# THE PHARMACOKINETICS OF METHSUXIMIDE AND A MAJOR METABOLITE IN THE DOG AND MAN

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(Under the supervision of Assoc. Prof. Peter G. Welling.)

Methsuximide, a succinimide derivative, is useful in the treatment of petit mal epilepsy. One of its major biotransformation products is the N-demethylated metabolite, 2-methyl-2-phenylsuccinimide, which is pharmacologically active. Methsuximide induces its own metabolism by stimulation of hepatic drug metabolizing enzyme activity. The metabolite is cleared from the body at a slower rate than is parent drug. Little information exists on the pharmacokinetic behavior of this drug in experimental animals or man. The present studies were undertaken to provide this information.

The dog was used as an experimental model, for man, since the qualitative metabolic pattern of methsuximide is similar in the two species. Moreover, the intravenous route of administration can be used in, and the metabolite can be administered to, the dog. Each of three dogs received single oral, single intravenous, and multiple oral daily doses of methsuximide. Following chronic oral doses, each dog received a second intravenous dose of drug. Hepatic enzyme induction was assessed by comparison of the data from the two intravenous doses of drug. Each dog also

received single oral and intravenous doses of metabolite to compare the bioavailability of this compound from doses of metabolite and/or methsuximide. One dog received multiple oral daily doses of metabolite to determine the induction potential of this compound.

Two normal, male, human volunteers each received single and multiple oral doses of methsuximide in order to compare results in dogs with those in humans.

The results indicate that after oral doses, the drug is well absorbed but undergoes extensive first-pass metabolism. In dogs and humans, induction results in an increased total body clearance of not only parent drug, but also the metabolite. After chronic oral 300 mg doses of drug, to dog or man, essentially no methsuximide can be detected in plasma, the entire dose being eliminated by first-pass metabolism. There is a quantitative shift in the metabolic pattern, as a result of induction, in the dog and man. The fraction of the dose of parent drug which is biotransformed to the metabolite decreases in the dog, but increases in man, between single and multiple doses of drug. Hence, the dog is an adequate model for man in a qualitative, but not quantitative sense.

The metabolite induces hepatic drug metabolizing enzymes independent of the parent drug, and the time course of this induction is similar to that after multiple doses of methsuximide. The metabolite is rapidly and almost completely absorbed after oral doses. The absorption half-life

averaged 1.3 hours with ca. 84% of the dose being absorbed.

The relative bioavailability of the metabolite from doses of parent drug compared to that from doses of metabolite averaged 45% in dogs, after chronic doses. In man, the relative bioavailability may be greater than in dogs, but the absolute value can not be determined without knowledge of the distribution volume of the metabolite in man.

These results strongly suggest that the therapeutic activity of methsuximide is associated with the metabolite rather than unchanged drug.

APPROVED: Peter G. Welling

Peter G. Welling, Assoc. Prof.

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BY

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To Elizabeth Margaret

and

To Peggy

for their love

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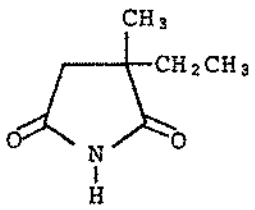
# I. INTRODUCTION

## A. Review of the Literature

In 1951, Miller and Long (1) introduced derivatives of succinimide of potential value in the treatment of petit mal epilepsy. In that same year, Chen et al. (2) confirmed the anticonvulsant activity of the α-phenylsuccinimide derivatives. The 2-phenyl, 2-methyl, and N-methyl groups on the succinimide ring increased the anticonvulsant activity while N-alkyl side chain lengths greater than methyl decreased activity. One derivative, without the N-alkyl group and with a 2-ethyl rather than 2-phenyl group, was highly effective in preliminary clinical studies (3,4). The anticonvulsant properties, toxicity (5), and metabolism (6) of the succinimides have been recently reviewed. Those succinimides currently in use in the United States as anti-epileptic agents are:

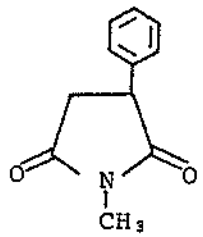
(±)-2-ethyl-2-methylsuccinimide
(ethosuximide, Zarontin®)[a]




(I)

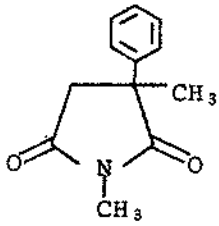
[a]Zarontin, Milontin, and Celontin are registered trademarks of Parke Davis and Company, Detroit, Mich. All drugs used in this thesis were the racemic mixtures.

(±)-N-methyl-2-phenylsuccinimide
(phensuximide, Milontin®)



(II)

(±)-N,2-dimethyl-2-phenylsuccinimide
(methsuximide, Celontin®)



(III)

There is little quantitative information on the metabolism, clinical pharmacology, or pharmacokinetics of the succinimides in experimental animals or man. While the original intent of this thesis was to examine the pharmacokinetics of the succinimides in general, it soon became apparent that the kinetics of methsuximide were complex and hence by limiting the investigation to this single compound, a more rigorous treatment of the subject could be realized.

Methsuximide has been shown to be well absorbed from the gastrointestinal (GI) tract of the rat, with absorption half-lives, $t_{1/2}^{abs}$, of 17.4 minutes and 52 minutes from the small intestine and stomach, respectively (7). Early studies on the tissue distribution of methsuximide in the rat are difficult to interpret due to the use of non-specific assays. After 100 mg $Kg^{-1}$ oral doses of methsuximide to rats (8), levels of drug were highest in body fat and heart

tissue, being, respectively, 10 and 6 times the level in plasma. Intermediate levels (3-4 times those in plasma) were found in liver, spleen, kidney, muscle, and brain tissues while levels of drug in plasma were lowest. The apparent biological half-life, $t_{1/2}$, of drug in plasma and in most tissues was ca. 2 hours. The decline in brain, liver, and lung drug concentrations was slower during the 8-24 hour period after dosing and this slower decline was attributed to the presence of a metabolite(s). In this study (8), the original colorimetric assay for methsuximide was used and parent drug could not be distinguished from metabolite(s). A similar distribution pattern was observed in rats when N-$^{14}CH_3$ labeled methsuximide was administered (7). Brain, spleen, heart, testes, lungs, salivary glands, and skeletal muscle had similar levels of total radioactivity through 6 hours after dose administration. The concentration of methsuximide in tissues at 60 minutes postdose were identical when determined by non-specific (liquid scintillation counting) and specific (gas chromatography) assays. The drug quickly traversed the blood-brain barrier but was also efficiently cleared from the brain (7). The onset of anticonvulsant activity paralleled the rise in the levels of methsuximide in blood and brain tissue. Peak activity and peak levels of drug both appeared at 60 minutes after dosing (7). The high concentrations of drug (or metabolites) in body fat reflects their lipophilic nature (7, 8). Since approximately 29% of the administered

dose of methsuximide appeared in expired air as $^{14}CO_2$ within 24 hours after dosing, it was suggested (7) that methsuximide was extensively N-demethylated to 2-methyl-2-phenylsuccinimide. This metabolite is very lipophilic and has a $pK_a$ of 8.52 (7). Since only 2.7% of the methsuximide dose was excreted into the 24 hour rat urine as the metabolite, while 29% was exhaled as $^{14}CO_2$ in the same time period (7), a long metabolite $t_{1/2}$ was expected. Rats which received oral 100 mg $Kg^{-1}$ doses of parent drug exhibited antileptazol (anticonvulsant) activity for up to six hours after dosing, yet there was little parent drug in the brain at this time. It was suggested that since the metabolite also possesses antileptazol activity (2,7,9), it may have been responsible for the extended pharmacological activity.[a]

A limited number of studies on the metabolism of methsuximide have appeared in the literature. Horning and coworkers (10) found N,2-dimethyl-2-(3,4-dihydroxy-1,5-cyclohexadien-1-yl)-succinimide, N,2-dimethyl-2-(4-hydroxyphenyl)-succinimide, N,2-dimethyl-2-(3-hydroxyphenyl)-succinimide, N-methyl-2-hydroxymethyl-2-phenylsuccinimide, two isomeric N,2-dimethyl-3-hydroxy-2-phenylsuccinimides, and a diol, presumably N,2-dimethyl-2-(4-hydroxyphenyl)-3-hydroxysuccinimide, as well as small amounts of parent drug in the urine of the rat, guinea pig, and man after oral

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[a] In this thesis, 'metabolite' refers to the pharmacologically active metabolite, 2-methyl-2-phenylsuccinimide, unless indicated otherwise.

doses of methsuximide. Assignment of structures was based on gas chromatography-mass spectroscopy (GC-MS) without the aid of pure reference standards and therefore the quantitative metabolic pattern of methsuximide could not be determined. Furthermore, the assay employed methylation and trimethylsilylation (TMS) and hence N-demethylated metabolites could not be distinguished from corresponding methylated metabolites. However, in a report describing the advantages of open tubular capillary gas chromatographic columns in drug metabolism research (11), the authors used ethylation and TMS in a GC assay to determine the metabolites of methsuximide in guinea pig urine. Importantly, for almost every N-methylated, hydroxylated metabolite found in urine, there was also the corresponding N-ethylated, hydroxylated metabolite. Hence, it appears that methsuximide and 2-methyl-2-phenylsuccinimide are metabolized by many of the same oxidative (hydroxylation) routes.

Dudley et al. (12) studied the metabolism of methsuximide in the dog and identified metabolites in the 48 hour urine by melting point determinations, mass spectroscopy, infrared (IR) and ultraviolet (UV) absorption spectroscopy, thin layer chromatography, and nuclear magnetic resonance (NMR) using pure synthesized metabolites as reference standards. Between 7 and 20% of the dose was recovered as 2-(p-hydroxyphenyl)-2-methylsuccinimide and N-methyl-2-(p-hydroxyphenyl)-2-methylsuccinimide after a one or two gram

dose of methsuximide. The former was the primary para-hydroxylated metabolite. Only small amounts of the N-demethylated compound were recovered from urine as was the case when 2-methyl-2-phenylsuccinimide itself was administered. No unchanged methsuximide was recovered and the authors concluded that the N-demethylation reaction is probably the primary biotransformation route for methsuximide.

After an oral 148 or 218 mg $Kg^{-1}$ dose of methsuximide to dogs (8), the peak levels of drug in plasma were 26 and 36 µg $ml^{-1}$, respectively, with an elimination $t_{1/2}$ of 3 hours. Glazko and Dill (13) reported methsuximide levels in plasma in twelve normal adult male humans at two oral, single dose levels of 0.6 g and 1.2 g in a study of cross-over design. Mean peak plasma levels of 3.1 $\pm$ 0.5 and 6.8 $\pm$ 0.6 (standard error) µg $ml^{-1}$ occurred at one hour after dose administration at the respective two dose levels. The mean plasma $t_{1/2}$ with both doses was 2.6 hours over the 1-12 hour post-dose period. The experiment was repeated at weekly intervals for four weeks and the plasma $t_{1/2}$ values during the first week were 75% greater than those during the fourth week. This was interpreted in terms of induction of hepatic drug metabolizing enzymes. Recent evidence has confirmed this interpretation since administration of I, II, or III for three days to the rat resulted in increases in the oxidation of hexobarbitone, hydroxylation of aniline, liver:body weight ratio, hepatic microsomal

cytochrome P-450, hepatic δ-aminolaevulinic acid synthetase and a reduction in hexobarbitone-induced hypnosis (14). These investigators also noted a decreased anticonvulsant activity after three days of administration of methsuximide to the rat. Although the anticonvulsant activity of the metabolite is at least one-half that of the parent drug (1, 2), it is active for up to six hours after oral metabolite administration to the rat (2).

In a group of five rats given methsuximide, 100 mg $Kg^{-1}$ intraperitoneally, at 0, 16, and 40 hours, and sacrificed 24 hours later, the mean liver weights were increased 11% over control (8). The N-demethylase activity of the 9000 x g supernatant of liver homogenates, determined by incubation with N-methylaniline, was increased with a 78% increase in enzyme activity and a 20% increase in hydroxylase activity (aniline as substrate) over control values. In another group of rats given daily, oral 100 mg $Kg^{-1}$ doses of methsuximide for four days (8), the concentrations of drug in liver and lung 2 hours post-dosing were 10 μg $g^{-1}$ compared to 70-90 μg $g^{-1}$ in single dose experiments. Plasma levels of drug were reduced from 23-28 μg $ml^{-1}$ to 1 μg $ml^{-1}$, between single and multiple doses of drug, with other tissues having very low concentrations of drug. The authors concluded that methsuximide may have increased the rate of its own metabolism (8).

The N-demethylated derivative has been identified as a major metabolite in both rabbit and human plasma after oral doses of methsuximide (15). The parent drug was rapidly

metabolized by the rabbit and within 30 minutes after dosing, the level of metabolite in plasma was much higher than that of the parent drug. The plasma of 17 patients on chronic methsuximide therapy was analyzed for methsuximide and metabolite content using the sensitive quadrupole mass fragmentography method (16). The metabolite levels in plasma averaged 700 times those of the parent drug and concentrations of metabolite in plasma of less than 10 $\mu g\ ml^{-1}$ were judged ineffective in seizure control while levels in excess of 40 $\mu g\ ml^{-1}$ were toxic. It is not clear whether this situation is secondary to auto-induction of hepatic enzymes since 16 of the 17 patients were on other drugs known to be inducers of hepatic enzymes. Importantly, this study also showed that the steady-state metabolite level in plasma ($\mu g\ ml^{-1}$) correlated well ($r = +0.6$) with the dose of methsuximide ($mg\ Kg^{-1}$) administered, the steady-state level being 1.62 times the dose over a dose range of 5-60 $mg\ Kg^{-1}$. Hence, the metabolite is eliminated by a first-order process(es) and no saturation of elimination routes occurs over a wide range of methsuximide doses. The plasma $t_{1/2}$ of the metabolite in humans has only recently been reported (17). In a cross-over design study from phensuximide to methsuximide with five patients on a daily 3 gram oral dose, the average $t_{1/2}$ of methsuximide was 1.4 hours (range 1.0-2.2 hours) while that of the metabolite was 38 hours. The metabolite accumulated to levels in excess of 40 $\mu g\ ml^{-1}$ in plasma (17). The significance of these $t_{1/2}$

values in terms of enzyme induction is not clear since phensuximide, a known inducer (14), was administered to these patients immediately prior to the methsuximide study. Hence, these $t_{1/2}$ values may represent the half-lives of methsuximide and metabolite in the hepatic enzyme induced state. In any event, as in the dog (18), the metabolite is rapidly formed and is persistent in the body relative to the parent drug. Since the metabolite is pharmacologically active it probably accounts for most if not all of the therapeutic activity of methsuximide (16,17).

In 1973 Karch reported an overdose case where an 18 year old female ingested about 10 grams of methsuximide (19). Sixteen hours postingestion, plasma levels of drug and metabolite were 18 and 44 $\mu g\ ml^{-1}$, respectively. At 64 hours postingestion, methsuximide levels had declined to 2 $\mu g\ ml^{-1}$ whereas the metabolite levels were only slightly lowered at 38 $\mu g\ ml^{-1}$. The clinical course was biphasic in nature with initial lethargy, from which the patient was easily aroused, followed by a period of improvement. However, this phase was followed by a period of extended, profound coma. Since the $t_{1/2}$ of methsuximide is less than 3 hours the coma is unlikely to have been due to the parent drug and was attributed to the metabolite, 2-methyl-2-phenylsuccinimide. The biphasic toxicity pattern experienced with this drug is potentially dangerous and close and constant scrutiny by the clinician is required in the treatment of methsuximide overdose victims. This case

report emphasizes the need for pharmacokinetic information, not only on the drug but also on the metabolite.

B. Statement of the Problem

While there is a considerable body of knowledge on the qualitative metabolic and tissue disposition of methsuximide in experimental animals, there is little information, other than $t_{1/2}$ values, on the pharmacokinetics of this compound or its active metabolite. What little information is available is often complicated, especially in humans, by the presence of other drugs. In earlier work on methsuximide by the author (18,20) it was established that the parent drug and the metabolite obey two- and one-compartment open model kinetics respectively after separate intravenous doses of the two compounds to the dog. Importantly, the distribution volumes of methsuximide and metabolite are similar (in dogs) so that the relative concentrations of each after doses of methsuximide may be considered to reflect the relative amounts of the compounds in the body at any time. Moreover, it was established that N-demethylation is an important metabolic step in the dog since approximately 40% ($f_m$) of the methsuximide dose was converted to the metabolite after single intravenous doses of the parent compound.

Many questions remain to be answered. For any substance which is extensively metabolized and has a short biological half-life, it is anticipated that only a

fraction of the absorbed dose of drug should reach the systemic circulation after an oral dose; i.e., a decreased bioavailability relative to an intravenous dose. This effect, called 'first pass metabolism', is due to the fact that the drug, after oral doses, must pass through the liver before it can reach the general circulation. Hence, it can undergo metabolism before reaching and being detected in the general circulation. The magnitude of first pass metabolism of methsuximide is unknown. If hepatic drug metabolizing enzymes are induced as a result of prior exposure of the body to methsuximide, then the bioavailability of parent drug would ostensibly decrease between single and multiple doses of drug. Perhaps more importantly, it is not known what effect first pass metabolism and enzyme induction have on the bioavailability of the pharmacologically active metabolite from doses of the parent drug. Similarly, there is no data on the time course of induction or what specific compound(s) (methsuximide or a metabolite?) causes induction after doses of methsuximide.

In light of first pass metabolism and enzyme induction it may be appropriate to examine the bioavailability characteristics of the metabolite as a drug of its own right. For the same reasons, it appears valid to question the meaningfulness of single dose studies in defining the bioavailability or kinetics of this compound since it is used clinically on a chronic, long-term basis.

Finally, the advantages and disadvantages of using the dog as an experimental model for humans need to be examined.

This thesis is the result of an in-depth, systematic analysis of the pharmacokinetics of methsuximide and its major metabolite in the dog and man. It is hoped that many of the above questions will be answered and thereby lead to a better understanding of how the body handles these compounds during chronic therapy and more rational therapy with this anticonvulsant agent.

# II. EXPERIMENTAL

## A. Materials and Subjects

Pure 5 gram samples of methsuximide and phensuximide were obtained from Parke-Davis and Company, Detroit, Mich.; the former drug was used in all methsuximide intravenous dose studies and as a standard, while the latter drug was used as an external standard in the assay. Methsuximide capsules 300 mg (Celontin Kapseals® 0.3 gram, Parke-Davis and Company, Detroit, Mich., lot number RM140) were obtained commercially and were used in all oral dosing studies. The N-demethylated metabolite of methsuximide, 2-methyl-2-phenylsuccinimide, was obtained from Aldrich Chemical Company, Milwaukee, Wisc. on two occasions: a) 1.5 grams, Custom-Synthesis, lot number 112837, m.p. 83-84° C; and, b) 6.0 grams in 1.0 gram amber vials, lot number 011367, m.p. 83-85° C, 99% purity. The metabolite was used as supplied to prepare all intravenous doses, and also as a chemical standard for assays. In all oral experiments it was packed undiluted into number 1 hard shell gelatin red/yellow capsules with a final weight of 299.0-301.0 mg of metabolite per capsule (Mettler Balance, Mettler Instrument Corp., Hightstown, N.J.). All administered drugs were racemic mixtures. All drugs were stored in the dark in an evacuated dessicator over anhydrous calcium sulfate (Drierite, W. A. Hammond Drierite Company, Xenia, Ohio), at ambient temperature.

Water was doubly distilled from alkaline permanganate in an all glass distillation apparatus. Normal saline solution and sodium heparin were obtained from University Hospitals, Madison, Wisc. All other chemicals used in these studies were either reagent or analytical grade and were used as received.

Three healthy, male, mongrel dogs were obtained from the Animal Care Center, University of Wisconsin, Madison, Wisc. The dogs were between one and two years of age and had received a complete veterinary medical examination prior to being released for research purposes. Dog A weighed 17.5 Kg, Dog B 18.6 Kg, and Dog C 13.8 Kg. Weights of the dogs varied slightly over the time span of these experiments, therefore the weight of each animal during each experiment is included in the raw data tables. The dogs were housed in a light and temperature controlled facility within the School of Pharmacy. The cages were constructed of metal, the floors of which were lined with absorbant papers so that the housing facilities were completely devoid of any hepatic enzyme inducing agents (i.e., cedar wood chip bedding (21-23)). The dogs had free access to water at all times but food (Purina Dog Chow) was withheld 12 hours prior to and 4 hours after dose administration in each experiment. In all multiple dose experiments this feeding routine was begun the week before commencement of a study in order to accustom the animals to eat during the remaining 8 hour period of the day.

Two healthy, male, human volunteers were administered methsuximide capsules 300 mg in a single and short-term multiple dose study. Both subjects underwent complete physical and laboratory examinations by a qualified physician at the Veteran's Administration Hospital, Madison, Wisc. The laboratory portion included a CBC, urinalysis and blood chemistry analysis (SMA-12 and SMA-4). Both subjects were deemed in excellent health including normal renal and hepatic functions. Informed consent was obtained from both subjects and this form is included for reference in Appendix A. Subject A was 28 years old, 89.1 Kg, and was 188 cm tall. Subject B was 45 years old, weighed 80.9 Kg and was 180 cm tall. After an overnight fast, subjects received the methsuximide dose of 300 mg with 250 ml of water. Intake of food and liquids was permitted 4 hours after dose administration. In the multiple dose study these restrictions were followed only on the last day of the study when blood samples were drawn. No known hepatic microsomal enzyme inducing agents or drugs other than methsuximide were ingested by the subjects one month prior to and during the studies. However, both subjects smoked and this activity was not restricted.

B. Methods

1. Methsuximide dissolution studies - Celontin Kapseals®. The dissolution of commercial 300 mg methsuximide capsules was determined using the standard U.S.P.

dissolution rotating basket apparatus. One capsule was placed in the basket and rotated at 100 rpm in 900 ml of 0.1 N hydrochloric acid. The temperature of the dissolution media was pre-equilibrated to 37.0 $\pm$ 0.5° C and controlled throughout each experiment by immersion of the vessel in a thermostatically controlled water bath. One ml samples of the dissolution media were rapidly withdrawn by a pipet fitted with a glass wool filter at 5.0 minute intervals over 90 minutes. These samples were then diluted with water to an appropriate concentration and analyzed, immediately at the end of the experiment, by gas chromatography.

2. Dog studies. During each experiment, the dog was placed in a restraining apparatus as previously described (18,20) for the initial period of the experiment, usually 2 hours, when frequent blood samples were drawn.

In experiments where the intravenous route of administration was used, two vein infusion sets (Miniset® Vein Infusion Set, Travenol Labs., Deerfield, Ill.) were positioned, one in each of the two front limbs. These sets were kept patent by infusing into them about 1 ml of heparin sodium solution 10 units/ml of normal saline every 15-30 minutes. One set was used to rapidly administer (30-60 seconds) the drug solution while the other was used to collect blood samples. The drug injection set was removed immediately after dose administration. In all dog studies

where the oral route of administration was used, only the blood collection set was positioned in a front leg. In the intravenous methsuximide experiments which immediately followed the multiple oral dose experiments it was essential to be able to draw multiple blood samples rapidly and efficiently, or risk repitition of the entire multiple dose study. In order to do this, a teflon catheter placement unit (Cathlon IV®, Jelco Labs, Raritan, N.J.) was positioned into a rear limb vein. This unit is a needle (18 gauge, 2 inches) with a teflon sheath around it. The entire unit was placed in the vein by direct venipuncture and the needle was withdrawn leaving the teflon tube in the vein. A three-way teflon stopcock was placed on the end of this teflon tube and the assembly securely taped to the leg. The insertion of this unit causes slightly more discomfort to the dog than does the vein infusion set. However, it assures that blood samples can be drawn rapidly when needed since the flexibility of the teflon sheath in the vein prevents its inadvertant removal due to movement of the animal. When used, this apparatus remained patent for over 2 hours with only infrequent infusions of heparin sodium solution. Regardless of the type of blood drawing equipment used, it was removed after two hours and the dog was freed of restraints. After this time, blood samples were drawn by separate venipunctures using a vein infusion set. Blood samples were drawn from any one of the four legs and the venipuncture sites were carefully managed to

insure proper wound healing.

Occasionally, blood samples were collected directly into an all glass syringe. There were no differences in the concentrations of drug or metabolite in plasma when blood was drawn through the infusion sets or into an all glass syringe (24).

The initial blood samples during each experiment were drawn using the following procedure: a) 3-5 ml of heparin diluted blood was withdrawn via the infusion set and put aside; b) 3.5-5.0 ml of blood was drawn over 5-15 seconds; c) 3.5-5.0 ml of sterile normal saline solution was injected; and, d) the heparin diluted blood was reinjected followed by 0.5 ml of heparin saline solution 10 units/ml. When blood was drawn by separate venipuncture, steps 'a' and 'd' were eliminated. During the first one hour of all experiments using the intravenous route of administration, blood samples were timed using an electronic timer started at the mid-point of the drug injection period. All other samples were drawn within a minute of the designated time. Sample times varied between each experiment and are listed in the appropriate tables in 'Results'.

Due to the limited aqueous solubility of methsuximide (25) (2.8 mg $ml^{-1}$, pH 7.0, 25° C) the intravenous dosage form consisted of 300 mg of methsuximide dissolved in 5.0 ml of an aqueous solution of 75% propylene glycol. For consistency, the metabolite was also administered in this dosage form except that one to two drops of 10% sodium

hydroxide solution was added to decrease the dissolution time (i.e., $pK_a$ of metabolite is 8.52 (7)). The drug solutions were visually clear and were used without sterilization. Each solution was prepared immediately before use and warmed to about 37° C before injection in order to prevent local vasoconstriction (26). The 5.0 ml injection was accomplished in approximately 30-60 seconds and was followed immediately by a ca. 20 ml rinse of warmed normal saline solution.

In all oral dose dog experiments, the capsule was manually placed on the posterior portion of the tongue and ingestion was facilitated by administration of 20 ml of tap water using an oral syringe accompanied by external throat massage.

All blood samples were immediately centrifuged for plasma and deep frozen (-20° C) until assayed for methsuximide and/or metabolite, usually within one week.

3. Human studies. Blood samples (10 ml) were withdrawn from a forearm vein into heparinized vacuum tubes (Vacutainers, green stoppered 10 ml, Bectin-Dickinson, Rutherford, N.J.) by a registered nurse or medical technologist. Plasma was obtained by centrifugation and was deep frozen (-20° C) until assayed. The times of blood sample collection are given in Tables XXII-XXIII, pages 105 and 112.

C. Assay

1. Procedures. The assay was a modification of that of Kinkel et al. (8). One ml samples of plasma, or of blank plasma containing known quantities of methsuximide and/or metabolite, were transfered by pipet into 13 ml glass stoppered centrifuge tubes and acidified with 0.5 ml of 0.5 N hydrochloric acid. Five ml of chloroform was added to each tube and the tubes were shaken horizontally (2 oscillations per second) on a flat-bed shaker for 15 min. The tubes were centrifuged at 2300 rpm (1000 g) for 15 minutes or until clean phase separation occurred. The aqueous layers were aspirated and 4.0 ml aliquots of the clear chloroform layers were transferred by pipet to clean, dry 13 ml screw cap centrifuge tubes. One or two ml, depending on the expected drug concentrations in the samples, of phensuximide solution, 1.0 µg/ml of chloroform, were added to the chloroform aliquots as an external standard. The chloroform was carefully evaporated to dryness in a $40^0$ C water bath under a gentle stream of nitrogen. Since drug loss has been reported due to this evaporation to dryness step (8), the last 0.1-0.2 ml of chloroform were evaporated to dryness outside the water bath. Thus, the temperature was allowed to decrease and this apparently prevents drug from volatilizing since no compound loss was observed. The residues were immediately redissolved in ca. 100 µl of carbon disulfide, mixed well using a vortex type shaker, and the samples were analyzed for methsuximide

and/or metabolite by gas chromatography on the same day. Each set of plasma sample unknowns was run with at least one sample of blank plasma containing known quantities of methsuximide and/or metabolite. The latter sample served as a check on the reproducibility of the GC response-concentration curves constructed at an earlier time.

The gas chromatograph used was a programmable dual column instrument equipped with dual flame ionization (FID) detectors (Hewlett-Packard, Model 5736A, Rolling Meadows, Ill.). A 183 cm (6 ft.) long, 2 mm internal diameter coiled glass column was silanized with 15% trichlorosilane in pyridine for 30 minutes, washed with acetone, dried, then packed with 3% OV-17 on Gas Chrome Q 100/120 mesh (Applied Science Labs., State College, Penn.). Silanized glass wool plugs were used to stopper both ends of the column. The column was initially conditioned at 210$^0$ C for 100 hours and overnight after assay of each series of plasma sample extracts.

The carrier gas was nitrogen flowing at 20 ml min$^{-1}$ while the flame gases were hydrogen and compressed air flowing at 30 and 240 ml min$^{-1}$, respectively. Flow rates of all gases were adjusted for maximum efficiency as previously described (18,20). The electrometer was set at a range setting of one with the attenuator varying between 8 and 512 depending on sample concentrations. The GC was operated in the single column mode at an isothermal column temperature of 180$^0$ C with the detector and the injection

port temperatures both at 250° C. Samples of 3 µl volume were introduced using the on-column injection technique with a 10 µl syringe (Hamilton Syringe, Hamilton Company, Reno, Nev.). The recorder (Perkin-Elmer, Model 023, Norwalk, Conn.) was used at zero recorder attenuation with the chart speed set at one cm $min^{-1}$. Under these instrument conditions the assay was sensitive to plasma compound concentrations of less than 0.10 µg $ml^{-1}$ of plasma. However, since the standard curves were constructed using 0.10 µg $ml^{-1}$ as the lowest concentration, this was considered the lowest detectable limit with lower instrument responses considered as 'trace' concentrations.

2. Standard curves. Compound recovery from plasma was previously reported to be 92 ± 3% for methsuximide and 88 ± 5% (standard deviation) for the metabolite (18,20). Peak height ratios (compound:external standard) were used as the measure of instrument detector response to injected compounds (18,20). Standard curves of response vs. concentration were constructed from the assay results of blank plasma samples containing added known quantities of drug and/or metabolite at 17 concentrations ranging from 0.10 to 50.0 µg $ml^{-1}$. Standard curves were done in triplicate on separate days. The regression equations of peak height ratio (y) vs. concentration (x) were: a) methsuximide, $y = 0.974x + 0.015$, $n = 51$, $r = +0.9996$, $P < 0.001$; b) metabolite, $y = 0.676x - 0.035$, $n = 51$, $r = +0.9988$, $P < 0.001$.

D. Summary of Experiments

1. Dog studies

a. Summary of experiments. Evidence will be presented in these studies suggesting that the metabolite (and possibly methsuximide) is an hepatic microsomal enzyme inducer. Induction is a time dependent phenomenon, thus it will be necessary to account for the time interval between any two doses of drug administered to the same dog regardless of whether the experiment was successfully completed or not. In several instances, doses were administered and for technical reasons, usually associated with blood sampling problems, the study was not completed. Each administered dose of drug is assigned a number referred to as a 'time index' (TI). To find the length of time in days between the administration of any two doses to the same dog one simply calculates the difference between the two TI's ($\Delta$TI) assigned to the doses.

The experiments conducted in each dog in ascending order of TI are summarized in the following tables:

Table I. Summary of Experiments in Dog A Including Dose, Dosage Regimen, and Time Index.

| Drug | Route[a] | Dose | Regimen | TI |
|---|---|---|---|---|
| methsuximide | po | 300 mg | single dose[b] | 1 |
| methsuximide | po | 300 mg | single dose | 7 |
| methsuximide | po | 300 mg | single dose | 39 |
| methsuximide | iv | 300 mg | single dose | 89 |
| methsuximide | po | 300 mg | multiple doses[c] | 117 thru 130 |
| methsuximide | iv | 300 mg | single dose | 131 |
| metabolite | iv | 300 mg | single dose | 192 |
| metabolite | po | 300 mg | single dose | 288 |

[a] Administration via the oral (po) or intravenous (iv) route.

[b] Study aborted after dose was administered.

[c] Dose administered once every 24 hours for 14 days.

Table II. Summary of Experiments in Dog B Including Dose, Dosage Regimen, and Time Index.

| Drug | Route[a] | Dose | Regimen | TI |
|---|---|---|---|---|
| methsuximide | po | 300 mg | single dose | 43 |
| methsuximide | po | 300 mg | single dose[b] | 75 |
| methsuximide | po | 300 mg | single dose | 84 |
| methsuximide | iv | 300 mg | single dose | 139 |
| methsuximide | po | 300 mg | multiple doses[c] | 160 thru 173 |
| methsuximide | iv | 300 mg | single dose | 174 |
| metabolite | iv | 300 mg | single dose | 226 |
| metabolite | po | 300 mg | single dose | 282 |
| metabolite | po | 300 mg | multiple doses[d] | 300 thru 309 |

[a]Administration via the oral (po) or intravenous (iv) route.

[b]Study was aborted after dose was administered.

[c]Dose administered every 24 hours for 14 days.

[d]Dose administered every 24 hours for 10 days.

Table III. Summary of Experiments in Dog C Including Dose, Dosage Regimen, and Time Index.

| Drug | Route[a] | Dose | Regimen | TI |
|---|---|---|---|---|
| methsuximide | po | 300 mg | single dose[b] | 70 |
| methsuximide | po | 300 mg | single dose | 82 |
| methsuximide | iv | 300 mg | single dose[b] | 153 |
| methsuximide | iv | 265 mg[c] | single dose | 183 |
| methsuximide | po | 300 mg | multiple doses[d] | 258 thru 271 |
| methsuximide | iv | 300 mg | single dose | 272 |
| metabolite | iv | 300 mg | single dose | 279 |
| metabolite | iv | 300 mg | single dose | 329 |
| metabolite | po | 300 mg | single dose | 352 |

[a] Administration via the oral (po) or intravenous (iv) route.

[b] Study was aborted after dose was administered.

[c] Drug injection set was pulled out of vein by dog before dose was completely injected. GC analysis of the remaining quantity of drug not administered indicated that 265 mg was actually given.

[d] Dose administered every 24 hours for 14 days.

b. Rationale. Since methsuximide is highly metabolized by the liver, is rapidly cleared from the body (18, 20), and reportedly stimulates its own metabolism by induction of hepatic microsomal enzymes (14), it was desirable to examine the change in kinetics of this compound and the resultant metabolite after single and multiple oral dose administration. Thus, each dog received at least one single oral dose of methsuximide and a multiple oral dose regimen of 0.3 g of drug every day for 14 days. In this latter study, blood samples were collected on days 1, 3, 5, 8, and 10. It was anticipated, with the low dose used and the expected high degree of first-pass metabolism, that after multiple oral doses the parent drug may not be detected over a long enough period of time to be able to accurately calculate its half-life or its clearance from the body. Thus, on the 15th day of the multiple oral dose study, an intravenous dose of methsuximide was administered. This then assures high blood levels of methsuximide since there can be no first-pass metabolism after intravenous injection into a peripheral vein. The kinetics of methsuximide and the resultant metabolite, administered at this time, could be compared to the kinetics obtained after a single intravenous injection (i.e., in the non-induced state).

The distribution volume of the metabolite was required in some calculations so that each dog received an intravenous dose of the pure metabolite as a single dose study.

Each dog also received a single oral dose of pure crystalline metabolite (in a capsule) to determine its bioavailability potential.

Additionally, one dog received an intravenous dose of metabolite near the end of the multiple oral methsuximide study to examine any change in its distribution volume as a consequence of hepatic enzyme induction. One dog participated in a multiple oral metabolite dose study consisting of once a day administration for 10 days with blood sampling on days 1, 3, 5, 8, and 10. The purpose of this study was to examine the possibility that the metabolite itself may be responsible for the stimulation of hepatic microsomal enzymes observed after methsuximide dose administration.

2. Human studies. The human studies consisted of a single oral dose of 300 mg of methsuximide in the two subjects followed by a multiple dose study with oral dose administration of 300 mg of methsuximide for seven days with blood sampling commencing on the seventh day. The ΔTI between the single and multiple dose studies were 114 and 49 for subjects A and B, respectively.

# III. RESULTS

## A. Dissolution of Methsuximide from Commercial Capsules

The dissolution rate of a drug from a hard shell gelatin capsule is a complex phenomenon dependent upon many factors including dissolution of the gelatin capsule, penetration of the dissolution medium into the powder plug, separation of the powder plug, and dissolution of the drug particles (27). Moreover, Celontin Kapseals®, in addition to the pure drug contain cornstarch, glyceryl mono-oleate, and polyethylene glycol.[a] An in-depth analysis of the contributions of each of these factors is beyond the scope of this thesis. However, under carefully controlled conditions, the dissolution half-lives of methsuximide capsules can be estimated.

The approach used here does not necessarily simulate the *in vivo* situation but rather provides *in vitro* information useful in assessing the potential for good absorbability of the drug. For instance, Kaplan (28) suggested that a sharp and early peak in an oral blood level-time profile of a drug with a reduced area under the curve, relative to the intravenous route of administration, may be falsely attributed to poor absorbability. If the *in vitro* results indicate rapid dissolution and high permeability

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[a]Product Analytical Review (PAR); Parke, Davis and Company, Detroit, Michigan.

characteristics, then the reduced availability (area under the curve) of the orally administered drug may be due to physiological* (i.e., first-pass metabolism) and not physiochemical* factors.

In these experiments virtual sink conditions prevailed since the final concentration of drug in the dissolution medium (300 mg/900 ml = 0.33 mg/ml) was less than 15% of the aqueous solubility of methsuximide (2.8 mg $ml^{-1}$). The dissolution half-lives ($t_{1/2}$) of five Celontin Kapseals$^{R}$ 0.3 gram were calculated from the slopes, obtained by linear regression analysis, of the logarithm of the amounts of drug remaining to be dissolved ($A_\infty - A$) versus time (t) profiles. The amount undissolved-time data are tabulated in Table IV. Figure 1 is the plot of the data from capsule 3 and represents a typical plot of $\log(A_\infty - A)$ vs. t. Table V gives the parameters from the linear regression analysis of the data for each capsule.

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*See Table I in reference 43 for additional factors.

Table IV. Summary of Methsuximide Capsule Dissolution Test Results ($A_\infty$ - A) vs. t. $A_\infty$ is the Amount of Drug Dissolved at Infinite Time (300 mg) and A is Amount Dissolved (mg) at any Time t (min).

| t (min) | ($A_\infty$ - A) (mg) | | | | |
|---|---|---|---|---|---|
| | Capsule no. | | | | |
| | 1 | 2 | 3 | 4 | 5 |
| 5 | 233 | 261 | 271 | 256 | 201 |
| 10 | 163 | 140 | 233 | 190 | 99 |
| 15 | 88 | 86 | 190 | 121 | 50 |
| 20 | 38 | 57 | 150 | 74 | 23 |
| 25 | 33 | 46 | 118 | 52 | 10 |
| 30 | 25 | 35 | 91 | 33 | ---[a] |
| 35 | 17 | 25 | 70 | --- | --- |
| 40 | 14 | 9 | 62 | --- | --- |
| 45 | --- | 5 | 43 | --- | --- |
| 50 | --- | --- | 38 | --- | --- |

[a]Not determined.

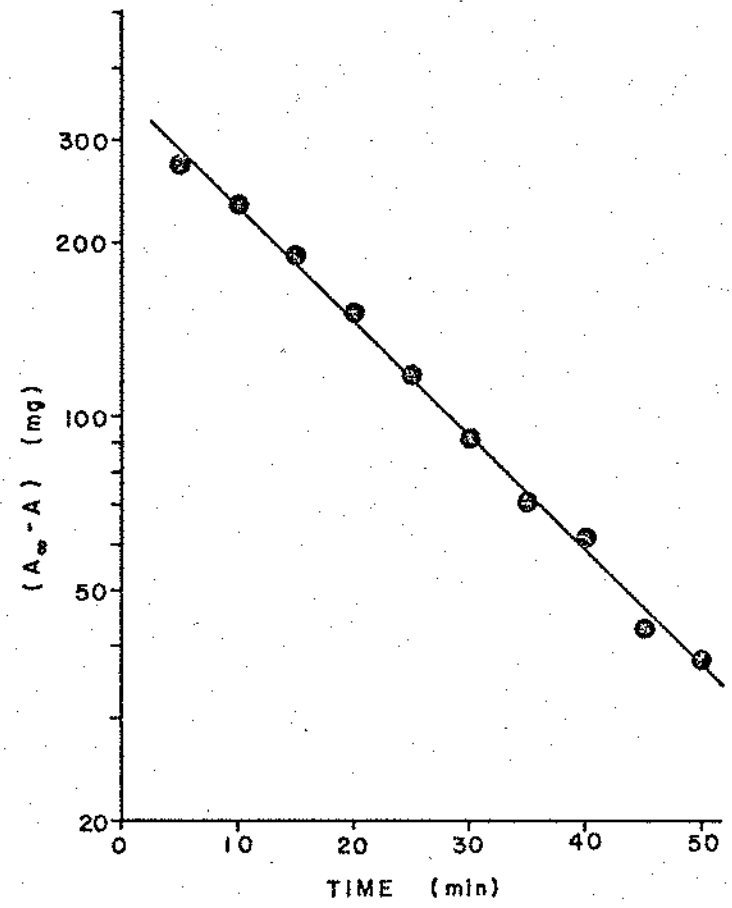



Figure 1. Plot of $\log(A_\infty - A)$ vs. t for Celontin Kapseal® Number 3.

Table V. Parameters of Linear Regression Analysis {$\ln(A_\infty - A)$ *vs.* t} on Methsuximide Capsule Dissolution Test Results.

| Capsule No. | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| r[a] | -0.9786 | -0.9820 | -0.9978 | -0.9994 | -0.9990 |
| slope ($min^{-1}$) | -0.0831 | -0.0905 | -0.0458 | -0.0865 | -0.1498 |
| EXP (intercept)[b] | 306 | 392 | 362 | 438 | 442 |
| $t_{1/2}$ (min)[c] | 8.3 | 7.7 | 15.1 | 8.0 | 4.6 |

[a]Correlation coefficient for regression.

[b]The base 'e' antilogarithm of the intercept.

[c]$t_{1/2} = 0.693/\text{slope}$.

It should be noted that calculation of $A_\infty$ from the values of the intercepts does not yield a value of 300 mg in any of the five cases studied. The theoretical basis for this observation has recently been delineated (29). The intercept is not only related to the amount remaining to be dissolved at time zero, but also to the length of time required for the particles first dislodged from the basket to completely dissolve.

The average dissolution $t_{1/2}$ of the five capsules was 8.7 minutes. Thus, on the average, after approximately 35 minutes 95% of the capsule drug contents was completely dissolved. This represents rapid dissolution (see ref. 27 for examples of dissolution times).

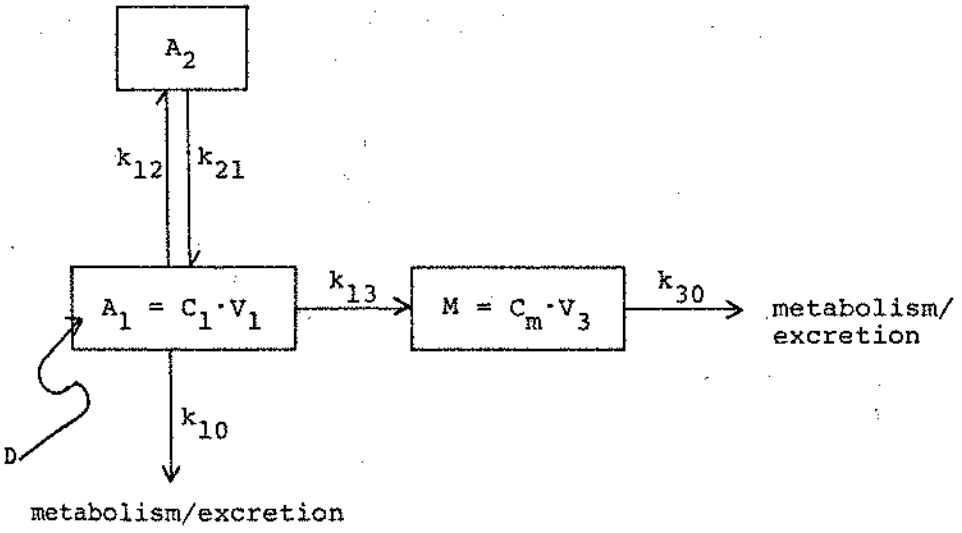
B. Dog Studies

1. Intravenous methsuximide doses

a. Theoretical. It was previously determined (18, 20) that methsuximide obeys two-compartment open model kinetics and the metabolite obeys one-compartment open model kinetics after intravenous administration. Scheme I depicts this model where D represents the bolus intravenous dose of methsuximide; $A_1$, $A_2$, and M are the amounts of drug in the central and peripheral compartments and the amount of metabolite in its central compartment, respectively. $C_1$ and $C_m$ are the concentrations of drug and metabolite in their respective central compartments. The rate constants $k_{12}$ and $k_{21}$ govern the rate of transfer of drug between the

two methsuximide compartments. The rate constant $k_{13}$ represents metabolism of methsuximide to 2-methyl-2-phenylsuccinimide, while $k_{10}$ represents metabolism of methsuximide to any other metabolite(s) and/or excretion. Further metabolism and/or excretion of the metabolite is represented by $k_{30}$. All rate constants are assumed to be first-order. $V_1$ and $V_3$ represent the central compartment volumes of methsuximide and metabolite, respectively.

Scheme I. Pharmacokinetic Model for Methsuximide After Intravenous Administration, Including Formation of Metabolite.




The equations describing the concentrations of drug and metabolite in plasma as a function of time, for this model (18,20), are:

$$C_1 = \frac{D}{V_1(\alpha - \beta)}\{(\alpha - k_{21})e^{-\alpha t} + (k_{21} - \beta)e^{-\beta t}\} \quad (1)$$

and,

$$C_m = \frac{WDk_{13}}{V_3}\{[\frac{k_{21} - \alpha}{(k_{30} - \alpha)(\beta - \alpha)}]e^{-\alpha t} + [\frac{k_{21} - \beta}{(k_{30} - \beta)(\alpha - \beta)}]e^{-\beta t} + [\frac{k_{21} - k_{30}}{(\beta - k_{30})(\alpha - k_{30})}]e^{-k_{30}t}\} \quad (2)$$

where:

$$\begin{matrix}\alpha \\ \beta\end{matrix} = \frac{1}{2}\{(k_{12} + k_{21} + k_{10} + k_{13}) \pm [(k_{12} + k_{21} + k_{10} + k_{13})^2 - 4(k_{21}k_{10} + k_{21}k_{10})]^{1/2}\} \quad (3)$$

The composite rate constant β represents the disposition phase and is the slope of the post-distributive log-linear phase of loss of drug from the body multiplied by -2.303; α represents the distribution phase and is the log-linear slope multiplied by -2.303 obtained by curve stripping (30).

In eq. 2, D is the dose of methsuximide administered. W, the molecular weight ratio of metabolite to methsuximide (189/203), is included in order to obtain $C_m$ in concentration units of μg $ml^{-1}$.

The areas under the $C_1$-t and $C_m$-t profiles (AUC) may be obtained by integrating eqs. 1 and 2 from time zero to time infinity and are, respectively:

$$*AUC^{meth}_{meth}(iv) = \frac{D}{V_1 K} \quad (4)$$

and,

$$AUC^{meth}_{meth}(iv) = \frac{k_{13}}{K} \cdot \frac{WD}{V_3 k_{30}} = \frac{fmWD}{V_3 k_{30}} \quad (5)$$

where K is the overall rate constant which describes loss of drug from the body ($K = k_{10} + k_{13}$) and $f_m$ is the fraction of the methsuximide dose which is converted to the metabolite ($f_m = k_{13}/K$).

Graphical estimates of each parameter in eqs. 1 and 2 were obtained as described earlier (18,20) and improved estimates, including confidence limits and coefficients of determination, were obtained using the iterative, nonlinear, least squares program NREG on a digital computer (Univac 1118, Madison Academic Computer Center, University of Wisconsin, Madison) as described previously (31). Each data point was weighted by the reciprocal of its concentration in the computer analysis (20,32).

In the three intravenous methsuximide experiments, which immediately followed the oral multiple dose experiments, there was some metabolite remaining in the plasma

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*Since many different AUC's will be considered, the following nomenclature is adopted: $AUC^{C}_{a}(b)$, and should read as the area under the C-t curve of compound 'a' after the 'b' route of administration of drug 'c'. The abbreviations meth, met, po, and iv indicate methsuximide, metabolite, oral, and intravenous, respectively.

from the last oral dose of methsuximide. In these cases, eq. 2 was modified to account for this 'residual' metabolite concentration, $C_{min}$, by adding to it the term $C_{min} \cdot e^{-k_{30}t}$. Here, $C_{min}$ is a constant and is the concentration of metabolite in plasma determined immediately prior to the administration of the intravenous methsuximide dose.

b. Results. The plasma level-time data, profiles, and computer determined kinetic parameters for the intravenous methsuximide experiments are given for each dog in Tables VI-XI and Figures 2-7.

2. Oral methsuximide doses

a. Theoretical. It is now well recognized, for many drugs which are rapidly and efficiently eliminated from the body through hepatic metabolism, that there is a reduced availability of the drug to the systemic circulation after oral dose administration compared to that after intravenous administration (33-36). This can happen even when the oral dose is totally absorbed from the gastrointestinal (GI) tract. The reduced oral availability occurs because the drug is absorbed directly into the hepatoportal circulation and must pass through the liver before it reaches the general circulation (blood sampling site). Thus, a large part of the dose can be metabolized during this 'first-pass' through the liver. The systemic

Table VI. Plasma Concentration (μg/ml)-Time (h) Data from Intravenous Methsuximide Experiments in Dog A from a Single Dose and Following Multiple Oral Doses of Methsuximide, Including Dog Weights and TI Values.

| Single Dose | | | Following Multiple Doses: | | |
|---|---|---|---|---|---|
| 17.5 Kg | TI 89 | | 17.5 Kg | TI 131 | |
| t | $C_l$ | $C_m$ | t | $C_l$ | $C_m$ |
| 0.08 | 21.0 | 2.9 | 0 | ----[b] | 1.9 |
| 0.17 | 13.1 | 3.0 | 0.083 | 13.2 | 6.6 |
| 0.25 | 10.8 | 4.1 | 0.17 | 9.7 | 6.6 |
| 0.50 | 8.1 | 4.8 | 0.25 | 10.9 | 6.95 |
| 0.75 | 7.9 | 4.9 | 0.50 | 6.6 | 6.4 |
| 1.0 | 5.2 | 7.7 | 0.75 | 6.1 | 6.0 |
| 1.5 | 4.9 | 7.3 | 1.0 | 5.9 | 6.4 |
| 2.0 | 3.7 | 5.8 | 1.5 | 3.8 | 6.6 |
| 3.0 | 2.1 | 6.8 | 2.0 | 4.1 | 6.8[c] |
| 4.1 | 1.1 | 6.8 | 3.0 | 2.1 | --- |
| 6.0 | 0.47[a] | 7.2 | 4.1 | 1.3 | 6.4 |
| 8.1 | ----[a] | 8.0 | 6.0 | 0.43 | 6.0 |
| 10.0 | ---- | 7.2 | 8.0 | 0.22[a] | 5.4 |
| 12.1 | ---- | 7.1 | 10.2 | ----[a] | 5.0 |
| 18.75 | ---- | 5.3 | 12.0 | ---- | 4.5 |
| 25.2 | ---- | 3.8 | 18.0 | ---- | 2.5 |
| 36.0 | ---- | 1.8 | 24.0 | ---- | 1.45 |
| 48.3 | ---- | 0.97 | 36.0 | ---- | 0.50 |
| 60.1 | ---- | 0.45 | 48.2 | ---- | 0.20 |

[a] Concentration was below detectable limit.

[b] Sample taken immediately prior to drug injection.

[c] 5.4 μg/ml, deleted - problem not identified.

Figure 2. Plasma concentration-time profile for dog A from single intravenous dose of 300 mg of methsuximide. Computer determined equations are, for methsuximide (●)

$$C_l = 27.2e^{-11.54t} + 10.4e^{-0.57t}$$

and for the metabolite (▲)

$$C_m = -1.3e^{-11.54t} - 10.7e^{-0.57t} + 12.0e^{-0.051t}.$$

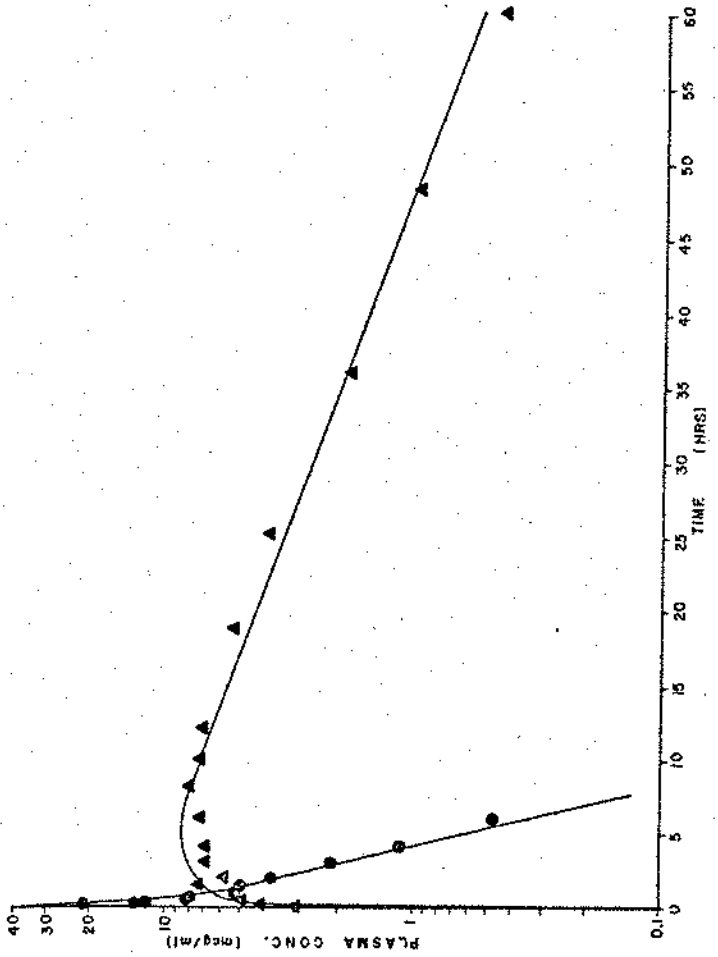

PLASMA CONC. (mcg/ml)
40
30
20
10
1
0.1
0
5
10
15
20
25
30
35
40
45
50
55
60
TIME (HRS)

Figure 3. Plasma concentration-time profile for dog A after intravenous dose of 300 mg of methsuximide immediately following multiple dose experiment. Computer determined equations are, for methsuximide (●)

$$C_l = 7.8e^{-6.18t} + 8.5e^{-0.54t}$$

and for the metabolite (▲)

$$C_m = -0.66e^{-6.18t} - 9.61e^{-0.54t} + 12.13e^{-0.0865t}.$$

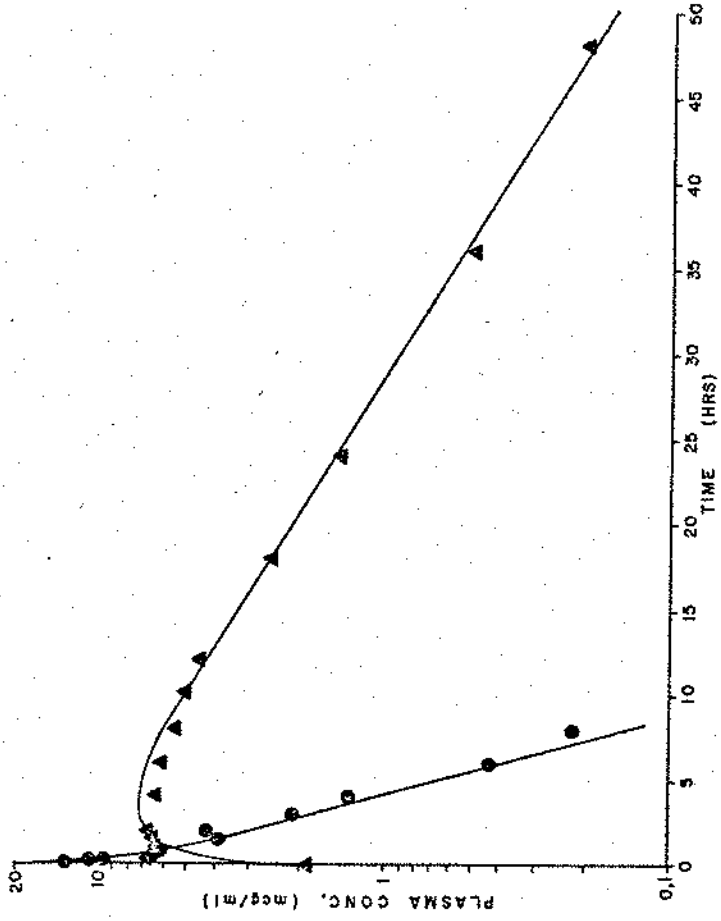

PLASMA CONC. (mcg/ml)
20
10
1
0,1
0
5
10
15
20
25
30
35
40
45
50
TIME (HRS)

Table VII. Summary of Pharmacokinetic Computer Estimated Scheme I Parameters and Parameters Derived Therefrom From Intravenous Methsuximide Doses in Dog A.

| Parameter | Single Dose | Following Multiple Doses |
|---|---|---|
| $k_{12}$ ($h^{-1}$) | 6.69 (4.00-9.38)[a] | 2.28 (-1.40-5.97) |
| $k_{21}$ ($h^{-1}$) | 3.61 (2.70-4.52) | 3.48 (2.27-6.74) |
| $k_{10}$ ($h^{-1}$) | 0.83 (0.65-1.02) | 0.51 (0.36-0.66) |
| $k_{13}$ ($h^{-1}$) | 0.98 (0.84-1.12) | 0.41 (0.31-0.52) |
| $k_{30}$ ($h^{-1}$) | 0.051 (0.041-0.062) | 0.0865 F |
| $V_1$ (L) | 7.99 F[b] | 18.39 (13.3-29.7)[c] |
| $V_3$ (L) | 13.70 F | 13.70 F |
| $C^0_{min}$ (μg/ml) | --- | 1.86 F |
| α ($h^{-1}$) | 11.54 | 6.18 |
| β ($h^{-1}$) | 0.57 | 0.54 |
| $t_{1/2}$ (methsuximide, h)[g] | 1.2 | 1.3 |
| $t_{1/2}$ (metabolite, h)[g] | 13.6 | 8.0 |
| cl (methsuximide, ml/min)[d] | 241 | 282 |
| cl (metabolite, ml/min)[d] | 12 | 20 |

| | | |
|---|---|---|
| $AUC^{meth}_{meth}$ (iv) ($\mu g\ ml^{-1}\ h$)[e] | 20.6 | 17.0 |
| $AUC^{meth}_{met}$ (iv) ($\mu g\ ml^{-1}\ h$)[e] | 215.1 | 100.8[h] |
| $R^2$ (methsuximide)[f] | 0.9976 | 0.9887 |
| r (methsuximide) | 0.9832 | 0.9459 |
| $R^2$ (metabolite) | 0.9557 | 0.8950 |
| r (metabolite) | 0.9817 | 0.9384 |
| $R^2$ (overall) | 0.9814 | 0.9435 |

[a] 95% confidence interval.

[b] Indicates parameter was fixed in regression analysis.

[c] Parameter used in regression analysis was $D/V_1$, thus this confidence interval was calculated with D = 300 mg.

[d] Plasma clearance calculated from $V_1K$ and $V_3k_{30}$.

[e] AUC calculated from regression equations, $C = Ae^{-\alpha t} + Be^{-\beta t} + \ldots$, as $\frac{A}{\alpha} + \frac{B}{\beta} + \ldots$.

[f] Coefficient of determination, $R^2 = \frac{\Sigma obs^2 - \Sigma dev^2}{\Sigma_{obs}{}^2}$.

[g] Half-lives calculated from $0.693/\beta$ and $0.693/k_{30}$.

[h] AUC due only to the dose administered. Thus, it is AUC in 'e' above minus AUC due to metabolite present a t = 0, $C^0_{min}/k_{30}$.

Table VIII. Plasma Concentration (μg/ml)-Time (h) Data from Intravenous Methsuximide Experiments in Dog B from a Single and Following Multiple Oral Doses of Methsuximide, Including Dog Weights and TI Values.

| Single Dose: 18.6 Kg TI 139 | | | Following Multiple Doses: 18.6 Kg TI 174 | | |
|---|---|---|---|---|---|
| t | $C_l$ | $C_m$ | t | $C_l$ | $C_m$ |
| 0.12 | 13.0 | 2.1 | 0 | -- | 0.63 |
| 0.23 | 8.1 | 3.7 | 0.08 | 8.75 | 3.0 |
| 0.54 | 6.6 | 4.4 | 0.15 | 7.2 | 4.0 |
| 1.0 | 4.6 | 6.1 | 0.25 | 5.3 | 4.6 |
| 2.0 | 3.1 | 6.6 | 0.50 | 3.8 | 4.8 |
| 3.0 | 1.2 | 7.8 | 0.75 | 2.6 | 4.2 |
| 4.1 | 0.67 | 7.6 | 1.03 | --[b] | 4.4 |
| 6.0 | 0.25 | 7.8 | 1.5 | 1.8 | 4.9 |
| 8.0 | --[a] | 7.1 | 2.0 | 0.94 | 4.8 |
| 10.0 | -- | 5.9 | 3.0 | 0.50 | 4.3 |
| 12.0 | -- | 5.4 | 4.0 | 0.27 | 4.1 |
| 18.0 | -- | 2.8 | 6.0 | --[a] | 3.5 |
| 24.2 | -- | 1.8 | 8.0 | -- | 3.0 |
| 36.0 | -- | 0.53 | 10.0 | -- | 2.0 |
| 48.2 | -- | 0.21 | 12.0 | -- | 1.4 |
| | | | 18.0 | -- | 0.63 |
| | | | 24.0 | -- | 0.29 |

[a]Concentration below detectable limit.

[b]5.8 μg/ml, blood sample drawn through drug injection infusion set, deleted.

Figure 4. Plasma concentration-time profile for dog B from single intravenous dose of 300 mg of methsuximide. Computer determined equations are, for methsuximide (●)

$$C_l = 26.8e^{-12.68t} + 7.9e^{-0.58t}$$

and for the metabolite (▲)

$$C_m = -1.6e^{-12.68t} - 12.1e^{-0.58t} + 13.7e^{-0.0893t}.$$

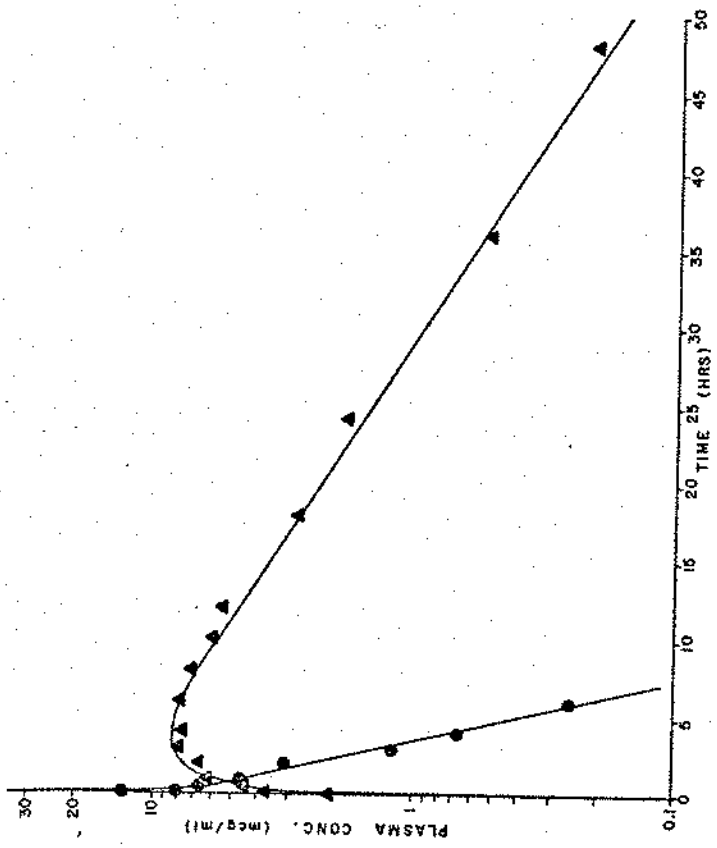

PLASMA CONC. (mcg/ml)
30
20
10
1
0.1
0
5
10
15
20
25
30
35
40
45
50
TIME (HRS)

Figure 5. Plasma concentration-time profile for dog B after intravenous dose of 300 mg of methsuximide immediately following multiple dose experiment. Computer determined equations are, for methsuximide (●)

$$C_l = 9.1e^{-5.23t} + 3.6e^{-0.77t}$$

and for the metabolite (▲)

$$C_m = -1.9e^{-5.23t} - 6.1e^{-0.77t} + 8.6e^{-0.1416t}$$

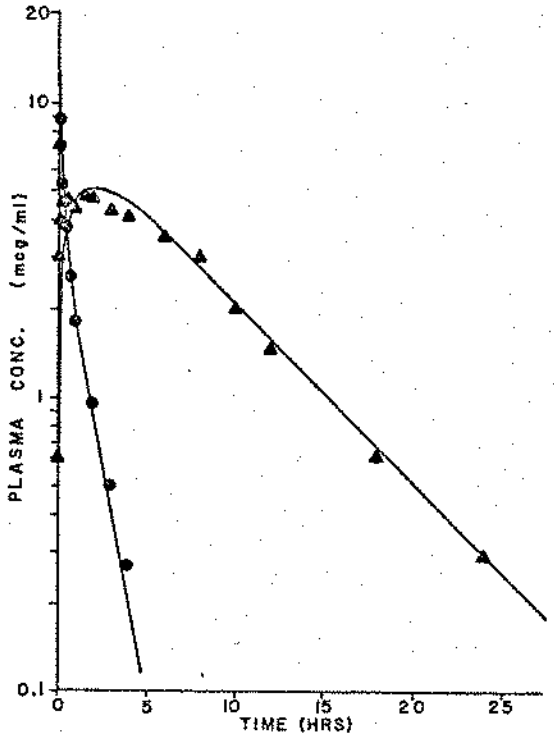

20
10
1
0.1
PLASMA CONC. (mcg/ml)
0
5
10
15
20
25
TIME (HRS)

Table IX. Summary of Pharmacokinetic Computer Estimated Scheme I Parameters[a], and Parameters Derived Therefrom, From Intravenous Methsuximide Doses in Dog B.

| Parameter | Single Dose | Following Multiple Doses |
|---|---|---|
| $k_{12}$ ($h^{-1}$) | 7.72 (6.27-9.18) | 1.98 (0.47-3.50) |
| $k_{21}$ ($h^{-1}$) | 3.34 (2.40-4.29) | 2.02 (-0.20-4.23) |
| $k_{10}$ ($h^{-1}$) | 0.86 (0.74-0.97) | 1.28 (0.98-1.59) |
| $k_{13}$ ($h^{-1}$) | 1.31 (1.19-1.42) | 0.68 (0.54-0.82) |
| $k_{30}$ ($h^{-1}$) | 0.0893 F | 0.1416 F |
| $V_1$ (L) | 8.65 F | 23.6 F |
| $V_3$ (L) | 14.08 F | 14.08 F |
| $c^0_{min}$ ($\mu g\ ml^{-1}$) | --- | 0.63 F |
| $\alpha$ ($h^{-1}$) | 12.68 | 5.23 |
| $\beta$ ($h^{-1}$) | 0.58 | 0.77 |
| $t_{1/2}$ (methsuximide, h) | 1.2 | 0.9 |
| $t_{1/2}$ (metabolite, h) | 7.8 | 4.9 |
| cl (methsuximide, ml $min^{-1}$) | 313 | 771 |
| cl (metabolite, ml $min^{-1}$) | 21 | 33 |

| | | |
|---|---|---|
| $AUC^{meth}_{meth}$ (iv) ($\mu g\ ml^{-1}\ h$) | 15.7 | 6.4 |
| $AUC^{meth}_{met}$ (iv) ($\mu g\ ml^{-1}\ h$) | 132.4 | 48.0 |
| $R^2$ (methsuximide) | 0.9974 | 0.9913 |
| r (methsuximide) | 0.9948 | 0.9732 |
| $R^2$ (metabolite) | 0.9902 | 0.9477 |
| r (metabolite) | 0.9935 | 0.9641 |
| $R^2$ (overall) | 0.9939 | 0.9677 |

[a]See footnotes for Table VII, p. 45.

Table X. Plasma Concentration (μg/ml)-Time (h) Data from Intravenous Methsuximide Experiments in Dog C from a Single and Following Multiple Oral Doses of Methsuximide, Including Dog Weights and TI Values.

| Single Dose 13.8 Kg TI 183 | | | After Multiple Doses 13.8 Kg TI 272 | | |
|---|---|---|---|---|---|
| t | $C_l$ | $C_m$ | t | $C_l$ | $C_m$ |
| 0.104 | 11.7 | 2.55 | 0 | -- | 1.1 |
| 0.168 | 9.5 | 4.6 | 0.083 | 14.0 | 5.2 |
| 0.257 | 7.6 | 4.4 | 0.168 | 11.2 | 6.4 |
| 0.51 | 7.2 | 5.5 | 0.25 | 8.6 | 6.9 |
| 0.75 | 5.0 | 6.0 | 0.50 | 6.4 | 7.0 |
| 1.0 | 3.8 | 7.2 | 0.75 | 4.4 | 6.4 |
| 1.5 | 2.2 | 6.6 | 1.0 | 3.7 | 7.3 |
| 2.0 | 1.5 | 9.1 | 1.5 | 2.2 | 7.3 |
| 3.0 | 1.4 | 9.7 | 2.0 | 1.3 | 6.8 |
| 4.0 | 0.68 | 8.9 | 3.0 | 1.05 | 7.3 |
| 6.0 | 0.29 | 10.3 | 4.0 | 0.66 | 6.3 |
| 8.0 | 0.16 | 9.2 | 6.0 | 0.19 | 5.8 |
| 10.0 | --[a] | 7.6 | 8.0 | --[a] | 5.2 |
| 12.0 | -- | 6.9 | 10.0 | -- | 4.1 |
| 16.0 | -- | 6.4 | 12.0 | -- | 3.1 |
| 24.0 | -- | 4.1 | 18.0 | -- | 1.4 |
| 36.0 | -- | 2.0 | 24.0 | -- | 0.92 |
| 49.0 | -- | 0.69 | 30.0 | -- | 0.54 |
| 60.0 | -- | 0.34 | 36.0 | -- | 0.32 |

[a]Concentration below detectable limit.

Figure 6. Plasma concentration-time profile for dog C from single intravenous dose of 265 mg of methsuximide. Computer determined equations are, for methsuximide (●)

$$C_l = 10.35e^{-1.78t} + 2.12e^{-0.31t}$$

and for the metabolite (▲)

$$C_m = -6.6e^{-1.78t} - 9.4e^{-0.31t} + 16.0e^{-0.0620t}.$$

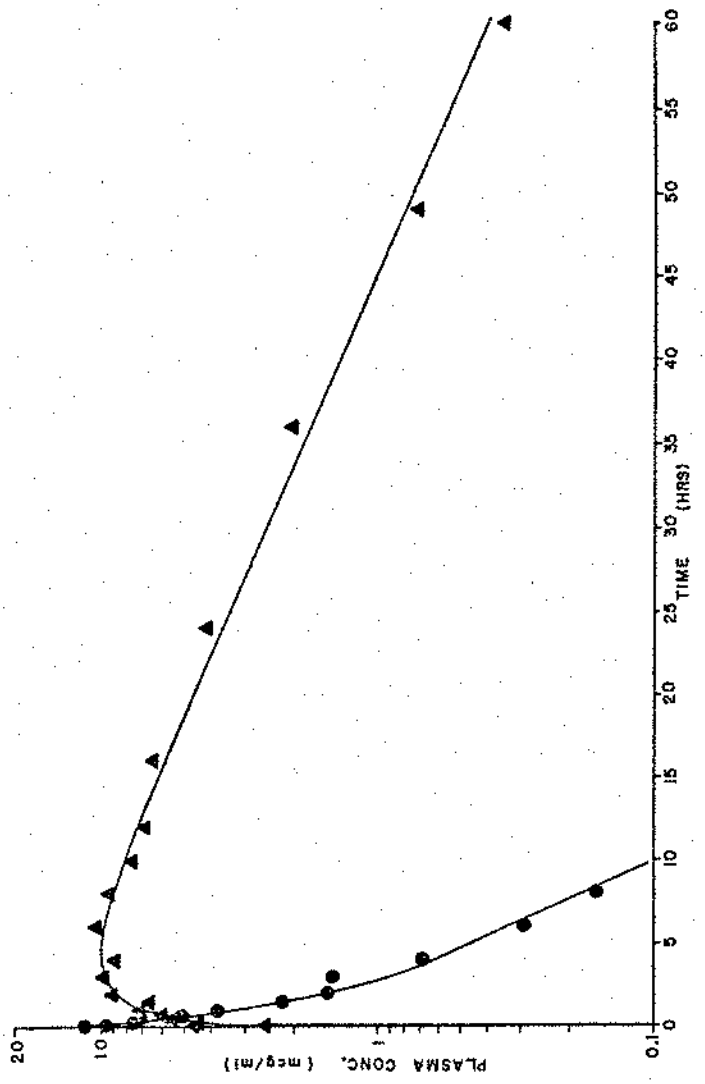

PLASMA CONC. (meg/ml)
20
10
1
0.1
0
5
10
15
20
25
30
35
40
45
50
55
60
TIME (HRS)

Figure 7. Plasma concentration-time profile for dog C after intravenous dose of 300 mg of methsuximide immediately following multiple dose experiment. Computer determined equations are, for methsuximide (●)

$$C_l = 12.52e^{-2.53t} + 3.30e^{-0.61t}$$

and for the metabolite (▲)

$$C_m = -4.57e^{-2.53t} - 5.73e^{-0.61t} + 11.40e^{-0.0991t}.$$

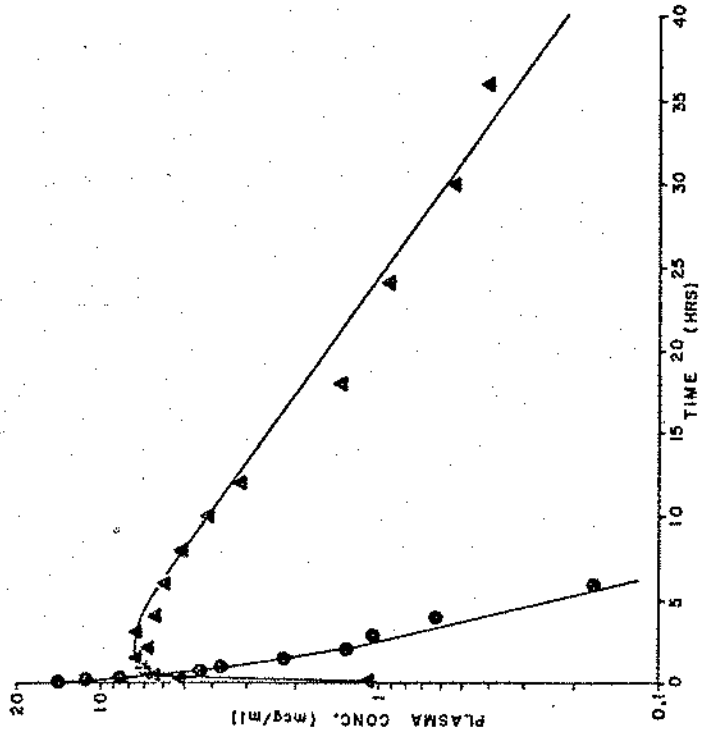

PLASMA CONC. (mcg/ml)
20
10
1
0.1
0
5
10
15
20
25
30
35
40
TIME (HRS)

Table XI. Summary of Pharmacokinetic Computer Estimated Scheme I Parameters[a], and Parameters Derived Therefrom, From Intravenous Methsuximide Doses in Dog C.

| Parameter | Single Dose | Following Multiple Doses |
|---|---|---|
| $k_{12}$ ($h^{-1}$) | 0.54 (0.17-0.91) | 0.61 (-0.11-1.32) |
| $k_{21}$ ($h^{-1}$) | 0.56 (-0.11-1.24) | 1.01 (-0.71-2.72) |
| $k_{10}$ ($h^{-1}$) | 0.37 (0.26-0.49) | 0.96 (0.72-1.20) |
| $k_{13}$ ($h^{-1}$) | 0.60 (0.51-0.68) | 0.54 (0.42-0.65) |
| $k_{30}$ ($h^{-1}$) | 0.0620 F | 0.0991 F |
| $V_1$ (L) | 21.25 F | 18.96 F |
| $V_3$ (L) | 10.75 F | 10.75 F |
| $C^0_{min}$ ($\mu g\ ml^{-1}$) | --- | 1.10 F |
| $\alpha$ ($h^{-1}$) | 1.78 | 2.53 |
| $\beta$ ($h^{-1}$) | 0.31 | 0.61 |
| $t_{1/2}$ (methsuximide, h) | 2.2 | 1.1 |
| $t_{1/2}$ (metabolite, h) | 11.2 | 7.0 |
| cl (methsuximide, ml $min^{-1}$) | 343 | 474 |
| cl (metabolite, ml $min^{-1}$) | 11 | 18 |

| | | |
|---|---|---|
| $AUC^{meth}_{meth}$ (iv) ($\mu g\ ml^{-1}\ h$) | 14.3[b] | 10.4 |
| $AUC^{meth}_{met}$ (iv) ($\mu g\ ml^{-1}\ h$) | 224 | 92.7 |
| $R^2$ (methsuximide) | 0.9894 | 0.9937 |
| r (methsuximide) | 0.9868 | 0.9693 |
| $R^2$ (metabolite) | 0.9798 | 0.9335 |
| r (metabolite) | 0.9775 | 0.9604 |
| $R^2$ (overall) | 0.9829 | 0.9617 |

[a]See footnotes for Table VII, p. 45.

[b]AUC normalized to a dose of 300 mg by multiplying by 300/265.

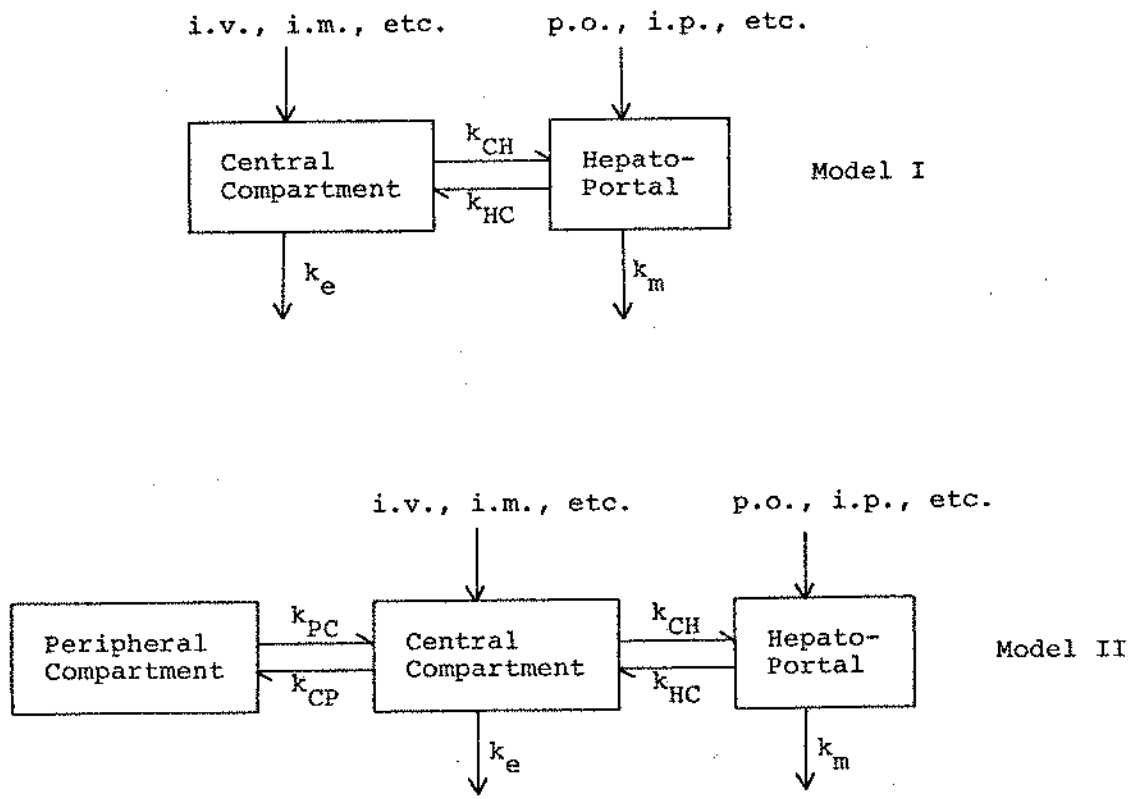
availability of a drug after oral dose administration is the product of the fraction of the dose which is absorbed from the GI tract F and the fraction of the absorbed dose which reaches the systemic circulation unchanged, f. The systemic availability of a drug after oral administration may be determined experimentally by separate administration of equal doses of drug by the oral and intravenous routes and comparing the AUC values as indicated in eq. 6.

$$fF = \frac{AUC\ (po)}{AUC\ (iv)} \qquad (6)$$

If the systemic availability of drug is less than one, then the drug is not totally absorbed, undergoes first-pass metabolism, or both. If $f < 1$, then the fraction of the absorbed dose which is metabolized on the first-pass is $(1 - f)$.

In the event that only oral or intravenous data are available, eq. 6 cannot be used to calculate the systemic availability of an oral dose of drug. However, two methods (37,38) have been independently developed which lead to identical equations useful in predicting the systemic oral availability of a drug in this case. The original derivation of the "first-pass equations" were presented by Gibaldi et al. (37) and a detailed analysis can be found in that author's text book (39). Two first-pass models were presented and are depicted in Scheme II.

Scheme II. First-Pass Pharmacokinetic Models Applicable to the One (I)- and Two (II)-Compartment Open Models with the 'Liver' Explicitly Separated from the Central Compartments.



As can be seen in this scheme, the oral (p.o.) and intraperitoneal (i.p.) routes of administration are hepatic routes while the intravenous (i.v.) and intramuscular (i.m.) routes are peripheral routes. Thus, orally or intraperitoneally administered compounds must pass through the hepato-portal system before drug can be observed in the sampled compartment (central). The rate constants $k_e$ and $k_m$ represent urinary excretion and hepatic metabolism, respectively, while all other k's are compartmental transfer rate constants. All rate constants are assumed to be first-order. With either model, the equations describing the AUC after p.o. or i.v. dose administration are (39):

$$\text{AUC (po)} = \frac{FD}{V_c}\left(\frac{k_{HC}}{k_{CH}k_m}\right) \tag{7}$$

and,

$$\text{AUC (iv)} = \frac{D}{V_c}\left(\frac{k_{HC} + k_m}{k_{CH}k_m}\right) \tag{8}$$

where $V_c$ represents the volume of distribution of the drug in the central compartment. Taking the ratio of eq. 7 to eq. 8 and substituting into eq. 6 results in eq. 9 which is useful in analyzing the extent of first-pass metabolism in terms of rate constants.

$$fF = \frac{Fk_{HC}}{k_{HC} + k_m} = \frac{\text{AUC (po)}}{\text{AUC (iv)}} \tag{9}$$

Examination of eq. 9 indicates that AUC (po) must be less than or equal to AUC (iv) and that the magnitude of the reduction in AUC (po) is governed by the relative values of $k_{HC}$ and $k_m$. If $k_{HC} >> k_m$, then f approaches 1.0 and virtually all of the drug escapes pre-systemic elimination. However, if $k_m >> k_{HC}$, then f approaches zero and essentially a total first-pass effect is seen.

Gibaldi has pointed out (39) that Models I and II and eq. 9 are of theoretical interest only since $k_{HC}$, $k_{CH}$, $k_m$, and $k_e$ cannot be determined from plasma level data, i.e., the liver cannot be separated from the rest of the rapidly perfused tissues of the body (central compartment) since it is probable that most drugs equilibrate very rapidly between blood and the hepato-portal system. To avoid this problem, eq. 9 was coupled with physiological considerations to obtain eqs. 10-11 useful in predicting the extent of first-pass metabolism using data obtained from either oral or intravenous dose administration.

$$f_{po} = \frac{Q_L}{Q_L + \frac{FD}{AUC\ (po)}} \qquad (10)$$

$$f_{iv} = 1 - \frac{D}{Q_L \cdot AUC\ (iv)} \qquad (11)$$

The details of the derivation can be found elsewhere (39) but the assumptions are: 1) the clearances of drug between the hepato-portal and central compartments are equal in either direction, and 2) this clearance is blood (or plasma)

flow rate limited, $Q_L$ being the hepatic blood (or plasma) flow rate. Equations 10 and 11 are useful in predicting the extent of first-pass metabolism from an oral dose based on oral $f_{po}$ or intravenous $f_{iv}$ data. Importantly, eq. 11 predicts what f should be after an oral dose if it is completely absorbed (F = 1) so that $f_{po}$ may not equal $f_{iv}$.

Equation 11 was used to predict the extent of first-pass metabolism which would be expected after single oral and multiple doses of methsuximide to dogs. Table XII gives the predicted values of f with the assumptions that the oral dose is completely absorbed (F = 1) and the average blood flow rate to the canine liver is 40 ml $min^{-1}$ $Kg^{-1}$ (40). The latter assumption may be tenuous since normal canine hepatic blood flow rates vary with the method of measurement and reported values range between 23.0 and 45.0 ml $min^{-1}$ $Kg^{-1}$ (41,42).

Table XII. Predicted Values of $f_{iv}$ Using Eq. 11 in Three Dogs After Single and Multiple Doses of Methsuximide.

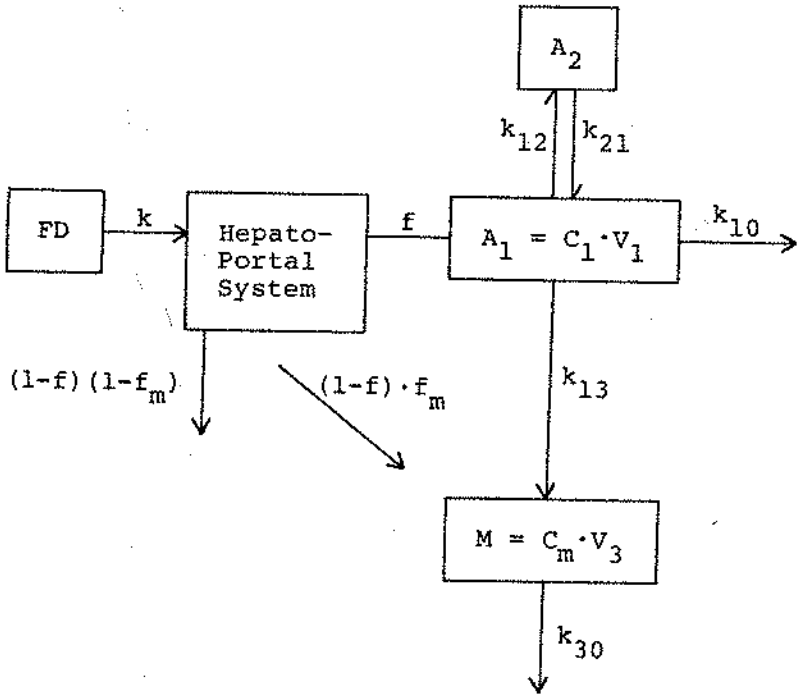
| | $f_{iv}$* | |
|---|---|---|
| Dog | Single Dose | After Multiple Doses |
| A | 0.28 | 0.16 |
| B | 0.18 | 0 |
| C | 0 | 0 |

*Values of AUC (iv) were obtained from Tables VII, IX, XI, pps. 44, 51, 58. Since AUC values are from levels of drug in plasma, the plasma flow rates were used in this calculation, $Q_P = (1-H)Q_B$ where H is the hematocrit and is 0.486 in Dogs B, C and 0.52 in Dog A.

The values of $f_{iv}$ in Table XII indicate that there should be a large first-pass effect after oral administration of methsuximide. Hence, any pharmacokinetic model proposed to describe the data from oral methsuximide doses must include this effect.

Since the pharmacokinetic models for methsuximide and metabolite have already been established from intravenous experiments, then the model for oral methsuximide is similar except for provisions for first-order absorption and first-pass metabolism. Scheme III is such a model:

Scheme III. Pharmacokinetic Model for Methsuximide After Oral Administration.



In Scheme III, FD is the dose of drug which is available for absorption, D being the dose administered and F the fraction of the dose absorbed from the GI tract. This dose is absorbed directly into the hepato-portal system with a first-order rate constant k. A fraction of the absorbed dose reaches the systemic circulation as unchanged drug f, while the remaining fraction (1 - f) is metabolized to 2-methyl-2-phenylsuccinimide $(1 - f) \cdot f_m$ or to other metabolite(s) $(1 - f)(1 - f_m)$. Hence, in this model, the hepato-portal system is an integral part of the central methsuximide and metabolite compartments ($A_1$ and M, respectively). Other symbols are as defined in Scheme I. It is also assumed in this model that $fm = k_{13}/(k_{13} + k_{10})$, i.e., that the fraction of the absorbed dose which is metabolized to the metabolite during the first-pass (fm) is the same as that after the drug reaches the systemic circulation $[k_{13}/(k_{13} + k_{10})]$ (43). Thus, the liver is assumed to be the only metabolic organ and metabolism during the first-pass is not saturated (43).

The derivations of the equations which describe the concentrations of drug $C_1$ and metabolite $C_m$ in their respective compartments as a function of time, and also the derivations of eqs. 12 and 13 for the total AUC of methsuximide and metabolite, are given in Appendix B.

$$AUC_{meth}^{meth} \text{ (po)} = \frac{fFD}{V_1 K} \qquad (12)$$

$$AUC_{met}^{meth}\ (po) = \frac{fmFWD}{V_3 k_{30}} \qquad (13)$$

b. Results

i. Single doses. The drug and metabolite levels in plasma-time data, following single oral doses of methsuximide are given for each dog in Tables XIII-XV and Figures 8-12. Table XVI presents the AUC and apparent $t_{1/2}$ values for methsuximide and the metabolite from these single dose studies.

ii. Multiple doses. The levels of methsuximide and metabolite in plasma-time data for the multiple oral dose methsuximide experiments are given for each dog in Tables XVII-XIX and Figures 13-15. For comparative reasons, the data from the two intravenous methsuximide experiments are also plotted in Figures 13-15.

3. Intravenous metabolite (2-methyl-2-phenylsuccinimide) doses

a. Theoretical. It was previously determined (18, 20) that the metabolite obeys one-compartment open model kinetics after intravenous administration. Scheme IV depicts this model where D' represents the bolus intravenous dose of metabolite, M is the amount of metabolite in its distribution volume $V_3$, at any time, giving a concentration of $C_m$. The first-order rate constant $k_{30}$ represents excretion and/or further metabolism of the metabolite.

Table XIII. Plasma Concentration (μg/ml)-Time (h) Data Following Single Oral Doses of Methsuximide in Dog A, Including Dog Weight and TI Values.

| 17.5 Kg | TI 7 | | 175. Kg | TI 39 | |
|---|---|---|---|---|---|
| t | $C_l$ | $C_m$ | t | $C_l$ | $C_m$ |
| 0.25 | -- | 0.27 | 0.25 | 0.12 | 0.10 |
| 0.50 | trace[b] | 1.3 | 0.50 | 0.25 | 0.17 |
| 0.75 | trace | 1.95 | 0.75 | 0.34 | 0.18 |
| 1.0 | -- | 5.6 | 1.00 | 0.50 | 0.67 |
| 2.0 | -- | 8.1 | 2.50 | 0.64 | 5.1 |
| 3.0 | -- | 8.2 | 4.25 | 0.31 | 6.8 |
| 6.0 | -- | 7.8 | 6.50 | 0.12 | 7.0 |
| 8.0 | -- | 7.7 | 8.25 | --[a] | 6.4 |
| 10.0 | -- | 7.1 | 10.25 | -- | 5.7 |
| 12.25 | -- | 6.1 | 12.25 | -- | 5.2 |
| 18.75 | -- | 4.5 | 16.25 | -- | 4.2 |
| 24.75 | -- | 3.1 | 25.0 | -- | 2.3 |
| 36.0 | -- | 1.2 | 36.25 | -- | 0.84 |
| 49.0 | -- | 0.60 | 49.0 | -- | 0.30 |
| 60.5 | -- | 0.27 | | | |

[a]Concentration was below detectable limit.

[b]Indicates that a peak was observed on the GC tracing at the correct retention time but was below 0.10 μg/ml.

Figure 8. Plasma concentration-time profile for dog A after single 300 mg oral dose of methsuximide, TI 7. Only traces of unchanged drug were detected. Key: (▲) metabolite.

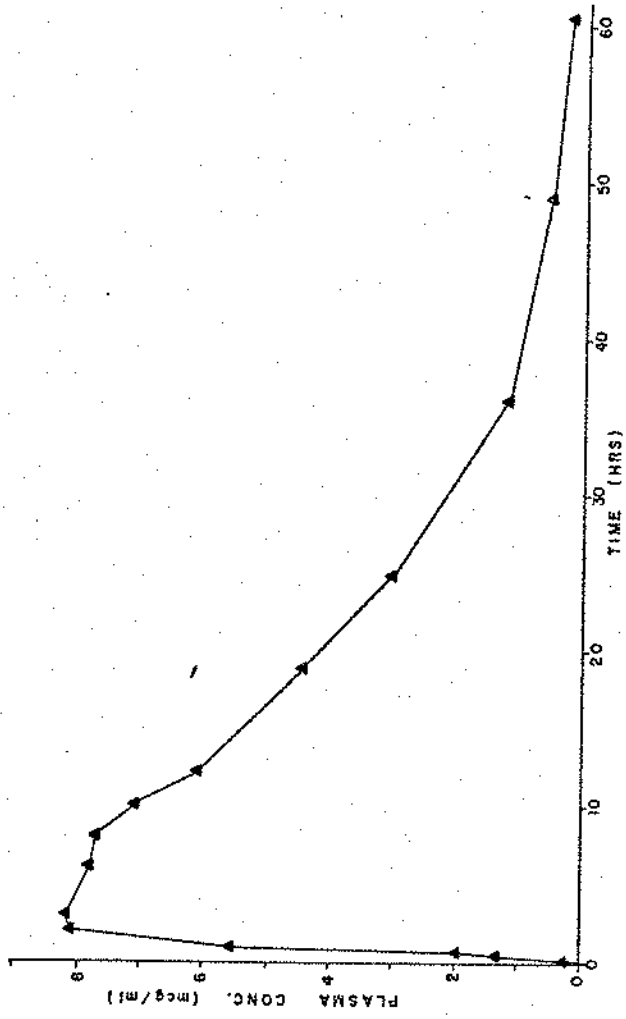

PLASMA CONC. (mcg/ml)
0
2
4
6
0
10
20
30
40
50
60
TIME (HRS)

Figure 9. Plasma concentration-time profile for dog A after single 300 mg oral dose of methsuximide, TI 39.

Key:

(●) methsuximide

(▲) metabolite.

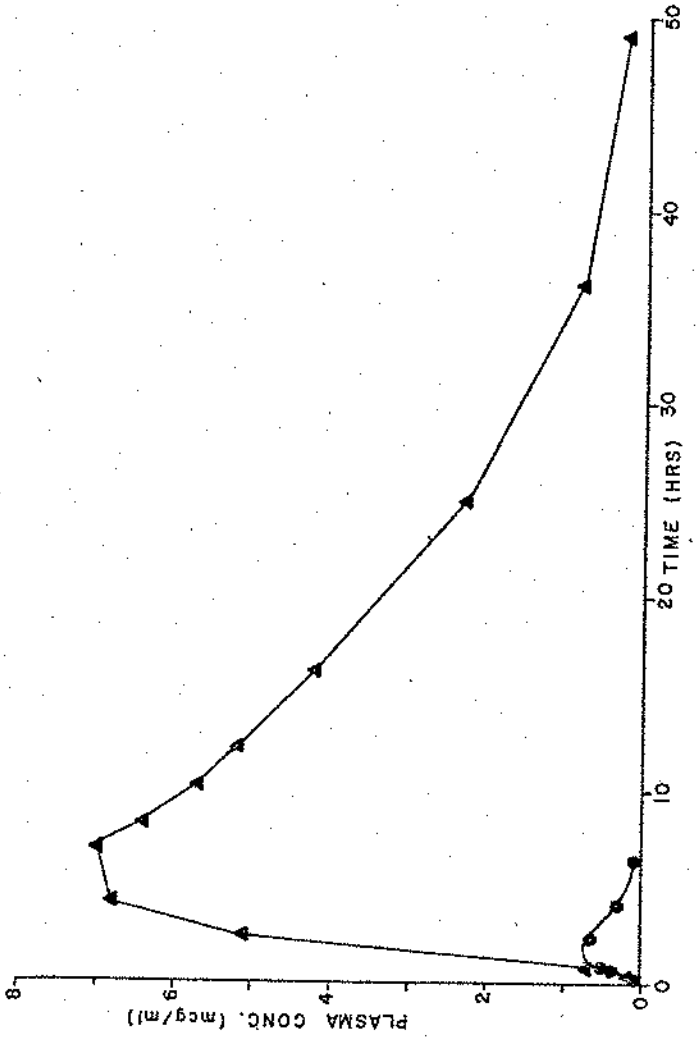

PLASMA CONC. (mcg/ml)
0
2
4
6
8
0
10
20
30
40
50
TIME (HRS)

Table XIV. Plasma Concentration ($\mu g\ ml^{-1}$)-Time (h) Data Following Single Oral Doses of Methsuximide in Dog B, Including Dog Weights and TI Values.

| 18.6 Kg | | TI 43 | 18.6 Kg | | TI 84 |
|---|---|---|---|---|---|
| t | $C_l$ | $C_m$ | t | $C_l$ | $C_m$ |
| 0.25 | 0.50 | 1.2 | 0.25 | --[a] | --[a] |
| 0.50 | 1.4 | 4.2 | 0.50 | 0.37 | 0.95 |
| 0.75 | 1.1 | 4.4 | 0.75 | 1.8 | 3.1 |
| 1.0 | 0.80 | 6.1 | 1.0 | 2.1 | 4.9 |
| 1.5 | 0.50 | 7.1 | 1.5 | 1.3 | 6.6 |
| 2.0 | 0.30 | --[a] | 2.0 | 0.9 | 6.8 |
| 3.0 | --[a] | 6.3 | 3.1 | 0.36 | 6.4 |
| 4.0 | 0.11 | 6.4 | 4.0 | 0.22 | 6.9 |
| 6.1 | --[b] | 5.8 | 6.25 | --[a] | 6.7 |
| 8.1 | -- | 5.4 | 8.1 | -- | 5.9 |
| 12.6 | -- | 4.1 | 10.1 | -- | 5.6 |
| 18.25 | -- | 1.8 | 12.0 | -- | 5.0 |
| 24.5 | -- | 1.3 | 25.4 | -- | 1.4 |
| 36.0 | -- | 0.3 | 36.6 | -- | 0.41 |
| 48.0 | -- | 0.11 | 48.3 | -- | 0.14 |
| 60.0 | -- | --[b] | | | |

[a]Interference with unidentified peaks in GC.

[b]Concentration was below detectable limit.

Figure 10. Plasma concentration-time profile for dog B after single 300 mg oral dose of methsuximide, TI 43.

Key:

(●) methsuximide

(▲) metabolite.

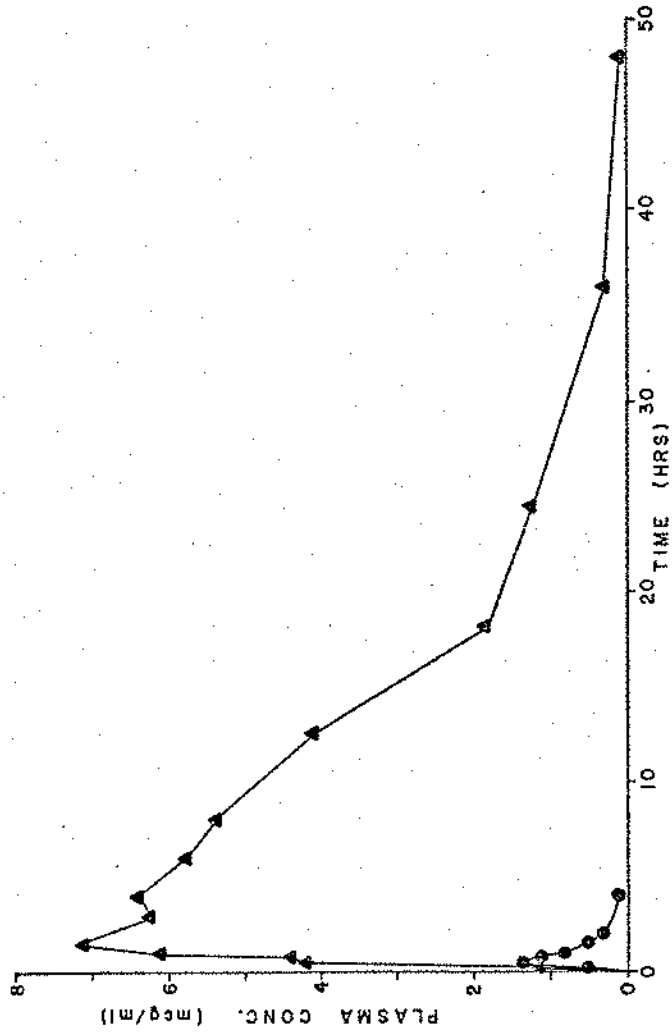

PLASMA CONC. (mcg/ml)
0
2
4
6
8
0
10
20
30
40
50
TIME (HRS)

Figure 11. Plasma concentration-time profile for dog B after single 300 mg oral dose of methsuximide, TI 84.

Key:

(●) methsuximide

(▲) metabolite.

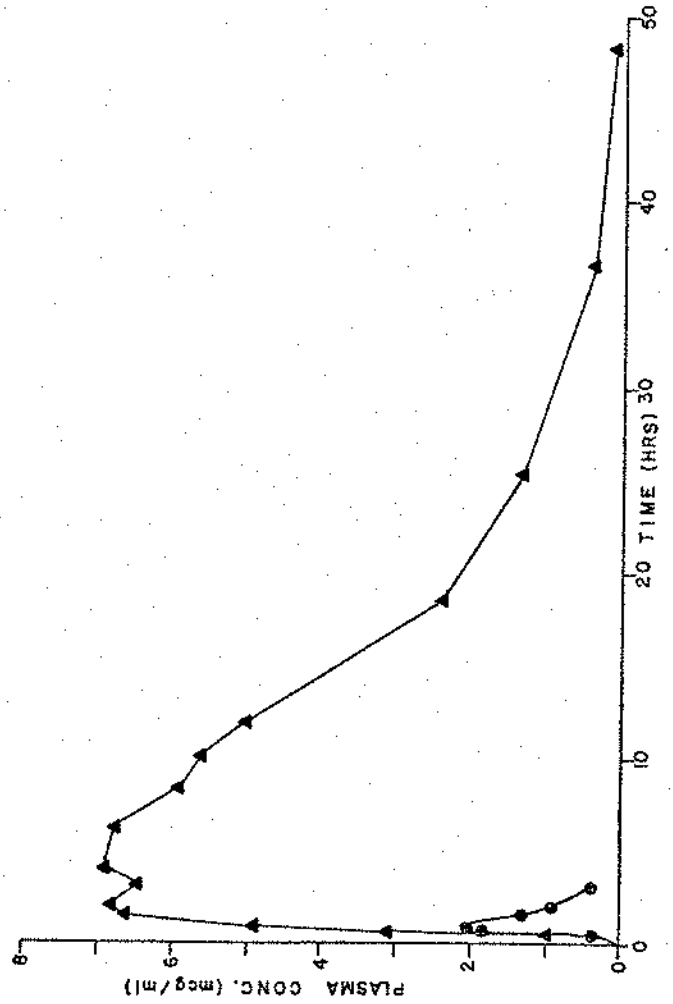

PLASMA CONC. (mcg/ml)
0
2
4
6
8
0
10
20
30
40
50
TIME (HRS)

Table XV. Plasma Concentration (μg $ml^{-1}$)-Time (h) Data Following Single Oral Dose of Methsuximide in Dog C, Including Dog Weight and TI Value.

| 13.8 Kg | TI 82 | |
|---|---|---|
| t | $C_l$ | $C_m$ |
| 0.25 | trace | 0.13 |
| 0.50 | 0.50 | 1.3 |
| 0.75 | 1.6 | 3.3 |
| 1.0 | 3.2 | 5.0 |
| 1.5 | 2.8 | 8.7 |
| 2.0 | 2.0 | 10.4 |
| 3.0 | 1.1 | 12.7 |
| 4.0 | 0.55 | 12.3 |
| 6.1 | 0.27 | 14.3 |
| 8.1 | 0.15 | 11.4 |
| 12.15 | --[a] | 8.5 |
| 19.0 | -- | 5.2 |
| 24.25 | -- | 3.6 |
| 36.0 | -- | 1.6 |
| 49.0 | -- | 0.48 |
| 60.0 | -- | 0.23 |

[a]Concentration was below detectable limit.

Figure 12. Plasma concentration-time profile for dog C after single 300 mg oral dose of methsuximide, TI 82.

Key:

(●) methsuximide

(▲) metabolite.

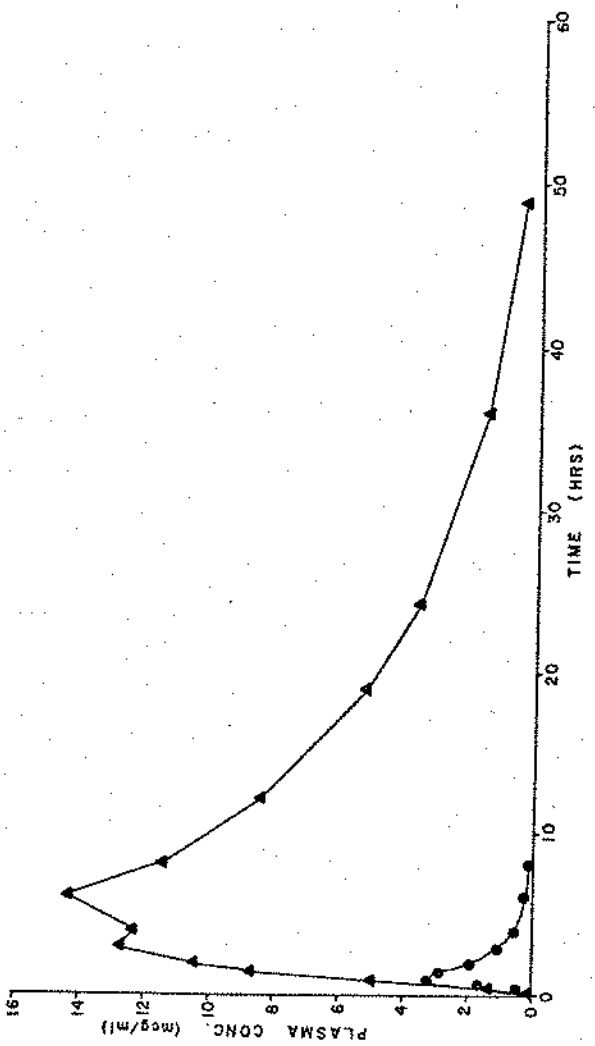

PLASMA CONC. (mcg/ml)
0
2
4
6
8
10
12
14
16
0
10
20
30
40
50
60
TIME (HRS)

Table XVI. Values for AUC and $t_{1/2}$ for Methsuximide and Metabolite after Single Oral Methsuximide Doses to Dogs.

| | Methsuximide | | Metabolite | |
|---|---|---|---|---|
| Dog | AUC[a] ($\mu g\ ml^{-1}\ h$) | $t_{1/2}$[b] (h) | AUC[a] ($\mu g\ ml^{-1}\ h$) | $t_{1/2}$[b] (h) |
| A | 0 | --[c] | 186.0 | 10.3 |
| | 2.7 | 1.7 | 141.2 | 9.3 |
| B | 2.0 | 1.2 | 108.7 | 6.7 |
| | 3.5 | 0.9 | 121.5 | 7.1 |
| C | 7.9 | 1.4 | 248.1 | 9.1 |
| Average | 3.2 | 1.0 | 161 | 8.3 |

[a]AUC for time zero to infinity was determined by use of the trapezoidal rule with the area from last data point to time infinity determined by the ratio of final concentration to the terminal slope, $C_1/\lambda$.

[b]Apparent half-life ($0.693/\lambda$) where $\lambda$ is the terminal log-linear slope of data points and is determined by linear regression analysis.

[c]No methsuximide was detected. In the average, this $t_{1/2}$ was considered zero.

Table XVII. Plasma Concentration (μg/ml)-Time (h) Data from Multiple Oral Doses of Methsuximide in Dog A Weighing 17.5 Kg. Drug was Administered Every Day for 14 Days With Blood Sampling on Days 1, 3, 5, 8, and 10.

| Day 1 | | TI 117 | Day 3 | | TI 119 | Day 5 | | TI 121 | Day 8 | | TI 124 | Day 10 | | TI 126 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| t | $C_l$ | $C_m$ | t | $C_l$ | $C_m$ | t | $C_l$ | $C_m$ | t | $C_l$ | $C_m$ | t | $C_l$ | $C_m$ |
| 0.25 | trace | trace | 0 | -- | 5.9 | 0 | -- | 3.7 | 0 | -- | 2.8 | 0 | -- | 1.95 |
| 0.53 | trace | trace | 0.28 | -- | 5.6 | 0.5 | 0.20 | 3.45 | 0.5 | -- | 1.5 | 0.5 | -- | 1.8 |
| 1.0 | 0.21 | 0.11 | 0.50 | -- | 5.8 | 1.1 | 0.60 | 4.6 | 1.0 | 0.6 | 2.6 | 1.0 | -- | 3.0 |
| 1.5 | 0.97 | 0.81 | 1.0 | -- | 5.8 | 2.0 | 0.72 | 6.8 | 2.0 | 0.46 | 7.5 | 2.0 | -- | 6.3 |
| 2.1 | 1.0 | 5.6 | 2.0 | -- | 16.0 | 3.0 | 0.37 | 11.4 | 3.0 | 0.35 | 10.4 | 3.0 | -- | 8.9 |
| 3.0 | 0.83 | 8.0 | 3.1 | -- | 14.5 | 4.0 | 0.25 | 10.9 | 4.0 | 0.20 | 8.3 | 4.0 | -- | 8.3 |
| 4.0 | 0.63 | 10.6 | 4.0 | -- | 15.0 | 6.0 | -- | 9.4 | 6.1 | -- | 6.7 | 6.0 | -- | 7.4 |
| 6.0 | 0.28 | 9.6 | 6.0 | -- | 12.7 | 8.0 | -- | 8.5 | 8.0 | -- | 5.7 | 8.0 | -- | 5.7 |
| 8.0 | 0.20 | 9.2 | 8.0 | -- | 11.3 | 12.0 | -- | 6.1 | 12.0 | -- | 5.6 | 12.0 | -- | 4.2 |
| 12.0 | -- | 7.7 | 12.0 | -- | 10.1 | 24.0 | -- | 2.4 | 24.0 | -- | 1.9 | 24.0 | -- | 1.4 |
| 24.0 | -- | 4.5 | 24.1 | -- | 4.5 | | | | | | | | | |

Figure 13. Plasma concentration-time profile for dog A during multiple 300 mg oral daily doses of methsuximide for 14 days. The two intravenous methsuximide profiles are also shown, for comparative purposes. Complete sets of blood samples were taken on days 1, 3, 5, 8, and 10. Low levels of methsuximide in plasma were observed on days 1, 5, and 8 only; these levels are not shown. Key:

(●) methsuximide

(▲) metabolite.

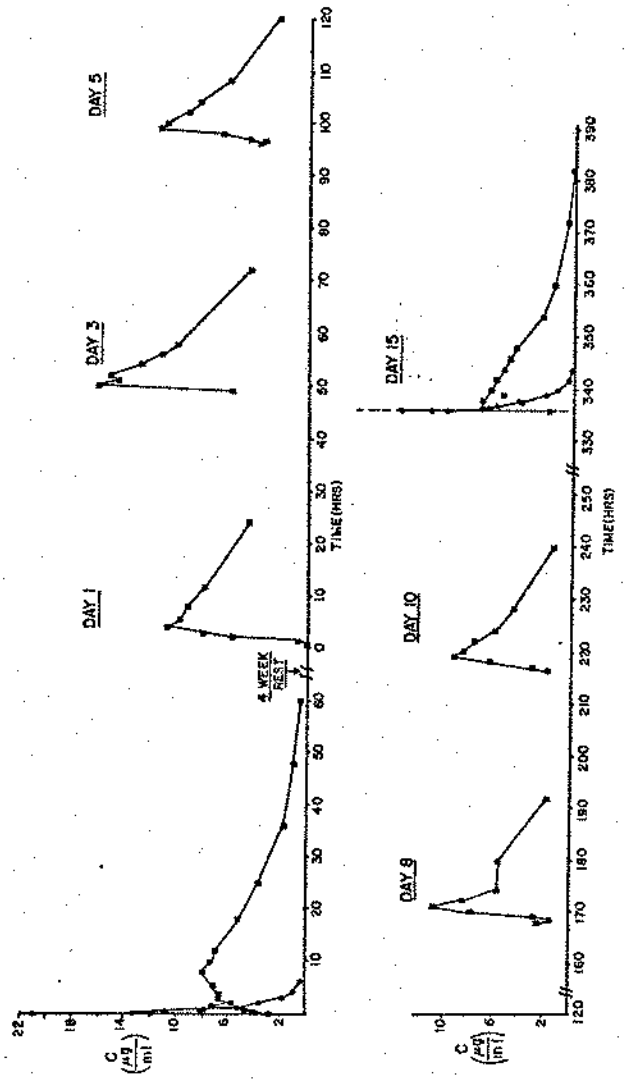

DAY 1
DAY 3
DAY 5
DAY 8
DAY 10
DAY 15
4 WEEK REST
TIME (HRS)
C (μg/ml)

Table XVIII. Plasma Concentration (μg/ml)-Time (h) Data from Multiple Oral Doses of Methsuximide in Dog B Weighing 18.6 Kg. Drug was Administered Every Day for 14 Days With Blood Sampling on Days 1, 3, 5, 8, and 10. On Days 2 and 4 Samples Were Only Taken Near the Plasma Level Peak.

| Day 1 | | TI 160 | Day 2 | | TI 161 | Day 3 | | TI 162 | Day 4 | | TI 163 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| t | $C_l$ | $C_m$ | t | $C_l$ | $C_m$ | t | $C_l$ | $C_m$ | t | $C_l$ | $C_m$ |
| 0.08 | -- | trace | 0 | -- | 1.6 | 0 | -- | 0.91 | 0 | -- | 0.75 |
| 0.25 | 0.14 | 1.4 | 1.0 | -- | 9.6 | 0.092 | -- | 0.87 | 1.0 | -- | 9.9 |
| 0.50 | 0.61 | 2.8 | 2.0 | -- | 9.0 | 0.57 | -- | 7.2 | 2.1 | -- | 8.4 |
| 0.78 | 0.73 | 4.1 | 3.0 | -- | 7.9 | 1.0 | -- | 10.9 | 3.22 | -- | 7.3 |
| 1.10 | 0.53 | 5.6 | 4.0 | -- | 6.9 | 1.5 | -- | 9.5 | 4.0 | -- | 6.2 |
| 1.50 | 0.40 | 6.5 | | | | 2.0 | -- | 9.2 | | | |
| 2.0 | 0.25 | 6.9 | | | | 3.08 | -- | 8.8 | | | |
| 3.0 | -- | 7.5 | | | | 4.0 | -- | 7.5 | | | |
| 4.0 | -- | 6.9 | | | | 6.08 | -- | 6.1 | | | |
| 6.0 | -- | 6.1 | | | | 8.0 | -- | 4.8 | | | |
| 8.3 | -- | 6.1 | | | | 10.08 | -- | 3.5 | | | |
| 10.0 | -- | 4.6 | | | | 12.0 | -- | 3.5 | | | |
| 12.1 | -- | 4.6 | | | | 24.0 | -- | 0.75 | | | |
| 24.0 | -- | 1.6 | | | | | | | | | |

| Day 5 | | TI 164 | Day 8 | | TI 167 | Day 10 | | TI 169 |
|---|---|---|---|---|---|---|---|---|
| t | $C_l$ | $C_m$ | t | $C_l$ | $C_m$ | t | $C_l$ | $C_m$ |
| 0 | -- | 0.73 | 0 | -- | 0.53 | 0 | -- | 0.64 |
| 0.092 | -- | 0.61 | 0.28 | -- | 0.39 | 0.30 | -- | 2.2 |
| 0.55 | -- | 9.7 | 0.53 | 0.31 | 0.97 | 0.53 | -- | 3.6 |
| 0.75 | -- | 10.2 | 0.75 | 0.70 | 1.3 | 0.83 | -- | 5.8 |
| 1.03 | -- | 9.0 | 1.0 | 0.85 | 1.8 | 1.03 | -- | 6.6 |
| 1.53 | -- | 7.6 | 1.5 | 1.1 | 2.7 | 1.63 | -- | 6.9 |
| 2.1 | -- | 8.3 | 2.03 | 0.62 | 5.0 | 2.07 | -- | 7.0 |
| 3.0 | -- | 7.0 | 3.07 | 0.26 | 5.6 | 3.0 | -- | 5.5 |
| 4.07 | -- | 6.3 | 4.0 | -- | 5.3 | 4.0 | -- | 5.3 |
| 6.0 | -- | 5.2 | 6.0 | -- | 4.7 | 6.0 | -- | 4.1 |
| 8.0 | -- | 4.6 | 8.0 | -- | 3.4 | 8.0 | -- | 3.4 |
| 10.0 | -- | 3.1 | 10.0 | -- | 2.6 | 10.0 | -- | 2.1 |
| 12.0 | -- | 2.5 | 12.0 | -- | 1.8 | 12.0 | -- | 1.4 |
| 24.0 | -- | 0.54 | 24.0 | -- | 0.36 | 24.0 | -- | 0.31 |

Figure 14. Plasma concentration-time profile for dog B during multiple 300 mg oral daily doses of methsuximide for 14 days. The two intravenous methsuximide profiles are also shown, for comparative purposes. Complete sets of blood samples were taken on days 1, 3, 5, 8, and 10 while on days 2 and 4 samples were only drawn near the peak plasma levels. Low levels of methsuximide in plasma were observed on days 1 and 8 only; these levels are not shown. Key:

(●) methsuximide

(▲) metabolite.

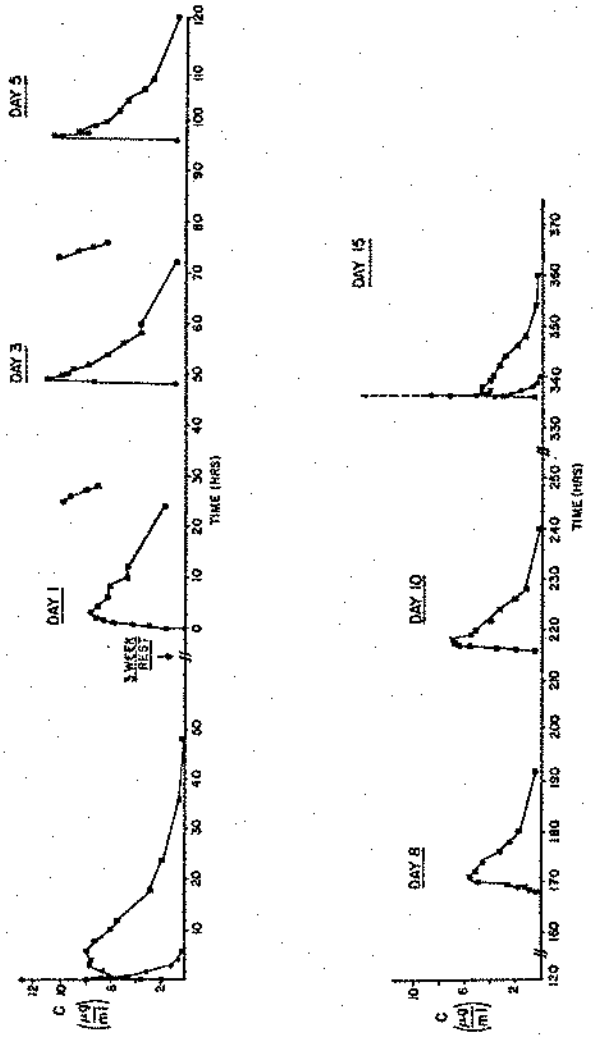

DAY 1
DAY 3
DAY 5
DAY 8
DAY 10
DAY 15
3 WEEKS REST
TIME (HRS)
C (μg/ml)

Table XIX. Plasma Concentration (μg/ml)-Time (h) Data from Multiple Oral Doses of Methsuximide in Dog C Weighing 13.5 Kg. Drug was Administered Every Day for 14 Days With Blood Sampling on Days 1, 3, 5, 8, and 10. On Days 2 and 4 Samples Were Only Taken Near the Plasma Level Peak.

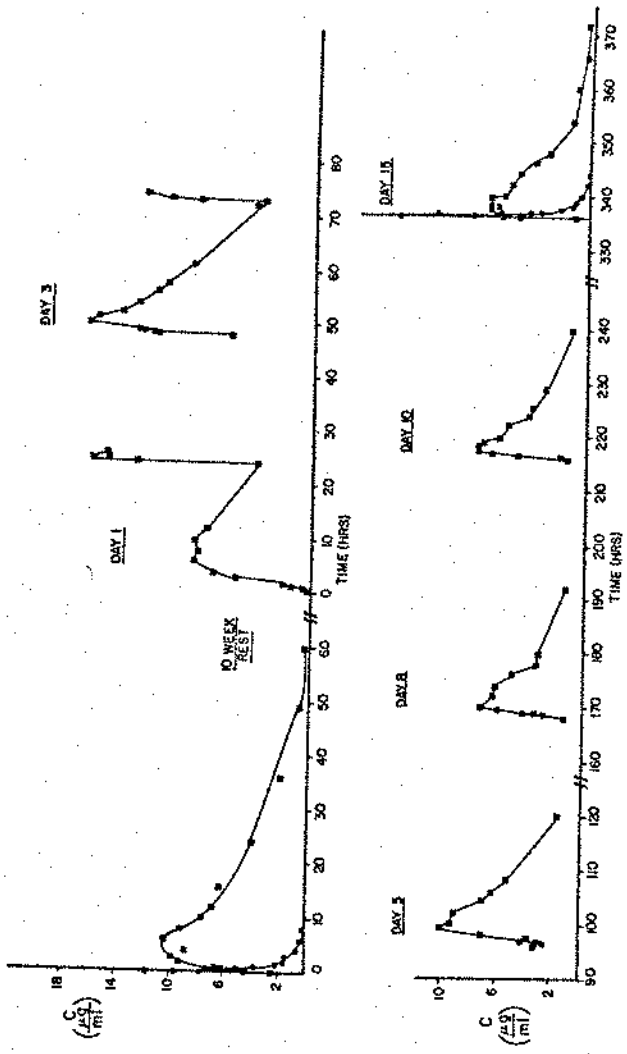
| Day 1 | | TI 258 | Day 2 | | TI 259 | Day 3 | | TI 260 | Day 4 | | TI 261 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| t | $C_l$ | $C_m$ | t | $C_l$ | $C_m$ | t | $C_l$ | $C_m$ | t | $C_l$ | $C_m$ |
| 0.25 | -- | -- | 0 | -- | 4.0 | 0 | -- | 6.3 | 0 | -- | 4.5 |
| 0.50 | -- | 0.21 | 0.5 | 2.3 | 13.2 | 0.25 | 0.13 | 11.8 | 0.6 | 0.14 | 3.9 |
| 0.75 | 0.10 | 0.67 | 1.0 | 1.5 | 16.2 | 0.505 | trace | 12.0 | 1.0 | 0.48 | 8.7 |
| 1.0 | 0.13 | 0.93 | 1.5 | 0.90 | 15.1 | 0.75 | trace | 13.2 | 1.5 | 0.18 | 11.0 |
| 1.5 | 0.19 | 1.5 | 2.0 | 0.76 | 15.4 | 1.0 | trace | 12.8 | 2.1 | trace | 12.6 |
| 2.0 | 0.24 | 2.1 | | | | 2.0 | trace | 16.8 | | | |
| 3.0 | 1.5 | 5.6 | | | | 3.0 | trace | 16.0 | | | |
| 4.0 | 1.0 | 7.2 | | | | 4.0 | trace | 14.3 | | | |
| 6.0 | 0.47 | 8.7 | | | | 6.0 | -- | 13.3 | | | |
| 8.0 | 0.3 | 8.5 | | | | 8.0 | -- | 11.7 | | | |
| 10.0 | 0.14 | 8.6 | | | | 9.5 | -- | 11.1 | | | |
| 12.0 | 0.10 | 7.9 | | | | 12.8 | -- | 9.0 | | | |
| 24.0 | -- | 4.0 | | | | 24.0 | -- | 4.5 | | | |

| Day 5 | | TI 262 | Day 8 | | TI 264 | Day 10 | | TI 266 |
|---|---|---|---|---|---|---|---|---|
| t | $C_l$ | $C_m$ | t | $C_l$ | $C_m$ | t | $C_l$ | $C_m$ |
| 0 | -- | 3.3 | 0 | -- | 1.3 | 0 | -- | 1.4 |
| 0.25 | -- | 3.1 | 0.25 | -- | 1.4 | 0.25 | -- | 1.2 |
| 0.50 | -- | 2.4 | 0.50 | -- | 2.8 | 0.50 | -- | 1.9 |
| 0.75 | -- | 2.9 | 0.75 | -- | 3.5 | 0.75 | -- | 5.0 |
| 1.0 | -- | 4.1 | 1.0 | -- | 4.4 | 1.0 | -- | 6.8 |
| 1.5 | -- | 3.8 | 1.5 | -- | 6.1 | 1.5 | -- | 7.9 |
| 2.0 | -- | 7.2 | 2.0 | -- | 7.3 | 2.0 | -- | 7.9 |
| 3.0 | -- | 10.1 | 4.0 | -- | 6.7 | 3.1 | -- | 7.6 |
| 4.15 | -- | 9.4 | 6.0 | -- | 6.5 | 4.0 | -- | 6.4 |
| 6.0 | -- | 9.2 | 8.0 | -- | 5.3 | 6.0 | -- | 5.7 |
| 8.1 | -- | 7.1 | 10.0 | -- | 3.4 | 8.0 | -- | 4.3 |
| 10.0 | -- | 6.4 | 12.0 | -- | 3.3 | 9.5 | -- | 4.0 |
| 12.0 | -- | 5.3 | 24.0 | -- | 1.3 | 12.7 | -- | 3.0 |
| 24.0 | -- | 1.7 | | | | 24.0 | -- | 1.1 |

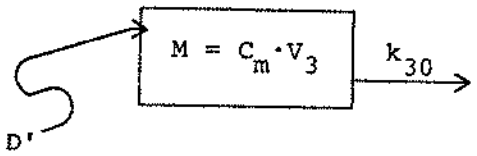
Figure 15. Plasma concentration-time profile for dog C during multiple 300 mg oral daily doses of methsuximide for 14 days. The two intravenous methsuximide profiles are also shown, for comparative purposes. Complete sets of blood samples were taken on days 1, 3, 5, 8, and 10 while on days 2 and 4 samples were only drawn near the peak plasma levels. Low levels of methsuximide in plasma were observed on days 1, 2, 3, and 4 only; these levels are not shown. Key:

(●) methsuximide

(▲) metabolite.


DAY 3
DAY 1
10 WEEK REST
DAY 5
DAY 8
DAY 12
DAY 15
C (μg/ml)
TIME (HRS)

Scheme IV. Pharmacokinetic Model for the Metabolite After Intravenous Administration.




With this model, the concentration of metabolite in plasma as a function of time is given by (18,20) eq. 14.

$$C_m = \frac{D'}{V_3} e^{-k_{30}t} \qquad (14)$$

The AUC of the $C_m$-t data is obtained by integrating eq. 14 from time zero to time infinity:

$$AUC_{met}^{met} \text{ (iv)} = \frac{D'}{V_3 k_{30}} \qquad (15)$$

The parameters $V_3$ and $k_{30}$ are determined from the intercept and slope, respectively, of the linear regression equation determined from the ln $C_m$ - t data.

b. Results. The plasma level-time data, profiles, and pharmacokinetic parameters obtained by linear regression analysis for the intravenous metabolite experiments are given for each dog in Tables XX-XXI and Figures 16-18.

Table XX. Plasma Concentration (μg/ml)-Time (h) Data from Intravenous Metabolite Doses in Three Dogs, Including Dog Weights and TI Values. Two Experiments Were Done in Dog C; One While the Hepatic Enzymes Were Uninduced, and One When They Were Partially Induced.

| Dog A | | Dog B | | Dog C | | | |
|---|---|---|---|---|---|---|---|
| 17.0 Kg | TI 192 | 19.5 Kg | TI 226 | 13.5 Kg | TI 279 | 14.5 Kg | TI 329 |
| t | $C_m$ | t | $C_m$ | t | $C_m$ | t | $C_m$ |
| 0.074 | 22.3 | 0.094 | 25.6 | 0.25 | 29.2 | 0.25 | 28.1 |
| 0.17 | 24.9 | 0.17 | 21.6 | 0.50 | 26.3 | 0.50 | 25.4 |
| 0.25 | 20.1 | 0.255 | 17.9 | 1.0 | 24.1 | 1.0 | 22.6 |
| 0.50 | 20.6 | 0.52 | 16.7 | 2.0 | 21.4 | 1.6 | 20.5 |
| 0.75 | 21.4 | 0.76 | 16.8 | 4.0 | 17.7 | 2.0 | 23.0 |
| 1.0 | 19.2 | 1.0 | 18.8 | 6.0 | 18.1 | 3.0 | 20.8 |
| 1.5 | 17.6 | 1.5 | 16.5 | 8.0 | 16.1 | 4.0 | 19.6 |
| 2.0 | 18.0 | 2.0 | 20.0 | 12.0 | 13.1 | 6.0 | 17.5 |
| 3.0 | 16.6 | 3.0 | 17.8 | 24.0 | 6.6 | 8.0 | 16.4 |
| 4.0 | 16.7 | 4.0 | 16.1 | 30.0 | 3.7 | 12.1 | 14.1 |
| 6.0 | 14.0 | 7.0 | 11.2 | 36.0 | 2.5 | 24.3 | 7.4 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 8.0 | 14.2 | 9.0 | 10.3 | 48.0 | 0.76 | 36.0 | 3.0 |
| 10.0 | 13.0 | 12.5 | 7.2 | 54.0 | 0.53 | 48.4 | 1.2 |
| 12.0 | 11.9 | 24.0 | 2.7 | | | 55.5 | 0.80 |
| 22.2 | 6.4 | 36.7 | 0.63 | | | | |
| 30.2 | 3.9 | 48.4 | 0.25 | | | | |
| 37.0 | 2.5 | | | | | | |
| 48.2 | 1.0 | | | | | | |
| 55.0 | 0.67 | | | | | | |

'95

Figure 16. Plasma concentration-time profile for dog A from single 300 mg intravenous dose of metabolite. Linear regression determined equation is:

$$C_m = 21.9e^{-0.061t} \quad (r = -0.9960).$$

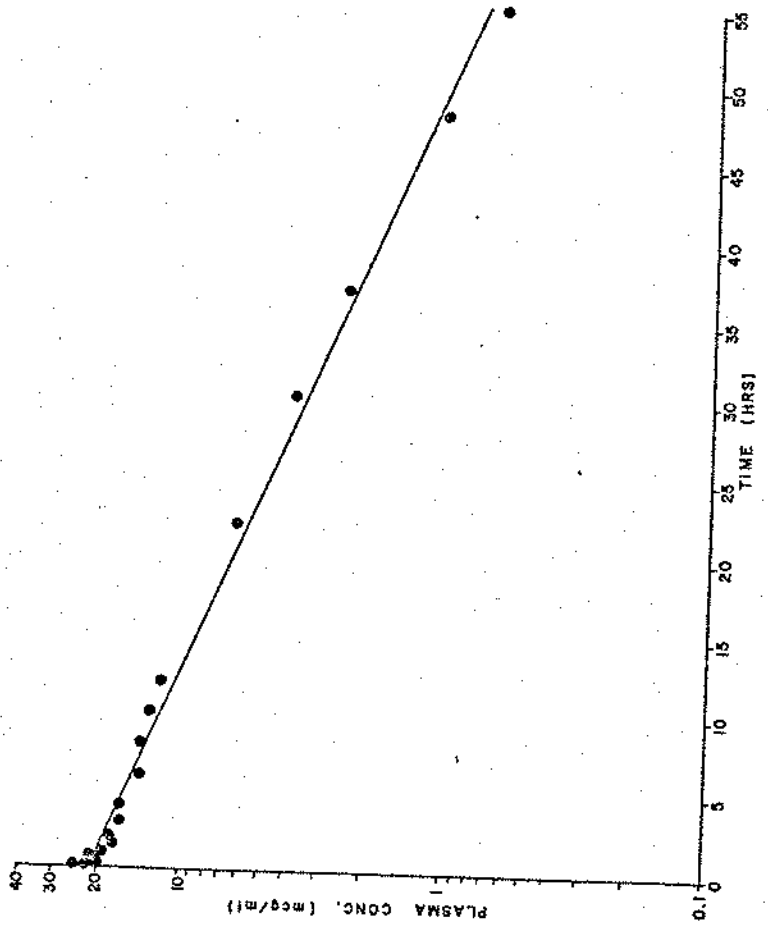

PLASMA CONC. (mcg/ml)
40
30
20
10
0.1
0
5
10
15
20
25
30
35
40
45
50
55
TIME (HRS)

Figure 17. Plasma concentration-time profile for dog B from single 300 mg intravenous dose of metabolite. Linear regression determined equation is:

$$C_m = 21.3e^{-0.092t} \quad (r = -0.9957).$$

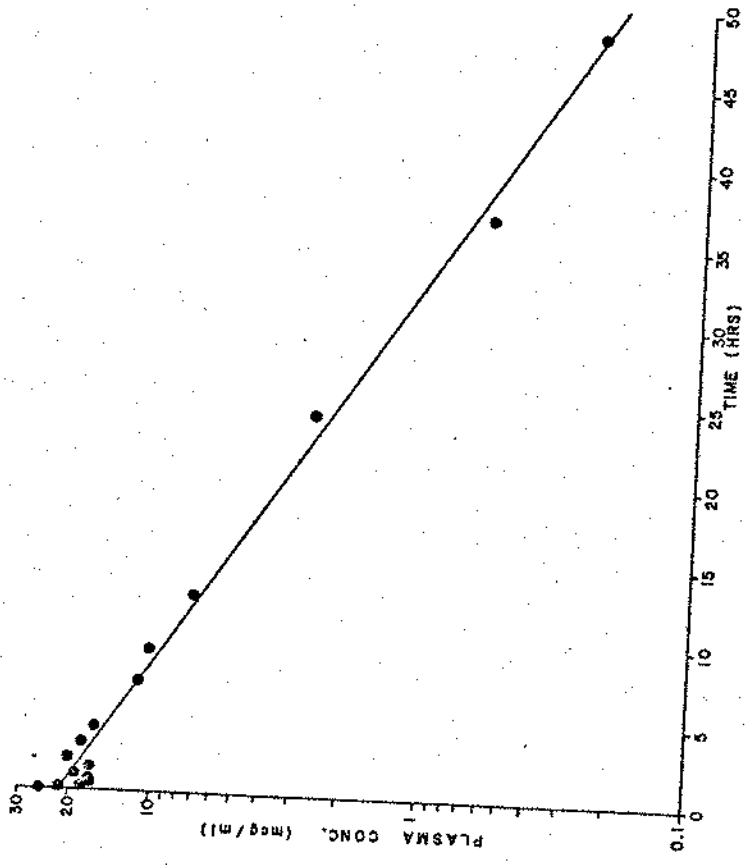

PLASMA CONC. (mcg/ml)
30
20
10
0.1
0
5
10
15
20
25
30
35
40
45
50
TIME (HRS)

Figure 18. Plasma concentration-time profiles for dog C from single 300 mg intravenous doses of metabolite in the uninduced (▲) and partially induced (●) hepatic microsomal enzyme state.

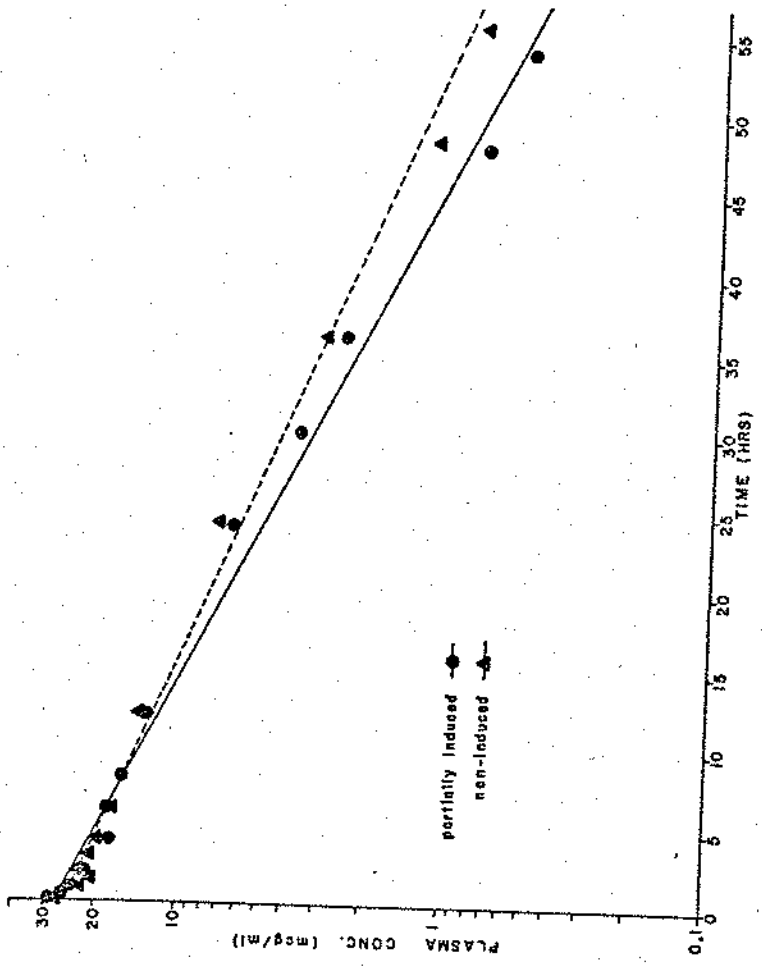

PLASMA CONC. (mcg/ml)
30
20
10
0
0.1
5
10
15
20
25
30
35
40
45
50
55
TIME (HRS)
partially induced
non-induced

Table XXI. Summary of Pharmacokinetic Scheme IV Parameters, after Intravenous Metabolite Doses in Dogs, as Determined by Linear Regression Analysis.

| | Dog (TI) | | | |
|---|---|---|---|---|
| Parameter | A (192) | B (226) | C (279) | C (329) |
| $V_3$ (L) | 13.70 | 14.06 | 10.75 | 11.20 |
| $V_3$ (L $Kg^{-1}$) | 0.81 | 0.72 | 0.80 | 0.77 |
| $k_{30}$ ($h^{-1}$) | 0.0611 | 0.0920 | 0.0712 | 0.0606 |
| $t_{1/2}$[a] (h) | 11.4 | 7.5 | 9.7 | 11.4 |
| $AUC_{met}^{met}$ (iv)[b] (µg $ml^{-1}$ h) | 358 | 232 | 392 | 442 |

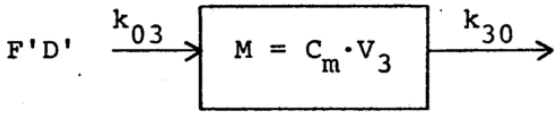
[a]Calculated from $t_{1/2} = 0.693/k_{30}$.

[b]Calculated from eq. 15, p. 93.

4. Oral metabolite doses

a. Theoretical. Since after intravenous administration of metabolite the $C_m$-t data are described by the one-compartment open model, then Scheme V describes the data after oral doses of metabolite.

Scheme V. Pharmacokinetic Model for the Metabolite After Oral Administration.




In this model, F' is the fraction of the dose D' of metabolite which is absorbed from the GI tract into the systemic circulation with a first-order rate constant $k_{03}$. The remaining symbols are as defined earlier. The equation describing the concentration of metabolite in plasma as a function of time is:

$$C_m = \frac{F'D'}{V_3}\left(\frac{k_{03}}{k_{03} - k_{30}}\right)\left(e^{-k_{30}t} - e^{-k_{03}t}\right) \qquad (16)$$

In one case, a time lag between dose administration and the appearance of drug in plasma was observed. These data were then analyzed by substituting $(t - t_{lag})$ in place of t in eq. 16. The area under the $C_m$ - t profile is obtained by integrating eq. 16 from zero to infinite time, eq. 17:

$$AUC_{met}^{met}\ (po) = \frac{F'D'}{V_3 k_{30}} \qquad (17)$$

Graphical estimates of each parameter in eq. 16 were obtained (18,20) and improved estimates, including confidence limits and coefficients of determination, were obtained using the iterative, nonlinear, least-squares program NREG on a digital computer (Univac 1118, Madison Academic Computer Center, University of Wisconsin, Madison) as described previously (18,20). Each data point was weighted by its reciprocal in the computer analysis (20,32).

b. Results

i. Single doses. The plasma level-time data, profiles, and computer determined kinetic parameters for the oral metabolite experiments are given for each dog in Tables XXII-XXIII and Figures 19-21.

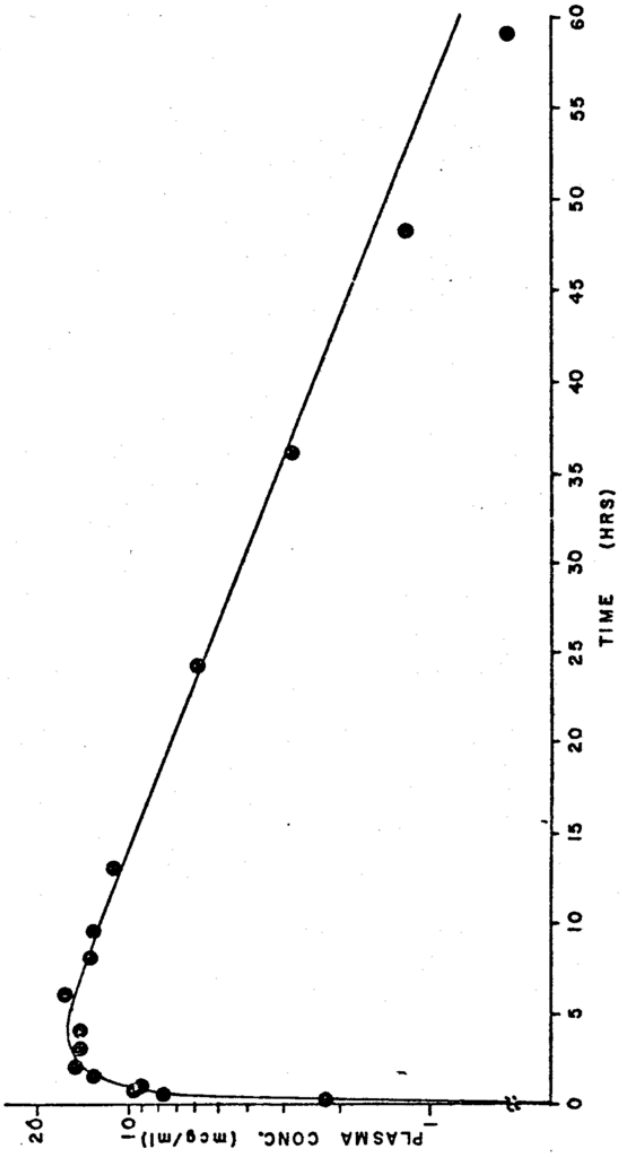
ii. Multiple doses. The plasma level-time data and profile during multiple oral 300 mg doses of metabolite to Dog B are given in Table XXIV and Figure 22.

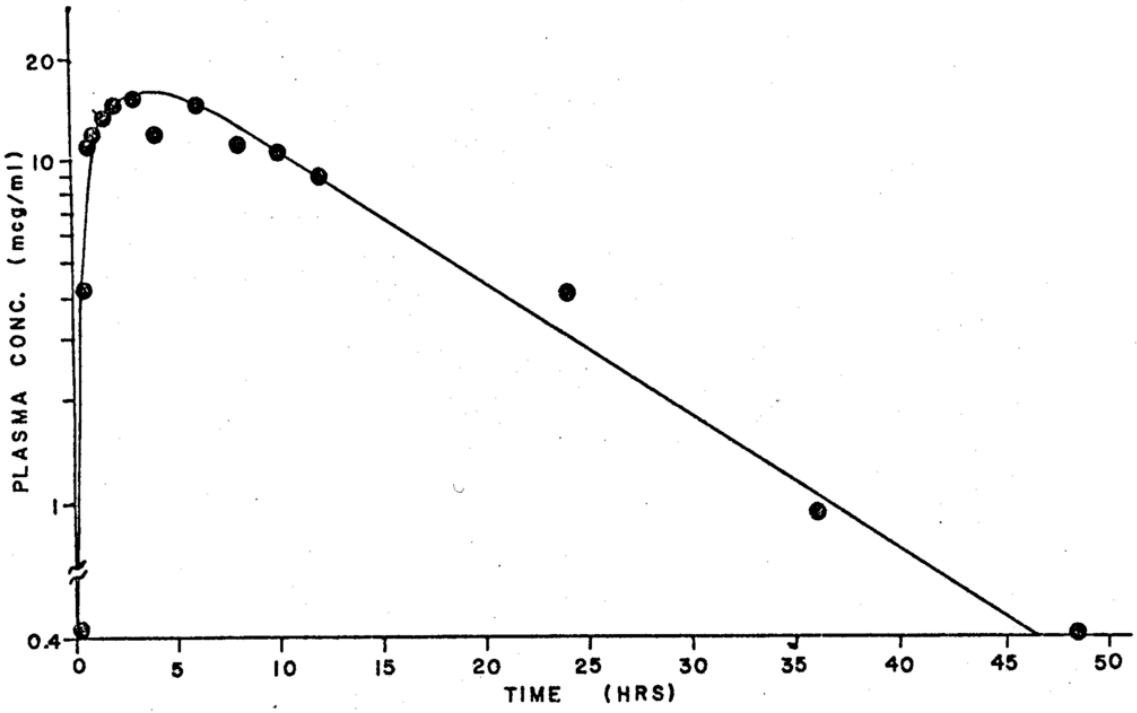
C. Human Studies - Oral Methsuximide Doses

Two volunteers participated in single and short term (7 days) multiple dose methsuximide experiments. In the latter study, blood samples were collected on the last day of the study and continued until the metabolite was cleared from the body. Thus, two complete blood level profiles

Table XXII. Plasma Concentration ($\mu g\ ml^{-1}$)-Time (h) Data from Single 300 mg Oral Metabolite Doses in Three Dogs, Including Dog Weights and TI Values.

| Dog A | | Dog B | | Dog C | |
|---|---|---|---|---|---|
| 16.8 Kg | TI 288 | 19.5 Kg | TI 282 | 14.5 Kg | TI 352 |
| t | $C_m$ | t | $C_m$ | t | $C_m$ |
| 0.25 | 2.2 | 0.25 | 0.42 | 0.25 | 0 |
| 0.50 | 7.7 | 0.50 | 4.2 | 0.50 | 0.32 |
| 0.75 | 9.6 | 0.75 | 11.1 | 0.75 | 2.6 |
| 1.0 | 9.1 | 1.0 | 12.0 | 1.0 | 5.0 |
| 1.5 | 13.2 | 1.5 | 13.3 | 1.5 | 15.4 |
| 2.0 | 15.2 | 2.0 | 14.7 | 2.1 | 18.7 |
| 3.0 | 14.6 | 3.0 | 15.1 | 3.0 | 18.5 |
| 4.0 | 14.5 | 4.0 | 11.9 | 4.0 | 18.0 |
| 6.0 | 16.7 | 6.0 | 14.5 | 6.0 | 15.1 |
| 8.0 | 13.5 | 8.0 | 11.1 | 8.1 | 16.3 |
| 9.5 | 13.3 | 10.0 | 10.6 | 10.1 | 14.6 |
| 13.0 | 11.3 | 12.0 | 8.8 | 12.0 | 13.7 |
| 24.25 | 5.9 | 24.0 | 4.0 | 24.3 | 8.6 |
| 36.0 | 2.9 | 36.0 | 0.90 | 36.1 | 4.3 |
| 48.25 | 1.2 | 48.5 | 0.30 | 48.0 | 1.7 |
| 59.0 | 0.56 | | | 62.0 | 0.56 |


PLASMA CONC. (mcg/ml)
20
10
1
0
5
10
15
20
25
30
35
40
45
50
55
60
TIME (HRS)





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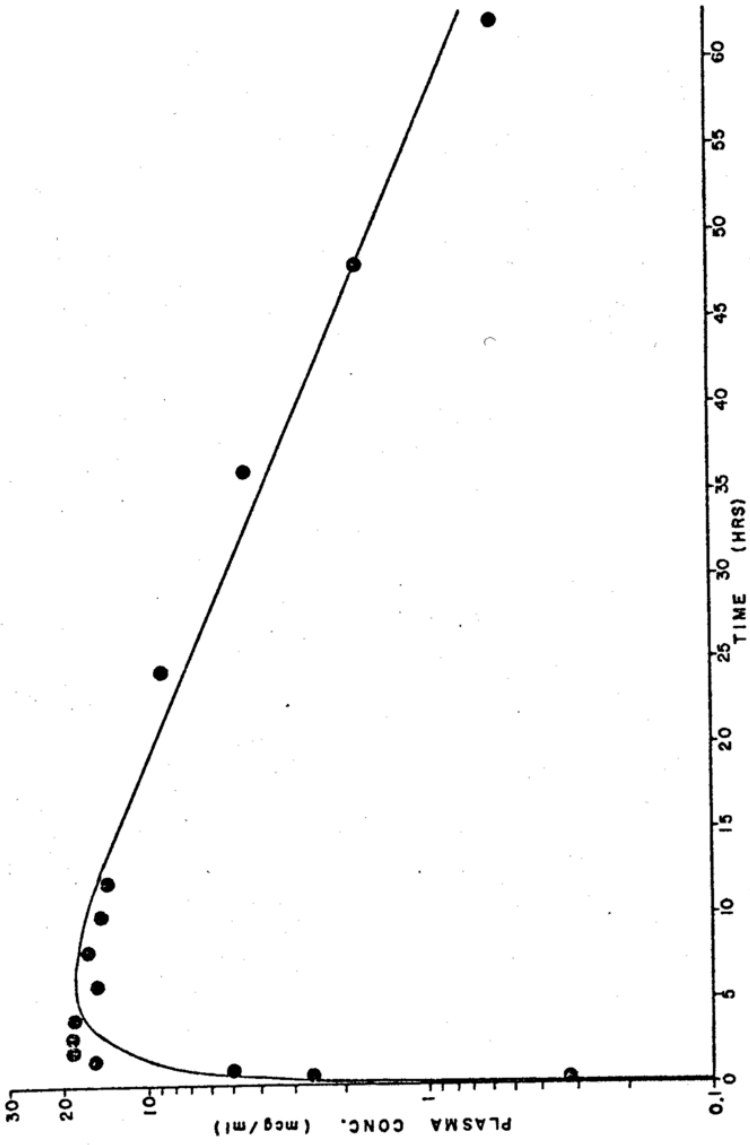
PLASMA CONC. (mcg/ml)
30
20
10
1
0.1
0
5
10
15
20
25
30
35
40
45
50
55
60
TIME (HRS)

| | | | |
|---|---|---|---|
| $R^2$ [d] | 0.9918 | 0.9816 | 0.9335 |
| r[e] | 0.9828 | 0.9664 | 0.9106 |

[a]Calculated from $0.693/k_{30}$.

[b]Calculated from $F'D'/V_3$ with D' = 300 mg and $V_3$ from Table XXI, p. 102.

[c]Calculated from eq. 17, p. 104.

[d]$R^2$ = (Σ squared observations - Σ squared deviations)/Σ squared observations.

[e]r is correlation coefficient.

[f]95% confidence interval.

[g]Indicates parameter was fixed in the regression analysis.

[h]No lag time observed in these experiments.

Table XXIV. Plasma Concentration (μg/ml)-Time (h) Data from Multiple Oral 300 mg Doses of Metabolite in Dog B Weighing 18.6 Kg. Dose was Administered Every Day for 10 Days With Blood Sampling on Days 1, 3, 5, 8, and 10. Blood Samples on Days 2 and 4 Were Drawn Only Near the Peak Concentration.

| Day 1 TI 300 | | Day 2 TI 301 | | Day 3 TI 302 | | Day 4 TI 303 | | Day 5 TI 304 | | Day 8 TI 307 | | Day 10 TI 309 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| t | $C_m$ | t | $C_m$ | t | $C_m$ | t | $C_m$ | t | $C_m$ | t | $C_m$ | t | $C_m$ |
| 0.25 | 11.7 | 0 | 2.5 | 0 | 1.8 | 0 | 1.1 | 0 | 0.86 | 0 | 0.66 | 0 | 0.65 |
| 0.50 | 12.3 | 0.5 | 12.8 | 0.38 | 12.4 | 1.5 | 14.7 | 0.25 | 12.8 | 0.25 | 12.3 | 0.25 | 10.4 |
| 0.75 | 11.6 | 1.5 | 14.2 | 1.0 | 14.4 | 3.0 | 13.8 | 0.50 | 13.8 | 0.50 | 11.5 | 0.50 | 16.2 |
| 1.0 | 14.8 | 3.0 | 13.7 | 2.0 | 12.5 | 4.0 | 10.7 | 0.75 | 15.3 | 0.75 | 12.9 | 0.75 | 14.4 |
| 1.5 | 14.4 | 4.1 | 11.2 | 3.0 | 12.3 | | | 1.0 | 15.0 | 1.0 | 12.0 | 1.5 | 14.2 |
| 2.0 | 14.6 | | | 4.0 | 12.7 | | | 1.5 | 16.1 | 1.5 | 11.2 | 2.0 | 14.2 |
| 3.0 | 13.5 | | | 6.1 | 9.3 | | | 2.0 | 13.2 | 2.0 | 11.7 | 3.1 | 11.0 |
| 4.0 | 11.5 | | | 8.0 | 8.2 | | | 3.0 | 12.5 | 3.0 | 9.8 | 4.0 | 9.6 |
| 6.1 | 11.3 | | | 9.5 | 4.9 | | | 4.0 | 10.0 | 4.0 | 8.4 | 6.0 | 8.0 |
| 8.0 | 10.5 | | | 13.3 | 3.6 | | | 6.0 | 7.9 | 6.4 | 6.3 | 9.0 | 5.1 |
| 10.0 | 8.6 | | | 24.0 | 1.1 | | | 8.0 | 5.5 | 8.0 | 5.2 | 13.0 | 2.7 |
| 12.0 | 7.1 | | | | | | | 10.0 | 5.2 | 10.0 | 3.4 | 24.3 | 0.58 |
| 24.0 | 2.5 | | | | | | | 12.0 | 3.2 | 12.0 | 2.6 | 30.1 | 0.28 |
| | | | | | | | | 24.0 | 0.81 | 24.0 | 0.46 | | |




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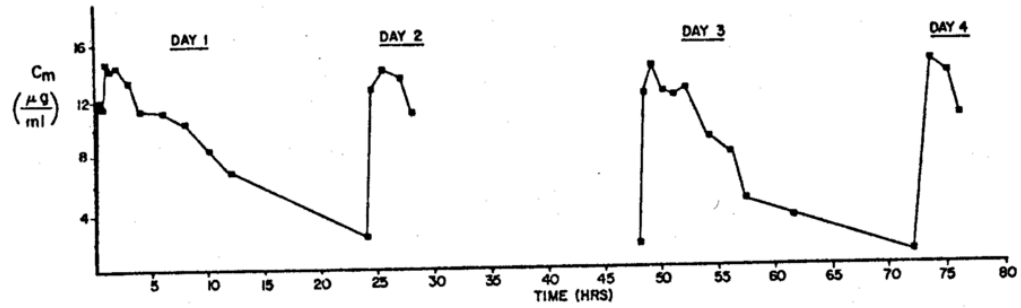
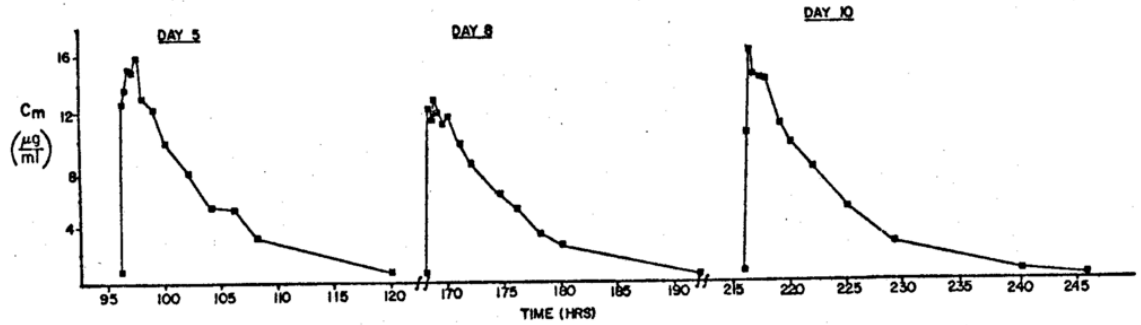


were obtained with each subject; one as a single dose study, and one, after multiple doses.

The plasma level-time data and profiles are given in Tables XXV-XXVI and Figures 23-24.

D. Summary of Area Under Drug and Metabolite Levels in Plasma vs. Time Profiles (AUC) Equations

Area analysis will be used frequently in the discussion section so, for convenience, all AUC equations will be summarized here:

I. Methsuximide AUC Equations

a. iv: $$AUC_{meth}^{meth}\ (iv) = \frac{D}{V_1 K} \qquad (18)$$

b. po: $$AUC_{meth}^{meth}\ (po) = \frac{fFD}{V_1 K} \qquad (19)$$

II. 2-Methyl-2-phenylsuccinimide (Metabolite) AUC Equations

a. iv: $$AUC_{met}^{meth}\ (iv) = \frac{fmWD}{V_3 k_{30}} \qquad (20)$$

$$AUC_{met}^{met}\ (iv) = \frac{D'}{V_3 k_{30}} \qquad (21)$$

b. po: $$AUC_{met}^{meth}\ (po) = \frac{fmFWD}{V_3 k_{30}} \qquad (22)$$

$$AUC_{met}^{met}\ (po) = \frac{F'D'}{V_3 k_{30}} \qquad (23)$$

Table XXV. Plasma Concentration ($\mu g\ ml^{-1}$)-Time (h) Data from Single and Multiple Doses of Methsuximide in Human Subject A.

| Single Dose TI 224 | | | Following Multiple Doses TI 345 | | |
|---|---|---|---|---|---|
| t | $C_l$ | $C_m$ | t | $C_l$ | $C_m$ |
| 0.25 | --[a] | -- | 0 | -- | 4.6 |
| 0.50 | 1.6 | 0.42 | 0.50 | -- | 5.7 |
| 1.0 | 2.0 | 2.2 | 1.1 | trace | 7.3 |
| 1.5 | 1.5 | 2.4 | 2.0 | trace | 8.3 |
| 2.0 | 0.87 | 2.3 | 4.0 | -- | 7.3 |
| 4.0 | 0.25 | 2.3 | 6.0 | -- | 7.9 |
| 6.0 | 0.11 | 2.1 | 8.2 | -- | 7.6 |
| 8.0 | trace[b] | 2.0 | 12.4 | -- | 6.8 |
| 12.0 | -- | 2.2 | 24.0 | -- | 4.5 |
| 24.0 | -- | 1.9 | 48.0 | -- | 3.1 |
| 36.0 | -- | 1.4 | 72.3 | -- | 1.8 |
| 48.0 | -- | 1.35 | 96.3 | -- | 1.2 |
| 72.5 | -- | 0.84 | 120.3 | -- | 0.75 |
| 97.0 | -- | 0.66 | | | |
| 121.0 | -- | 0.44 | | | |

[a] Indicates concentration was below detectable limit.

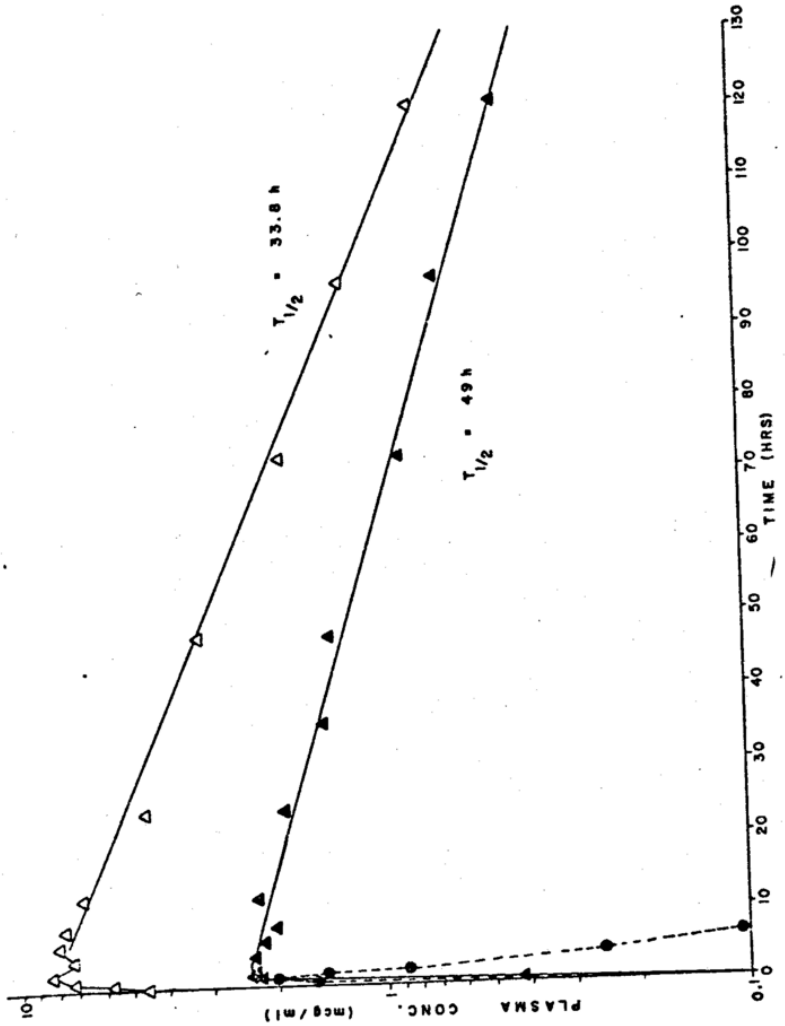
[b] Indicates peak was observed in GC at correct retention time but was below detectable limit.

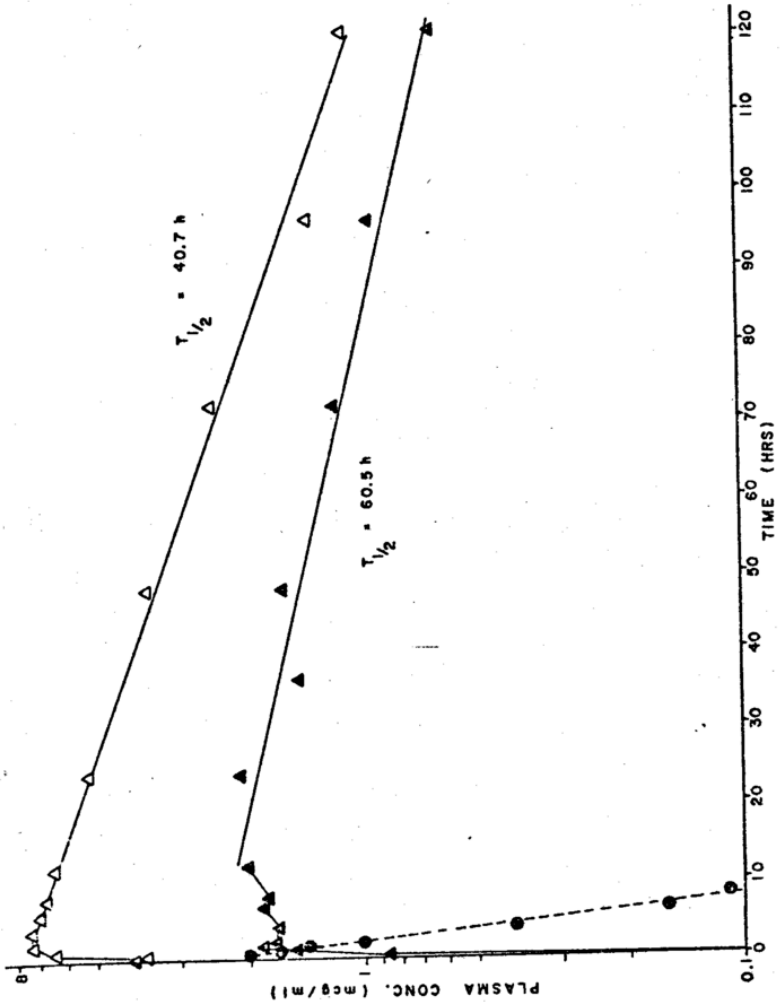
Table XXVI. Plasma Concentration ($\mu g\ ml^{-1}$)-Time (h) Data from Single and Multiple Doses of Methsuximide in Human Subject B.

| Single Dose TI 289 | | | Following Multiple Doses TI 345 | | |
|---|---|---|---|---|---|
| t | $C_l$ | $C_m$ | t | $C_l$ | $C_m$ |
| 0.50 | 2.0 | 0.86 | 0 | --[b] | 4.0 |
| 1.15 | 1.7 | 1.5 | 0.50 | -- | 3.7 |
| 1.6 | 1.4 | 1.9 | 1.15 | -- | 6.4 |
| 2.1 | 1.0 | 1.7 | 2.0 | -- | 7.3 |
| 4.0 | 0.40 | 1.7 | 4.0 | -- | 7.3 |
| 6.4 | 0.16 | 1.8 | 6.1 | -- | 7.0 |
| 8.0 | 0.11 | 1.8 | 8.1 | -- | 6.8 |
| 12.0 | trace[a] | 2.0 | 12.0 | -- | 6.4 |
| 24.0 | --[b] | 2.1 | 24.3 | -- | 5.2 |
| 36.25 | -- | 1.5 | 48.2 | -- | 3.6 |
| 48.0 | -- | 1.6 | 72.3 | -- | 2.4 |
| 72.0 | -- | 1.2 | 96.2 | -- | 1.4 |
| 96.0 | -- | 0.81 | 120.3 | -- | 1.1 |
| 120.5 | -- | 0.61 | | | |

[a]Indicates peak was observed on GC at correct retention time but was below detectable limit.

[b]Indicates concentration was below detectable limit.





# IV. DISCUSSION

An attempt will be made in this discussion to integrate all of the information obtained in the above studies and to present a concise picture of how the 'biological system' handles methsuximide and its active metabolite. The philosophical approach of this discussion is not merely to enumerate the pharmacokinetic parameter values associated with methsuximide, but rather to investigate various phenomena using pharmacokinetics as a tool and methsuximide as a model compound. However, the reader interested in numerical parameter values is provided this information, wherever possible.

The two main phenomena to be considered are hepatic enzyme induction and bioavailability. Hopefully, the methods developed here will be applicable in studies concerning other drugs with pharmacokinetic characteristics similar to the model compound.

## A. Dog Studies

1. Bioavailability of methsuximide from single oral doses of drug. Bioavailability is a term used to indicate measurement of both the relative amount of an administered dose which reaches the general circulation and the rate at which this occurs (44). Comparisons may be made between a) the same drug administered by two different routes (ex. i.m. vs. p.o.), b) two different dosage forms administered

by the same route (ex. brand A vs. brand B), or c) a non-intravenous dosage form of one drug with the intravenous dosage form of the same drug. In the last case, the absolute availability of the drug can be established. Since methsuximide was administered by both intravenous and oral routes in these studies, then the absolute availability of methsuximide from the commercial dosage form can be established.

If a drug is capable of undergoing first-pass metabolism, the absolute systemic availability from an oral dose is dependent on two factors. Namely, the fraction of the dose which is absorbed from the GI tract F, and the fraction of the absorbed dose which reaches the systemic circulation as intact drug f. For example, if 80% of the dose is absorbed from the GI tract (F = 0.80) and 50% of the absorbed dose reaches the systemic circulation after passing through the hepato-portal system (f = 0.50), the remainder being metabolized during the first-pass through the liver, then the fraction of the dose which reaches the systemic circulation is 0.40 (fF).

The absolute availability of methsuximide, eq. 6, is given by the ratio of eq. 19 to eq. 18 (p. 117).

$$fF = \frac{AUC^{meth}_{meth}\ (po)}{AUC^{meth}_{meth}\ (iv)} \qquad (6)$$

In Table XXVII are listed the values of fF for each single oral dose of methsuximide administered to dogs.

Table XXVII. Values of Absolute Systemic Availability (fF) Calculated from AUC Data from Single Oral and Intravenous Doses of Methsuximide to Dogs.

| Dog | $\Delta TI$[a] (d) | $AUC^{meth}_{meth}$ (po)[b] ($\mu g\ ml^{-1}\ h$) | $AUC^{meth}_{meth}$ (iv)[c] ($\mu g\ ml^{-1}\ h$) | fF[d] |
|---|---|---|---|---|
| A | 6 | 0[e] | 20.6 | 0 |
| | 32 | 2.7 | 20.6 | 0.13 |
| B | 43 | 2.0 | 15.7 | 0.13 |
| | 9 | 3.5 | 15.7 | 0.23 |
| C | 12 | 7.9 | 14.3 | 0.55 |

[a] $\Delta TI$ is the length of time between this oral dose and any previous dose to the same dog; i.e., time since last exposure to drug.

[b] From Table XVI, p. 81.

[c] From Tables VII, IX, and XI, pp. 44, 51, and 58.

[d] Calculated using eq. 6.

[e] Methsuximide not detected after oral dose.

From Table XXVII it is apparent that in dogs A and B, the systemic availability is very poor. In dog C, however, some 55% of the dose reaches the systemic circulation unchanged. Hence, there appears to be some inter-subject variability in the value of fF. It should also be noted that, in Table XXVII, there is one case where $AUC^{meth}_{meth}$ (po) is zero. This is a surprising observation from a supposedly single dose. It means that no drug was observed ($C_1 < 0.10$ µg/ml) at any time after a dose of 300 mg was administered. Since high concentrations of metabolite were observed in plasma after this dose (Fig. 8, p. 70) it is apparent that the drug was absorbed and hence, all of the absorbed dose was metabolized before it could reach the systemic circulation. Examination of the ΔTI values in Table XXVII show that this dog was exposed to methsuximide 6 days before this experiment was begun. It is possible that dog A was still in an hepatic enzyme induced state at the time this experiment commenced. Based on these ΔTI values, it appears that a single dose might be able to induce hepatic drug metabolizing enzymes and that this induced state lasts for at least 6 days following exposure to drug but returns to the normal state around 9 days after drug exposure. The time course of hepatic enzyme induction is discussed later (Sec. IV.A.2.).

Since $fF < 1$ in every case, there must be a large first-pass effect ($f < 1$), incomplete absorption of the drug from the GI tract ($F < 1$), or both of these. When a

drug is extensively metabolized, the value of F could be determined directly by use of mass balance considerations. This would require measurement of amounts of every metabolite in all excretory fluids of the body and would be a difficult task, especially when many metabolites are involved. An alternative method must therefore be used to separate the relative contributions of f and F to the absolute availability of methsuximide. Perhaps it is not inappropriate to ask "is it even necessary to separate these contributions?". An affirmative answer can be advocated from two standpoints. First, it is a scientific question and is of fundamental interest; and second, from a more practical standpoint, F may be controlled by physiochemical principles and therefore is under partial control of the formulator. Hence, a low value of fF may be increased by increasing F through modifications of the dosage form. On the other hand, f is under physiological control and its effect can only be eliminated by changing drug administration to a non-hepatic route.

One method to separate the relative contributions of f and F on the systemic availability of a drug is to use the first-pass equation (eqs. 10 or 11, p. 63) to predict a value of f and then calculate the value of F from the observed value of fF. However, at least two experimental problems are involved in the use of the first-pass equations. First, since drug is delivered to the clearing organ (liver) by the blood rather than plasma, $Q_L$ should be

the hepatic blood flow rate and the AUC values should be from blood level-time data in order to make physiological interpretations of the data (45). However, it is impractical to measure the concentrations of drug in blood in most instances. It is now common to determine the blood: plasma distribution ratio of drug, using radiolabeled compounds, and multiply this times the AUC from plasma level data to obtain the area under the blood level-time curve (46,47). Second, blood flow rates to the canine liver vary over a wide range (41,42) and hence these should be experimentally measured in each dog rather than using an assumed average value from the literature. Moreover, it has become apparent that some drugs can alter hepatic blood flow rate through their intrinsic pharmacological action (48,49), a subject which has recently been reviewed (50).

In the light of the experimental problems which may be involved in the use of the first-pass metabolism equations this approach is not used here to separate the relative contributions of f and F on the systemic availability of methsuximide. There is an alternate method which can be used to separate these contributions which is based on the metabolite levels in plasma after doses of parent drug. Since the concentration of metabolite in plasma is directly related to the dose of parent drug over the range of 5-60 mg $Kg^{-1}$ (16), it is apparent that the first-pass effect is not saturated with normal therapeutic doses of methsuximide. The fraction of the absorbed dose of methsuximide which is

converted to the metabolite (fm) is assumed independent of the route of administration; i.e., in Scheme III, fm due to first-pass metabolism is equal to $k_{13}/(k_{10} + k_{13})$. Hence, the AUC of the metabolite after intravenous and oral administration of the parent drug is defined by eqs. 20 and 22, respectively.

$$AUC_{met}^{meth}\ (iv) = \frac{fmWD}{V_3 k_{30}} \tag{20}$$

$$AUC_{met}^{meth}\ (po) = \frac{fmFWD}{V_3 k_{30}} \tag{22}$$

The ratio of these two equations will provide a solution for F as long as fm and $V_3$ are invariant between doses. Changes in $k_{30}$ between doses are readily measurable from terminal slopes of the metabolite levels in plasma-time data so this variance can be accounted for in the calculation. The major problem in the use of this ratio, as will be shown later, is that hepatic enzyme induction occurs after multiple doses of methsuximide. Hence, fm and possibly $V_3$ could be variable quantities dependent on how much prior drug exposure has occurred. The distribution volume of the metabolite $V_3$ was obtained after intravenous metabolite doses in one animal (dog C) both in the uninduced ($\Delta TI=50$) and in the induced ($\Delta TI=5$) states (see Table XX, p. 94). The results of these experiments are shown in Figure 18 and summarized in Table XXI (p. 102) and indicate that $V_3$ does not change (0.80 vs. 0.77 L/Kg) as a result of

hepatic enzyme induction. However, comparison of fm values $[k_{13}/(k_{13} + k_{10})]$, after single and multiple doses of methsuximide, listed in Tables VII, IX, and XI (pps. 44, 55, 58) indicate that this parameter does indeed change with repeated doses and fm is therefore dependent on prior drug exposure. The use of eqs. 20 and 22 to calculate F therefore requires a knowledge of how fm changes with the number of doses of drug administered. This information will be obtained from the multiple dose studies and the absolute availability parameters f and F will then be calculated (Sec. IV.A.3.).

2. Methsuximide multiple dose studies

a. Auto-induction of the biotransformation of parent drug and metabolite during chronic doses of methsuximide. In the previous section, it was shown that, after oral doses, methsuximide is poorly available to the general circulation. Examination of the data from single oral doses of methsuximide (Figs. 8-12, pps. 70, 72, 75, 77, 80) indicate that the pharmacologically active metabolite of methsuximide (2-methyl-2-phenylsuccinimide), is cleared much more slowly from the body than is the parent drug. The $t_{1/2}$ of the metabolite after single doses of methsuximide averages 8.3 hours versus 1.0 hour for the parent drug (Table XVI, p. 81). Thus, during repeated doses of methsuximide (300 mg every 24 hours) only the metabolite will accumulate in the body. If hepatic enzyme induction occurs

on multiple dosing, then it is probable that little, if any, methsuximide will be observed in the plasma. Therefore, at the end of the oral methsuximide study (day 15), an intravenous dose of methsuximide was given to assure high enough drug levels in plasma so that the $t_{1/2}$ and clearance could be accurately measured. These parameters can then be compared with those from the single intravenous doses.

The plasma levels during multiple doses of methsuximide are shown in Figures 13-15 (pps. 84, 88, 92) for dogs A, B, and C. Tables XXVIII-XXX summarize some of the pertinent information obtained from these figures. As anticipated, after multiple oral doses of methsuximide, parent drug could only be detected over short periods of time, if at all, as indicated by the $t_{1/2}$ and AUC values for methsuximide in Tables XXVIII-XXX. Hence, the oral methsuximide levels in plasma are of little value in assessing whether or not hepatic enzyme induction has occurred. However, the methsuximide AUC values decrease between the first and last intravenous doses in all three dogs. This indicates that the hepatic clearance of methsuximide has increased. Table XXXI compares the clearances of methsuximide after the two intravenous doses. Thus it appears that methsuximide has induced its own metabolism. This phenomenon has been well documented both in rats and in humans. In a study of 12 human subjects, at dose levels of 0.6 and 1.2 g of methsuximide, the $t_{1/2}$ of methsuximide after the

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| $t_{max}$ (h) | | | | | | | |
| meth | -- | 2.1 | -- | 2.0 | 1.0 | I | -- |
| met | 6-8 | 4.0 | 2.0 | 3.0 | 3.0 | 3.0 | 2.0 |
| AUC·$k_{30}$ (μg $ml^{-1}$) | | | | | | | |
| met | 11.0 | 11.8 | 11.9 | 10.1 | 9.8 | 8.8 | 8.7 |

[a]Methsuximide was not detected.

[b]Insufficient number of data points $\geq$ 0.10 μg $ml^{-1}$ to do required calculation or observation.

[c]Comparisons must be made between AUC values for each day which are due only to the dose administered on that day. Using day 5 as an example, this is calculated as follows: 1) use trapezoidal rule (51) and calculate AUC from day 5 data points, 2) subtract out AUC due to metabolite left from day 4, $C_{min}$ (day 4)/$k_{30}$, 3) add AUC from time of last data point to infinity, $C_{min}$ (day 5)/$k_{30}$. The value of $k_{30}$ is obtained from the log-linear slope of the metabolite data.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| $t_{max}$ (h) | | | | | | | |
| meth | -- | 0.8 | -- | -- | 1.5 | -- | -- |
| met | 3-6 | 3.0 | 1.0 | 0.75 | 3.1 | 2.1 | 1-2 |
| $AUC \cdot k_{30}$ ($\mu g\ ml^{-1}$) | | | | | | | |
| met | 11.8 | 10.4 | 11.2 | 10.4 | 7.8 | 7.9 | 6.8 |

[a]See footnotes to Table XXVIII.





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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| $t_{max}$ (h) | | | | | | | |
| meth | -- | 3.0 | -- | -- | -- | -- | -- |
| met | 6.0 | 6.0[b] | 2.0 | 3.0 | 2.0 | 1.5 | 2.0 |
| $AUC \cdot k_{30}$ ($\mu g\ ml^{-1}$) | | | | | | | |
| met | 13.9 | 12.5 | 12.3 | 9.4 | 7.7 | 7.8 | 9.2 |

[a]See footnotes to Table XXVIII.

[b]Plasma levels of metabolite from day 1 rise slowly during first 2 hours then rise rapidly (see Table XIX, p. 89). After dose administration dog had deep, hoarse cough and it appears that capsule was lodged in his throat for about 2 hours. Thus, one would expect a very delayed peak time.

Table XXXI. Plasma Clearances[a] of Methsuximide after First and Last Intravenous Doses in Dogs.

| | Plasma Clearance (ml/min) | |
|---|---|---|
| | First Dose | Last Dose |
| Dog A | 241 | 282 |
| Dog B | 313 | 771 |
| Dog C | 343 | 474 |

[a]Clearances obtained from Tables, VII, IX, and XI, pps. 44, 51, and 58.

first dose (week 1) averaged 2.6 hours yet after the fourth dose (week 4) the average $t_{1/2}$ had increased by 75% (13). Studies in the rat have confirmed that this increased rate of biotransformation is due to induction of oxidative drug metabolizing enzymes in the liver. Rats given methsuximide 100 mg/Kg po for 4 consecutive days had 2 hour post-dose liver drug concentrations of 10 µg/g compared to 70-90 µg/g after single doses (8). Drug levels in plasma were 1 µg/g compared to 23-28 µg/ml after single doses. Methsuximide was administered intraperitoneally 100 mg/Kg to a group of 5 rats at 0, 16, and 40 hours and, after sacrifice 24 hours later, the mean liver weight had increased 11% over control (8). The 9000 x g supernatant of liver homogenates exhibited an increased N-demethylase activity. Aniline was used as a probe and there was a 78% increase in enzyme activity and a 20% increase in hydroxylase activity over control values (52). The increased activity of hepatic drug metabolizing enzymes after oral administration of methsuximide for 3 days to rats was also confirmed by standard tests used to measure this activity (14). Hexobarbital induced hypnosis was decreased, while increases were observed in hexobarbitone and aniline oxidations, liver: body weight ratio, liver microsomal cytochrome P450, and hepatic δ-aminolaevulinic acid synthetase. There was a proliferation of smooth endoplasmic reticulum and a reduced anticonvulsant activity. Incubation of methsuximide with hepatic microsomes results in a type I spectral shift and

thus the interaction of methsuximide and cytochrome P450 is similar to that seen with phenobarbital (14).

Examination of the data for the metabolite in Tables XXVIII-XXX (pps. 133-138) reveals a phenomenon which has not previously been reported in the literature. Since the metabolite has an average $t_{1/2}$ of 8.3 hours after single oral doses of methsuximide, it would be anticipated that the metabolite will accumulate when methsuximide is given every 24 hours ($\tau$ = 24 hours), i.e., four half-lives are required to clear approximately 95% of the metabolite from the body (53). Accumulation can be qualitatively assessed by observation of the maximum ($C_{max}$) and minimum ($C_{min}$) concentrations of metabolite during each dosage interval. $C_{max}$ is the peak concentration of metabolite in the plasma while $C_{min}$ is the minimum concentration, i.e., that present immediately prior to administration of the next methsuximide dose. Both $C_{max}$ and $C_{min}$ should increase up to a steady-state value during multiple doses of drug. However, Figures 13-15 and Tables XXVIII-XXX indicate that $C_{max}$ increases only up to day 3, then slowly decreases to a final value which is even smaller than that after the first dose. A similar pattern is observed with the $C_{min}$ values. It appears then, that the induction results in an increased rate of biotransformation not only of methsuximide, but also of metabolite. This observation is confirmed by examination of $t_{1/2}$ values for the metabolite during the multiple dose study (Tables XXVIII-XXX). In each of the three

dogs, the $t_{1/2}$ of the metabolite progressively decreases. Moreover, there is a large progressive decrease in the AUC values for the metabolite - a parameter which may be taken as a measure of the "exposure" of the metabolite to the body.

These observations on the metabolite may be put on a more quantitative basis. After oral doses of methsuximide, the AUC of the metabolite is given by eq. 22.

$$AUC_{met}^{meth}\ (po) = \frac{fmFWD}{V_3 k_{30}} \tag{22}$$

It has been observed that the AUC progressively decreases and this effect is attributed to hepatic enzyme induction. If induction (increase in $k_{30}$) is the only phenomenon which occurs, then the value of AUC x $k_{30}$ should be constant, i.e., the inductive effect is removed from the AUC value. Tables XXVIII-XXX list the values of AUC x $k_{30}$ for each dose of parent drug. From day 1 through day 10, it is not constant; there is a small but progressive decrease in the value in each dog. There is a trend in the values as opposed to normal 'scatter' and thus one or more parameters, besides $k_{30}$, are varying during multiple doses of methsuximide. Eq. 22 can be rearranged to give eq. 24 and each 'constant' in the right hand side examined for its potential to change during multiple doses.

$$AUC_{met}^{meth}\ (po) \cdot k_{30} = \frac{fmFWD}{V_3} \tag{24}$$

The factors W and D are true constants and can be removed from consideration. The distribution volume of metabolite $V_3$ has also been observed to be constant between single dose (0.80 L/Kg) and multiple dose (0.77 L/Kg) studies (Table XXI, p. 102). Of the two remaining factors fm and F it appears that, although the fraction of the dose absorbed F need not be a constant, there is little reason to believe that F should progressively decrease with an increasing number of doses. Hence, the fraction of drug biotransformed to metabolite fm must decrease. That fm does indeed decrease can be confirmed by referring to the intravenous studies (F = 1) at the beginning and end of each oral repeated dose methsuximide study. In Table XXXII are listed the fm values obtained from the results of the computer regression analysis of the intravenous methsuximide experiments. In summary, it has been shown that chronic administration of methsuximide results in stimulation of drug metabolizing enzymes in the liver and leads to: 1) an increased biotransformation rate of methsuximide; 2) an increased biotransformation rate of the active metabolite; and 3) a quantitative shift in the metabolic pattern of methsuximide. Importantly, this shift results in a smaller fraction of the dose being converted to the active metabolite and a greater fraction presumably being converted to the more polar, hydroxylated metabolites.

A quantitative shift in metabolic pattern during multiple doses of a drug would not be difficult to rationalize

Table XXXII. Values of Fraction of Methsuximide Dose Which is Biotransformed to the Metabolite fm after Intravenous Doses of Drug in the Uninduced (Single Dose) and Induced (Following Multiple Doses) States.

| | fm* | |
|---|---|---|
| Dog | Single Dose | Following Multiple Doses |
| A | 0.54 | 0.45 |
| B | 0.60 | 0.35 |
| C | 0.62 | 0.36 |

*Ratio of $k_{13}$ to $(k_{13} + k_{10})$ from Tables VII, IX, and XI, pps. 44, 51, and 58.

if several oxidative drug metabolizing enzymes were involved. Preservation of the overall metabolism pattern from the uninduced to the induced state would require the activity of each enzyme system to be increased equally. Several studies indicate that this does not happen. In rats or mice, 3,4-benzpyrene (type II inducer) pretreatment stimulates the 2- but not the 4-hydroxylation of biphenyl. However, phenobarbital (type I inducer) pretreatment causes a large increase in the 4-hydroxylation but only a small increase in the 2-hydroxylation (54). This suggests at least two enzyme systems which are under independent control. There is evidence that three separate hydroxylating enzymes are involved in the metabolism of chlorobenzene (55). Likewise, in dogs, treatment with phenylbutazone markedly increases the 6β- and 16α-hydroxylations of testosterone but has no effect on the 7α-hydroxylation reaction (56). In the present case, methsuximide has numerous hydroxylated metabolites (see Introduction) and therefore, it is probable that several enzyme systems are involved in its metabolism. Moreover, it appears that the same systems hydroxylate methsuximide and its metabolite since for every hydroxylated methsuximide metabolite found in the urine, there is also the corresponding N-demethylated, hydroxylated metabolite (10,11). On this basis, a quantitative change in the metabolic pattern of methsuximide during repeated doses might not be unexpected.

b. Analysis of problem areas. In the above discussion, a number of conclusions have been drawn based on selected observations or results. It is the hope of the writer, that this work may be of value not only to pharmacokineticists, but also to scientists who may not be thoroughly familiar with the current literature of pharmacokinetics. Hence, a systematic search of the results and observations reported above has been made to identify those which may seemingly contradict the conclusions which have been made. It is the purpose of this section to analyze these problem areas.

One conclusion which has been made is that multiple doses of methsuximide results in an increased elimination of drug through induction of hepatic drug metabolizing enzymes. Based on this conclusion, the observation should be that the $t_{1/2}$ of methsuximide decreases after multiple doses of drug. However, the $t_{1/2}$ values in Tables XXVIII-XXX (pps. 133-138) indicate minimal decreases in these values and in fact, with dog A, the $t_{1/2}$ has increased slightly. The solution to this problem is inherent in the definition of $t_{1/2}$. In any multi-compartment model (methsuximide) the $t_{1/2}$ is equivalent to $0.693/\beta$ and $\beta$ is dependent not only on elimination ($K = k_{10} + k_{13}$) but also on distribution (see eq. 3, p. 36). The relationship between $t_{1/2}$ and K is curvilinear and therefore rather large increases in K (enzyme induction) may be accompanied by little or no increase in $t_{1/2}$ (57,58). This is the

principal reason why plasma clearances (VK) are used extensively in this thesis. On the other hand, with the single compartment model (metabolite) the $t_{1/2}$ is defined by 0.693/K and hence the $t_{1/2}$ will directly reflect hepatic enzyme induction. This can be observed in Tables XXVIII-XXX (pps. 133-138).

The next identifiable problem involves relative changes in the plasma clearance of methsuximide. Table XXXIII compares the clearances of methsuximide and metabolite in the uninduced and induced states. It is noted that there is very little change in the clearance of methsuximide (17% increase) in dog A as compared to dogs B and C. Yet, there is a similar increase in clearance of the metabolite in all three dogs. If the same enzyme systems are responsible for hydroxylating both methsuximide and the metabolite, then it seems illogical that the clearance of metabolite could increase by 67% while that of the parent drug increases by only 17%, in dog A. That this indeed can happen may be demonstrated by use of a hypothetical example. Scheme VI depicts the metabolic pathways of methsuximide including theoretical rate constants for each step in the uninduced (k) and induced states (k*).

Table XXXIII. Plasma Clearances of Methsuximide and Metabolite after Intravenous Administration of Methsuximide to the Dog in the Uninduced (U) and Induced (I) States.

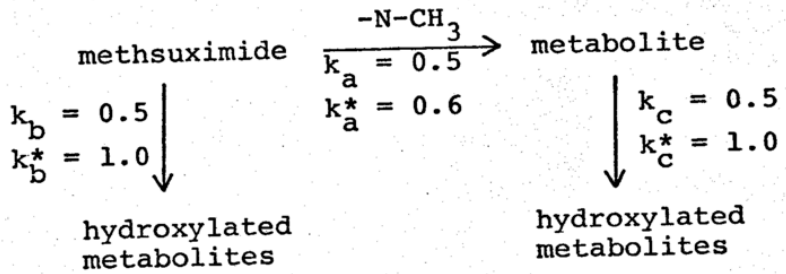
| | Dog A | | Dog B | | Dog C | |
|---|---|---|---|---|---|---|
| State[a]: | U | I | U | I | U | I |
| $cl_{meth}$[b] (ml $min^{-1}$) | 241 | 282 | 313 | 771 | 343 | 474 |
| | | (17)[c] | | (146) | | (38) |
| $cl_{met}$[b] (ml $min^{-1}$) | 12 | 20 | 21 | 33 | 11 | 18 |
| | | (67) | | (57) | | (64) |

[a] U and I represent the uninduced and induced hepatic drug metabolizing states, respectively.

[b] Data is from Tables VII, IX, and XI, pps. 44, 51, and 58.

[c] Numbers represent the percent increase in clearance.

Scheme VI. Metabolic Pathways for Methsuximide with Hypothetical Rate Constants for each Step When in the Uninduced (k) and Induced (k*) States.




In the normal, uninduced state, half of the methsuximide dose would be N-demethylated to the metabolite (which is then totally hydroxylated), and the other half would be hydroxylated directly. In the induced state, the rate constants for the hydroxylation steps double, while the N-demethylation rate constant increases, but to a lesser extent, i.e., a quantitative shift in the metabolic pattern. Comparison of the uninduced and induced states in terms of clearances ($V \cdot k$) indicates that N-demethylation ($k_a$) has increased by 20%, the clearances of methsuximide and metabolite, due to hydroxylations ($k_b$, $k_c$) have increased by 100%, however, the overall clearance of methsuximide ($k_a + k_b$) has increased by only 60%. Thus, hepatic enzyme induction may have a greater effect on the metabolite than on the parent drug and this occurs whenever the hydroxylation reactions are increased to a greater extent than is the N-demethylation step. Scheme VI also shows that even though N-demethylation activity increases, there can be a decrease

in the fraction of drug biotransformed to the metabolite (50% vs. 37.5%).

A third problem is simply whether the observation of an increased clearance of methsuximide and metabolite after multiple doses necessarily proves that hepatic enzymes are being induced. An alternate hypothesis is that clearance of a drug with a high hepatic extraction ratio (clearance/blood flow) will increase if the drug, through its intrinsic pharmacological action, causes an increased blood flow to the liver. Such hemodynamic drug interactions have been recently reviewed (50).

The most convincing evidence, to support the induction theory, is direct in vitro measurements showing increased hepatic enzyme activity after multiple doses of methsuximide (8,14). There is also considerable evidence in these studies supporting the induction theory. If a drug is poorly extracted by the liver, then hepatic clearance is independent of the liver blood flow rate (see Fig. 2, ref. 50). The average clearance of the metabolite (Table XXXIII) in the uninduced state is 15 ml $min^{-1}$ while the average blood flow rate to the canine liver is approximately 700 ml $min^{-1}$. The hepatic extraction ratio (15/700) is extremely small at 0.02, and it is unlikely that the clearance of the metabolite would be influenced by changes in hepatic blood flow.

A fourth problem deals with the computer analysis of the intravenous data (Tables VII, IX, and XI, pps. 44, 51,

59). In these tables, the overall elimination rate constant for methsuximide K, ($k_{10} + k_{13}$), actually decreases in dogs A and B, but not in dog C, between the single and multiple dose studies. This result is in conflict with the concept of induction of hepatic enzymes. In dogs A and B there was a considerable change in all distribution parameters, $k_{12}$, $k_{21}$, $V_1$, and $\alpha$, between the two studies, while in dog C the distribution parameters remain relatively unchanged.

There is no a priori reason why the distribution of methsuximide should change between single and multiple doses of drug. In this case, the distribution phase is very rapid and thus the computer must provide a fit to the data based on relatively few data points. The solution to this problem is to obtain more data points during the distribution phase (first one hour of study). However, in practice this is extremely difficult to do.

The problem of variations in the distribution of methsuximide does not prevent realistic analysis of the data. The clearance of methsuximide after intravenous doses can be accurately determined since it is the ratio of the dose to the AUC. This definition of clearance is model independent (59,60). The clearance determined from K and $V_1$ must give an equivalent answer, i.e.,

$$V_1 K = \frac{D}{AUC^{meth}_{meth\ (iv)}} = cl$$

Hence, if the calculated value of K is too small, then $V_1$ must be larger to obtain the same clearance. Comparison of the data in Tables VII, IX, and XI indicate that this has occurred in dogs A and B between the single and multiple dose studies. This is the primary reason why clearances are compared in these studies rather than the K values. Additionally, the value of fm must be independent of the relative magnitudes of $k_{10}$ and $k_{13}$. Table XXXIV compares the values of fm determined by two independent methods. Thus, while the absolute values of $k_{10}$ and $k_{13}$ are deceptive, the ratio of $k_{13}$ to $(k_{10} + k_{13})$ still provides correct values for fm.

In summary, computer generated results should not be taken as a panacea. It remains up to the experimenter to decide whether or not the generated results are real, even when the results apparently provide excellent fits to the data.

c. Time course of hepatic enzyme induction. The history of hepatic enzyme induction by exogeneous chemicals is relatively brief. The phenomenon was first observed in 1954 by Brown, Miller, and Miller (61). These workers studied the influence of dietary factors on hepatic amino-azo dye N-demethylase activity. Since that time, there have been reports that a wide variety of drugs and chemicals have the ability to stimulate their own metabolism during chronic administration. Table XXXV lists a few of

Table XXXIV. Fraction of the Methsuximide (iv) Dose Converted to the Metabolite, fm, Determined by Two Independent Methods.

| Dog | Dose[a] | fm | |
|---|---|---|---|
| | | $k_{13}/(k_{10} + k_{13})$[b] | AUC Analysis[c] |
| A | S | 0.54 | 0.55 |
| | M | 0.45 | 0.42 |
| B | S | 0.60 | 0.63 |
| | M | 0.35 | 0.36 |
| C | S | 0.62 | 0.60 |
| | M | 0.36 | 0.35 |

[a] S = single dose (uninduced), M = following multiple doses (induced).

[b] From Tables VII, IX, and XI, pps. 44, 51, and 58.

[c] Ratio of eqs. 20 and 21 corrected for variations in $k_{30}$:

$$fm = \frac{AUC^{meth}_{met}\ (iv)}{W \cdot AUC^{met}_{met}\ (iv)} \times \frac{k_{30}}{k_{30}}.$$

these:

Table XXXV. Some Drugs and Other Chemicals Which Stimulate Their Own Metabolism During Chronic Administration (from ref. 62).

| | |
|---|---|
| aminopyrene | glutethimide |
| benzene | hexobarbital |
| 9,10-dimethyl-1,2-benzanthracene | methoxyflurane |
| 3,4-benzpyrene | meprobamate |
| citrus red #2 | phenobarbital |
| chlordiazepoxide | pentobarbital |
| chlorpromazine | probenicid |
| chlorcyclizine | phenylbutazone |
| DDT | tolbutamide |

Although the induction phenomenon is now well documented, there is very little information on the time course of induction. In light of the large number of drugs which are capable of inducing metabolism it would be appropriate to determine at what rate this induction occurs. Such information may be clinically useful. For example, if a patient is to receive the drug for the first time, then the pharmacological and toxicological responses may vary over the induction period and dose adjustments may not be warranted until the metabolic 'steady-state' has been reached.

Until recently, there have been few studies on the

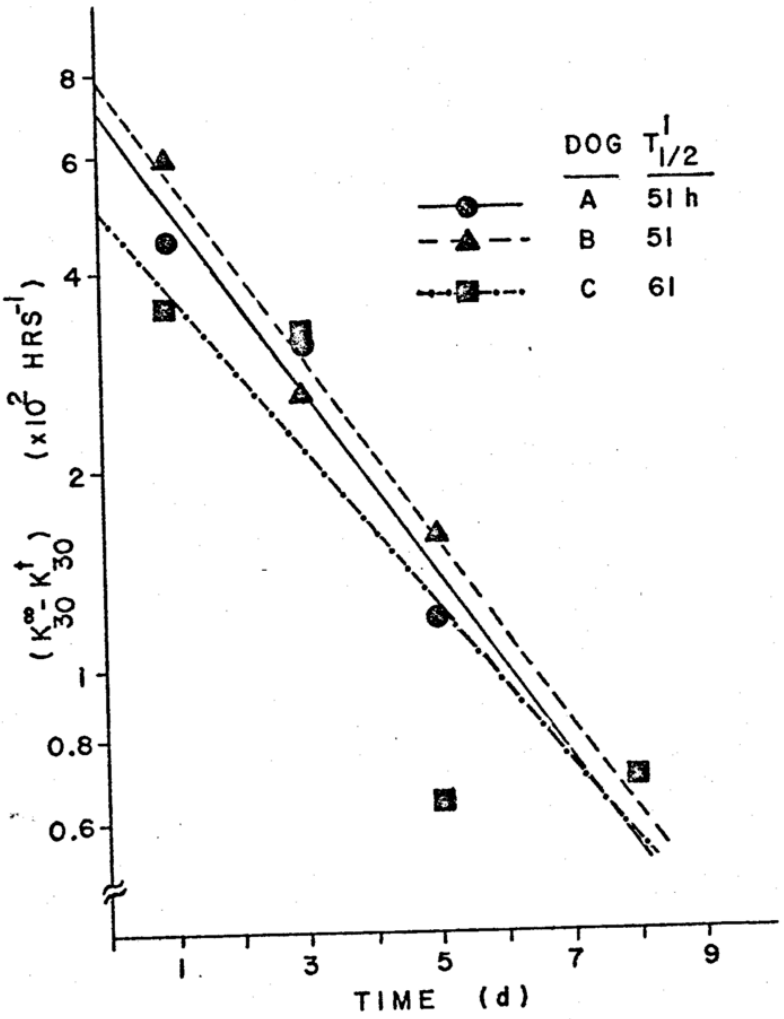
time course of induction. In rats, it appears that the maximum increase in enzyme activity (3-10x) is not reached for at least 3 days after daily phenobarbital administration (63). Levy has pioneered work in this area, using a pharmacokinetic approach, with both laboratory animals and humans. It was observed that after multiple doses of carbamazepine, the serum levels of drug were consistently below those predicted from a single dose of drug (64). A self-induction model was proposed and resulted in excellent prediction of the observed results. In this model, the elimination rate constant was considered to be time dependent as in eq. 25:

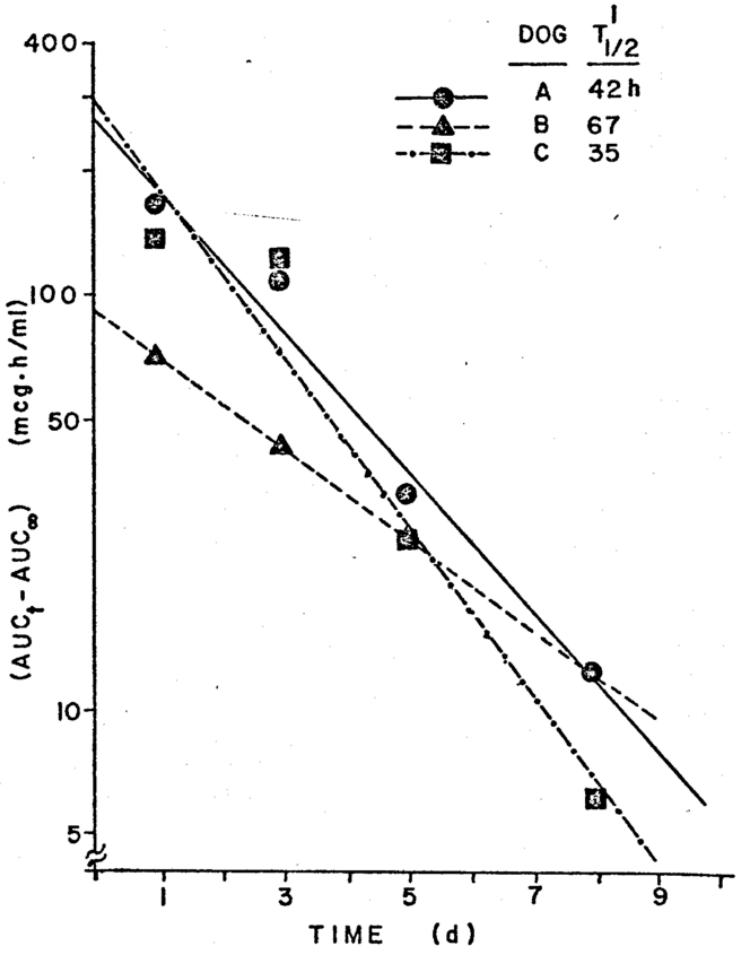
$$K_E(t) = K_E^{\infty} - (K_E^{\infty} - K_e^0)e^{-K_I t} \qquad (25)$$

where $K_E^0$ and $K_E^{\infty}$ are the elimination rate constants after a single dose and after the last chronic dose, respectively. $K_I$ is a first-order "induction rate constant". It was found that $K_E(t)$ increases exponentially between single and multiple doses (65). Unfortunately, the $K_I$ values were not reported. Exponentially decreasing serum concentrations of carbamazepine where observed in the monkey during long term (7 day) intravenous infusions of the drug (66). The induction half-lives $t_{1/2}^i$, $(0.693/K_I)$ were 5.8, 11.6, and 7.6 hours in three monkeys. From these data, it appears that, in the monkey, some 24-48 hours $(4 \cdot t_{1/2}^i)$ are required for hepatic enzyme activity to reach a steady-state value.

Although long-term intravenous infusion experiments have the potential for providing excellent estimates of $K_I$ values (thus also the induction rate) these studies generally are not experimentally feasible. Such infusions require close supervision by the experimenter and that the animal be stationary for a long time period (7 days), and thus would be extremely difficult in ambulatory dogs and virtually impossible in humans. In the present study, a method will be presented to obtain information on the time course of induction which has the potential for routine use in experimental animals and in humans. The only experimental obstacle is that a rather large number of blood samples are required over a relatively short time period.

Information on the time course of hepatic enzyme induction due to chronic administration of methsuximide can be obtained from the values of $k_{30}$, $(0.693/t_{1/2})$, and the AUC of the metabolite after methsuximide doses. Each of these values changes as a function of the number of doses of drug administered (Tables XXVIII-XXX, pps. 133-138). Since the dose is given once per day, then the logarithms of the AUC and $k_{30}$ can be plotted versus days of administration as in Figures 25 and 26. Since neither $k_{30}$ nor AUC are zero at infinite time, then the quantities plotted on the ordinate are respectively, $(k_{30}^{\infty} - k_{30}^{t})$ and $(AUC_t - AUC_{\infty})$ where t refers to any time and $\infty$ to infinite time (day when either parameter first ceases to change, day 8 or 10 in all experiments). Excellent regression correlation




DOG
$T_{1/2}^{l}$
A 42 h
B 67
C 35
400
100
50
10
5
$(AUC_t - AUC_8)$ (mcg·h/ml)
1
3
5
7
9
TIME (d)

coefficients (r) were obtained for the data from each dog in Figures 25 and 26 and all but one of the regressions are significant at the $P \leq 0.05$ level. Thus it appears that both the AUC and the elimination rate constant of the metabolite change exponentially upon repeated doses of methsuximide. The only variable quantity in the AUC (eq. 22) other than $k_{30}$ is fm. Hence, it appears that fm also declines exponentially. The average induction half-lives $t^i_{1/2}$ determined from the $k_{30}$ and AUC values are 54 and 48 hours, respectively. Thus, the time course of hepatic enzyme induction appears to progress exponentially with maximal enzyme activity occurring, on the average, approximately 8 days ($4 \times t^i_{1/2}$) after initiation of daily methsuximide doses to the dog. This time period is much longer than that observed in rats (62) and monkeys (66) and leads to the hypothesis that the time course of hepatic enzyme induction may be drug (potency) and also species (sensitivity) dependent.

There are at least two limitations to the general application of $k_{30}$ and AUC data to assess the time course of induction. The first limitation is that a large number of blood samples* are required to accurately determine these parameters. In humans, this may be possible only in the most willing of subjects. One alternative may be to examine only the terminal log-linear decline in drug concentration

*In these studies approximately 60 blood samples per dog were obtained.

in plasma over 5-10 days. Thus, the elimination rate constant may be adequately estimated from 3-5 data points on the appropriate days. Another alternative may be to administer the drug only every second or third day however this is not desirable if it is not a dosage regimen which is used clinically.

The second limitation to this method is more elusive. Hepatic enzyme induction is a continuously changing process over time, i.e., $k_{30}$ should not be constant over each daily period. However, in the present studies, the $k_{30}$ and AUC values appear to be constant over each daily period because $k_{30}$ is very small and changes slowly. In order to plot the logarithm of AUC or $k_{30}$ versus time one must assign a time value to the AUC or $k_{30}$. In these studies, the AUC or $k_{30}$ values were determined from data over a discreet time period (24 hours) and thus the AUC or $k_{30}$ value due to the first dose is assigned a time value of day 1 and so forth. Thus, the y-intercepts of the lines in Figures 25 and 26 have no physical meaning.

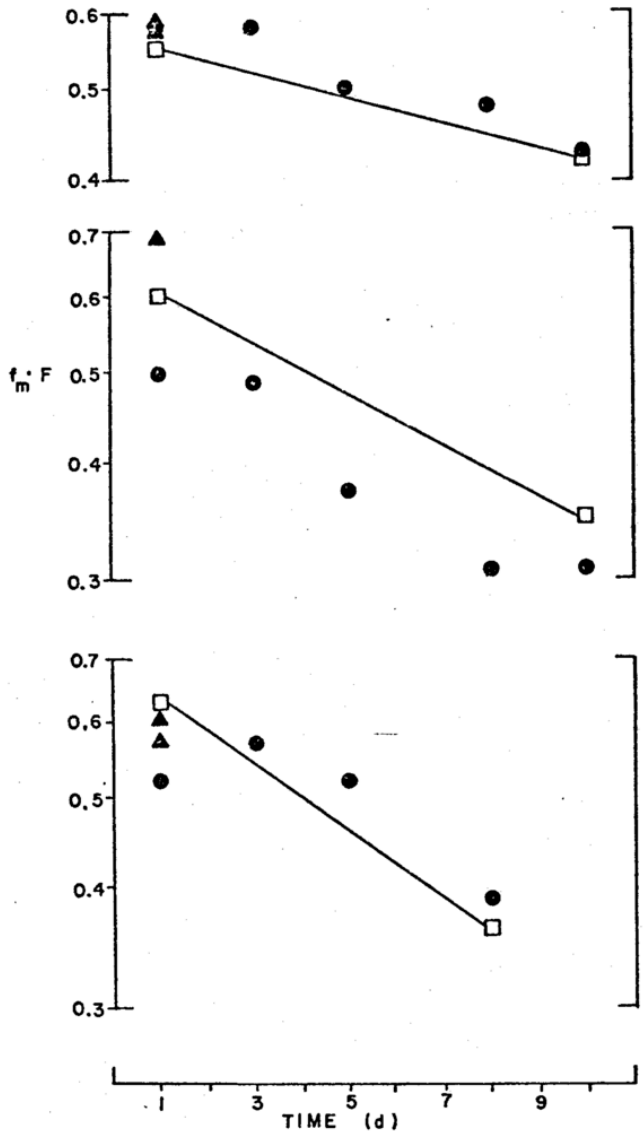
It should be noted that the lag time between first exposure of drug to the liver and the initiation of induction must be less than 24 hours, since after day 1 (Figures 25-26) both AUC and $k_{30}$ start to change. The time between first drug exposure and induction of hepatic enzymes with carbamazepine was 16, 12, and 16 hours in three monkeys (66). As pointed out by Levy, this time delay probably corresponds to hepatic protein synthesis rates (66).

3. Use of metabolite data to calculate contributions of individual parameters to the bioavailability of methsuximide after single and multiple doses. In section III.D. (p. 117) are summarized all of the AUC equations which are useful in calculating the extent of availability of methsuximide from the oral dosage form. The ratio of eqs. 19 and 18 gives an estimate of fF, the fraction of the dose which reaches the systemic circulation unchanged. However, from the methsuximide data alone, there is no way to separate the individual contributions of f and F to the product fF. A low value of fF may be due to extensive first-pass metabolism (f) or poor absorption (F) or both. Since the formulation scientist can have some control over F it is desirable to be able to separate its contribution to fF. The first method proposed to accomplish this was deemed unacceptable for methsuximide since blood flow rates to the liver and distribution ratios of drug between blood and plasma were not determined in these experiments. A second method was proposed but not implemented since information on enzyme induction was required, i.e., how fm changes during multiple doses of drug. This information has now been established (section IV.2.c.) and this method can now be utilized.

Referring to section III.D. (p. 117), it is observed that F, after single and multiple doses of methsuximide, may be obtained from eqs. 20 and 22 provided that it is known how fm changes with time. Figures 25 and 26 indicate

that $k_{30}$ and $AUC_{met}^{meth}$ (po) change exponentially with time during chronic dose administration. Therefore fm must also decrease exponentially over this same time period. The time course of this change in fm can be obtained by plotting the logarithm of fm, during the uninduced and induced states, obtained from the intravenous doses (F = 1) on the same time scale as in Figures 25 and 26. The values of fmF from any oral dose can be calculated from eq. 22 since all other parameters (in eq. 22) are known. Hence, fmF from each daily dose can be plotted on the same figure and F can be obtained by comparison. The data are plotted on Figure 27. To calculate F from any single or multiple dose of methsuximide using this method one simply: a) calculates, using eq. 22, the value of fmF (solid symbols); b) plots this point with the value of the x-axis being the day the dose was administered (i.e., 6 for a dose administered on day 6 of a multiple dosing regimen, single doses are considered day 1); c) determine the value of fm from the solid line for that day, and d) calculate F from values determined in 'a' and 'c' above.

The values of fmF, fm, and F from each oral dose of methsuximide in each dog, calculated by this method, are summarized in Table XXXVI. The F values in Table XXXVI indicate that methsuximide is very well absorbed from hard shell gelatin capsules with an overall (all 3 dogs) F of $0.98 \pm 0.14$ (S.D.). The official compendia (N.F.) requires that the drug content of methsuximide capsules be 92-108%



| | | | | | |
|---|---|---|---|---|---|
| | | 10 | 0.40 | 0.36 | 1.11 |
| | | | | | $\bar{F}$ = 1.01 |
| Dog C | Single dose | 1 | 0.69 | 0.60 | 1.15 |
| | Multiple doses | 1 | 0.49 | 0.60 | 0.82 |
| | | 3 | 0.48 | 0.53 | 0.91 |
| | | 5 | 0.37 | 0.47 | 0.79 |
| | | 8 | 0.31 | 0.49 | 0.63 |
| | | 10 | 0.31 | 0.35 | 0.89 |
| | | | | | $\bar{F}$ = 0.87 |

[a]Indicates day that dose was administered during multiple dose study. Single doses are considered as day 1.

[b]Calculated from eq. 22, $fmF = \frac{V_3 k_{30}}{WD} \cdot AUC_{met}^{meth}$ (po).

[c]From Figure 27 (solid line) or Table XXXIV, p. 153.

(276 to 324 mg) of label claim. Thus, F values greater than 1.00 may be expected. However, it is improbable that every capsule received by dog A was over label claim and hence some of the variability in these values may be due to the method of calculation.

Since the value of F may be determined, then the contribution to fF due to first-pass metabolism can be obtained. The values of fF, f, and F from single and multiple oral doses are summarized in Table XXXVII. Several points concerning the f values in Table XXXVII may be made. After single oral doses of methsuximide (uninduced state) there is a significant amount of absorbed drug which reaches the systemic circulation. In dogs A and B this ranges between 12 and 24% of the absorbed dose. In dog C, 46 and 48% of the absorbed dose escapes first-pass metabolism. However, after multiple doses of drug, hepatic drug metabolizing enzymes are induced and f should decrease. The f values from multiple doses to dog A do not change in any consistent pattern and this correlates with the only slight increase in methsuximide metabolism (clearance) observed after the two intravenous doses of methsuximide. However, in dogs B and C the f values decrease to zero in all but one case, again consistent with increased hepatic enzyme activity. Dog C had the largest change in f between single and multiple doses consistent with the fact that the time course of induction was fastest in this dog ($t^{i}_{1/2}$ = 35 hours).

| | | | | | |
|---|---|---|---|---|---|
| Dog C | Single doses | 1 | 0.55 | 1.15 | 0.48 |
| | Multiple doses | 1 | 0.38 | 0.82 | 0.46 |
| | | 3 | 0.00 | 0.91 | 0.00 |
| | | 5 | 0.00 | 0.79 | 0.00 |
| | | 8 | 0.00 | 0.63 | 0.00 |
| | | 10 | 0.00 | 0.89 | 0.00 |

[a]From Table XXXVI, p. 167.

[b]All single dose data is from Table XXVII, p. 126.

[c]All multiple dose data is calculated using eq. 6 and data in Tables XXVIII-XXX, pps. 133, 135, 137.

[d]Zero values of fF indicate that no drug appeared in the general circulation after an oral dose.

[e]$\Delta$TI value for this dose is 6 so that the animal appears to have been in the induced state.

In summary, methsuximide, in the dog, is well absorbed after oral doses but, on chronic dosing schedules, all of the drug is metabolized on the first-pass through the liver. The poor availability of methsuximide to the systemic circulation is due to first-pass metabolism and hence cannot be increased through dosage form modifications.

4. Metabolite multiple oral dose study

a. Auto-induction of the biotransformation of metabolite after chronic oral doses of metabolite. There are three components to the mammalian hepatic microsomal drug metabolizing mono-oxygenase system (67):

1. cytochrome P450 or $P_1$450 (P448)
2. NADPH-P450 reductase
   (also NADPH-cytochrome c reductase)
3. phosphatidylcholine

Maintenance of enzyme activity(s) requires the presence of all three components. The reactions catalyzed by microsomal mono-oxygenase which may be important in the biotransformation of methsuximide and 2-methyl-2-phenylsuccinimide are (68): a) aromatic hydroxylation, b) aliphatic hydroxylation, and c) nitrogen oxidation (N-dealkylation).

Two types of induction of mono-oxygenase activity have been observed: phenobarbital pretreatment causes an increase in cytochrome P450 while cytochrome P448 is increased after 3-methylcholanthrene (3-MC) pretreatment (63,69). Methsuximide appears to be a phenobarbital type

inducer since both compounds accelerate the metabolism of hexobarbital (14,63) while 3-MC does not (69). A large number of chemicals induce cytochrome P450-dependent monooxygenases and these compounds generally possess three common properties: a) lipophilic character, b) ability to interact with the cytochrome, and c) a reasonable residence time in the liver (70). Auto-induction of the metabolism of non-allyl containing barbiturates correlates well with the logarithm of the 1-octanol/water partition coefficient (71) and hydrophobic interactions appear to be the single most important factor in the binding of substrates to microsomes in aqueous suspension (72). Phenobarbital, the classic inducer, has an extremely long residence time in the body, the approximate $t_{1/2}$ being 100 hours (73). Both methsuximide and the metabolite are highly lipophilic since both attain high concentrations in body fat (7-9). Methsuximide interacts with hepatic microsomes (14) but this information is not available for the metabolite. Although methsuximide possesses the first two properties common to enzyme inducers, it does not possess the third. After multiple doses of drug, in the dog, no methsuximide is observed in plasma, virtually all of it being metabolized during the first-pass through the liver. Even after single oral doses in the uninduced dog, the residence time of methsuximide in the body is very short. However, the metabolite has a long $t_{1/2}$ in the dog and thus it is probable that the enzyme induction observed after chronic

methsuximide administration is due to the metabolite and not the parent drug. To test this hypothesis, multiple oral doses of metabolite were administered to dog B, one dose (300 mg) per day for 10 days. The experimental design was similar to that in the chronic methsuximide experiments and the levels of metabolite in plasma as a function of time are shown in Figure 22. The AUC due to each dose on the sampling days, $k_{30}$, and $t_{1/2}$ values for this experiment are listed in Table XXXVIII. Since the metabolite obeys one-compartment open model kinetics, the $k_{30}$ and $t_{1/2}$ values are a direct reflection of hepatic elimination rates. The data in Table XXXVIII indicate that the metabolite is capable of stimulating its own metabolism apparently through auto-induction of hepatic drug-metabolizing enzymes.

The exposure of metabolite to the body (AUC) is almost halved, while the $t_{1/2}$ is reduced by 43%, between the first and last doses of metabolite. Thus, it appears that the induction of hepatic enzymes observed during multiple doses of methsuximide is due to the metabolite which is formed rather than from the parent drug. However, from these data, it can not be concluded that methsuximide is devoid of enzyme inducing activity since it is impossible to administer methsuximide without the metabolite being formed and at the same time measure enzyme activity through classical means (i.e., hydroxylation of aniline, etc.).

Table XXXVIII. Summary of Results During Chronic Oral 300 mg Doses of Metabolite in Dog B.

| Day | $AUC_{met}^{met}$ (po) [a] ($\mu g\ ml^{-1}\ h$) | $k_{30}$ [b] ($h^{-1}$) | $t_{1/2}$ [c] (h) |
|---|---|---|---|
| 1 | 221 | 0.079 | 8.8 |
| 3 | 135 | 0.121 | 5.7 |
| 5 | 126 | 0.131 | 5.3 |
| 8 | 101 | 0.147 | 4.7 |
| 10 | 119 | 0.139 | 5.0 |

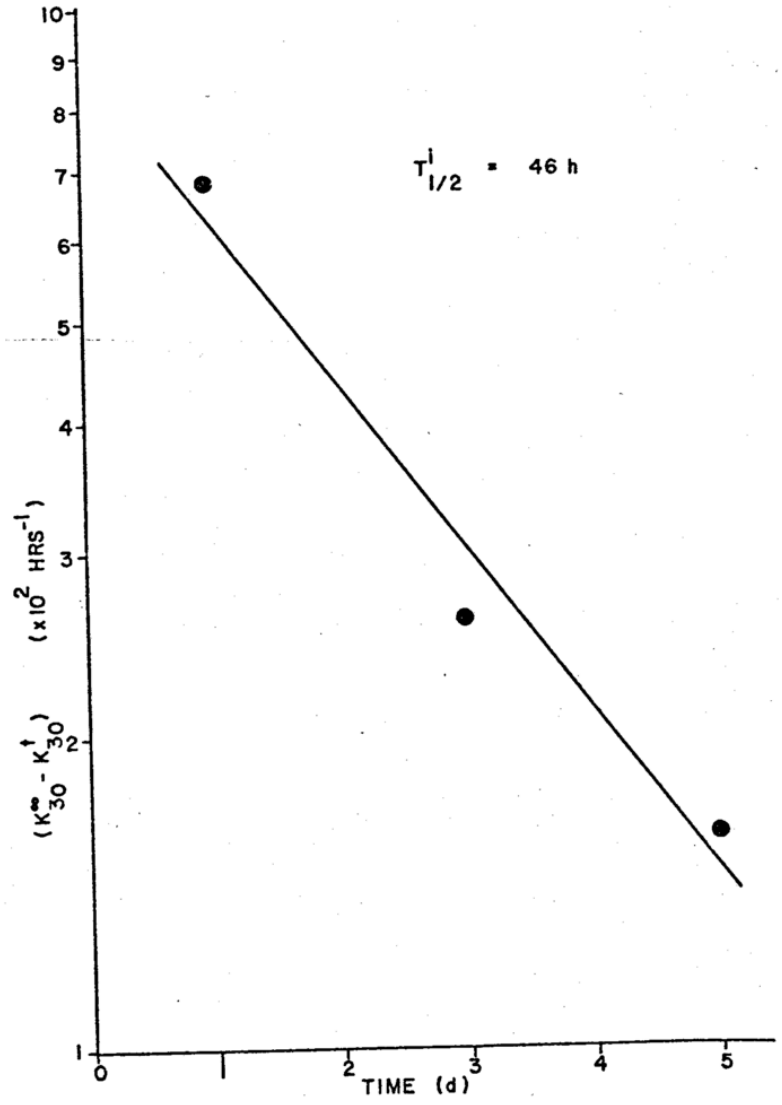
[a] AUC due only to the dose administered on that day, area for day n is calculated by trapezoidal rule minus area due to previous dose $C_f^{(n-1)}/k_{30}$ plus area $C_f^n/k_{30}$ where $C_f$ is the final data point on day n or n-1.

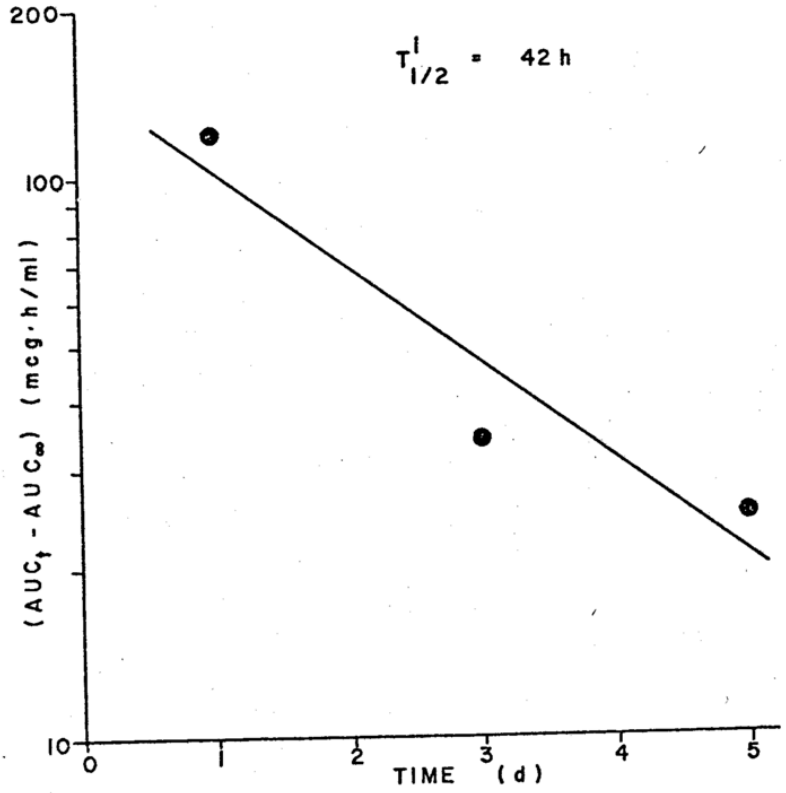
[b] Calculated by linear regression analysis on terminal log-linear data points.

[c] $t_{1/2} = 0.693/k_{30}$.

b. Time course of hepatic enzyme induction. The time course of hepatic enzyme induction has been discussed previously for the case of methsuximide in section IV.A.2.c., p. 152. The same methods can be used to obtain the time course of induction during multiple doses of the metabolite. Figures 28 and 29 graphically depict this time course in terms of AUC and $k_{30}$, respectively. The induction half lives ($t^{i}_{1/2}$) by either method (Figs. 28-29) are approximately the same, 46 vs. 42 hours. The activity of the drug metabolizing enzymes reaches a steady-state approximately 7 days ($4 \times t^{i}_{1/2}$) after commencement of daily doses of metabolite. Induction during chronic metabolite administration is only slightly faster than that after methsuximide (8 days to reach a steady-state). Thus, it appears that the time course of induction is independent of the parent drug. The question of whether or not methsuximide is an inducer of its own right is immaterial, from a practical point of view, since after chronic doses of methsuximide the animal is in the induced state. This state must be maintained by the metabolite because no methsuximide is present - all of it being metabolized on the first-pass through the liver.

5. Bioavailability of metabolite from single and multiple oral doses of metabolite. Metabolism by N-demethylation is a very common route among drugs used in epilepsy (74) and in other diseases (75,76). As with methsuximide


$T^{i}_{1/2}$ = 46 h
$(K^{\infty}_{30} - K^{t}_{30})$ $(x10^{2}\ HRS^{-1})$
TIME (d)
10
9
8
7
6
5
4
3
2
1
0
1
2
3
4
5


200
100
10
$T^{1}_{1/2}$ = 42 h
$(AUC_t - AUC_\infty)$ (mcg·h/ml)
0
1
2
3
4
5
TIME (d)

(9,16,18), all of these antiepileptic agents (74) are N-demethylated to produce a pharmacologically active, slowly cleared metabolite. After multiple doses of methsuximide, or in patients on other inducing drugs (16,17), the levels of metabolite in plasma accumulate in excess of 40 µg/ml with little, if any, parent drug being present. It has been suggested that the metabolite accounts for all of the pharmacological activity of methsuximide during chronic doses and the proposed therapeutic level for the metabolite in plasma is 10-40 mcg/ml (16). Furthermore, there is a reasonably good correlation ($r = +0.6$) between the methsuximide dose and level of metabolite in plasma; the steady-state concentration ($\mu g\ ml^{-1}$) being equal to 1.62 times the dose ($mg\ Kg^{-1}$) (16).

Observations such as those described above have led several research groups to voice their opinions on the subject. Baty (77) has stated that:

> "It is now well recognized that the use of animal models in drug evaluation requires investigation of not simply the structure of metabolites but also their pharmacokinetic behaviour and the correlation of dosage and blood levels."

Similarly, Butler and Waddell (74) state that

> ". . .it appears dubious whether anything is accomplished therapeutically by administration of one of these methylated drugs that cannot be accomplished by administration of the non-methylated compound into which it is converted in the body. Neither is there evidence that the use of methylated drugs permits a lower incidence of toxic reactions."

In a small clinical trial with the metabolite, both methsuximide and metabolite had similar spectra of toxic effects and therapeutic activity (56% decrease in number of seizures per week with the metabolite) but 20% of the patients treated with metabolite experienced GI effects including nausea, vomiting, and drowsiness (78). Strong and coworkers (16) suggest that further evaluation of N-desmethylmethsuximide as an anticonvulsant drug in its own right seems warranted. Any such evaluation would be incomplete without bioavailability information on the metabolite. This is the subject of the following discussion. Each of the three dogs received single oral and intravenous doses of metabolite while dog B also received multiple oral doses. Thus, the absolute bioavailability of the pure crystalline metabolite from a hard shell gelatin capsule can be assessed. The kinetic results from these studies are summarized in Tables XXI (p. 102) (single intravenous doses), XXIII (p. 112) (single oral doses), and XXXVIII (p. 175) (multiple oral doses).

The metabolite is rapidly absorbed after single oral doses with an average absorption half-life $t_{1/2}^{abs}$ $(0.693/k_{03})$ of 1.3 $\pm$ 0.6 (S.D.) hours (Table XXIII, p. 112). The absolute availabilities of the metabolite F' after single and multiple doses are summarized in Table XXXIX. The average F' for metabolite is 0.84 $\pm$ 0.11 (S.D.) while that for methsuximide is 0.98 $\pm$ 0.14 (S.D.). While there is some variability in the extent of absorption, both compounds are

Table XXXIX. Summary of Absolute Availabilities F' of Metabolite after Single and Multiple Oral Doses of Metabolite.

| | F' | | |
|---|---|---|---|
| Dosage Regimen | Dog A | Dog B | Dog C |
| Single dose[a] | 0.904 | 1.03 | 0.958 |
| Multiple doses[b] | | | |
| Day 1 | | 0.81 | |
| 3 | | 0.76 | |
| 5 | | 0.77 | |
| 8 | | 0.69 | |
| 10 | | 0.78 | |
| Average F' | 0.84 | ±0.11 (S.D.) | |

[a] From Table XXIII, p. 112.

[b] Calculated from eq. 23 with AUC and $k_{30}$ values from Table XXXVIII and $V_3$ from Table XXI, p. 102.

very well absorbed.

Since the metabolite is primarily responsible for the pharmacological activity of methsuximide after chronic multiple oral doses of parent drug, it would be instructive to compare the bioavailability of the metabolite from doses of methsuximide with that from doses of the metabolite. These comparisons are made in Table XL. The $t_{1/2}$ and $t_{max}$ values for the metabolite, in Table XL, after doses of either methsuximide or parent drug are approximately the same, hence, the qualitative shapes of the $C_m$-t profiles do not vary. Moreover, this observation provides circumstantial evidence that methsuximide must be absorbed from the canine GI tract at approximately the same rate as is the metabolite.* The $C_{max}$ and AUC values are much lower from the parent drug than from the metabolite. The reason for this is as follows: after oral doses of methsuximide, only the fraction Ffm of the dose appears in the body as metabolite while after doses of metabolite the fraction appearing in the body is F'. The relative bioavailability of the metabolite may be defined as the availability of the metabolite after doses of methsuximide (Ffm) divided by that after oral doses of metabolite (F'). Most importantly, since methsuximide is used on a chronic basis, and since fm

*The absorption rate constant for methsuximide, in these studies, is difficult to obtain directly from oral dose data because of the large first-pass effect. Methsuximide is rapidly absorbed from the rat stomach and small intestine with absorption half-lives of 52 and 17.4 min., respectively (7).

| | | | | |
|---|---|---|---|---|
| Metabolite | $AUC_{met}^{met}$ (po)[d] ($\mu g\ ml^{-1}\ h$) | 367 | 245 | 426 |
| | $t_{1/2}$[d] (h) | 12.8 | 7.7 | 11.3 |
| | F'[d] | 0.904 | 1.03 | 0.958 |
| | $t_{max}$[e] (h) | 3.8 | 3.5 | 6.1 |
| | $C_{max}$[e] ($\mu g\ ml^{-1}$) | 16.1 | 16.1 | 17.9 |

[a]From Table XVI, p. 81.

[b]Fraction of the absorbed dose which is converted to the metabolite after single and after multiple doses of methsuximide; obtained from Table XXXVI, p. 167.

[c]The time $t_{max}$ when the metabolite reaches its observed maximum concentration in plasma, $C_{max}$. Both $t_{max}$ and $C_{max}$ are from Tables XIII-XV, pps. 68, 73, and 78.

[d]From Table XXIII, p. 112.

[e]The time $t_{max}$ is obtained by taking the derivative of eq. 16 (p. 103) with respect to time and equating the result with zero. $C_{max}$ is obtained by substituting the value of $t_{max}$ into eq. 16. Parameter values needed for these calculations are in Table XXIII, p. 112.

decreases during chronic doses, then the relative availability should be Ffm (multiple)/F' (multiple) where 'multiple' refers to the values after repeated doses of the respective compounds. The average relative availability* of metabolite after chronic doses of methsuximide to three dogs is 0.45. This relative availability indicates that only 45% of the methsuximide dose is being effectively utilized in a therapeutic sense. Hence, in dogs, methsuximide may be viewed as an inefficient prodrug.

Based on this bioavailability analysis, and the previously discussed clinical pharmacology study (78), there is no apparent reason why 2-methyl-2-phenylsuccinimide could not be used as an effective therapeutic agent in the treatment of petit mal epilepsy.

B. Human Studies

It is well documented in the literature that the metabolism of methsuximide is stimulated after chronic doses of drug to humans (8,16) and that the metabolite is primarily responsible for the pharmacological activity of methsuximide (15,16,19). There has hitherto been no evidence that the biotransformation of the metabolite may also be induced or that the quantitative pattern of metabolism changes in humans during chronic doses of the parent drug, as occurs

*Ffm (multiple) is from the average of the results in Table XL, while F' is from the average of the results in Table XXXIX.

in the dog. It appears that the dog is a good model for predicting the qualitative metabolic disposition of methsuximide in humans (10). However, since one of the metabolites is pharmacologically active, then the metabolic pattern should be known on a quantitative basis, at least in terms of the active metabolite.

Methsuximide is approved only as an oral dosage form in humans. Under this constraint, and with the large body of information obtained from the dog experiments, it is possible to compare quantitatively the pharmacokinetics of both drug and metabolite in dogs and humans.

The drug and metabolite concentrations in plasma versus time profiles after single and multiple doses of methsuximide in the two subjects are given in Figures 23-24. The pharmacokinetic parameters necessary in evaluating these data are summarized in Table XLI.

Figures 23 and 24 show that unchanged drug reaches the systemic circulation after the first dose but not after multiple doses. In the absence of information from intravenous dose studies, the fraction of the dose which escapes first-pass metabolism can only be estimated through use of eq. 10. The AUC observed after single oral doses of drug, due to methsuximide, appears to represent some 50% of the dose ($f_{po}$, Table XLI). Even after single oral doses, methsuximide is rapidly cleared from the body, has a small AUC value, and hence probably contributes minimally to the therapeutic action relative to the metabolite. After

| | | | |
|---|---|---|---|
| $f_{po}$ | 0.50 | 0.0 | -100 |
| $t_{1/2}$ (met) (h) | 60.5 | 40.7 | -33 |
| $AUC_{met}^{meth}$ (po) ($\mu g\ ml^{-1}\ h$) | 212 | 229[c] | +8 |
| $AUC_{met}^{meth}$ (po) x $k_{30}$ ($\mu g\ ml^{-1}$) | 2.42 | 3.89 | +61 |
| $fm/V_3$ ($10^2\ L^{-1}$) | 0.87 | 1.39 | +60 |

[a]All $t_{1/2}$ and AUC values were calculated as in Table XXXVIII.

[b]Calculated using eq. 10 (p. 63) with F = 1 (assumption supported by studies in dogs), and with plasma flow rate to liver of 11.4 ml $min^{-1}$ $Kg^{-1}$ body weight (39).

[c]AUC due only to dose administered on day 7. See footnotes to Table XXVIII (p. 133) for method of calculation.

[d]Calculated from eq. 22 (p. 117).

multiple 300 mg doses, the first-pass effect is complete and essentially no methsuximide appears in plasma. The $t_{1/2}$, AUC, and $f_{po}$ values for methsuximide, in the two subjects are virtually identical.

The following observations may be made from the data for the metabolite. First, biotransformation of the metabolite, as in dogs, is induced in humans. The degree of reduction in the $t_{1/2}$ between single and multiple doses is identical in the two subjects (32-33%). This phenomenon has not been reported previously. The $t_{1/2}$ of the metabolite reportedly averages 38 hours (17) in humans. This study however, was of a cross-over design from phensuximide to methsuximide and therefore the value probably represents the induced state $t_{1/2}$ and is very similar to the induced metabolite $t_{1/2}$ values reported here.

The second observation is that the AUC values do not decrease between single and multiple doses, as in the dog, but they actually increase slightly. The factors contributing to the AUC can be analyzed using eq. 22, as was done with the multiple dose methsuximide data in dogs.

$$AUC_{met}^{meth} \text{ (po)} = \frac{fmFWD}{V_3 k_{30}} \qquad (22)$$

Since $k_{30}$ increases between the doses, its effect on the AUC can be removed as indicated in eq. 26.

$$AUC_{met}^{meth}\ (po) \times k_{30} = \frac{fmFWD}{V_3} \qquad (26)$$

The values of AUC x $k_{30}$ increase by 52 and 61% in the two subjects (Table XLI), and assuming that FWD remains constant[a] the change must be due to fm, $V_3$, or both. Since $V_3$ did not change in dogs with repeated doses of drug (Table XXI, p. 102), then the change must be due to fm. The absolute values of fm and $V_3$ can not be determined since $V_3$ can only be obtained from plasma level-time profiles resulting from oral or intravenous doses of the metabolite and the metabolite is not an approved drug for human use. However, the values of the ratio $fm/V_3$ do indicate that fm increases substantially (52 and 60%), assuming $V_3$ is constant, between single and chronic doses in the two subjects.[b] This phenomenon is the opposite of what happens in the dog. While chronic doses of methsuximide results in increased body clearances of methsuximide and the metabolite in both dogs and humans, the fraction of the dose biotransformed to the

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[a] F can not be responsible for increases of this magnitude since, in dogs, F was very high after single doses and no significant change occurred as a result of repeated doses.

[b] If the assumption can be made that the volume of distribution of the metabolite is the same in dogs and humans on a volume per Kg of body weight basis, then fm increases between single and multiple doses of drug from 0.60 to 0.91 and from 0.54 to 0.87 in the two subjects. After multiple doses of drug, most of the dose is N-demethylated and methsuximide would be a very efficient 'pro-drug'. However, the assumption that $V_3$ is the same in dogs and man is tenuous and would have to be proven by direct measurement of $V_3$ in human subjects.

metabolite fm decreases in dogs but increases in humans. This species dependency reflects quantitative rather than qualitative differences in the metabolic pattern of methsuximide between the uninduced and induced states. Namely, fm can increase in humans as a result of induction if N-demethylase activity is increased to a greater extent than is the activity of the hydroxylating enzymes, and fm can decrease in dogs if the opposite occurs. Thus, the sensitivity of the multiple enzyme systems to induction appears to be species dependent.

In summary, since the metabolite is primarily responsible for therapeutic activity of methsuximide, then hepatic enzyme induction is a deleterious effect, in dogs, in two ways: a) the body clearance of the metabolite increases, and b) less active metabolite is formed. In humans, however, induction causes an increased clearance of metabolite but is accompanied by an increase in the fraction of metabolite formed. Importantly, in the two subjects studied, these two effects appear to counteract one another since the AUC values do not change significantly between the doses (Table XLI). Hence, it appears that the 'exposure' of the metabolite to the body does not change significantly as a result of hepatic enzyme induction in the human. Finally, the relative bioavailability of the metabolite after doses of methsuximide and doses of the metabolite, a measure of the efficiency of metabolite delivery after doses of parent drug, remains unknown until

the metabolite can be administered to humans. Dependent on the absolute value of fm during chronic dose administration, methsuximide may or may not be an efficient 'prodrug'.

# V. CONCLUSIONS

A number of major conclusions concerning the pharmacokinetics of methsuximide and its major active metabolite, 2-methyl-2-phenylsuccinimide, in dogs and humans can be drawn from the results of this thesis. These are enumerated as follows:

1. The dissolution of methsuximide from its commercial dosage form was rapid. 95% of the dose dissolved in 35 minutes on the average.

2. Methsuximide was well absorbed from the GI tract with the fraction of the dose absorbed being 98 $\pm$ 14 (S.D.) %.

3. After oral doses, methsuximide undergoes extensive first-pass metabolism. The fraction of the absorbed dose of methsuximide which reached the systemic circulation, f, after single oral 300 mg doses ranged between 12 and 48%. After multiple doses, f was effectively reduced to zero, i.e., all of the drug was metabolized during the first-pass through the liver. Similarly, in humans f was predicted to be approximately 0.5 after single oral 300 mg doses but this value decreased to zero after multiple doses.

4. Induction of hepatic drug metabolizing enzymes occurs after repeated doses of methsuximide in dogs and humans. This induction results in an increase in not only the total body clearance of methsuximide, but also that of the metabolite. There is adequate evidence in the literature that the metabolism of methsuximide can be induced but this is the first report that the biotransformation of the metabolite is also induced after chronic doses of methsuximide to dogs or humans.

5. Hepatic enzyme induction results in a quantitative shift in the metabolic pattern of methsuximide. Most importantly, the magnitude and direction of this shift is species dependent. In the dog, the average fraction of the absorbed dose of methsuximide which was converted to the active metabolite, fm, reduced from 59% after a single dose, to 39% after multiple doses. However, in humans fm increased by 50-60% between single and multiple doses. The absolute values of fm in humans could not be established from the available data.

6. During the induction period in dogs, the elimination rate constant of the metabolite $k_{30}$ increased and the area under the metabolite concentration in plasma-time curve (AUC) decreased semilogarithmically with the

number of daily doses of parent drug administered. The induction half-life averaged 51 hours so that the activity of drug metabolizing enzymes in the liver reached a steady-state value approximately 8 days after the commencement of administration of 300 mg daily doses of parent drug.

7. In a therapeutic sense, the AUC of the metabolite after doses of parent drug is the single most important parameter in comparing single and multiple doses of methsuximide. In dogs, fm decreased and $k_{30}$ increased leading to an AUC value, after multiple doses, which was less than half that after single doses. However, in humans, both fm and $k_{30}$ increased and, in the two subjects studied, the AUC remained relatively constant between single and multiple doses. Hence, the dog is a poor model for humans when studying the quantitative effects of hepatic enzyme induction on the pharmacokinetics of methsuximide and its major metabolite, 2-methyl-2-phenylsuccinimide.

8. The metabolite is capable of inducing hepatic drug metabolizing enzymes and probably is responsible for the induction observed during multiple doses of methsuximide. The time course of induction during chronic doses of the metabolite is only slightly faster than during chronic doses of parent drug. The steady-state

activity of the hepatic enzymes was reached approximately 7 days after the start of daily oral doses of the metabolite.

9. The metabolite is rapidly and almost completely absorbed after oral doses (crystals in hard shell gelatin capsules) to the dog. The absorption half-life averaged 1.3 hours with ca. 84% of the dose being absorbed.

10. The relative bioavailability of the metabolite from doses of parent drug compared to doses of metabolite averaged 45% in the three dogs. Since the metabolite is primarily responsible for the therapeutic activity of methsuximide, only 45% of the dose of parent drug is being effectively utilized. In man, the relative bioavailability of the metabolite cannot be determined, without data from administration of the metabolite to humans, but could be greater than that in the dog if the distribution volume of the metabolite $V_3$ is similar in dog and man on a volume per Kg body weight basis. Hence, the evidence that the N-demethylated metabolite, rather than methsuximide, may be a more efficient way of delivering metabolite to the human body, although compelling, has yet to be proven.

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# APPENDIX A

## Glossary of Terms

$A_1$ The amount of methsuximide in its central body compartment at any time after dose administration.

$A_2$ The amount of methsuximide in its peripheral body compartment at any time after dose administration.

$AUC_a^c$ (b) From time zero to time infinity, the area under the plasma level-time curve of compound 'a' after the 'b' route of administration of a single dose of compound 'c'.

$AUC_t$ The area under the metabolite level in plasma-time curve from time zero to time infinity due only to a single dose administered on day t during a multiple dose regimen.

$AUC_\infty$ The area under the metabolite level in plasma-time curve from time zero to time infinity due only to a single dose administered on a day, during a multiple dose regimen, when induced hepatic drug metabolizing enzyme activities have stabilized.

$\alpha$ The composite first-order rate constant which is defined by eq. 3 (p. 36) and represents the distribution phase of methsuximide after intravenous administration of drug.

$\beta$ The composite first-order rate constant which is defined by eq. 3 (p. 36) and represents the post-distributive elimination of methsuximide after intravenous administration of drug; $\beta = 0.693/t_{1/2}$.

$C_1$ The concentration of methsuximide in the plasma (central compartment).

$C_m$ The concentration of metabolite in the plasma.

$C_{max}$ The maximum concentration of metabolite in the plasma during a dosage interval $\tau$ during oral doses of methsuximide or metabolite.

$C_{min}$ The minimum concentration of metabolite in the plasma during a dosage interval $\tau$ during doses of methsuximide or metabolite.

cl The plasma clearance of drug ($V_1K$ or $\frac{D}{AUC^{meth}_{meth}\ (iv)}$) or metabolite ($V_3k_{30}$ or $\frac{D'}{AUC^{met}_{met}\ (iv)}$).

D The dose of methsuximide administered.

D' The dose of metabolite administered.

$f$ The fraction of the absorbed oral dose of methsuximide which reaches the systemic circulation as unchanged drug.

$f_{po}$ The fraction of the absorbed oral dose of methsuximide (using eq. 10, p. 63) predicted to reach the systemic circulation as unchanged drug when only oral drug data are available.

$f_{iv}$ The fraction of the oral dose of methsuximide predicted (using eq. 11, p. 63) to reach the systemic circulation as unchanged drug, assuming the oral dose is completely absorbed, when only intravenous drug data are available.

$F$ The fraction of the oral dose of methsuximide which is absorbed from the gastrointestinal tract.

$fF$ The fraction of the oral dose of methsuximide which is available to the systemic circulation as unchanged drug.

$fm$ The fraction of the absorbed oral or intravenous dose of methsuximide which is biotransformed to the metabolite, 2-methyl-2-phenylsuccinimide.

$F'$ The fraction of the oral dose of metabolite which is absorbed from the gastrointestinal tract.

$G$ The amount of methsuximide in the gut at any time after an oral dose of methsuximide.

$H$ The blood hematocrit defined as the volume fraction of red blood cells in total blood.

$k$ The first-order absorption rate constant for methsuximide absorption from the gut into the hepato-portal system.

$k_{03}$ The first-order absorption rate constant for metabolite absorption from the gut into the central body compartment.

$k_{10}$ The first-order elimination rate constant for excretion or metabolism of methsuximide to compounds other than 2-methyl-2-phenylsuccinimide.

$k_{13}$ The first-order rate constant for formation of metabolite from methsuximide (or metabolism of methsuximide to 2-methyl-2-phenylsuccinimide).

$k_{CH}$, $k_{HC}$ The first-order rate constants describing the transfer of a compound between the central (C) and hepato-portal (H) compartments in Scheme II.

$k_{CP}$, $k_{PC}$ The first-order rate constants describing the transfer of a compound between the central (C) and peripheral (P) compartments in Scheme II.

$k_e$, $k_m$ The first-order rate constants for urinary excretion (e) of drug and metabolism (m) during the first-pass through the liver.

$k_{30}^{t}$ The first-order rate constant for elimination of the metabolite on day t during a multiple dose regimen.

$k_{30}^{\infty}$ The first-order rate constant for elimination of the metabolite, during a multiple dose regimen, when induced hepatic drug metabolizing enzyme activities have stabilized.

K The overall first-order elimination rate constant for methsuximide ($k_{10} + k_{13}$).

$K_I$ The first-order rate constant for the induction of hepatic drug metabolizing enzyme activities.

$K_E$ (t) The first-order rate constant for elimination of drug at any time during the hepatic drug metabolizing enzyme induction process.

$K_E^0$ The first-order rate constant for elimination of drug when hepatic drug metabolism enzyme activities are not induced.

$K_E^\infty$ The first-order rate constant for elimination of drug when hepatic drug metabolizing enzyme activities are fully induced.

$\lambda$ The slope of the apparent log-linear decline in the concentration of methsuximide in plasma-time profile after oral drug administration.

M The amount of metabolite in the body at any time after administration of drug or metabolite.

met An abbreviation for metabolite.

meth An abbreviation for methsuximide.

$Q_L$ The blood or plasma flow rate to the liver.

$t_{1/2}$ The half-life of a compound in plasma.

$t^{i}_{1/2}$ The induction half-life describing the time course of induction of hepatic drug metabolizing enzyme activities, $0.693/K_I$.

$t^{abs}_{1/2}$ The absorption half-life of methsuximide.

$t_{lag}$ The time period (lag time) between dose administration and first appearance of drug or metabolite in plasma.

$t_{max}$ The time after dose administration when drug or metabolite concentration in plasma is maximal.

TI, ΔTI The time index, which is an integer, assigned to each dose of drug administered so that the length of time in days between administration of any two doses of drug to the same subject can be obtained by difference (ΔTI).

$\tau$ The dosage interval, which is the length of time between consecutive doses in a multiple dose regimen.

$V_1$ The volume of distribution of methsuximide in its central compartment.

$V_3$ The volume of distribution of metabolite in its central compartment.

$V_c$ The central compartment volume of distribution of any drug in Scheme II.

W The molecular weight ratio of metabolite and methsuximide (189/203).

# APPENDIX B

## Consent Form for the Study of Factors Influencing Gastrointestinal Drug Absorption (Methsuximide) in Human Volunteers.

CONSENT FORM

FOR THE STUDY OF FACTORS

INFLUENCING GASTROINTESTINAL DRUG ABSORPTION (METHSUXIMIDE)

A study is being conducted by the School of Pharmacy, in conjunction with the University Hospitals, University of Wisconsin, on the absorption and also the accumulation in the blood stream of some orally administered drugs during single and repeated doses.

The study and consent form, described below, are concerned with the absorption and accumulation in the blood stream of the anticonvulsant drug methsuximide. The reasons for us carrying out this study are that, although methsuximide is used in clinical practice, very little is known about how efficiently oral doses of methsuximide are absorbed into the blood stream and also what blood levels of methsuximide are obtained during repeated (one dose each day) doses. Methsuximide breaks down in the body to form other compounds, one of which is known to be therapeutically active. We are interested in what types of blood levels of these compounds are achieved after repeated doses of methsuximide.

We intend to obtain information about methsuximide absorption and accumulation during repeated doses by administering an oral dose of methsuximide to you each day for seven days. During that time, as described in detail later, we will take blood samples (5 ml, about one

teaspoonful) from your arm. We will then measure the amounts of methsuximide, and also compounds that it has broken down to, in the blood.

Before describing the study in detail, here is some information you should know about methsuximide:

Methsuximide is one of several drugs used for the control of seizures in certain forms of epilepsy. It is quite a potent drug.

The most common side effects associated with repeated doses of methsuximide are nausea, vomiting, stomach pain, and constipation. Methsuximide may also cause changes in your red cell count and white cell count in your blood. Methsuximide has caused various conditions such as skin rashes, drowsiness, dizziness, irritability, nervousness, headache, blurred vision and hiccups.

Warning: Owing to the possibility that methsuximide may cause drowsiness, you will not be permitted to drive a motor vehicle or handle any type of moving machinery on the days you receive methsuximide. If you develop any reaction to methsuximide during the study you must immediately discontinue participation.

The dose of methsuximide you will receive each day is about the same as that used in initial therapy with this drug. It is lower than that used in regular maintenance therapy.

Study Details

If you are accepted as a candidate for the study, you will receive a physical examination by Dr. Harold Booker (or another qualified physician) to see whether you are in good physical shape and have nothing wrong with you to preclude you from the study. The physical examination is free. If you pass the physical (your record is of course confidential, and will be given to you on completion of the study) you will then be advised as to the precise study details and sampling schedules. The program will be as follows:

1. You will not be permitted to take any drug known to induce your drug metabolizing enzymes (liver enzymes) for one month before the study. You will not take any drug other than the required doses of methsuximide, and that includes alcohol, for one week before and also during the study.

2. You will come to the School of Pharmacy, or the Neurological and Rehabilitation (N and R) Hospital at 8 am each day for seven (7) days to receive your daily dose of methsuximide (one 300 mg capsule). When you get up in the morning you will drink 150 ml (about 5 ounces) of water. You will take your methsuximide capsule with an additional 150 ml of water. You will take the capsule on an empty stomach after overnight fast, and you will not be permitted to eat any food until 11 am. So you will take one capsule each morning under these conditions for seven days.

3. On days one, three & five of the study you will give blood samples (5 ml) at 8 am, 8.30 am, 9 am, 10 am, 4 pm and 8 pm.

4. On day seven you will give more blood samples (5 ml each time) at 8 am, 8.30 am, 9 am, 10 am, noon, 2 pm, 4 pm and 8 pm. You will then give more blood samples at 8 am each day for five more days. We need to take this many blood samples after your last dose as one therapeutically active metabolite of methsuximide stays in the body for a long time and we want to monitor the rate at which it disappears from your blood stream. Altogether you will give 31 blood samples (not including the one for your physical) during the study, i.e., during a period of about 12 days.

5. We will assay all your blood samples for methsuximide and its break down products to find out the levels of these compounds in your blood stream and how they accumulate in your body during repeated dosing.

THE FOLLOWING POINTS ARE IMPORTANT:

1. There will be no immediate benefits to you by taking the drug methsuximide in this study.

2. Apart from the risks already mentioned, the only other major risk to you will be that of infection due to repeated blood sampling. This risk is extremely small as all blood samples will be drawn in the School of Pharmacy or at the N and R Hospital by a fully qualified technician

under aseptic conditions.

3. You will receive a copy of this consent form which you may retain.

4. If there is anything you do not understand about this consent form, or the study in general, please ask Dr. Booker or Dr. Welling to explain it to you.

5. You are free to withdraw your consent and to discontinue participation in the study at any time.

CONSENT

I have read and understood the information provided in this consent form.

Anything that I have not fully understood has been explained to me.

I understand that this study will be of no benefit to me. I further understand that I am free to withdraw from the study at any time.

I hereby give consent for Dr. Harold Booker, Dr. Peter Welling, or a qualified assistant, to administer the methsuximide to me as indicated in the study protocol. I also consent to Dr. Booker or a qualified assistant to obtain venous blood samples from me at the times and using the procedures described to me.

Subject_____ Witness_____

Date_____ Date_____

# APPENDIX C

Derivation of Equations for Scheme III Which Describe:

a) The Concentration of Drug and Metabolite in Plasma as a Function of Time, and

b) The Area Under the Concentration-Time Profiles of Drug and Metabolite;

After Oral Doses of Methsuximide

The pharmacokinetic model after oral doses of methsuximide, including formation of the metabolite, is a composite of the models for methsuximide and metabolite after intravenous doses of each compound with provisions for first-order absorption and first-pass metabolism of methsuximide. The model in Scheme III and the appropriate equations, are complex. After oral drug administration the equations describing the concentration-time profiles will be triexponential for methsuximide and quadraexponential for the metabolite. (Absorption phase adds one more exponent to the equations which describe the data after intravenous doses.) It is apparent from the oral dose profiles, Figures 8-12, that it is impossible to obtain this number of exponents from these data. It appears that, even with the simplest two-compartment model (no first-pass metabolism), it is difficult to determine three exponents from oral blood level data. In fact, data simulated using the two-compartment model with oral absorption often are generally adequately described by the one-compartment model equations (79). The problem is compounded further when first-pass metabolism occurs since the amounts of drug reaching the systemic circulation may be a small fraction of the dose, so that only few measurable blood-level data points may be obtained. This is the situation with methsuximide after oral doses. Even when Scheme III is simplified, by assuming that methsuximide obeys one-compartment model kinetics, it is difficult to obtain meaningful values for

each of the parameters. The difficulty is principally due to the large number of parameters and too few data points. However, one approach which may be used is area analysis. This approach is used here and it enables the calculation of values for all bioavailability parameters except the absorption rate constant.

The differential equation describing the amounts of drug in the gut (G) and body (A) compartment, assuming methsuximide obeys single compartment kinetics, as a function of time are:

$$\frac{dG}{dt} = -kG \tag{C1}$$

$$\frac{dA}{dt} = fkG - KA \tag{C2}$$

and that for the metabolite in its compartment is:

$$\frac{dM}{dt} = k(1 - f)fm \cdot G + k_{13}A - k_{30}M \tag{C3}$$

The application of the La Place transform to linear differential equations is useful since it converts them into linear algebraic equations. The La Place transform of a time dependent function f(t) is F(s) as defined by eq. C4,

$$F(s) = \int_0^\infty e^{-st} f(t)dt \tag{C4}$$

where s is a real number such that the integral converges

for some finite value of s and all greater values. The La Place transforms of functions common to pharmacokinetics do not have to be calculated with equation C4 but are tabulated in La Place transform tables (80).

The La Place transforms of eqs. C1-C3 are:

$$\bar{G} = \frac{FD}{(s + k)} \tag{C5}$$

$$\bar{A} = \frac{fk\bar{G}}{(s + K)} \tag{C6}$$

$$\bar{M} = \frac{k(1 - f)fm\bar{G} + k_{13}\bar{A}}{(s + k_{30})} \tag{C7}$$

where the bar represents the La Place transform of that quantity. Substitution of C5 into C6, and substitution of the resulting equation into C7 for $\bar{A}$, yields equations where the La Place transforms are only functions of s and model parameters:

$$\bar{A} = \frac{fkFD}{(s + k)(s + K)} \tag{C8}$$

$$\bar{M} = \frac{k(1 - f)(fmFDs + k \cdot k_{13}FD}{(s + k)(s + K)(s + k_{30})} \tag{C9}$$

The anti-La Place of eqs. C8 and C9, found in tables (80), yields the desired equations when both sides are divided by the appropriate distribution volumes V and $V_3$:

$$C = \frac{fFD}{V}\left(\frac{k}{k - K}\right)\left(e^{-Kt} - e^{-kt}\right) \qquad (C10)$$

and

$$C_m = \frac{kWFD}{V_3}\left\{\frac{k(1 - f)fm - fmK}{(k_{30} - k)(k - K)} \cdot e^{-kt} + \frac{-f \cdot fmK}{(K - k_{30})(k - K)} \cdot e^{-Kt}\right.$$

$$\left. + \frac{k_{30}(1 - f)fm - fmK}{(K - k_{30})(k_{30} - k)} \cdot e^{-k_{30}t}\right\} \qquad (C11)$$

The area under the plasma level-time curves for methsuximide and metabolite may be obtained by integrating equations C10 and C11 from zero to infinite time. This integration is tedious in that it requires a large number of algebraic manipulations to simplify the resultant expressions. A simpler method of obtaining the AUC is to make use of a basic property of La Place transforms as recently reiterated by Wagner (81). From the definition of the La Place transform, eq. C4, it can be seen that a time dependent function f(t) {C(t) or $C_m(t)$} can be integrated between the limits of zero and infinite time simply by setting s equal to zero in the La Place transformed differential equations (eqs. C8 and C9). Thus,

$$AUC^{meth}_{meth}\ (po) = \left.\frac{fkFD}{(s + k)(s + K)}\right|_{s = 0} \qquad (C12)$$

and

$$AUC_{met}^{meth}\ (po) = \left.\frac{k(1 - f)fmFDs + k \cdot k_{13}FD}{(s + k)(s + K)(s + k_{30})}\right|_{s = 0} \qquad (C13)$$

which after simplification becomes:

$$AUC_{meth}^{meth}\ (po) = \frac{fFD}{VK} \qquad (C14)$$

and

$$AUC_{met}^{meth}\ (po) = \frac{fmFD}{V_3 k_{30}} \qquad (C15)$$

# APPENDIX D

## Pharmacokinetics of Methsuximide and a Major Metabolite in Dogs

by

Michael R. Dobrinska and Peter G. Welling