factor from μmol to mg i.e. 1 μmol = 0.232 mg phenobarbitone]

$$Dose = Cl/f \times C_P \times Dosing\ Interval \times 0.232 \dots\dots\dots\dots (17)$$

Using the above equation, therefore, indicates that a dose of 5 $mg.kg^{-1}.24\ hrs^{-1}$ should maintain an average serum concentration in the region of 65 $\mu mol.l^{-1}$ which is within the therapeutic range determined in man (40 - 110 $\mu mol.l^{-1}$) (*Saunders and Penry,1981; Gal,1986; Shell,1984*). Chapter four will examine the practical application of predicting the serum concentration for a desired therapeutic effect in dogs.

The relatively small fluctuation in peak and trough serum concentrations at steady state (5.44 ± 1.22 $\mu g.ml^{-1}$ as shown in Table 10) found in this study would support once daily dosing. This would then enhance owner compliance thus hopefully achieving better epileptic control.

3.5 CONCLUSIONS

From the results of this study, it can be concluded that:-

1. An oral dose of 5 mg.kg^{-1} should give average serum PHB concentrations of 65 μmol.l^{-1} in adult dogs.

2. Once daily dosing may be adequate since relatively small peak-trough fluctuations were observed (5.44 ± 1.22).

3. The dose may be given with food although the extent of absorption is approximately 10 % less than when given on an empty stomach. This is probably of little clinical significance especially if serum levels are monitored.

4. Side effects of polyphagia, polydipsia, sedation and ataxia are commonly observed in the first two to nine days but disappear thereafter. This is probably due to tolerance to the effects.

5. After initiation of therapy, dosing should continue unchanged for three weeks (steady state) before measuring serum concentrations and adjusting the dose accordingly.

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Chapter Four

The assessment of therapeutic drug monitoring as an aid in the treatment of clinically diagnosed epileptic dogs.

4.1 INTRODUCTION

The therapeutic range of serum concentrations of phenobarbitone has been reasonably well defined in man (*Welty et al,1983; Shell,1984; Painter,1989*). The term "therapeutic range" describes the relationship between the desired pharmacologic activity and the drug concentration in the plasma or serum (*Welty et al,1983*). Therapeutic ranges, determined from clinical trials, are not rigidly defined but, rather, represent average values.

Patients vary in their response to antiepileptic drugs. Some benefit with serum concentrations that are low, while others require concentrations above the therapeutic range. Tolerance to phenobarbitone may also develop, whereby the response is diminished with time for a given concentration (*Rowland and Tozer,1989*). There may also be differences in the absorption and metabolism of the drug. Thus different patients may require highly varying dosages of the same drug to achieve and maintain serum concentrations in the therapeutic range. Use of standard dosage regimens may, therefore, often result in unpredictable serum concentrations and response (*Welty et al,1983*).

For man, a considerable body of information describing the variable absorption and elimination pattern of phenobarbitone is available (See Chapters 1 & 2). Due to to the variability of the kinetics of the drug in the individual and the seizure pattern irregularity, antiepileptic drug therapy should be tailored to the individual patient (*Welty et al,1983; Kutt and Kiffin Penry,1974; Painter,1989*).

Monitoring the blood levels of antiepileptic drugs has increased the efficiency and safety of drug therapy in epilepsy. It can facilitate individualization of dosage regimen, reveal irregular drug intake or non-compliance, and can identify the responsible agent in intoxicated patients on multiple drug therapy (*Kutt and Kiffin Penry,1974*). It has been noted, however, by some authors that before the development of methods to determine anticonvulsant drug blood levels, many patients were managed successfully on clinical grounds alone (*Livingstone et al,1975*).

Historically, much of the information upon which the veterinarian has relied was derived from human medicine and the veterinary application of a considerable portion of this is subject to serious question. For example, *Shell(1984)* states that blood levels are clinically effective 12 to 24 hours after oral administration. However, in the preceding study on healthy dogs (Chapter 3), the peak serum concentration levels after 24 hrs were approximately 35% lower than that observed three weeks later at steady state. It may be possible to achieve immediate effective control by giving a large enough loading dose, but this tends to cause pronouced sedation which is often as alarming to the client as are the seizures. The same author also states that effective blood levels in the dog are similar to that of man, with toxicity developing with blood levels of phenobarbitone greater than 40 $\mu g.ml^{-1}$ (*Shell,1984*). However, clinical trials have provided conflicting evidence.

In a study on dogs given phenobarbitone by *Farnbach (1984)*, a sixfold variation was observed between dosage and achieved serum concentration. In this study, 20 of 42 dogs having seizures and receiving phenobarbitone were controlled at serum concentrations ranging from 14.3 to 43.1 $\mu g.ml^{-1}$. This author concluded that there was a need for serum concentration monitoring as an adjunct to any drug protocol in seizure control since effectiveness was correlated far better with serum concentrations than with oral dosage.

In a recent study describing a consultative service to veterinary surgeons to treat epileptic dogs, it was concluded that, whilst there was little confirmation of the diagnosis of idiopathic epilepsy in most of the cases involved and no direct measurement of hepatic function in 16 of the cases, their results strengthened the case for the use of therapeutic drug monitoring in canine epilepsy (*Morton and Honhold,1988*). Their results obtained in the first two years of drug monitoring suggest that this service can be valuable in the improved control of seizures.

The purpose of this study was to investigate the practical application of therapeutic monitoring as an aid in the treatment of canine epilepsy.

4.2   MATERIALS AND METHODS

4.2.1   Selection and treatment of patients

Patients were selected from epileptic dogs presented for treatment at the Durban Veterinary Clinic over a 24 month period. Other veterinary practices in the Durban and surrounding area were invited to participate in this study and an appeal for uncontrolled epileptic dogs was placed in the local newspaper. Despite frequent contact with the other practices, only one patient from another practice was referred for serum monitoring, and only one person responded to the newspaper appeal.

All presenting patients were given a thorough clinical examination which included screening for internal parasites, and the determination of serum electrolyte and enzyme levels to evaluate the presence of organ disease or failure which may be the aetiological cause of the seizures. Two dogs were eliminated on the basis of uremia due to renal failure, and one dog had evidence of hepatic cirrhosis.

Those dogs having seizures from no other discernible cause were diagnosed as suffering from idiopathic epilepsy and, with the permission of the client, were selected for serum monitoring. Details of the patient's breed, age, mass, sex, seizure frequency at the time of sample, current drug regime, time and method of drug administration were recorded (see Fig. 6 in Appendix C). The owners of these dogs were given an "Epileptic Diary" to record the seizure frequency and interval, daily medication times, any change in dose, and any other observation or comment. This diary was used to evaluate the dogs progress and also appeared to increase the owners enthusiasm in assisting in the project. Concurrent records were kept by the investigator, noting seizure frequency, medication, any clinical evaluations for drug toxicity, anecdotal comments from the owner, and reasons for any change in medication. On return of the patients for blood collection to determine the drug serum concentration, the clients were questioned as to the seizure frequency and further verification was by phone.

Dogs diagnosed as suffering from idiopathic epilepsy, and who had not received any previous medication, were placed on a phenobarbitone dosage rate of approximately 5 mg.kg^{-1} body mass given once daily. This dosage level was shown to be able to, with reasonable accuracy, achieve a drug serum concentration which was in the therapeutic range for man (refer Chapter 3). It was stressed to the owners to administer the daily dose at the same time each day and to record whether it was given with or without food. All observations were to be recorded in the diary. Owners were cautioned as to the probability of the appearance of side effects and the expected duration thereof. All patients were requested to return in 3 weeks for evaluation of efficacy of treatment and a blood sample was drawn to determine the serum drug level at the current steady state in order to provide a base line. This was useful in judging compliance or for estimating the extent of a future change in dosage, if this became necessary. Dogs who had recorded no further seizures on commencement of therapy were maintained on the same dose. Those dogs where seizures were still present, were placed on a higher dose based

on the serum concentration of the drug and again requested to
return after 3 weeks for re-evaluation. Blood levels were deter-
mined after each change in dosage.

Dogs that were currently on phenobarbitone therapy for idiopathic
epilepsy and still uncontrolled were initially monitored to
assess the serum concentration of the drug and then the dosage
was increased. They too returned after 3 weeks in order to assess
efficacy of treatment and to determine the serum concentration.

A total of 14 dogs were evaluated comprising a variety of breeds,
mass, and of both sex (Table 11). From these dogs , 20 serum
samples were analyzed to determine the drug serum concentration.

The importance of compliance with the recommendations was
stressed and, on the basis of the results of the drug serum con-
centrations obtained, the drug regimens appear to have been com-
plied with in all cases.

4.2.2 Blood sampling

Blood samples were taken aseptically from the cephalic vein and
the serum separated by centrifugation. The serum was then stored
at -20°C until analyzed.

4.2.3 Drug assay

All serum samples were assayed by the Drug Studies Unit of the
University of Durban Westville. Serum concentrations of phenobar-
bitone were determined by the use of fluorescence polarization
immunoassay (TDx System, Abbott Laboratories described in Chapter
2.2.8)

4.2.4 Data analysis

Logistic Regression

A common form of representing concentration-response is a plot of the intensity of response against the logarithm of the drug concentration. In the treatment of epilepsy, a graded response with concentration may occur but usually the response is all or nothing. Logistic regression is used to assess the frequency of patients reponding or not responding to a stimulus, which, in this case is phenobarbitone serum concentration.

Each patients clinical state was categorized by the binary dependent variable of "responsive" or "nonresponsive" on the basis of seizure control. Phenobarbitone serum concentrations *versus* response/non-response were analyzed by logistic regression to determine the Cp_{50} of phenobarbitone for the control of seizures. The Cp_{50} (analogous to ED_{50}) is the phenobarbitone serum concentration at which there is a 50% probability of suppressing seizures (*Ausems et al.,1988*).

Logistic regression is a method for analyzing proportions analogous to multiple regression for continuous variables, where proportion is the outcome variable which in this case is controlled or not controlled. It allows both the inclusion of continuous explanatory variables and the assessment of interaction between the variables(*Kirkwood,*). Logistic regression is so called because it investigates the linear dependence of the logistic transformation of the outcome variable on several explanatory variables, where the logistic transformation, or logit for short, is defined as:

$$\text{Logit}(p) = \log_e \left[\frac{\text{proportion}}{1 - \text{proportion}} \right] \quad \ldots\ldots\ldots\ldots (17)$$

The model is fitted using a mathematical technique called maximum likelihood, which also takes account of the fact that the variation of a proportion has a binomial distribution. Both theoretical and empirical considerations suggest that when the dependent variable is an indicator variable, the shape of the response curve will frequently be curvilinear(*Armitage and Berry,*).

Predictive performance

Based on the pharmacokinetic values for phenobarbitone in normal healthy dogs, the population value (n = 10) for drug clearance was obtained (Chapter 3.3.5) and by rearranging Equation 12, a steady state serum concentration could be predicted. This could then be extrapolated to the group of epileptic patients. In order to assess the accuracy of predicting the serum concentration in this naive group, the proximity of the prediction to the true (observed) value is the difference between the two. The quantity that results from the prediction minus the true value is termed the prediction error (*pe*)(*Sheiner and Beal,1981; Miller and Rheeders,1989*). From this value, the bias (average predictive error) and precision (root mean squared error) of the predictions can be determined.

A two-tailed Student's *t* test is used to assess whether the mean prediction error was different from zero, indicating bias. The Null Hypothesis, H_0, states that mpe = 0 i.e. the mean prediction error does not significantly deviate from zero. Rejection of H_0 at the 95% confidence level using an unpaired t-test indicates the presence of significant bias (*Fotheringham,1982*).

4.3 RESULTS

4.3.1 Therapeutic drug monitoring

The results of the serum concentrations attained and the clinical state achieved is presented in Table 11. Of the 14 dogs examined,

10 dogs were controlled after primary dosing, 1 dog was controlled after adjusting the dose twice, and 3 dogs remained uncontrolled despite therapeutic drug monitoring and the consecutive increase in dosage. The lowest drug serum concentration at which control was achieved was 12.64 μg.ml^{-1} and the highest serum concentration where control was not achieved was 48.64 μg.ml^{-1}. There appeared to be a great individual variation in the effective concentration.

4.3.2 Statistical analysis by logistic regression

The interaction of the binary dependent variables of responsive and nonresponsive clinical states were analyzed by logistic regression for concentration, breed of dog, age, and sex. The following results were obtained:

Table 12: Maximum likelihood analysis of variance for 4 variables

Variable	Chi-Square	Probability
Conc	0.55	0.4565
Breed	0.79	0.3728
Age	0.03	0.8725
Sex	0.39	0.5331

A probability factor of $P < 0.05$ is taken to be statistically significant. It can therefore, be seen that no significance was observed between concentration and effect, breed and effect, sex and effect, and age and effect. The dose divided by the mass was also analyzed and P was found to be 0.2085 ($P = 0.2085$), which is also not statistically significant.

Population profiles of the variables dose and concentration were examined which yielded probabilities of 0.2555 and 0.3001 respectively and are, therefore, also not statistically significant.

4.3.3 Predictive performance

In the assessment of predictive performance, the mean prediction error was positively biased but this was not significantly different or deviant from zero (-5.0937 < 2.47 > 5.2577, 95% CI). The predicted concentration was within 5 $\mu g.ml^{-1}$ of the observed concentration with 95% confidence (Table 13). This means that on average the concentration predicted using population pharmacokinetic parameters of phenobarbitone determined in healthy mixed breed dogs was only slightly higher than the concentration measured in the epileptic dogs.

In the assessment of absolute precision using mse, the square root of mse was 10 $\mu g.ml^{-1}$ indicating that, at 95% confidence limits, the precision of prediction would be within 10 $\mu g.ml^{-1}$.

4.4 DISCUSSION

In a study done by *Farnbach(1984)*, it was postulated that serum concentrations should be maintained between 15 and 45 $\mu g.ml^{-1}$ to achieve clinical efficacy in 50% to 70% of epileptic dogs. This study tends to confirm this statement. The therapeutic range of phenobarbitone, as shown in this study and in others, is fairly wide with clinical control occurring at varying concentrations. Some dogs were controlled at relatively low serum concentrations, whilst other dogs were not controlled at high and seemingly toxic levels (Table 11) (*Morton and Honhold, 1988*). Some authors support the monitoring of serum drug concentrations as an essential aid in the treatment of idiopathic epilepsy on the basis that there appeared to be a sixfold variation between dosage and achieved serum concentration. This implied that effectiveness is

correlated far better with serum concentrations than with oral dosage (*Morton and Honhold,1988; Cunningham et al,1983; Farnbach,1984*). Our results, and in particular the results of the predictive performance , does not support the above variation which is probably due to noncompliance. Although the sample size in our study was small (n = 14), there would appear to be poor relationship between effect and serum concentration and no EC_{50} could be calculated. However, the predictive performance indicates that the prediction error is statistically unbiased and that serum concentrations at steady state can be achieved within 5 $\mu g.ml^{-1}$ using population pharmacokinetics of clearance. This, combined with clinical assessment of efficacy of treatment, would appear to be a more practical approach to achieving control of epilepsy in the dog.

Morton and Honhold (1988) also observed a large difference in the dosage required to achieve therapeutic levels. In this study, this large difference was not observed. However, it was noted that where a high dosage was given in an attempt to achieve control, the predictive value was in most cases considerably different to the observed value (Table 11).

Drug serum monitoring does, however, have its place in the assessment of side-effects, where seizures recur in patients whose attacks were previously well controlled, and, also in the determination of noncompliance (*Kutt and Kiffin Penry,1974*).

As was mentioned previously, although a relatively common ailment of dogs, very few epileptic cases were forthcoming despite a wide local appeal. Those cases that were forthcoming were often epileptics that were not adequately controlled despite incremental doses of phenobarbitone administered in the first place. There is, therefore, a certain degree of bias in the results obtained and presented. The relatively small sample size (n = 20) was insufficient to statistically differentiate between those dogs that would have readily responded to therapy at average doses, and those that would be non-responsive. It was determined by a statistician (Professor Clark, University of Natal, personal

communication,1990), that, in the order of 100 patients would be
required to provide results that would have sufficient power
(small probability of concluding no relationship when there in
fact is one).

4.5   CONCLUSIONS

Improper prescription by the veterinarian and the improper ad-
ministration of phenobarbitone by the owners, are the most common
causes of anticonvulsant failure. A true anticonvulsant failure
is failure to control seizures when the drug has been correctly
prescribed and correctly administered and the dose slowly in-
creased until toxic signs are seen. Tolerance to phenobarbitone
can occur and the dosage then needs to be increased. In this
case, a serum concentration would be of interest. Drug interac-
tions with concomitant drug therapy should also be examined where
tolerance is suspected.

This study does not, therefore, support that therapeutic drug
monitoring (TDM) should be done routinely in the treatment of
canine epilepsy. However, as discussed previously, more patients
would be required before this statement can be completely jus-
tified. Therapeutic control of epilepsy should, therefore, be
based on the frequency of seizures, the client concern for the
degree of control, and the time to onset of control. Predictive
serum concentrations can be made with reasonable confidence, with
follow-up examinations after 3 weeks. In the event of uncon-
trolled seizures, TDM may be advantageous in the control of dogs
not conforming to normal population pharmacokinetics, and in the
identification of noncompliance.

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APPENDIX A

TABLE   2:   MINIMUM AND MAXIMUM ENVIRONMENTAL TEMPERATURES
              RECORDED OVER THE TRIAL PERIOD

DAY	MIN °C	MAX °C
0	21	30
1	22	33
2	21	30
3	18	25
4	18	30
5	17	28
6	18	31
7	18	23
8	18	30
9	18	31
10	18	30
11	18	31
12	19	31
13	20	28
14	19	28
15	20	33
16	20	30
17	20	31
18	21	30
19	19	26
20	19	22
21	18	22
22	19	28
23	20	32
24	19	27

TABLE 3: HAEMATOLOGY VALUES FOR THE EXPERIMENTAL DOGS RECORDED PRE - AND POST-TRIAL (POST TRIAL VALUE IN BOLD)

PARAMETER	UNITS	1	2	3	4	5	6	7	8	9	10
WBC	9	13.7	22.0	18.6	11.6	18.0	8.6	7.1	14.8	8.9	9.5
	x 10.1^{-1}	**11.5**	**22.6**	**15.2**	**26.1**	**21.6**	**14.0**	**4.6**	**15.7**	**13.5**	**11.7**
RBC	12	6.84	5.26	6.78	6.14	7.46	5.96	6.57	6.31	7.12	6.67
	x 10.1^{-1}	**6.02**	**4.76**	**7.66**	**6.28**	**6.62**	**5.53**	**5.93**	**4.44**	**6.48**	**4.83**
Hgb	g.dl^{-1}	16.9	10.6	15.5	13.0	18.1	12.8	13.8	15.2	17.1	15.1
		15.1	**10.1**	**17.3**	**13.6**	**16.0**	**12.6**	**13.6**	**11.6**	**16.7**	**11.6**
Hct	1.1^{-1}	0.480	0.386	0.452	0.384	0.517	0.383	0.398	0.431	0.483	0.429
		0.413	**0.327**	**0.50**	**0.383**	**0.458**	**0.350**	**0.372**	**0.316**	**0.451**	**0.314**
MCV	fl	70.1	58.1	66.6	62.4	69.3	64.3	60.5	68.2	67.7	64.3
		68.6	**59.9**	**65.1**	**60.9**	**69.1**	**63.2**	**62.6**	**71.0**	**69.6**	**64.9**
MCH	-	24.7	20.1	22.9	21.2	24.3	21.5	21.0	24.1	24.0	22.7
		25.0	**21.1**	**22.6**	**21.6**	**24.2**	**22.7**	**22.9**	**26.1**	**25.7**	**24.1**
MCHC	g.dl^{-1}	35.2	34.6	34.4	34.0	35.0	33.4	34.7	35.3	35.4	35.3
		36.5	**35.3**	**34.7**	**35.4**	**35.0**	**35.9**	**36.6**	**36.7**	**36.9**	**37.0**
RDW	%	13.1	17.3	13.9	16.7	13.1	15.9	20.8	13.8	14.1	10.9
		12.0	**22.4**	**13.5**	**14.8**	**13.6**	**15.7**	**19.6**	**13.9**	**13.7**	**11.1**
PLT	9	209.0	229.0	238.0	clumped	clumped	clumped	105.0	279.0	239.0	276.0
	x 10.1^{-1}	**181.0**	**231.0**	**289.0**	**72.0**	**69.0**	**177.0**	**107.0**	**153.0**	**255.0**	**clumped**
PLT CRIT		0.261	0.281	0.336	-	-	-	0.125	0.337	0.333	0.279
		0.249	**0.288**	**0.388**	**0.089**	**0.090**	**0.256**	**0.161**	**0.250**	**0.393**	**6.112**
MPV	fl	12.5	12.2	14.1	7.7	13.9	-	11.9	12.1	14.0	10.1
		13.8	**12.5**	**13.4**	**14.9**	**14.3**	**14.4**	**15.0**	**16.3**	**15.4**	**15.0**
PDW		14.2	15.5	15.1	16.9	15.5	-	15.0	14.6	15.0	14.8
		14.9	**15.4**	**15.5**	**15.6**	**17.5**	**15.1**	**15.5**	**14.9**	**15.2**	**17.1**
LYMPH	%	70.0	80	-	69.0	78.0	-	82.0	81.0	78.0	71.0
		69.0	-	**80.0**	**79.0**	**75.0**	**77.0**	-	**82.0**	-	-
LYMPH	9	10.0	17.5	-	7.9	14.0	-	5.8	12.0	6.9	6.8
	x 10.1^{-1}	**7.9**	-	**25.1**	**20.5**	**16.4**	**10.8**	-	**12.9**	-	-
NEUTROPHILS	%	47	53	53	45	69	48	63	59	46	56
		46	**56**	**73**	**62**	**74**	**63**	**82**	**78**	**70**	**56**
LYMPHOCYTES	%	27	26	26	23	14	34	18	26	25	23
		18	**18**	**15**	**16**	**13**	**15**	**11**	**7**	**12**	**24**
MONOCYTES	%	7	12	6	13	13	9	10	5	5	5
		9	**10**	**8**	**4**	**3**	**13**	**6**	**3**	**6**	**14**

TABLE 4:   SERUM BIOCHEMISTRY VALUES FOR THE EXPERIMENTAL DOGS RECORDED PRE – AND POST-TRIAL (POST TRIAL VALUE IN BOLD)

PARAMETER	UNITS	1	2	3	4	5	6	7	8	9	10
UREA	mmol.l⁻¹	5.5	7.2	3.2	4.1	5.6	4.2	5.2	5.9	3.7	5.3
		5.9	**6.8**	**3.6**	**5.1**	**5.2**	**3.3**	**4.5**	**5.7**	**5.4**	**5.1**
CREATINE	umol.l⁻¹	90	81	72	68	78	102	90	90	94	96
		102	**102**	**104**	**89**	**99**	**90**	**79**	**95**	**104**	**71**
GLUCOSE	mmol.l⁻¹	–	4.0	4.1	4.2	4.4	4.5	4.0	3.9	3.7	3.8
		3.8	**4.0**	**4.5**	**4.1**	**4.3**	**4.5**	**3.8**	**4.5**	**3.9**	**4.3**
ALK PHOS	i.u	99	86	78	107	30	61	55	72	67	38
		193	**132**	**97**	**95**	**77**	**283**	**289**	**116**	**93**	**103**
TOTAL BILI.	umol.l⁻¹	1	1	1	1	1	1	1	1	5	2
		2	**1**	**3**	**3**	**7**	**1**	**2**	**5**	**2**	**1**
T.S.P.	g.l⁻¹	62	89	63	72	84	68	66	63	54	67
		72	**86**	**68**	**65**	**86**	**67**	**67**	**65**	**64**	**63**
ALBUMIN	g.l⁻¹	24	27	32	26	28	26	24	29	28	26
		24	**27**	**31**	**26**	**28**	**21**	**22**	**26**	**27**	**20**
GLOBULIN	g.l⁻¹	38	62	31	46	56	42	42	34	26	41
		48	**59**	**37**	**39**	**58**	**45**	**45**	**39**	**37**	**43**
GammaGT	i.u.	3	4	2	1	2	0	2	2	2	3
		4	**10**	**4**	**4**	**4**	**3**	**2**	**3**	**3**	**3**
AST	i.u.	16	21	42	16	21	22	15	18	15	15
		19	**24**	**29**	**19**	**22**	**22**	**28**	**27**	**29**	**31**
ALT	i.u.	37	18	38	16	18	17	28	25	34	45
		63	**15**	**33**	**16**	**20**	**63**	**46**	**18**	**48**	**50**

TABLE 5:   CANINE PHENOBARBITONE DRUG
            TRIAL - SERUM STORAGE
            TUBE CODE

SAMPLE TIME	CODE
DAY 0	
Before medication	A
1/2 hour post.	B
1 hour post.	C
2 hours post.	D
4 " "	E
6 " "	F
8 " "	G
12 " "	H
24 " "	I
DAY 7	J
DAY 14	K
DAY 21	L
DAY 22	
Before medication	M
1/2 hour post.	N
1 hour post.	O
2 hours post.	P
4 " "	Q
6 " "	R
8 " "	S
12 " "	T
24 " "	U

DAY 24

Before medication	V
1/2 hour post.	W
1 hour post.	X
2 hours post.	Y
4 " "	Z
6 " "	AA
8 " "	BB
12 " "	CC
24 " "	DD

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TABLE 5A   INDIVIDUAL PHENOBARBITONE CONCENTRATIONS ($\mu g.l^{-1}$) FOR ALL THE DOGS OVER THE SAMPLING PERIOD

	SAMPLE	DOG 1	DOG 2	DOG 3	DOG 4	DOG 5	DOG 6	DOG 7	DOG 8	DOG 9	DOG 10
Day 1											
predose	A	0.29	LOW	LOW	0.17	0.30	0.17	0.04	0.14	LOW	0.24
$\frac{1}{2}$	B	2.85	3.77	6.56	5.78	1.50	2.31	3.26	6.90	7.09	0.76
1	C	6.19	5.65	7.94	6.84	1.82	3.21	3.45	7.82	7.01	4.16
2	D	6.02	6.43	7.44	6.80	1.92	5.87	6.05	7.48	6.79	7.07
4	E	5.71	6.10	6.30	6.90	1.81	6.67	5.88	6.82	7.58	6.80
6	F	5.77	6.01	6.43	6.60	2.01	6.60	5.93	7.44	6.23	6.90
8	G	5.94	6.17	6.85	6.59	1.94	6.50	6.92	7.84	6.60	6.64
12	H	4.98	5.46	5.21	5.48	1.84	4.87	4.87	6.18	6.26	6.00
24	I	4.43	4.78	5.28	5.81	1.55	5.44	4.43	5.15	5.42	5.48
Day 7	J	19.72	16.42	16.63	15.89	19.74	16.86	20.20	4.69	14.87	17.68
Day 14	K	17.36	12.69	13.92	13.41	15.71	17.12	14.21	14.02	11.22	15.54
Day 21	L	15.51	12.61	7.98	11.42	13.32	14.99	14.09	11.51	10.11	12.89

TABLE 5A continued...

	SAMPLE	DOG 1	DOG 2	DOG 3	DOG 4	DOG 5	DOG 6	DOG 7	DOG 8	DOG 9	DOG 10
Day 22											
prehours	M	14.16	9.36	11.18	11.02	12.83	14.74	12.48	12.07	10.00	15.05
$\frac{1}{2}$	N	17.29	15.28	16.55	14.96	13.89	15.82	10.93	13.98	10.16	15.49
1	O	19.68	17.60	17.20	18.70	15.18	16.74	15.16	13.91	12.36	16.80
2	P	20.24	19.30	17.80	17.93	17.13	19.96	18.37	16.85	15.52	21.15
4	Q	21.01	17.84	17.12	17.59	19.05	20.84	18.69	18.13	16.63	20.22
6	R	20.02	17.71	16.70	17.58	19.35	20.71	19.74	15.99	15.09	20.35
8	S	20.39	17.16	15.33	19.23	18.35	19.87	19.12	18.17	15.67	19.83
12	T	19.17	15.15	13.48	15.15	15.73	19.91	16.97	15.34	13.27	17.92
24	U	14.94	11.41	10.87	10.85	13.36	15.18	13.36	12.13	10.28	13.98
Day 24											
prehours	V	14.62	11.65	13.77	9.90	13.58	15.37	13.57	11.92	9.64	12.67
$\frac{1}{2}$	W	15.54	12.48	15.27	11.06	13.77	16.51	13.17	11.66	10.27	15.27
1	X	15.43	16.51	15.46	11.45	13.69	19.55	13.38	12.64	13.00	17.42
2	Y	14.65	18.26	15.71	14.07	13.60	20.57	13.56	12.53	15.23	19.46
4	Z	14.02	16.73	16.45	15.87	11.94	17.78	12.47	13.29	15.56	19.39
6	AA	15.62	16.60	18.18	14.84	11.09	17.80	12.69	14.59	14.93	18.70
8	BB	17.87	15.51	17.68	14.80	11.15	16.37	14.12	16.28	13.77	18.17
12	CC	16.91	14.54	16.18	13.24	9.86	13.55	16.20	14.80	13.28	16.91
24	DD	15.36	11.87	17.49	11.36	8.24	13.60	12.75	12.53	10.80	14.72

FIGURE 2: CLINICAL DATA COLLECTION FORM

NUMBER OF DOG: _____

SPECIES OF DOG: _____

SEX OF DOG: _____

DESCRIPTION: _____

MASS: _____

Date	A	H	F	Medication given & time
	(A = APPETITE H = HABITUS F = FAECES)			

FIGURE 2A:

NUMBER OF DOG: 1   KENNEL NO: 21   COLLAR NO: 160

BREED OF DOG: Labrador X German Shepherd Dog

SEX: Male

COLOUR: Black

MASS: 24.25kg - 19/1/88   DOSE: 4,00 tabs.(120 mg)
      26.30kg - 26/1/88   4,25 tabs.(127.5 mg)
      27.00kg - 02/2/88   4,50 tabs.(135 mg)
      27.15kg - 09/2/88   same
      27.55kg - 17/2/88   same

DESCRIPTION: Large, lean, placcid temperament.

DAY	OBSERVATIONS & PROCEDURES
0	06h00 jugular cath. inserted. Dog weighed. Initial blood sample taken for base-line. 08h00 dose given with 20ml water. Blood samples taken by veni-puncture of cephalic as cath. pulled out at 0,5; 1,0; 2,0; 4,0; 6,0; 8,0; 12,0; & 24hrs post dosing. Serum collected and frozen within 14 hours. No problems. Dog fed at 15h00.
1	07h45 took blood sample as·above. 08h00 dose given with water. 15h00 fed. A+++ F+++ W+ H noisy.
2	08h00 dose given in capsule with water. Fed 15h00.
3	08h00 dose given. HQ ataxia++. A+++F+++W+.Fed 15h00.
4	08h00 dose given. HQ ataxia+. A+++F+++W+. Fed 15h00.
5	08h00 dose given. HQ ataxia++. A+++F+++W+H v.quiet. Fed 15h00.
6	08h00 dose given. HQ ataxia+. A+++F+++W+H+. Fed 15h00.
7	07h30 blood sample taken and weight recorded. 08h00 dose given c/w. A+++F+++W+ H quiet & sleeps alot in the sun. Dose adjusted due to weight gain. Fed at 15h00.

8   08h00 dose given c/w. A+++F+++W++ H quiet. Fed 15h00.

---

9   08h00 dose given c/w. A+++F+++W+ H alert but quiet. Fed 15h00.

---

10   08h00 dose given c/w. A+++F+++W+ H++. Fed 15h00.

---

11   08h00 dose given c/w. A+++ F diarrhoea yellow/brown colour H++. Vomited white bile. Fed 15h00.

---

12   08h00 dose given c/w. A+++ F+ W++ H++. Fed 15h00.

---

13   08h00 dose given c/w. A+++ F+++ W++ H++. Fed 15h00.

---

14   07h30 blood sample taken and dog weighed. 08h00 dose given c/w. Dose adjusted due to weight gain. A+++ F+++ W+++ H++

---

15   08h00 dose given c/w. A+++ F+++ W+++ H quiet but alert. Urine normal. Fed 15h00.

---

16   08h00 dose given c/w. A+++ F+++ W+++ H quiet. Urine normal. Fed 15h00.

---

17   08h00 dose given c/w. A+++ F+++ W++ H quiet but alert. Urine normal. Fed 15h00.

---

18   08h00 dose given c/w. A+++ F+++ W++ H quiet but alert. Urine normal. Fed 15h00.

---

19   08h00 dose given c/w. A+++ F+++ W+++ H quiet but alert. Fed 15h00.

---

20   08h00 dose given c/w. A+++ F+++ W+++ H quiet but alert. Fed 15h00.

---

21   07h30 blood sample taken. Dose given at 08h00 c/w. Dog weighed.

FIGURE 2B: .

NO. OF DOG: 2   KENNEL NO: 20   COLLAR NO: 171

BREED of DOG: Non-Descript (Cross Breed)

SEX: Male

COLOUR: Light brown with grey muzzle.

MASS: 16.85kg - 19/1/88   DOSE: 2.75 tabs.(82.5 mg)
      16.80kg - 26/1/88   same
      17.65kg - 02/2/88   3.00 tabs.(90 mg)
      17.25kg - 09/2/88   same
      17.75kg - 17/2/88   same

DESCRIPTION: Medium-sized dog. Lean. Placcid. Approx. 9 years.

DAY	OBSERVATIONS & PROCEDURES
0	As described in DOG 1.
1	Blood sample taken at 07h30. 08h00 dose given with water. A+++F+++W+H noisy. Fed 15h00.
2	08h00 dose given in caps. with water. A+++F+++W++. Fed 15h00.
3	08h00 dose given in caps. with water. A+++F+++W++H noisy. Fed 15h00.
4	08h00 dose given in caps. with water. A+++ F+++ W++ H quiet. Fed 15h00.
5	08h00 dose given c/w. A+++ F+++ W++ H quiet. Fed 15h00.
6	08h00 dose given c/w. A+++ F+++ W++ H quiet. Fed 15h00.
7	07h30 blood sample taken and dog weighed. 08h00 dose given c/w. A+++ F+++ W+ H quiet. Fed 15h00.
8	08h00 dose given c/w. A+++ F+++ W++ H v.quiet/drowsy. Fed 15h00.
9	08h00 dose given c/w. A+++ F+++ W++ H alert but v.quiet. Fed 15h00.

10	08h00 dose given c/w. A+++ F+++ W+++ H lively/panting. Fed 15h00.
11	08h00 dose given c/w. A+++ F+++ W+ H walking slowly/quiet. Fed 15h00.
12	08h00 dose given c/w. A+++ F+++ W++ H lively/alert. Fed 15h00.
13	08h00 dose given c/w. A+++ F+++ W++ H v.alert. Fed 15h00.
14	07h30 blood sample taken and dog weighed. 08h00 dose given c/w. Dose adjusted due to weight gain. A+++ F+++ W++ H alert. Fed 15h00.
15	08h00 dose given c/w. A+++ F+++ W+++ H quiet but alert. Fed 15h00.
16	08h00 dose given c/w. A+++ F+++ W++++ H v.quiet. Urine excessive and clear. Fed 15h00.
17	08h00 dose given c/w. A+++ F+++ W++ H quiet but alert. Fed 15h00.
18	08h00 dose given c/w. A+++ F+++ W+++ H quiet but alert. Urine normal. Fed 15h00.
19	08h00 dose given c/w. A+++ F+++ W+++ H quiet but alert. Fed 15h00 .
20	08h00 dose given c/w. A+++ F+++ W+++ H quiet but alert. Fed 15h00.
21	07h30 blood sample taken. 08h00 dose given c/w. Dog weighed.

FIGURE 2C:

NUMBER OF DOG: 3   KENNEL NO: 19   COLLAR NO: 564

BREED OF DOG: Non-Descript (Cross Breed)

SEX: Male

COLOUR: Brown

MASS: 14.90kg - 19/1/88   DOSE: 2.5 tabs.(75 mg)
     14.25kg - 26/1/88   same
     14.50kg - 02/2/88   same
     14.65kg - 09/2/88   same
     14,65kg - 17/2/88   same

DESCRIPTION: Medium, lean, excitable. 11 months.

DAY	OBSERVATIONS & PROCEDURES
0	As descibed in DOG 1.
1	08h00 dose given with water. A+++W+F+++ H noisy. Fed 15h00.
2	08h00 dose given in caps. with water. A+++W+F+++ H++. Fed 15h00.
3	08h00 dose given c/w. A+++W+F+++ H noisy. Fed 15h00.
4	08h00 dose given c/w. A+++W+++F+++ H++. Concentrated urine passed. Fed 15h00.
5	08h00 dose given c/w. A+++W++F+++ H alert/active. Fed 15h00.
6	08h00 dose given c/w. A+++W++F+++ H quiet/alert. Fed 15h00.
7	07h30 blood sample taken and dog weighed. 08h00 dose given c/w. A+++ F+++ W+ H alert/ licking paws. Fed 15h00.
8	08h00 dose given c/w. A+++ F+++ W+++ H v.quiet & sleepy. Fed 15h00.
9	08h00 dose given c/w. A+++ F diarrhoea W++ H v.quiet/panting. Urine v.clear. Fed 15h00.

10   08h00 dose given c/w. A+++ F+ W+ H boisterous. Fed 15h00.

11   08h00 dose given c/w. A+++ F+++ W+ H panting alot/ lively &
     friendly/ excitable. Fed 15h00.

12   08h00 dose given c/w. A+++ F+++ W+ H lively/ v.alert.
     Fed15h00.

13   08h00 dose given c/w. A+++ F+++ W+ H quiet but alert. Fed
     15h00.

14   07h30 blood sample taken and dog weighed. 08h00 dose given
     c/w. A+++ F+++ W+ H quiet but alert. Urine clear. Fed 15h00.

15   08h00 dose given c/w. A+++ F+++ W+++ H quiet but alert.
     Urine normal. Fed 15h00 .

16   08h00 dose given c/w. A+++ F+++ W++ H quiet but alert. Urine
     normal. Fed 15h00.

17   08h00 dose given c/w. A+++ F+++ W+++ H quiet but alert.
     Urine normal. Fed 15h00.

18   08h00 dose given c/w. A+++ F+++ W+++ H quiet but alert.
     Urine normal. Fed 15h00.

19   08h00 dose given c/w. A+++ F+++ W+++ H quiet but normal. Fed
     15h00.

20   08h00 dose given c/w. A+++ F+++ W+++ H quiet but v.alert.
     Urine normal. Fed 15h00.

21   07h30 blood sample taken. 08h00 dose given c/w. Dog weighed.

FIGURE 2D:

NUMBER OF DOG: 4   KENNEL NO: 18   COLLAR NO: 51

BREED OF DOG: Fox Terrier X Collie

SEX: Male

COLOUR: White with black spots

MASS: 15.45kg - 19/1/88   DOSE: 2.5 tabs.(75 mg)
      14.70kg - 26/1/88   same
      14.50kg - 02/2/88   same
      14.75kg - 09/2/88   same
      14.90kg - 17/2/88   same

DESCRIPTION: Medium, lean, excitable. Age 2 years.

DAY	OBSERVATIONS & PROCEDURES
0	As described for DOG 1.
1	08h00 dose given with water. A+++ W+ F- H active. Fed 15h00.
2	08h00 dose given in caps. with water. A+++W+F+++ H v.active. Fed 15h00.
3	08h00 dose given c/w. A+++ W++F+++ H active/noisy. Fed 15h00.
4	08h00 dose given c/w. A+++ W++ F++ H ++. Fed 15h00.
5	08h00 dose given c/w. A+ W++ F khaki-coloured & soft H++. Fed 15h00. Still had remaining pellets from previous nights feed but removed before medication.
6	08h00 dose given c/w. A+ W++ F still loose/khaki. T=38.7°C. Fed 15h00.
7	07h30 blood sample taken and dog weighed. 08h00 dose given c/w. A+++ F+++ W+ H quiet. HQ ataxia +. Fed 15h00.
8	08h00 dose given c/w. A+++ F soft with **TAPEWORM** W+ H+ T=39.3°C. Dog has a purulent occular discharge. Fed 15h00.

9   08h00 dose given c/w. A+ F+ W+ H v.quiet. T=39.2°C. Still has occular discharge from R eye. Fed 15h00.

10 08h00 dose given c/w. A++ F++ W++ H quiet. Fed 15h00.

11   08h00 dose given c/w. A+++ F+++ W++ H v.v.excitable. T=38.5°C. Fed 15h00.

12 08h00 dose given c/w. A+++ F+++ W++ H v.excitable & lively. T=38.6°C. Fed 15h00.

13 08h00 dose given c/w. A+++ F+++ W++ H alert but quiet. Occular discharge again. Fed 15h00.

14 07h30 blood sample taken and dog weighed. 08h00 dose given c/w. A+++ F+++ W++ H quiet. Urine normal. Fed 15h00.

15 08h00 dose given c/w. A+++ F+++ W++ H quiet but alert. Urine normal. Fed 15h00.

16 08h00 dose given c/w. A+++ F+++ W+ H quiet but alert. Urine normal. Fed 15h00.

17 08h00 dose given c/w. A+++ F+++ W++ H quiet but alert. Urine normal. Fed 15h00.

18 08h00 dose given c/w. A+++ F+++ W+++ H quiet but alert. Urine normal. Fed 15h00.

19 08h00 dose given c/w. A+++ F+++ W+++ H quiet but alert. Fed 15h00.

20 08h00 dose given c/w. A+++ F+++ W+++ H quiet but alert. Fed 15h00.

21 07h30 blood sample taken and dog weighed. 08h00 dose given.

FIGURE 2E:

NUMBER OF DOG: 5   KENNEL NO: 17   COLLAR NO: 689

BREED OF DOG: Bull Terrier

SEX: Male

COLOUR: Black with white & tan

MASS: 22.65kg - 19/1/88   DOSE: 3.75 tabs.(112.5 mg)
      21.50kg - 26/1/88   3.50 tabs.(105 mg)
      21.65kg - 02/2/88   same
      22.30kg - 09/2/88   same
      21.65kg - 17/2/88   same

DESCRIPTION: Medium, well muscled, excitable, needle-shy.
             Age approx. 2 years.

DAY	OBSERVATIONS & PROCEDURES
0	As described in DOG 1.
1	08h00 dose given with water. A+++ W++ F+ H quiet but alert. Fed 15h00.
2	08h00 dose given in caps. with water. A+++ W++ F putty-coloured and loose. stool examined but NAD. H++. Fed 15h00.
3	08h00 dose given c/w. A+++ W++ F++(normal) H quiet. Passed concentrated urine. Fed 15h00.
4	08h00 dose given c/w. A+++ W+ F+++ H++. Fed 15h00.
5	08h00 dose given c/w. A+++ W++ F+++ H boisterous but later quieter. Fed 15h00.
6	08h00 dose given c/w. A+++ W++ F+++ H boisterous. Fed 15h00.
7	07h30 blood sample taken and dog weighed. 08h00 dose given c/w. Dose adjusted due to weight loss. A+++ F+++ W+ H quiet. Fed 15h00.
8	08h00 dose given c/w. A+++ F+++ W+ H quiet but alert. Fed 15h00.

9   08h00 dose given c/w. A+++ F+++ W+ H quiet but alert. Fed 15h00.

10   08h00 dose given c/w. A+++ F+++ W+ H panting but lively. Fed 15h00.

11   08h00 dose given c/w. A+++ F+++ W++ H alert/ v.lively/ panting alot -(hot day). Fed 15h00.

12   08h00 dose given c/w. A+++ F+++ W++ H lively. Fed 15h00.

13   08h00 dose given c/w. A+++ F+++ W++ H alert but quiet. Fed 15h00.

14   07h30 blood sample taken and dog weighed. 08h00 dose given c/w. A+++ F+++ W++ H quiet but v.alert. Urine normal. Fed 15h00.

15   08h00 dose given c/w. A++ F+++ W++ H quiet but v.alert. Urine normal. Fed 15h00.

16   08h00 dose given c/w. A+++ F+++ W+++ H quiet but v.alert. Urine normal. Fed 15h00.

17   08h00 dose given c/w. A+++ F+++ W++ H quiet but v.alert. Urine normal. Fed 15h00.

18   08h00 dose given c/w. A+++ F+++ W++ H quiet but v.alert. Urine normal. Fed 15h00.

19   08h00 dose given c/w. A+++ F+++ W++ H quiet but v. alert. Fed 15h00.

20   08h00 dose given c/w. A+++ F+++ W++ H quieter but still alert. Urine normal. Fed 15h00.

21   07h30 blood sample taken and dog weighed. 08h00 dose given c/w.

FIGURE   2F:

NUMBER OF DOG: 6   KENNEL NO: 15   COLLAR NO: 264

BREED OF DOG: Mastiff X

SEX: Male

COLOUR: Brown with white

MASS: 22.55kg - 19/1/88   DOSE: 3.75 tabs.(112.5 mg)
      22.25kg - 26/1/88   same
      22.45kg - 02-2/88   same
      21.45kg - 09/2/88   same
      21.05kg - 17/2/88   same

DESCRIPTION: Large, thin, placcid. Age uncertain (± 6 years).

DAY	OBSERVATIONS & PROCEDURES
0	As described in DOG 1.
1	08h00 dose given with water. A+++ W+ F+++ H quiet. Fed 15h00.
2	08h00 dose given in caps. with water. A++ W+ F+++ H v.quiet-T=39,7°C. Fed 15h00.
3	08h00 dose given c/w. T=39,5°C. Blood smear shows WBC increase esp. N+++(immatures). Suspect reaction locally at multiple veni-puncture site. Inject 3cc Duplocillin i/m. A+++ W++ F+++ H v.quiet. Fed 15h00.
4	08h00 dose given c/w. T=39,5°C. Walking okay. A+++ W++ F+++ H+. Fed 15h00.
5	08h00 dose given c/w. T=39,0°C. Happy dog. A+++ W++ F+++ H v.quiet. Fed 15h00.
6	08h00 dose given c/w. A+++ W++ F+++ H+. Fed 15h00.
7	07h30 blood sample taken and dog weighed. 08h00 dose given c/w. A+++ F+++ W+ H quiet. Fed 15h00.
8	08h00 dose given c/w. A+++ F+++ W++ H v.quiet/ appears slightly weak. Urinating normal. Fed 15h00.

9   08h00 dose given c/w. A+++ F+++ W+ H quiet but alert. Fed 15h00.

10   08h00 dose given c/w. A+++ F+++ W+ H panting but appears fine. Fed 15h00.

11   08h00 dose given c/w. A+++ F+++ W++ H quiet/ lethargic. Fed 15h00.

12   08h00 dose given c/w. A+++ F+++ W++ H good. Fed 15h00.

13   08h00 dose given c/w. A+++ F+++ W++ H quiet but alert. Urine normal. Fed 15h00.

14   07h30 blood sample taken and dog weighed. 08h00 dose given c/w. A+++ F+++ W+++ H quiet but alert. Urine normal. Fed 15h00.

15   08h00 dose given c/w. A+++ F+++ W+++ H quiet but alert. Urine normal. Fed 15h00.

16   08h00 dose given c/w. A+++ F+++ W++ H quiet but alert. Urine normal. Fed 15h00.

17   08h00 dose given c/w. A+++ F+++ W++ H alert. Urine normal. Fed 15h00.

18   08h00 dose given c/w. A+++ F+++ W++ H quiet but alert. Urine normal. Fed 15h00.

19   08h00 dose given c/w. A+++ F+++ W+++ H quiet but alert. Fed 15h00.

20   08h00 dose given c/w. A+++ F+++ W+++ H quiet but alert. Urine normal. Fed 15h00.

21   07h30 blood sample taken and dog weighed. 08h00 dose given c/w.

FIGURE 2G:

NUMBER OF DOG: 7   KENNEL NO: 14   COLLAR NO: 75

BREED OF DOG: Bull Dog X Ridgeback

SEX: Male

COLOUR: Tan

MASS: 27.90kg - 19/1/88   DOSE: 4.5 tabs.(135 mg)
     23.25kg - 26/1/88   4.0 tabs.(120 mg)
     23.45kg - 02/2/88   same
     23.15kg - 09/2/88   same
     23.05kg - 17/2/88   same

DESCRIPTION: Medium, barrel-framed, lean. Age 8 years.

DAY	OBSERVATIONS & PROCEDURES
0	As described in DOG 1. Dog however becomes viscious when forcing tablets down. Therefore tabs. given in a tiny piece of mince which it takes readily.
1	08h00 dose given in meat. No water is given but tabs. well swallowed. A+++ W+ F+++ H quiet. Fed 15h00.
2	08h00 dose given in caps. in meat. A+++ W+ F- H quiet. Fed 15h00.
3	08h00 dose given c/m. A+++ W++ F- H quiet. Fed 15h00.
4	08h00 dose given c/m. A+++ W++ F+ H quiet. Passed concentrated urine. Fed 15h00.
5	08h00 dose given c/m. A+++ W++ F+++ H quiet/sleeps alot. Fed 15h00.
6	08h00 dose given c/m. A+++ W++ F+++ H quiet. Licking paws. Fed 15h00.
7	07h30 blood sample taken anddog weighed. 08h00 dose given c/m. A+++ F+++ W+ H quiet/ resting. Fed 15h00.
8	08h00 dose given c/m. A+++ F+++ W++ H v.lethargic/ appears weak. Urinating normal. Fed 15h00.

9   08h00 dose given c/m. A+++ F+++ W+ H alert & bright. Urinating normal. Fed 15h00.

10 08h00 dose given c/m. A+++ F+++ W+ H quiet but alert. Fed 15h00.

11 08h00 dose given c/m. A+++ F+++ W++ H quiet/ walking slowly. Fed 15h00.

12 08h00 dose given c/m. A+++ F+++ W++ H++. Fed 15h00.

13 08h00 dose given c/m. A+++ F normal but has **TAPEWORM** W++ H quiet but alert. Fed 15h00.

14 07h30 blood sample taken and dog weighed. 08h00 dose given c/m. A+++ F+++ W++++ H v.quiet. Fed 15h00.

15 08h00 dose given c/m. A+++ F+++ W+ H quiet but alert. Urine normal. Fed 15h00.

16 08h00 dose given c/m. A+++ F+++ W+++ H v.quiet. Urine normal. Fed 15h00.

17 08h00 dose given c/m. A+++ F+++ W+++ H alert. Urine normal. Fed 15h00.

18 08h00 dose given c/m. A+++ F+++ W++ H quiet. Urine normal. Fed 15h00.

19 08h00 dose given c/m. A+++ F+++ W+++ H quiet. Fed 15h00.

20 08h00 dose given c/m. A+++ F+++ W+++ H quiet. Urine normal. Fed 15h00.

21 07h30 blood sample taken and dog weighed. 08h00 dose given c/m.

FIGURE 2H:

NUMBER OF DOG: 8   KENNEL NO: 12   COLLAR NO: 636

BREED OF DOG: Staffie X Labrador

SEX: Female

COLOUR: Brindle & black

MASS: 18.70kg - 19/1/88   DOSE: 3 tabs.(90 mg)
      18.25kg - 26/1/88   same
      19.05kg - 02/2/88   same
      19.20kg - 09/2/88   same
      18.95kg - 17/2/88   same

DESCRIPTION: Medium, well muscled, excitable, Age 1 year.

DAY	OBSERVATIONS & PROCEDURES
0	As described in DOG 1.
1	08h00 dose given with water. A+++ W+ F- H noisy. Fed 15h00.
2	08h00 dose given in caps. with water. A+++ W++ F+++ H noisy. Fed 15h00.
3	08h00 dose given c/w. A+++ W++ F+++ H noisy. Fed 15h00.
4	08h00 dose given c/w. A+++ W+ F- H quiet. Fed 15h00.
5	08h00 dose given c/w. A+++ W++ F soft H noisy. Fed 15h00.
6	08h00 dose given c/w. A+++ W++ F+++ H noisy. Fed 15h00.
7	07h30 blood sample taken and dog weighed. 08h00 dose given c/w. A+++ F+++ W+ H v.quiet. Fed 15h00.
8	08h00 dose given c/w. A+++ F normal but has **TAPEWORM** W+++ H v. quiet. Urinating normal. Fed 15h00.
9	08h00 dose given c/w. A+++ F+++ W++ H++. Fed 15h00.

10 08h00 dose given c/w. A+++ F+++ W+ H panting alot/ v.quiet. Fed 15h00.

11 08h00 dose given c/w. A+++ F+++ W++ H alert & lively. Fed 15h00.

12 08h00 dose given c/w. A+++ F+++ W+++ H boisterous. Fed 15h00.

13 08h00 dose given c/w. A+++ F+++ W++ H v.alert. Fed 15h00.

14 07h30 blood sample taken and dog weighed. 08h00 dose given c/w. A+++ F+++ W+++ H quiet. Urine normal. Fed 15h00.

15 08h00 dose given c/w. A+++ F+++ W++ H alert. Fed 15h00.

16 08h00 dose given c/w. A+++ F+++ W++ H v.alert. Urine normal. Fed 15h00.

17 08h00 dose given c/w. A+++ F+++ W+++ H alert. Urine normal. Fed 15h00.

18   08h00 dose given c/w. A+++ F+++ W++ H quiet but v.alert. Urine normal. Fed 15h00.

19   08h00 dose given c/w. A+++ F+++ W++ H quiet but alert. Fed 15h00.

20   08h00 dose given c/w. A+++ F+++ W++ H quiet but v.alert. Urine normal. Fed 15h00.

21 07h30 blood sample taken and dog weighed. 08h00 dose given.

FIGURE 2I:

NUMBER OF DOG: 9   KENNEL NO: 13   COLLAR NO: 455

BREED OF DOG: GERMAN SHEPHERD DOG (GSD)

SEX: Female

COLOUR: Black & Tan

MASS: 21.90kg - 19/1/88   DOSE: 3.5 tabs.(105 mg)
      21.45kg - 26/1/88   same
      22.45kg - 02/2/88   3.75 tabs.(112.5 mg)
      22.95kg - 11/2/88   same
      22.35kg - 17/2/88   same

DESCRIPTION: Large, thin, placcid. Age uncertain (± 2 years)

DAY	OBSERVATIONS & PROCEDURES
0	As described in DOG 1.
1	08h00 dose given with water. A+++ W+ F- H quiet. Fed 15h00.
2	08h00 dose given in caps. with water. A+++ W+ F+ H quiet. Fed 15h00.
3	08h00 dose given c/w. A+++ W+ F+ H noisy. Fed 15h00.
4	08h00 dose given c/w. A+++ W+ F+++ H restless. Fed 15h00.
5	08h00 dose given c/w. A+++ W+ F+++ H quiet. Fed 15h00.
6	08h00 dose given c/w. A+++ W++ F+++ H noisy. Fed 15h00.
7	07h30 blood sample taken and dog weighed. 08h00 dose given c/w. A+++ F+++ W+ H quiet. Fed 15h00.
8	08h00 dose given c/w. A+++ F+++ W++ H drowsy. Urinating normal. Fed 15h00.
9	08h00 dose given c/w. A+++ F+++ W+ H alert. Fed 15h00.

10   08h00 dose given c/w. A+++ F+++ W+ H panting a lot/ lethar-
      gic. Fed 15h00.

---

11   08h00 dose given c/w. A+++ F+++ W++ H alert. Fed 15h00.

---

12   08h00 dose given c/w. A+++ F+++ W+ H v. alert. Fed 15h00.

---

13   08h00 dose given c/w. A+++ F+++ W++ H alert. Fed 15h00.

---

14   07h30 blood sample taken and dog weighed. 08h00 dose given
      c/w. Dose adjusted due to weight gain. A+++ F+++ W+++ H
      quiet but alert. Fed 15h00.

---

15   08h00 dose given c/w. A+++ F+++ W+++ H quiet but alert. Fed
      15h00.

---

16   08h00 dose given c/w. A+++ F+++ W++ H quiet but alert. Urine
      normal. Fed 15h00.

---

17   08h00 dose given c/w. A+++ F+++ W++ H quiet but alert. Urine
      normal. Fed 15h00.

---

18   08h00 dose given c/w. A+++ F+++ W+++ H quiet but alert.
      Urine normal. Fed 15h00.

---

19   08h00 dose given c/w. A+++ F+++ W+++ H quiet but alert. Fed
      15h00.

---

20   08h00 dose given c/w. A+++ F+++ W++ H quiet but alert. Urine
      normal. Fed 15h00.

---

21   07h30 blood sample taken and dog weighed. 08h00 dose given
      c/w.

FIGURE 2J:

NUMBER OF DOG: 10   KENNEL NO: 11   COLLAR NO: 118

BREED OF DOG: Doberman

SEX: Male

COLOUR: Black with tan

MASS: 27.10kg - 19/1/88   DOSE: 4.5 tabs.(135 mg)
      26.15kg - 26/1/88   same
      26.60kg - 02/2/88   same
      26.20kg - 09/2/88   same
      25.85kg - 17/2/88   same

DESCRIPTION: Large, thin, placcid. Age 8 years.

DAY	OBSERVATIONS & PROCEDURES
0	As described in DOG 1.
1	08h00 dose given with water. A+++ W+ F++ H noisy. Fed 15h00.
2	08h00 dose given in caps. with water. A+++ W+ F+++ H drowsy, seems to cross-over hind legs, looks bewildered. Fed 15h00.
3	08h00 dose given c/w. A+++ W+ F+++ H scrapes hind feet when walking, paws appear puffy, otherwise quiet. Fed 15h00.
4	08h00 dose given c/w. A+++ W+ F++ H quiet, walking better, not so inco-ordinated, paws still puffy. Fed 15h00.
5	08h00 dose given c/w. A+++ W+ F++ H v.quiet, knuckling and scraping hind paws when walking. Fed 15h00.
6	08h00 dose given c/w. A+++ W+ F+++ H v.quiet T=39,0°C. Fed 15h00.
7	07h30 blood sample taken and dog weighed. 08h00 dose given c/w. A+++ F+++ W+ H v.quiet/ drowsy. Fed 15h00.
8	08h00 dose given c/w. A+++ F+++ W+++ H weak T=40.8°C. Urinating normal. Fed 15h00.

9   08h00 dose given c/w. A+++ F+++ W+++ H alert/ brighter T=38.4°C. Fed 15h00.

10   08h00 dose given c/w. A+++ F+++ W++ H v.quiet. Fed 15h00.

11   08h00 dose given c/w. A+++ F+++ W+ H lethargic. Fed 15h00.

12   08h00 dose given c/w. A+++ F+++ W++ H v.quiet. Occular purulent discharge. Fed 15h00.

13   08h00 dose given c/w. A+++ F+++ W+ H quiet. Discharge stopped. Fed 15h00.

14   07h30 blood sample taken and dog weighed. 08h00 dose given c/w. A+++ F+++ W+++ H quiet. Fed 15h00.

15   08h00 dose given c/w. A+++ F+++ W+++ H quiet. Fed 15h00.

16   08h00 dose given c/w. A+++ F+++ W+ H quiet. Urine normal. Fed 15h00.

Table 6 : Absorption rate constant (K_A) and elimination rate constant (K_E)
for the dogs in the phenobarbitone pharmacokinetic trial for
Day 1, Day 22, and Day 24

	K_A			K_E		
DOG NO	DAY 1	DAY 22	DAY 24	DAY 1	DAY 22	DAY 24
1	1.71	0.08	0.26	0.017	0.019	0.015
2	1.82	1.34	1.03	0.014	0.029	0.021
3	4.00	2.73	0.51	0.019	0.049	0.008
4	3.64	1.28	0.44	0.010	0.025	0.032
5	2.58	0.43	1.30	0.009	0.024	0.025
6	0.79	0.36	1.76	0.015	0.022	0.020
7	0.99	0.50	1.13	0.019	0.028	0.034
8	4.00	0.50	0.52	0.017	0.022	0.037
9	9.81	0.50	0.62	0.012	0.030	0.024
10	0.76	0.58	1.23	0.017	0.021	0.024

TABLE   8:   THE ELIMINATION HALF-LIFE CALCULATED FOR EACH
DOG FOR DAY 0 AND DAY 22

DOG	DAY 0	DAY 22
1	40.3	37.2
2	48.5	24.2
3	35.7	31.4
4	69.3	27.2
5 *	–	–
6	47.1	31.1
7	35.9	24.8
8	39.8	31.6
9	59.2	23.3
10	41.3	32.7
MEAN	46.3	29.3

* full dosage on day 0 suspect

TABLE 9: AREA UNDER CURVE VALUES FOR THE DOGS FOR DAY 22 AND DAY 24 CALCULATED BY THE LINEAR TRAPEZOIDAL RULE

DOG	DAY 22	DAY 24
1	443.53	385.30
2	364.37	349.66
3	337.36	402.92
4	365.49	317.60
5	385.08	248.78
6	447.16	367.89
7	397.65	339.13
8	363.97	335.27
9	318.42	313.47
10	423.70	407.35
X	384.62	346.74
SD	43.30	47.70

TABLE 10:   PEAK AND TROUGH SERUM CONCENTRATIONS   (μg.ml^{-1})
                FOR PHENOBARBITONE AT STEADY STATE IN HEALTHY DOGS
                (N=10)

DOG No.	tmax	Cmax	Cmin	Cmax-Cmin
1	8hr	17.87	14.02	3.85
2	2hr	18.26	11.65	6.61
3	6hr	18.18	13.77	4.41
4	4hr	15.87	9.90	5.97
5	0,5	13.77	8.24	5.53
6	2hr	20.57	13.55	7.02
7	12hr	16.20	12.47	3.73
8	8hr	16.28	11.66	4.62
9	4hr	15.56	9.64	5.92
10	2hr	19.46	12.67	6.79
X	4.85	17.20	11.75	5.44 (± 1.22)

tmax = time to peak serum concentration
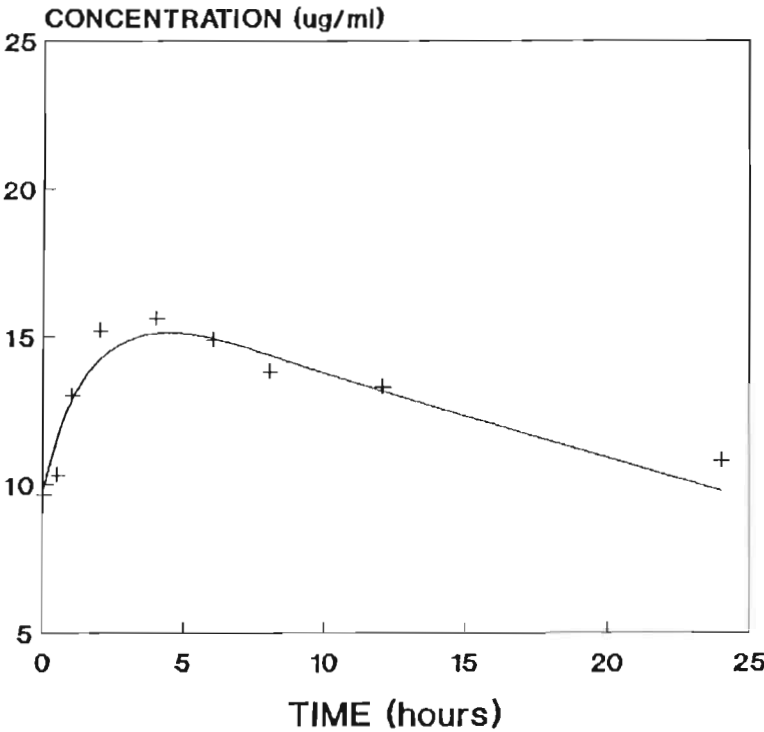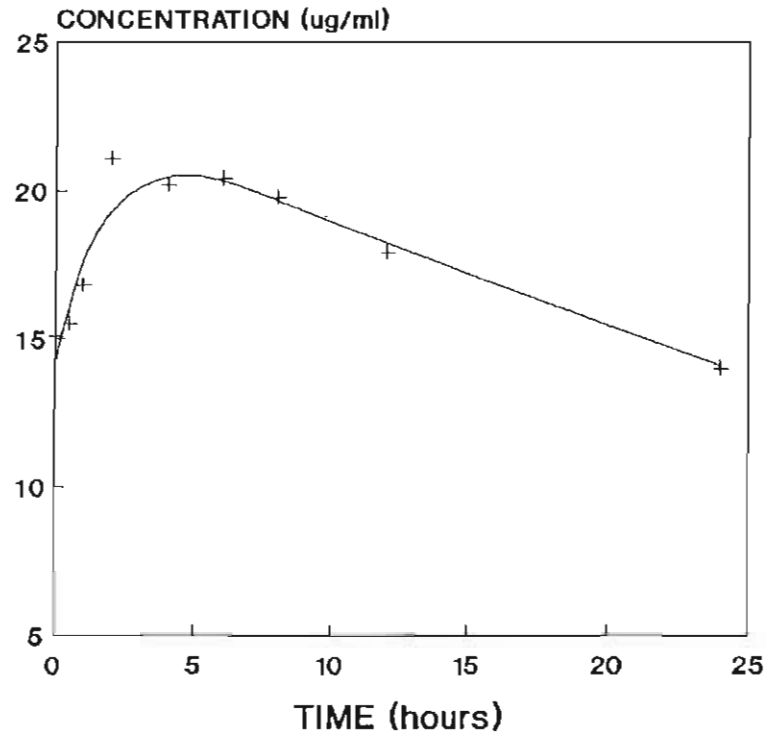Cmax = maximum serum concentration
Cmin = minimum serum concentration

APPENDIX B

FIGURE 3A

PHENOBARBITONE SERUM CONCENTRATIONS
AT STEADY-STATE WITHOUT FOOD
DOG 1

PHENOBARBITONE SERUM CONCENTRATIONS
AT STEADY-STATE WITH FOOD
DOG 1
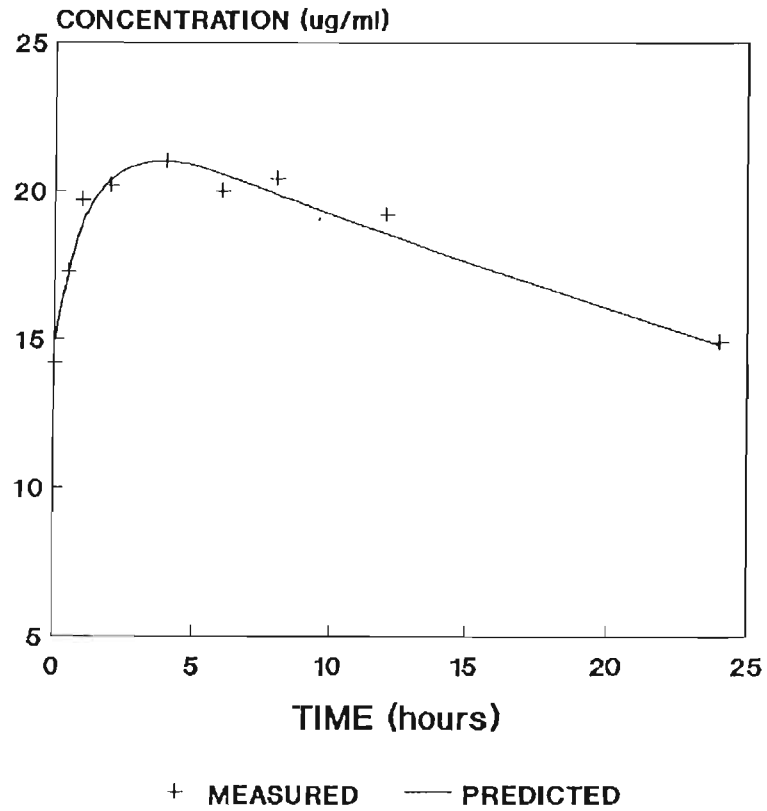
+ MEASURED   —— PREDICTED

+ MEASURED   —— PREDICTED
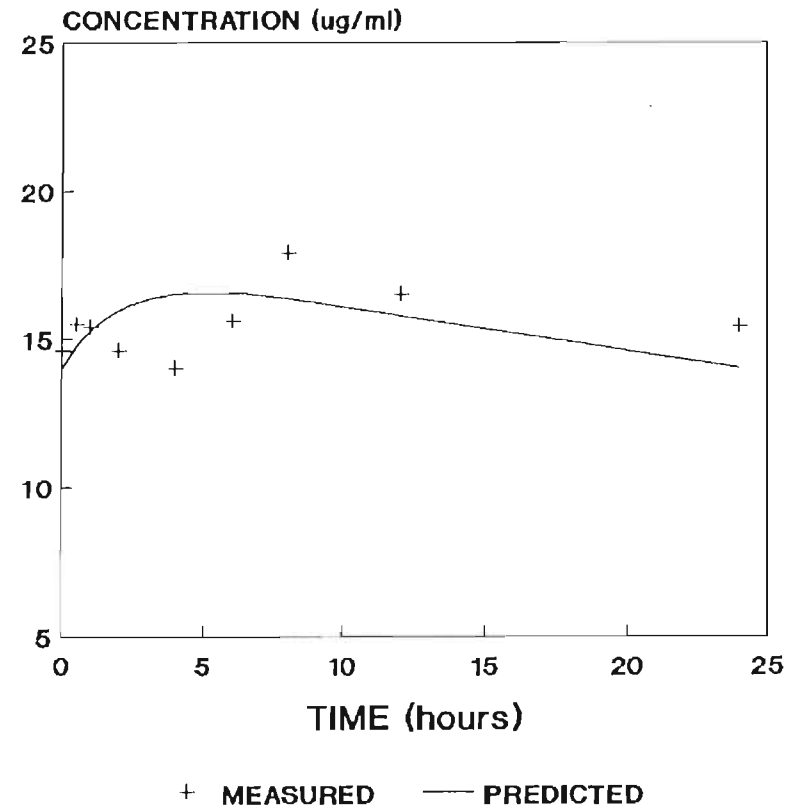
FIGURE 3B

PHENOBARBITONE SERUM CONCENTRATIONS
AT STEADY-STATE WITHOUT FOOD
DOG 2
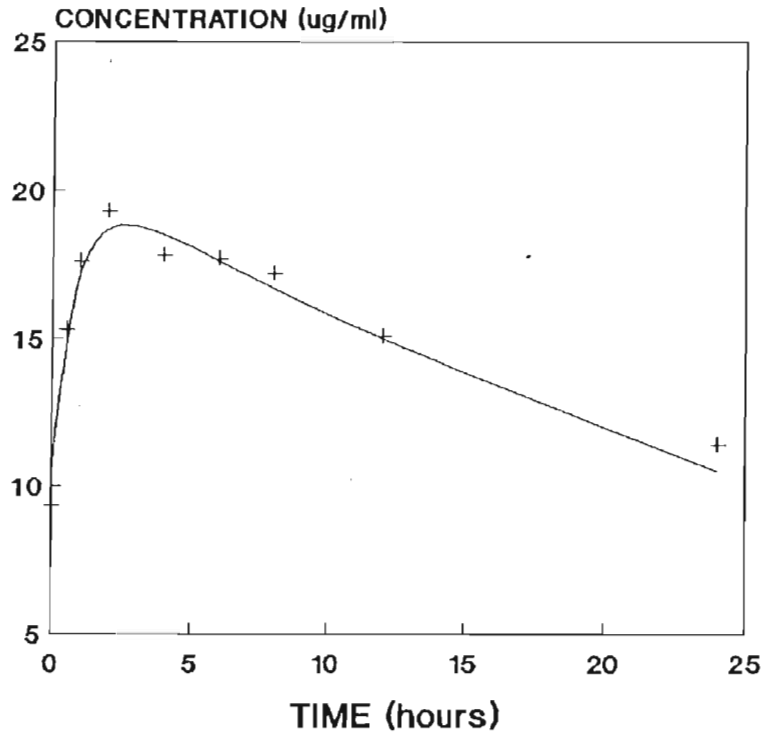
CONCENTRATION (ug/ml)

TIME (hours)

+ MEASURED ——— PREDICTED

PHENOBARBITONE SERUM CONCENTRATIONS
AT STEADY-STATE WITH FOOD
DOG 2

CONCENTRATION (ug/ml)

TIME (hours)
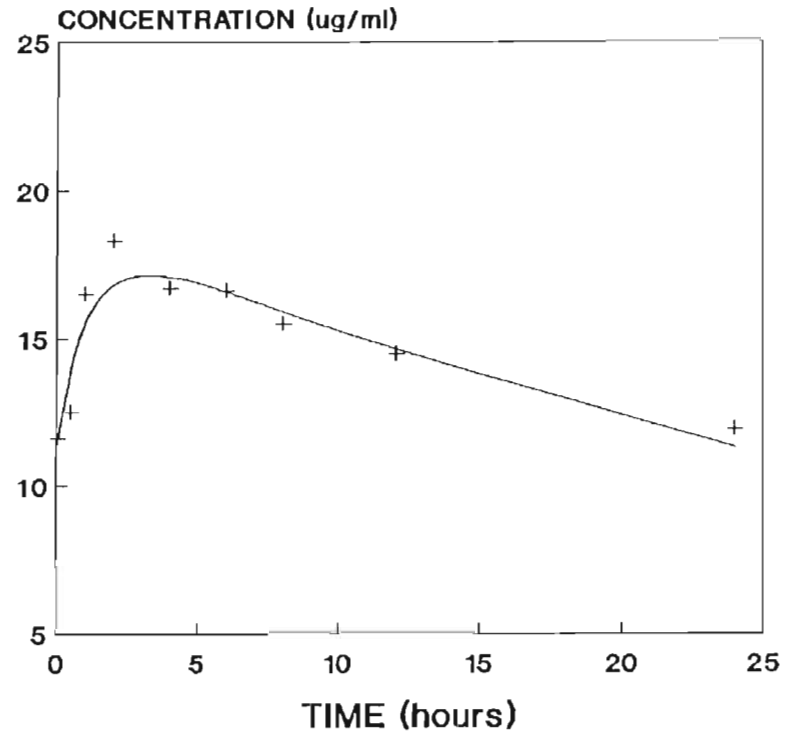
+ MEASURED ——— PREDICTED

FIGURE 3C

PHENOBARBITONE SERUM CONCENTRATIONS
AT STEADY-STATE WITHOUT FOOD
DOG 3

CONCENTRATION (ug/ml)

TIME (hours)
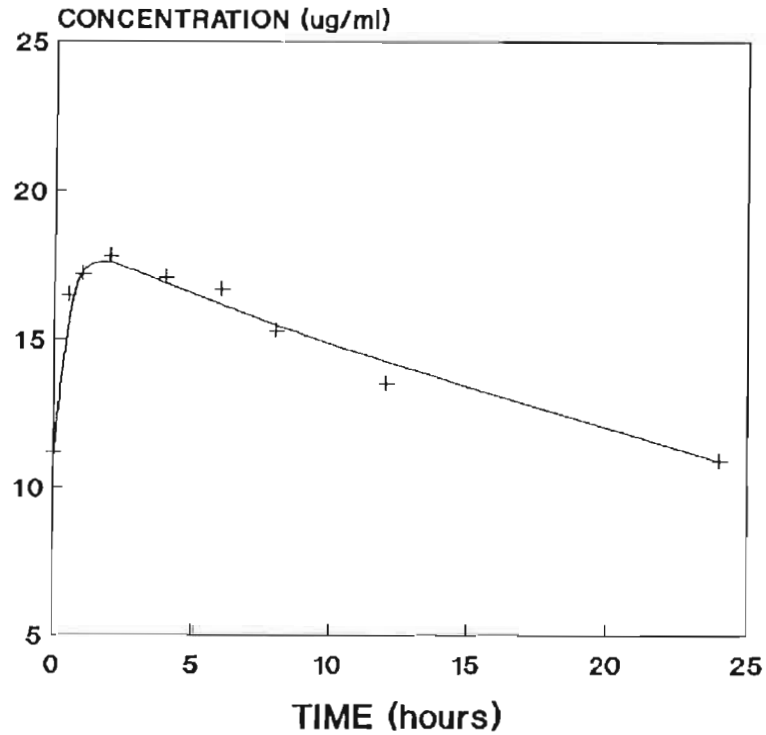
+ MEASURED   —— PREDICTED

PHENOBARBITONE SERUM CONCENTRATIONS
AT STEADY-STATE WITH FOOD
DOG 3

CONCENTRATION (ug/ml)

TIME (hours)
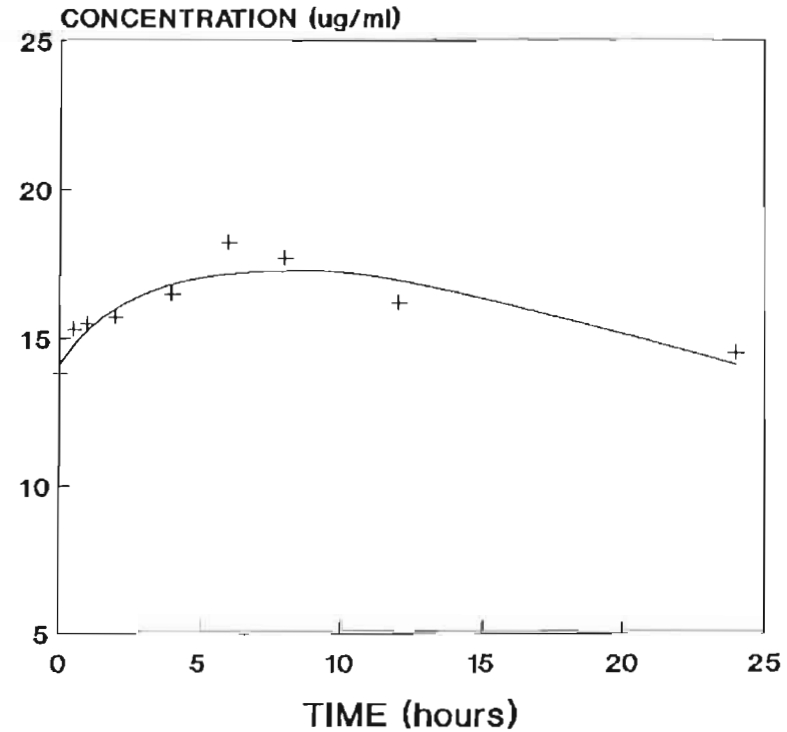
+ MEASURED   —— PREDICTED

FIGURE 3D

PHENOBARBITONE SERUM CONCENTRATIONS
AT STEADY-STATE WITHOUT FOOD
DOG 4
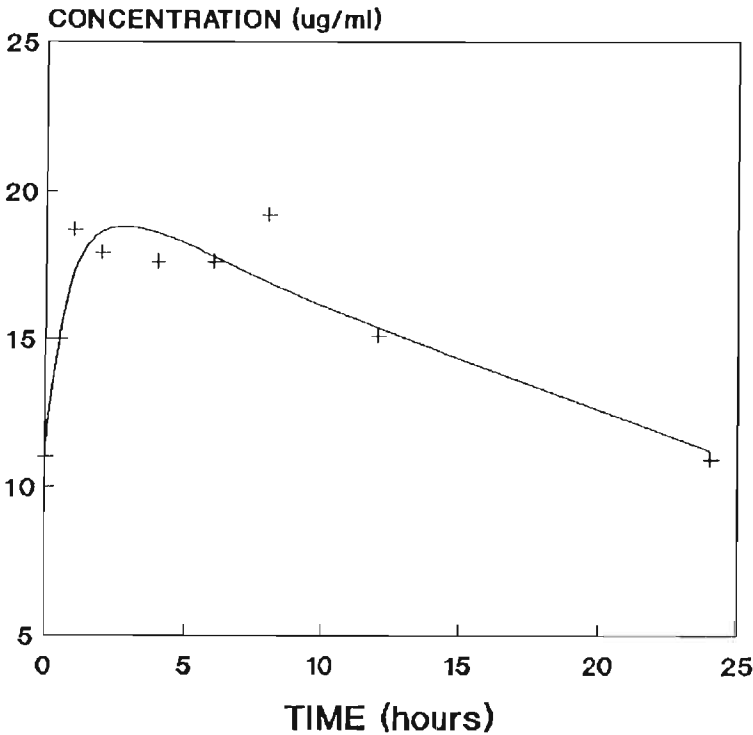
PHENOBARBITONE SERUM CONCENTRATIONS
AT STEADY-STATE WITH FOOD
DOG 4
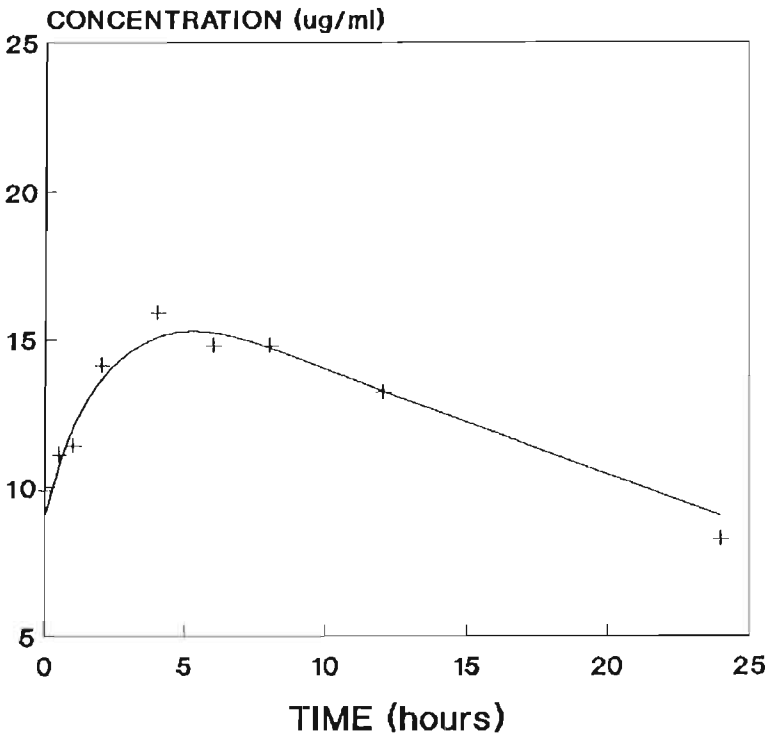
+ MEASURED   —— PREDICTED   + MEASURED   —— PREDICTED

FIGURE 3E

PHENOBARBITONE SERUM CONCENTRATIONS
AT STEADY-STATE WITHOUT FOOD
DOG 5
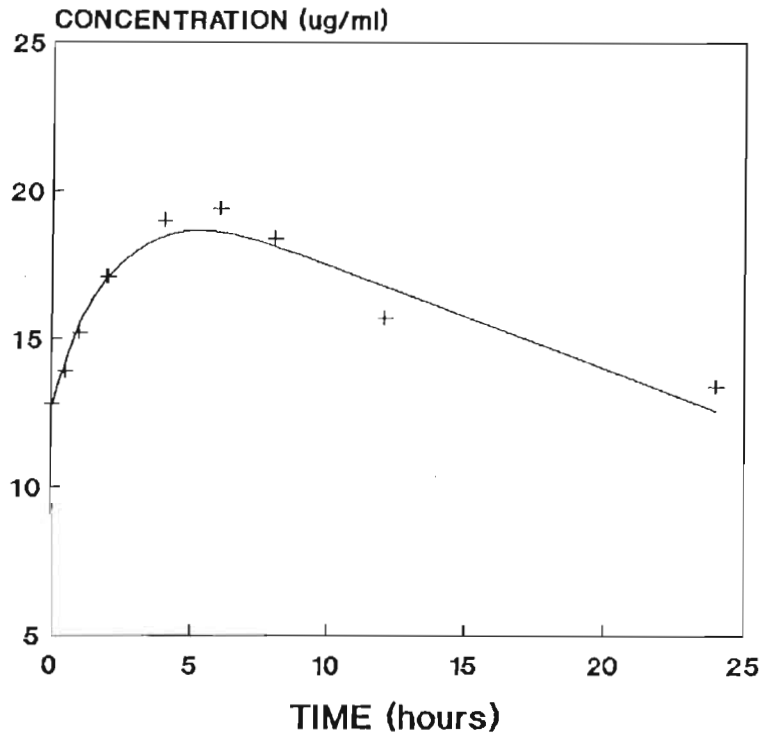
CONCENTRATION (ug/ml)

TIME (hours)

+ MEASURED   —— PREDICTED

PHENOBARBITONE SERUM CONCENTRATIONS
AT STEADY-STATE WITH FOOD
DOG 5

CONCENTRATION (ug/ml)

TIME (hours)
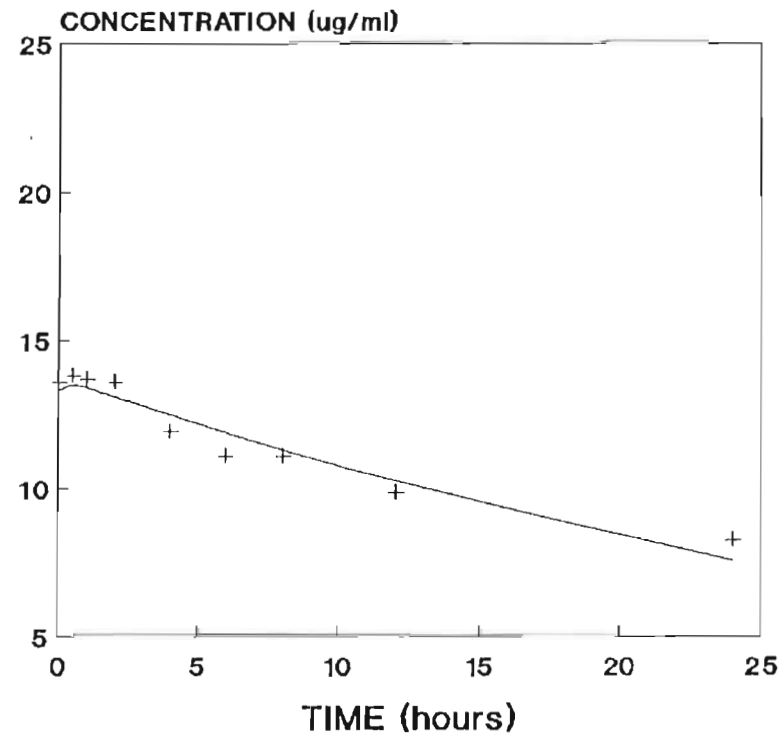
+ MEASURED   —— PREDICTED

FIGURE 3F

PHENOBARBITONE SERUM CONCENTRATIONS AT STEADY-STATE WITHOUT FOOD DOG 6
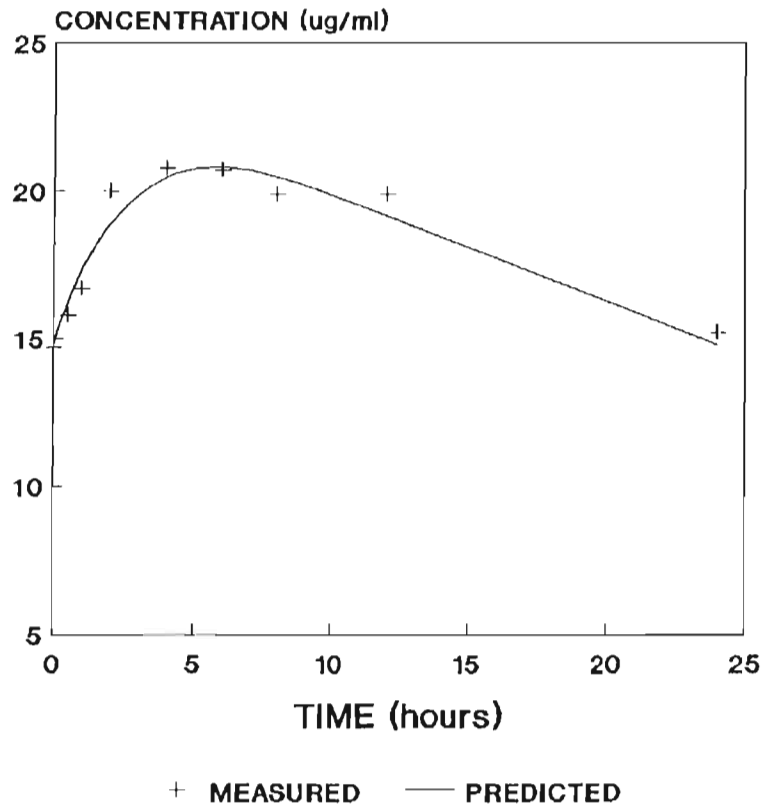
PHENOBARBITONE SERUM CONCENTRATIONS AT STEADY-STATE WITH FOOD DOG 6

+ MEASURED — PREDICTED

+ MEASURED — PREDICTED
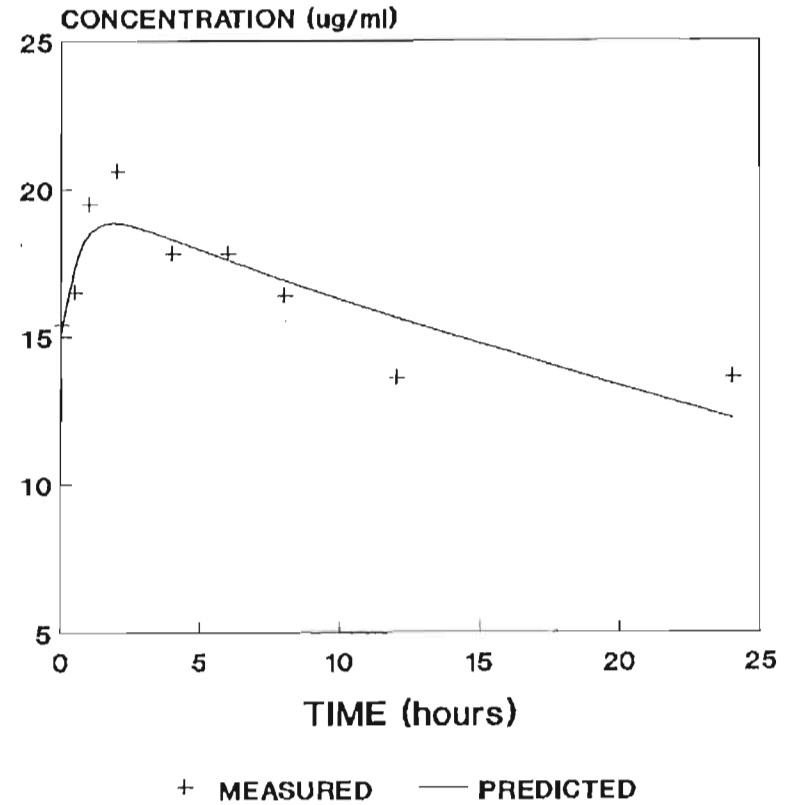
FIGURE 3G

PHENOBARBITONE SERUM CONCENTRATIONS
AT STEADY-STATE WITHOUT FOOD
DOG 7
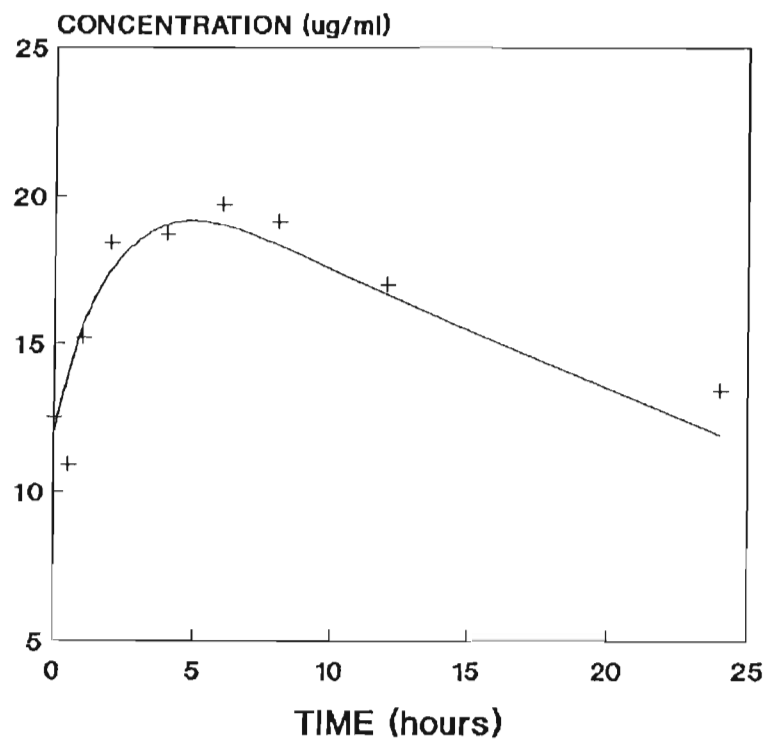
PHENOBARBITONE SERUM CONCENTRATIONS
AT STEADY-STATE WITH FOOD
DOG 7
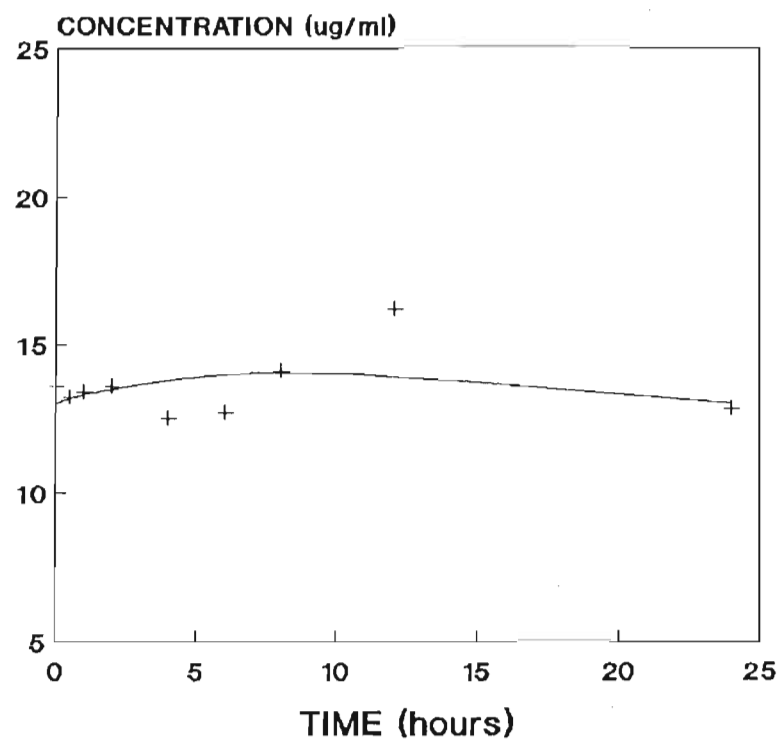
+ MEASURED ——— PREDICTED

FIGURE 3H

PHENOBARBITONE SERUM CONCENTRATIONS
AT STEADY-STATE WITHOUT FOOD
DOG 8
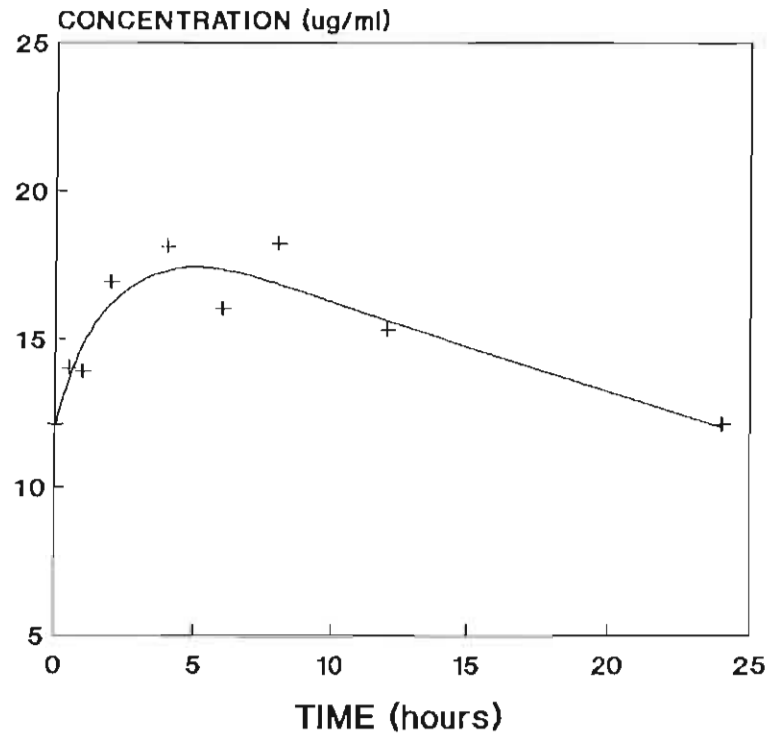
PHENOBARBITONE SERUM CONCENTRATIONS
AT STEADY-STATE WITH FOOD
DOG 8
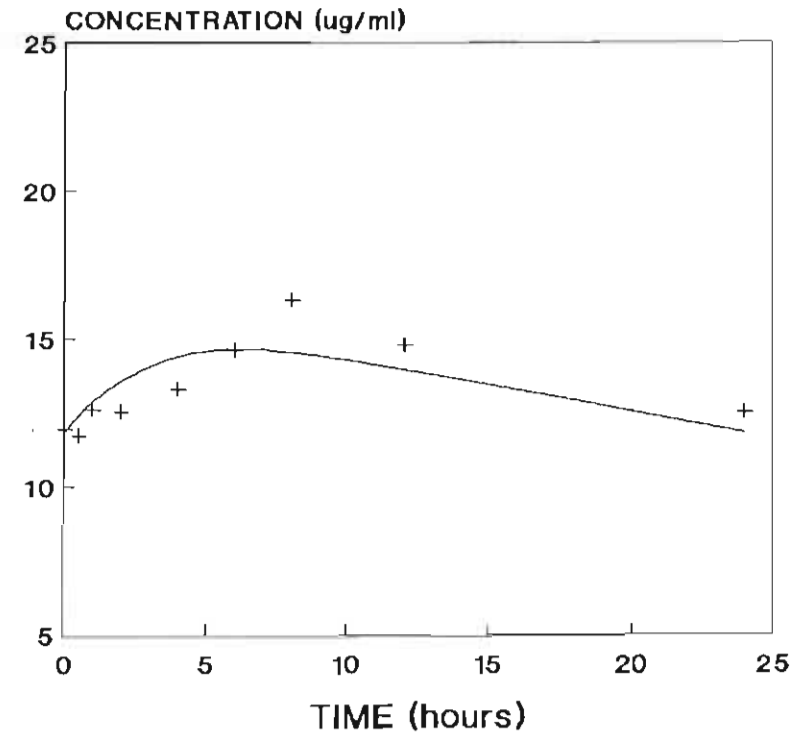
CONCENTRATION (ug/ml)

TIME (hours)

+ MEASURED ——— PREDICTED

FIGURE 31

PHENOBARBITONE SERUM CONCENTRATIONS AT STEADY-STATE WITHOUT FOOD DOG 9
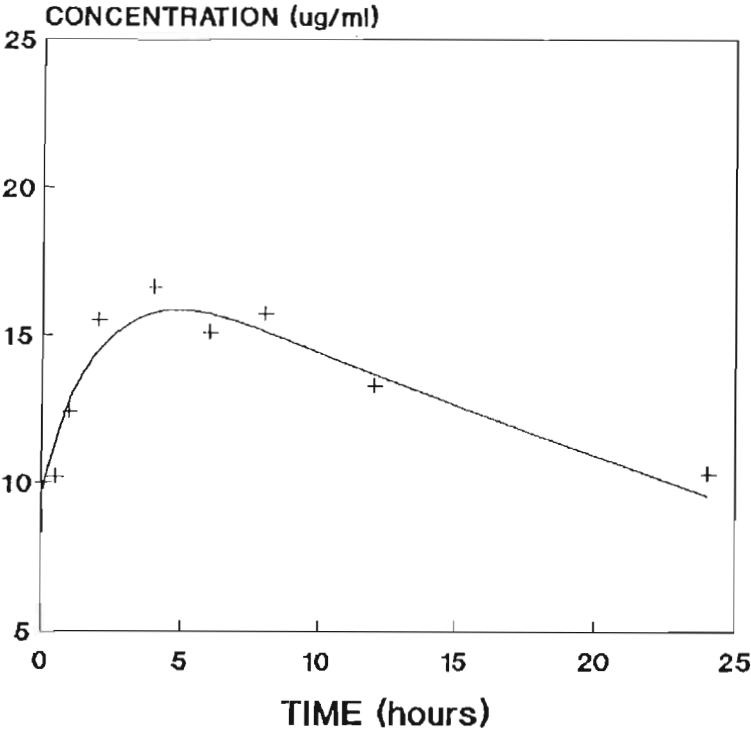
PHENOBARBITONE SERUM CONCENTRATIONS AT STEADY-STATE WITH FOOD DOG 9

+ MEASURED — PREDICTED

FIGURE 3J

PHENOBARBITONE SERUM CONCENTRATIONS
AT STEADY-STATE WITHOUT FOOD
DOG 10

PHENOBARBITONE SERUM CONCENTRATIONS
AT STEADY-STATE WITH FOOD
DOG 10
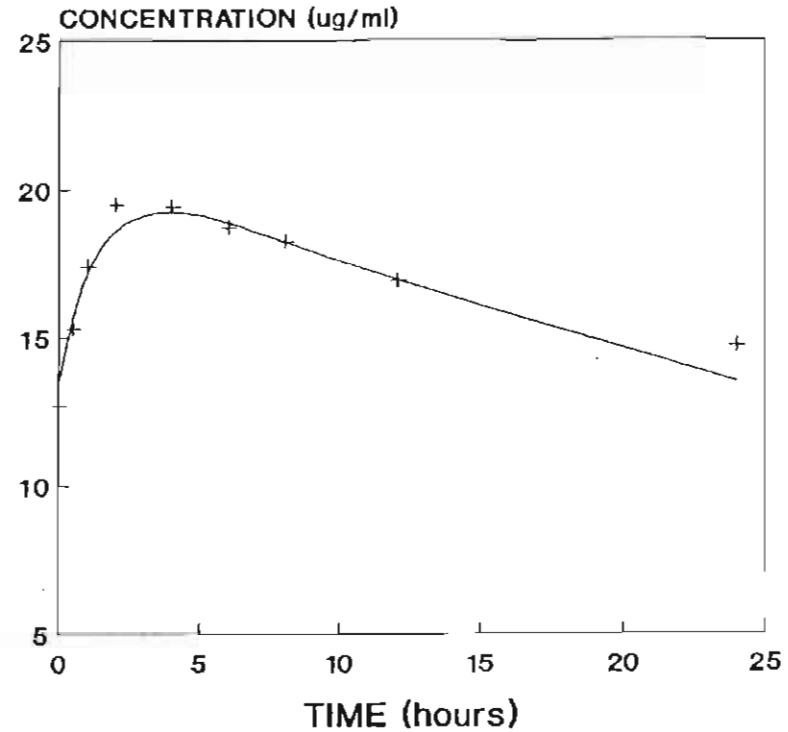
APPENDIX C

FIG 6: CANINE EPILEPSY DATA FORM

VETERINARY HOSPITAL:_____   DATE:_____

NAME OF DOG:_____

NAME OF OWNER:_____   ADDRESS:_____

_____

TEL.NO:_____

AGE: |____|____| YR |____|____| MTHS   SEX: | M | F |

MASS: _____   BREED:_____

CONCURRENT ILLNESS: | Y | N | Specify:_____

NUMBER OF SEIZURES: DAY |_____| WEEK |_____| MONTH |_____|

PRESENT ANTI-EPILEPTIC MEDICATION

DRUG	DOSE	DOSING REGIME	DATE COMMENCED	SERUM CONC. ug/ml	WITH MEALS

TIME AND DATE OF LAST DOSE: _____
TIME OF LAST SAMPLE: _____

Concurrent Medication during last month

DRUG	DOSE	DATE COMMENCED

CLINICAL PHARMACOKINETICS LABORATORY
DEPARTMENT OF PHARMACOLOGY
UNIVERSITY OF DURBAN WESTVILLE
PRIVATE BAG X54001
DURBAN
4000

Telephone: 8202671 8202356

Table 11: Description of epileptic patients treated (n=14) and the results of therapeutic drug monitoring

PATIENT NO	SPECIES	AGE (yrs)	MASS (kg)	SEX	DOSE (mg)	SERUM CONC* (μg.ml^{-1})	CLINICAL STATE
1	MIN DOB	14	4.5	F	30	16.56	RESPONSIVE
2	PEKE	10	4	F	60	24.52	RESPONSIVE
3	PEKE X	12	4	F	15	17.76	RESPONSIVE
4	MALTESE	2	3.5	F	60	28.74	RESPONSIVE
5	POODLE	12	7	M	60	21.68	RESPONSIVE
6	PUG	6.5	14	F	45	29.74	RESPONSIVE
7	DAXIE	4	6	M	45	14.54	NON RESPONSIVE
	DAXIE	4	6	M	60	31.89	NON RESPONSIVE
8	DAXIE	10	8	F	30	17.79	NON RESPONSIVE
	DAXIE	10	8	F	60	32.3	NON RESPONSIVE
	DAXIE	10	8	F	90	48.64	NON RESPONSIVE
9	NDS DOG	–	8	F	210	74.57	RESPONSIVE
10	DOB	12	28	F	30	2.31	NON RESPONSIVE
	DOB	12	28	F	60	5.26	NON RESPONSIVE
11	MIN DOB	6	7	M	30	16.8	NON RESPONSIVE
	MIN DOB	6	7	M	60	26.87	NON RESPONSIVE
	MIN DOB	6	7	M	180	70.47	RESPONSIVE
12	ST BERNARD	3.5	80	M	300	23.16	RESPONSIVE
13	MALTESE X	14	5.5	F	30	12.64	RESPONSIVE
14	FOX T X	4.5	7	F	30	18.71	RESPONSIVE

* = measured serum concentration

Table 13: The prediction error between the predicted and
the observed concentration in the epileptic dogs

DOG NO	DOSE GIVEN (mg.kg^{-1})	PREDICTED CONC (µg.ml^{-1})	OBSERVED CONC (µg.ml^{-1})	PREDICTED ERROR
1	6.7	20.99	16.56	−4.43
2	15	46.99	24.52	−22.47
3	3.75	11.75	17.76	+6.01
4	17.1	53.57	28.74	−24.83
5	8.6	26.94	21.68	−5.26
6	3.2	10.02	29.74	+19.72
7	7.5	23.49	14.54	+8.95
8	10	31.33	31.89	+0.56
9	3.75	11.75	17.79	+6.04
10	7.5	23.50	32.3	+8.80
11	11.2	35.09	48.64	+13.55
12	26.2	82.08	74.57	−7.51
13	1.1	3.45	2.31	−1.14
14	22.2	6.89	5.26	−1.63
15	4.3	13.47	16.8	+3.33
16	8.6	26.94	26.87	−0.07
17	25.7	80.51	70.47	−10.04
18	3.75	11.75	23.16	+11.41
19	5.5	17.23	12.64	−4.59
20	4.3	13.47	18.71	+5.24

APPENDIX D

Front page/Voorblad:

Black-shouldered kite
with chicks (Courtesy
Information Services)

Contents ● Inhoud

THE PHARMACOKINETICS OF PHENOBARBITONE IN FASTING AND NON-FASTING DOGS

G D THURMAN*, M L McFADYEN** and R MILLER**

ABSTRACT

The pharmacokinetics of oral phenobarbitone was studied in 10 clinically healthy adult dogs. The drug was given once daily in tablet form, at a dose of 5 mg kg^{-1} of body mass. Serial venous blood samples (n = 9) were collected from each dog on Day 1 (the first day of drug dosing), on Day 22, and on Day 24 after continuous dosing. Trough serum concentrations were determined on Day 7, Day 14 and Day 21. The drug was administered to the dogs on an empty stomach, except on Day 24, when it was given with food, in order to assess the influence of food on its absorption. Drug serum concentrations were described by a one-compartmental open model with first order absorption and elimination. An average steady-state trough serum level of phenobarbitone of 52,96 ± 8,40 mmol ℓ^{-1} was achieved after 3 weeks of daily dosing. The mean elimination half-lives for Day 1 and Day 22 were 46,3 ± 11,3 h and 29,3 ± 4,6 h respectively. The area under the curve for Day 22 was 1 656,17 ± 186,45 μmol h^{-1} ℓ^{-1} and for Day 24 was 1 493,06 ± 205,4 μmol h^{-1} ℓ^{-1}. The mean clearance value for Day 22 was 0,0133 ± 0,0016 ℓ h^{-1} kg^{-1}. Side effects of polyphagia, polydipsia, sedation and ataxia were commonly observed in the first 2-9 d, but disappeared thereafter.

It was concluded that a dose of 5 mg kg^{-1} would achieve an average serum concentration of 64,59 μmol ℓ^{-1} in adult dogs. Once daily dosing should be adequate, since relatively small peak-through fluctuations were observed (27,6 μmol ℓ^{-1}). The dose may be given with food for convenience, even though the extent of absorption is approximately 10% less than when given on an empty stomach. If the 10% difference is considered clinically significant, the dose can be adjusted accordingly. Dosing should continue unchanged for at least 3 weeks to allow enzyme induction to settle and to achieve steady-state before adjusting doses based on serum concentrations.

Key words: Canine, epilepsy, pharmacokinetics, phenobarbitone.

INTRODUCTION

Seizure disorders are amongst the most frequently seen neurological problems in small animal practice. Seizures often present a diagnostic dilemma because they are episodic, frequently not observed by the veterinarian and often present with no other clinical abnormality. Seizures, epilepsy, fits or convulsions have been described as collective terms used to describe a disorder of brain function characterised by paroxysmal, stereotyped alterations in behaviour[3]. The term epilepsy indicates that the seizures are recurring, but does not imply the cause of the seizures. The most common form of epilepsy in the dog presents as a tonic-clonic seizure, with petit-mal and Jacksonian forms of seizures being rarely diagnosed[3].

Epileptic seizures in dogs may be controlled by a variety of drugs, the most common of which is phenobarbitone alone or in combination with other anticonvulsant drugs which is administered orally[7].

Barbiturates were the first class of drugs to be used for treatment of epilepsy. Phenobarbitone was first introduced as an effective anticonvulsant in 1912, providing therapeutic control against most varieties of seizures except absence (petit-mal) seizures[6].

In order to achieve a reasonable therapeutic effect without marked adverse effects in man, phenobarbitone plasma concentrations of 40 to 110 μmol ℓ^{-1} are considered necessary[7]. In order to achieve a therapeutic plasma level, the recommended oral maintenance dose of phenobarbitone in adults and adolescents is from 1,5 to 2 mg kg^{-1} per day[5]. Infants and children require approximately twice the adult dosage, or 3 to 4,5 mg kg^{-1} per day, because they clear the drug faster than adults[5]. Because of its long half-life in man, dosing once daily at bedtime is adequate in most patients. It requires 2-3 weeks (5 half-lives) for phenobarbitone to reach steady-state therapeutic levels in man.

The control of epileptic seizures in dogs is, however, not as well-described as is the case in man. The literature offers dosage ranges for dogs of 2 to 20 mg kg^{-1} phenobarbitone per day which may be divided into twice daily or even 3 times daily dosing, or given "to effect"[3,7]. As phenobarbitone requires only once daily administration in man because of its long half-life (50-120 hours), it would therefore appear that, in dogs, the clearance of phenobarbitone is more rapid than in humans.

The purpose of this study was to monitor and evaluate plasma serum phenobarbitone concentrations over a period of 3 weeks on daily dosing. The effect of food on the absorption of the drug was also investigated.

MATERIALS AND METHODS

Ten adult dogs of mixed breeds and sex were selected for the purpose of the drug trial (Table 1). All were short-haired and without excess body fat. Two weeks prior to commencement of the trial, the dogs

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**Department of Pharmacology, University of Durban-Westville

Table 1: Breed, sex, age, body mass and condition of experimental dogs

No.	Breed	Sex	Age (yrs)	Mass (kg)	Condition
1	Lab. X G.S.D.	M	4	24	lean
2	Mixed	M	9	17	lean
3	Mixed	M	1	15	lean
4	Fox Terrier X	M	2	24	lean
5	Bull Terrier	M	2	23	well-muscled
6	Mastiff X	M	7	23	lean
7	Bull Mastiff X	M	8	28	lean
8	Lab. X Bull Terrier	F	1	19	well-muscled
9	G.S.D.	F	2	22	lean
10	Doberman	M	8	27	lean

M = Male; F = Female; X = Mixed breed.

were examined and evaluated for tractability and clinical soundness. This involved recording the body mass of each animal, a thorough physical examination, and the collection of venous blood for haematological and biochemical screening (full blood count, plasma (P)-glucose, serum (S)-urea, serum (S)-creatinine, alanine transaminase (ALT), serum (S)-bilirubin, serum alkaline phosphatase (ALP), total serum proteins (S-TSP), serum (S)-albumin and serum (S)-globulins). Animals showing any deviation from the accepted norm, were excluded from the test group. The selected animals were inoculated and dewormed in accordance with standard veterinary preventive medicine and quarantined individually in kennels for a period of 2 weeks prior to the study. For the duration of the drug trial, the animals were provided with water ad lib. and were fed dry commercial, pelleted dog food daily at 15:00. Following the quarantine period, the dogs were again clinically examined for soundness and their mass was recorded.

Sodium phenobarbitone (PHB) (Lethyl, Lennon Laboratories, 30 mg per tablet) was administered orally in tablet form at a dosage rate of 5 mg kg⁻¹ of body mass per day. It was administered with 20 ml tap water to all the dogs on an empty stomach at 08:00 each day, until the last day of the trial. On Day 24, food was given directly after dosing, in order to evaluate the effects of food on drug absorption, as this would probably simulate the clinical situation.

On Day 1 (the first day of drug dosing), Day 22 (empty stomach) and Day 24 (with food), serial venous blood samples (n = 9) were withdrawn from the jugular vein, over a 24 h period following drug administration (i.e. at 0,5; 1; 2; 4; 8; 12; and 24 h after dosing). On Day 7, 14, and 21, one blood sample was withdrawn from the cephalic vein just prior to dosing (i.e. trough-level). All blood samples were withdrawn into sterile, dry 3 ml syringes

and placed into capped test tubes without anti-coagulant (Venoject-plain). The serum was separated by centrifugation at 1 500 rpm for 5 min at 20°C and then stored at -20°C until assayed for PHB concentration.

The animals were monitored daily for signs of poor health or side-effects, in particular for polyphagia, polydipsia, polyuria, sedation, ataxia, and hyperkinesis which have been reported in the literature[3 7].

The concentration of phenobarbitone in the serum samples was determined, using the TDx System which uses the fluorescence polarisation immunoassay (FPIA) technology (Abbot Laboratories Diagnostic Division, Irving Texas, United States of America).

The serum concentration time pairs for each dog were fitted for single dose, steady state without food, and steady state with food using nonlinear least squares regression (STATIS (Version 2.1) 1987 Aydesoft statistical and scientific software, Larkhall). A one- and a two-compartment open model were compared for effectiveness of fit by the residual sum of squares and Akaike's Information Criterion (AIC)[9]. Estimations of the absorption rate constant (K_A) and the elimination rate constant (K_E) were obtained.

The first-order elimination constant (K_E) was used to calculate the biological half-life using the following equation:

$$t^{1/2} = 0.693/K_E \qquad (1)$$

The area under the curve (AUC) was calculated by the linear trapezoidal rule. The total body clearance at steady state can be calculated in a model-independent fashion by using the following equation:

$$Cl = F \times dose/AUC \ (\ell \ hr^{-1} \ kg^{-1}) \qquad (2)$$

Analysis of variance was used on the half-life and AUC values in order to test variation between subjects and also between treatments (P < 0,05 taken to be significant).

RESULTS

A one-compartment open model was adequate to describe the data. Calculated pharmacokinetic values indicating the absorption, accumulation and excretion of PHB from Days 1, 22 and 24 are presented in Table 1 and Fig. 1. On Day 1 there was a fairly rapid absorption of PHB with average peak serum concentrations of 28.03 ± 2.84 µmol ℓ⁻¹ occurring 2 h after dosing. Absorption rate constants (Table 2) showed a large variation.

Serum levels measured on Day 22, showed a trough concentration of 52.96 ± 8.40 µmol ℓ⁻¹. Following drug administration, there was again a fairly rapid absorption with an average peak concentration of 80.52 ± 6.63 µmol ℓ⁻¹ attained within 2 to 4 h of dosing.

Following Day 1, there was a slow accumulation in the PHB plasma concentration followed by a decline to a stable trough level of 52.96 ± 8.18 µmol ℓ⁻¹ which occurred 22 d from commencement of initial dosing (Fig. 2).

The mean elimination half-life ($t^{1/2}$) for Day 1 was 46.3 ± 11.3 h and for Day 22 it was 29.3 ± 4.6 h. The results are given in Table 2. Dog 5, a bull terrier, could not be used in this comparison as there was doubt as to the dose it had received on Day 1. The results obtained were compared by two-way analysis of variance, which showed that there was no significant difference between subjects (P < 0.5), but there was a significant difference between Day 1 and Day 22 (P < 0.0066).

The area under the curve (AUC) is a measure of extent of drug absorption if clearance is constant. A difference of approximately 10% in the AUC was found when Day 22 and Day 24 were compared (P = 0.0496), suggesting that a lesser amount of the drug had been absorbed when given with the food (Table 2).

The mean clearance value for the sample group on Day 22, was 0.0133 ± 0.0016 ℓ h⁻¹ kg⁻¹.

The majority of dogs showed mild to moderate side-effects to the drug. The side-effects of polyphagia, polydipsia and sedation were initially observed from Day 2 in all test animals. In addition, 3 dogs showed hindquarter ataxia. All these side-effects, however, were of short duration and were no longer evident by Day 10. All haematological and biochemical parameters measured remained within normal physiological limits and no significant changes were recorded.

DISCUSSION

Phenobarbitone distributes into most body tissues fairly rapidly, permitting the

Table 2: Phenobarbitone pharmacokinetics in dogs given phenobarbitone once daily per os

	$t\frac{1}{2}$ h		K_A h^{-1}		K_E h^{-1}		AUC μmol h ℓ$^{-1}$		Cl/F ℓ h^{-1} kg^{-1}	ΔC_p μmol ℓ$^{-1}$
	Day 1	Day 22	Day 1	Day 22	Day 1	Day 22	Day 22	Day 24	Day 22	Day 22
MEAN	46.3	29.3	3.0085	0.9068	0.0156	0.0242	1656.17	1493.06	0.0133	27.60
SD	11.31	4.6	2.7085	0.7287	0.0032	0.0036	186.45	205.4	0.0016	7.49
CV%	24.4	15.7	90.02	80.35	20.5	14.9	11.3	13.8	12.0	27.18

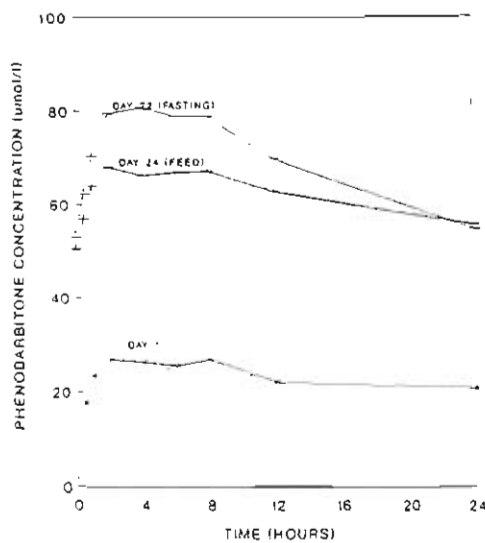ΔC_p = average peak - trough serum concentrations

Fig. 1: **Average serum phenobarbitone concentrations in dogs for the initial day of dosing (Day 1), 22 days after dosing (Day 22) and after the intake of food on Day 24**

plasma pharmacokinetics to be described adequately by a one-compartment open model. Absorption rate constants showed a 80-90% variation, but were within the ranges found in other studies[1]. The time required to reach peak levels after oral PHB, was between 2 to 4 h on Day 1, Day 22, and Day 24. It would therefore appear that the administration of food, together with the tablets, has little effect on rate of absorption. However, if one compares the extent of absorption using the AUC values, there is a statistically significant difference between administration with and without food. The lower AUC on Day 24 is unlikely to be the result of further enzyme induction and increased clearance, as trough levels on Days 21, 22 and 24 are similar. Thus, it would appear that the feeding of food together with the tablets, reduces the extent of PHB absorption by approximately 10%. Continuous dosing with food therefore, would be expected to produce serum level profiles, on average 10% lower, than if dosing were given on an empty stomach.

In general, a difference of less than 20% is considered to be bio-equivalent[3]. Therefore it is unlikely that feeding and simultaneously dosing the animals, will have a clinically significant effect on the control of epilepsy in the dog.

In this particular study, the mean elimination half-life of phenobarbitone sodium declined from 46.3 ± 11.6 h calculated for Day 1, to 29.3 ± 4.6 h after 3 weeks of daily oral dosing. It is assumed that this decline is due to microsomal enzyme induction[5-7]. The initial rise in trough levels from Days 1 to 7 and the decline and stabilisation by 3 weeks, supports this assumption. Both the initial and final half-lives in this study were considerably shorter than those reported in a single dose study by Pedersoli et al.[1], where the $t\frac{1}{2}$ for oral dosing was 72.3 ± 15.5 h. The half-life of 55.0 ± 15 h reported in another study[5] after 5 d of oral dosing, is comparable to the initial half-life reported in this study. Five d is probably not long enough for enzyme induction to reach a maximum. Another possible reason for the relatively short half-lives found in the present study could be the relatively short sampling interval of only 24 h after dosing, whereby distribution may still be operative. The other studies sampled over 288 and 131 h respectively[4,5]. Considerable intersubject variation in the half-life has also been reported[4,5] which was not as apparent in this study (coefficient of variation for Day 22 = 15.7%) possibly because of our larger numbers.

Steady-state is assumed to be close to 5 times the elimination $t\frac{1}{2}$. Using this to estimate the time necessary to reach steady-state in this study would have given a period of 6 or 9 d, utilising our calculated $t\frac{1}{2}$ for Day 1 and Day 22 respectively. Ravis et al[5]. in their study, assumed steady-state would be reached at approximately 11 d. However, in this study where dosing was continued over a longer period of time, trough levels only stabilised between 14 to 21 d, as indicated in Fig. 2.

It therefore is apparent that 3 weeks of daily dosing is required before enzyme induction is complete (14 d at least) and steady state at a constant clearance can be achieved.

Since phenobarbitone was not administered intravenously, it was not possible to estimate the relative bioavailability (F) of oral phenobarbitone. Clearance in this situation is therefore more correctly termed oral clearance, since it includes the oral bioavailability factor (Cl/F). In humans and dogs, bioavailability has been shown to be essentially complete (88-100%)[2,4]. Oral clearance (Cl/F) will therefore closely approximate total body clearance (Cl).

Clearance of phenobarbitone in dogs was calculated as 0.0133 ± 0.0016 ℓ h^{-1} kg^{-1} which is more than 4 times that for humans (0.0032 ℓ h^{-1} kg^{-1})[1]. Using oral clearance, the following equation can be used to calculate a dose for a desired concentration (C_p). [Note 0.232 is the conversion factor from mmol to mg]

$$\text{Dose} = \text{Cl/F} \times C_p \times \text{Dosing Interval} \times 0.232 \qquad (3)$$

Using the above equation, therefore, indicates that a dose of 5 mg kg^{-1} 24 h^{-1} should maintain an average serum concentration in the region of 65 μmol ℓ$^{-1}$ which is within the therapeutic range determined in man (40-110 μmol ℓ$^{-1}$)[1,3]. Further studies are being undertaken to determine the therapeutic range in epileptic dogs.

The small fluctuation in the serum concentration (ΔC_p) as shown in Table 2, combined with the relatively long $t\frac{1}{2}$ (29,3 ± 4.6 h) would support that once daily dosing is probably adequate to maintain reasonable therapeutic effects. This would then enhance owner compliance, thus hopefully achieving better epileptic control.

In conclusion, our recommendations subject to determination of the therapeutic range in dogs, are as follows:
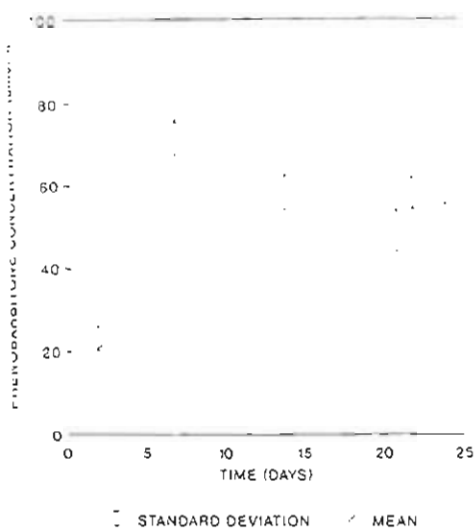
Fig. 2: **Average trough serum pheno-barbitone concentrations in dogs (n = 10) over 24 days**

1) An oral dose of 5 mg kg⁻¹ should give average serum PHB concentrations of 65 μmol ℓ^{-1} in adult dogs. Once daily dosing may be adequate since relatively small peak-trough fluctuations were observed.

2) The dose may be given with food, although the extent of absorption is approximately 10% less than when given on an empty stomach. This is probably of little clinical significance, especially if serum levels are monitored.

3) Side-effects of polyphagia, polydipsia, sedation and ataxia are commonly observed in the first 2-9 d but disappear thereafter. This is probably due to tolerance to the effects.

4) After initiation of therapy, dosing should continue unchanged for 3 weeks (steady-state) before measuring serum concentrations and adjusting the dose accordingly.

ACKNOWLEDGEMENTS

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