THE PHARMACOKINETICS OF D- AND L-α-ACETYLMETHADOL AND THEIR N-DEMETHYLATED METABOLITES IN THE DOG

Francis L. S. Tse

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

L-α-acetylmethadol (LAAM) is currently being investigated as a substitute for methadone in the treatment of narcotic abstinence symptoms. It is known to have a slower onset and longer duration of pharmacologic activity than its d- enantiomer (DAAM), and repeated dosing of LAAM has caused cumulative toxicities in some cases. Both LAAM and DAAM undergo extensive biotransformation in man or experimental animals to form active metabolites, with only a small fraction of the dose being excreted unchanged. It has been postulated that the difference in time course of pharmacologic activity between LAAM and DAAM is due mainly to differences in the pharmacokinetic behavior of their active metabolites.

The present study was undertaken to provide a relatively complete pharmacokinetic profile for LAAM and its N-demethylated metabolites, l-α-noracetylmethadol (NORLAAM) and l-α-dinoracetylmethadol (DINORLAAM). The pharmacokinetic behavior of drug and metabolites after administering LAAM was then compared with that after dosing DAAM. Finally, the accumulation characteristics and drug metabolizing enzyme inducing properties of LAAM and DAAM and

their respective major metabolites during repeated doses of LAAM and DAAM were examined.

Each of three dogs was administered single intravenous doses of LAAM, DAAM, NORLAAM and DINORLAAM, and single oral doses of LAAM and DAAM. Two of the dogs also received LAAM and DAAM orally on a chronic basis, one dose every two days for 14 days. Pure samples of the nor- and dinor- metabolites of DAAM (NORDAAM and DINORDAAM, respectively) were not available for experimentation.

Plasma level data indicated that the pharmacokinetics of LAAM, DAAM and their N-demethylated metabolites after intravenous doses to dogs can be adequately described by a two compartment open model. Apparent distribution volumes at steady state were 1260, 1260, 500 and 300% body wt for LAAM, NORLAAM, DINORLAAM and DAAM, respectively, indicating extensive tissue uptake of these compounds. Average biological half-lives were 6.6 hr for LAAM, 12 hr for NORLAAM and 3.6 hr for DINORLAAM. After intravenous administration of LAAM, plasma NORLAAM levels were very low, fluctuating but persistent. Higher levels of NORLAAM and DINORLAAM were observed after oral doses of LAAM, probably because of rapid metabolism of LAAM in the liver during first-pass. Although LAAM had a shorter half-life than NORLAAM, the conversion of LAAM to NORLAAM appeared to be less efficient than that of NORLAAM to DINORLAAM.

After intravenous administration, the half-life of

DAAM (ca 3.5 hr) was shorter than that of LAAM, but increased significantly (to ca 6.2 hr) after the drug was given orally. The half-life of NORDAAM showed a similar increase from 5 hr after intravenous DAAM to 8 hr after orally administered DAAM.

Both LAAM and DAAM were rapidly and almost completely absorbed after oral doses. However, the two isomers differed in the extent of first-pass metabolism, the data indicating more efficient hepatic extraction of DAAM than LAAM. DINORDAAM appeared to resemble DINORLAAM in having a shorter true half-life than the nor- metabolite. Hence, for both LAAM and DAAM, the rate of elimination of the nor-metabolite was believed to be the limiting factor in the loss of both the nor- and dinor- metabolites. The longer half-lives of both LAAM and NORLAAM than those of the corresponding d- isomers may partially reflect the different time courses of pharmacologic effects of LAAM and DAAM.

The pharmacokinetics of LAAM and DAAM, as well as their nor- and dinor- metabolites, were virtually unchanged by repeated dosing of the parent drugs. The dosage regimen employed in this study resulted in no accumulation of LAAM or DAAM, and very little accumulation of the metabolites.

APPROVED: Peter G. Welling

Peter G. Welling, Assoc. Prof.

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BY

FRANCIS L. S. TSE

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To Irene

and

To My Parents

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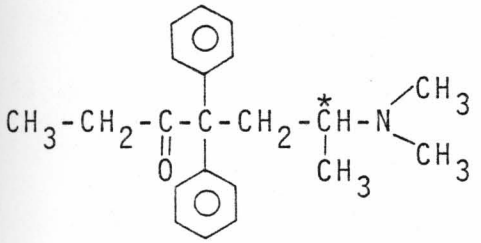
I. INTRODUCTION

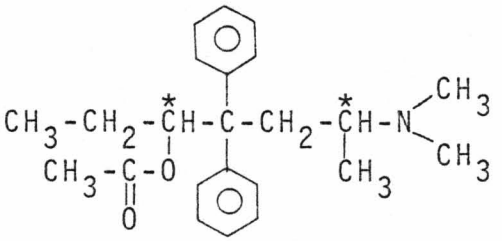
A. Background

1. The Isomers

Acetylmethadol (6-dimethylamino-4,4-diphenyl-3-acetoxyheptane), a closely related derivative of methadone (6-dimethylamino-4,4-diphenyl-3-heptanone), was first synthesized by Bockmühl and Ehrhart in 1948 (1) and was further described by Speeter and associates (2) and Pohland and associates (3) in 1949.



Methadone



Acetylmethadol

Due to the presence of two asymmetric carbon atoms in the molecule, two pairs (α and β) of enantiomers exist. The Bockmühl-Ehrhart synthesis by sodium and propanol reduction of dl-methadone yields the β acetylmethadols, while the Speeter and Pohland procedures which utilized platinum oxide hydrogenation of dl-methadone give the α isomers. It was also noted (3) that the α-methadols, and hence their acetylated derivatives, exhibit optical rotations that are opposite in sign to the parent ketones. Thus l-α-acetylmethadol (LAAM), which has the

S-configuration at C_3 and at C_6, is derived from d-methadone with the 6S configuration, and d-α-acetylmethadol (DAAM) from l-methadone which has the 6R configuration.

When comparing the morphine-like activities and also the toxicities of the various isomers of methadone, methadol and acetylmethadol in mice, Eddy and associates (4,5) found that both α and β dl-methadols were less toxic and had less analgesic effects when dosed orally or subcutaneously than dl-methadone. The acetylmethadols, on the other hand, were similar to dl-methadone in toxicity, but had greater analgesic effect. Other investigators (6,7) found dl-α-acetylmethadol to be more constipating than morphine in rats and rabbits when given in equivalent analgesic doses, though there is less likelihood of developing drug tolerance. A more recent study (8) showed that the duration of antinociceptive action of LAAM, determined in the rat tail pinch test, was six times that of morphine and three times that of methadone. The onset of activity was considerably later after LAAM than after morphine or methadone.

Of the α and β diastereoisomers, the α-dl-acetylmethadols have been shown to have more analgesic activity and to be less toxic in mice than the β-dl forms (5). David and associates (9) studied the control of chronic pain by α-dl-acetylmethadol and reported that oral or subcutaneous doses of 5 to 10 mg given three or four times daily were well

tolerated and highly effective, with relief of pain occurring within 20 to 30 minutes of dosing and lasting between four and five hours.

In tests of presumptive analgesia in rodents, Chen (10) observed that LAAM had a delayed onset and prolonged duration of analgesic activity when compared with the d-isomer. Using the rat tail burn method, Pohland and associates (3) found that the analgesic activity of LAAM and DAAM were 37% and 200%, respectively, of that of dl-methadone. These observations are in good agreement with those made by Smith (11), who showed that DAAM was more potent as an analgesic agent than LAAM. In rats, DAAM had double the analgesic activity of racemic methadone, whereas LAAM was about one-fourth as active. The levo forms also exhibited delayed onset and prolonged duration of pharmacologic effects. Clinical studies by Keats and Beecher (12) confirmed the superior analgesic activity of DAAM to LAAM and also indicated delayed toxicity after repeated doses of LAAM in the form of unexplained coma somewhat similar to morphine poisoning. Other reports of clinical toxicities of the α acetylmethadols, particularly the long duration and cumulative toxicities of the dl- and l- forms, caused them to be abandoned as analgesics (13,14).

The first clinical study on the addicting characteristics of α acetylmethadol was reported by Fraser and Isbell (15). In ten morphine addicts, 30 mg of the racemic

mixture or 15 mg of DAAM given subcutaneously induced intense euphoria, starting within 30 minutes and lasting 30 hours post dose. Thirty mg of LAAM produced no effect until nine hours after subcutaneous or intravenous injection, while euphoria appeared within two hours upon administering equal doses orally. The duration of euphoria was longer after dosing LAAM than DAAM. Further studies in man (13) showed that morphine-like effects were observed within 30 minutes of a subcutaneous injection of DAAM, whereas injection of LAAM by the same route resulted in a four to six hour delay in onset of activity, with prolonged action lasting up to 72 hours. Both the delay in onset and duration of action were somewhat shorter following oral doses of LAAM. After repeated doses of LAAM, 30 mg b.i.d. for two days, cumulative toxic effects manifested as depression approaching coma, severe nausea and mental confusion appeared in some patients. These were prevented, however, and previously narcotic addicted patients were well maintained, when the LAAM dosage interval was increased to 72 or even 96 hours.

Many subsequent clinical studies have been conducted to compare the efficacy of either α-dl-acetylmethadol or LAAM with that of methadone in the treatment and rehabilitation of narcotic addicts. The results indicated no observable differences in the safety and effectiveness of the two drugs (14,16-22). Methadone patients usually

experience a "rush"-like effect within 2 hr of taking medication. This effect does not occur with LAAM, which appears to have a smoother action and a more consistent inner state associated with it. This difference between methadone and LAAM appears to be an important factor in patient preference and acceptance (22-24). A major advantage of LAAM, however, is that it will suppress abstinence symptoms for approximately twice as long as methadone (23), and some investigators (18,19) suggested 80 mg oral doses of LAAM three times a week as a substitute for daily administration of 100 mg methadone. The established efficacy and prolonged activity of LAAM provide particular advantages in permitting less frequent doses, less frequent visits by patients to the clinic and the possibility of more effective control over illegal opiate distribution.

2. Metabolism and Pharmacokinetics of α-Acetylmethadols

In an attempt to explain the delayed onset and prolonged activity of LAAM, as observed in the earlier investigations, many experiments were conducted to study the metabolism and pharmacokinetics of this drug. A number of accurate and sensitive chemical assays have been reported for determining LAAM and its major metabolites in biological fluids (25-31). However, difficulties still exist in assay specificity and hence in the simultaneous determination of these compounds.

Sung and Way (32) studied the metabolism and physiological disposition of both enantiomers of α-acetylmethadol in the rat and showed that both are extensively metabolized with little, if any, drug being excreted unchanged. They noticed that the time course of morphine-like effects did not correlate with tissue levels of LAAM, and suggested that observed LAAM activity was due to an active metabolite. It was further postulated that the delayed and prolonged activity of LAAM compared to that of the more potent dextro-rotatory isomer was due to a slower rate of metabolism and greater secretion into stomach followed by reabsorption.

Indeed, it was subsequently shown that the analgesic effect of LAAM after intraventricular injection was significantly reduced in mice pretreated with the metabolic enzyme inhibitor SKF-525A, except during the first hour (33). The lack of inhibition during the first hour was thought to be due to sufficiently high levels of LAAM to provide maximum pharmacologic response despite reduced metabolite formation.

In a detailed study of the metabolism of dl-α-acetylmethadol in male albino rats, McMahon and associates (34) used oral and intravenous doses of 2.5 mg/Kg of acetylmethadol and a derivative, noracetylmethadol, with ^{14}C in the N-methyl or the O-acetyl groups. Comparison of data from the two routes of administration indicated efficient absorption

of both acetylmethadol and noracetylmethadol from the gastrointestinal tract. Based on the rates of excretion of radiocarbon dioxide from rats receiving labelled compounds, these authors showed that the major route of acetylmethadol metabolism was sequential N-demethylation. The half-time of removal of the first N-methyl group was approximately two hours, a rate roughly three times faster than removal of the second N-methyl group. Hence, they proposed that the active metabolite of LAAM is l-α-noracetylmethadol (NORLAAM), which is probably responsible for the delayed and prolonged effects of LAAM. Hydrolysis of the acetyl group was shown also to be a significant pathway of dl-α-acetylmethadol metabolism, to the extent of about 30% following intravenous administration. However, the resulting carbinol could not be detected in tissues. Tissue distribution studies in rats after intravenous dosing of labelled acetylmethadol demonstrated peak levels of radioactivity in lung, muscle and brain within one to five minutes, and later in liver and kidney. Plasma levels were low, reached minimum values at about 30 minutes, but rose to a new maximum at eight hours or later. It was suggested that the second peak represented re-entry of final metabolites into the circulation from tissue sites. After oral administration, much lower levels were obtained in all tissues except liver, where maximum levels were generally reached at 0.5 to 1.0 hour after dosing. Specific identification of

acetylmethadol and noracetylmethadol showed that at 0.5 hour after intravenous dosing, the unchanged drug predominated over the metabolite in all tissues. At four hours, it still predominated in brain, while both compounds were present in equal quantities in lung tissue. However, in the liver, the major site of metabolism, noracetylmethadol predominated. Total body radioactivity was shown to be persistent in rats dosed with N-methyl labelled noracetylmethadol, the rate of disappearance indicating a half-life of about 14 days.

Billings and coworkers (27) identified both NORLAAM and α-l-dinoracetylmethadol (DINORLAAM) in rats dosed orally with LAAM HCl (5 mg/Kg), and reported half-times of disappearance of 13 and 21 hr, respectively, for the nor- and dinor- metabolite. The peak plasma level of both compounds occurred at about 4 hr, which could account for the delayed onset of activity following administration of the tertiary amine.

Two subsequent studies have compared the morphine-like activity of the nor- and dinor- metabolites with that of the parent compound. Nickander and associates (35) showed that both metabolites were approximately 15 times more effective than LAAM in depressing electrically induced twitch of longitudinal muscle of the guinea pig ileum *in vitro*, and concluded that they are responsible for the prolonged *in vivo* effects of LAAM. However, in an *in vivo*

mouse writhing test, Smits (36) obtained less morphine-like activity from the dinor- than from the nor- metabolite. Noracetylmethadol is also an active analgesic in man. In a double-blinded study involving 300 patients, Gruber and Baptisti (37) showed that this compound was approximately three times as potent as morphine when administered in single oral doses to patients with postpartum uterine cramps. Noracetylmethadol also had a greater duration of effectiveness, and caused fewer untoward effects and slower development of drug tolerance.

Billings and associates (38) confirmed the same metabolic pathway of acetylmethadol via N-demethylation in man. Plasma levels of both nor- and dinor- metabolites were observed shortly after the initial dose of LAAM, and considerable accumulation of DINORLAAM occurred after repeated dosing. These authors reported a six- to eight-fold increase in urinary excretion of both LAAM and the demethylated metabolites during repeated doses. They postulated that these compounds are extensively tissue bound following the initial dose, thereby limiting excretion levels, whereas the ability of the tissue to bind amines becomes saturated during repeated dosage, resulting in substantial increase of the excretion rate. Nevertheless, urinary excretion of total amines accounted for only a small percentage of the dose.

Based on a study in rats, Henderson and coworkers (39)

reported that less than 20% of LAAM and metabolites was excreted in the urine, regardless of the dose or route of administration. These authors suggested that the elimination of drug via the feces may result from biliary excretion of LAAM and metabolites, rather than from lack of absorption of parent drug.

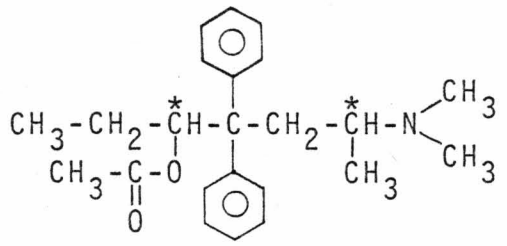
Further evidence of the importance of the fecal elimination route for LAAM and its metabolites has been provided by Misra and Mulé (40). These authors administered 2 mg/Kg of [2-^{3}H]-LAAM to female monkeys by subcutaneous injection and obtained only 20 to 23 percent of total radioactivity in urine compared to 34 to 39 percent in the feces during one week.

In addition to the N-demethylated metabolites, Kaiko and Inturrisi (25) reported methadol and normethadol as metabolites of LAAM in human urine, and summarized the LAAM metabolic pathway as shown in Scheme I (26). Methadol is the product of hydrolysis of the acetyl group, which has previously been observed in the rat by McMahon and associates (34). α-l-Methadol possessed much greater analgesic effectiveness than d-methadone orally or subcutaneously in mice, and was one-third to one-half as effective as LAAM (41). α-l-Methadol was progressively N-demethylated in the rat to α-l-normethadol and α-l-dinormethadol, which was then N-acetylated to form α-l-N-acetyldinormethadol (42). Both α-l-normethadol (43,44) and α-l-dinormethadol (44)

Scheme I

Acetylmethadol Metabolic Pathway in Man,

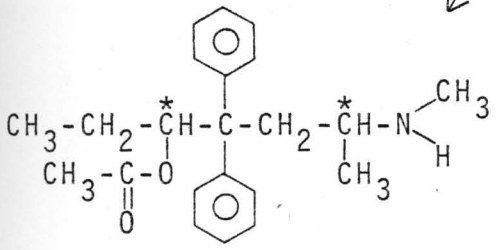
as Proposed by Kaiko *et al.* (26).



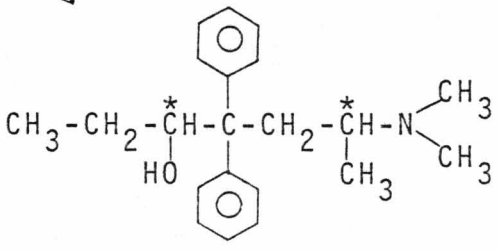
Acetylmethadol

N-demethylation

Deacetylation



Noracetylmethadol

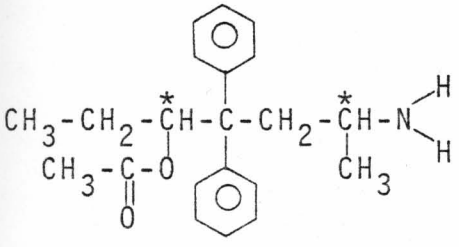


Methadol

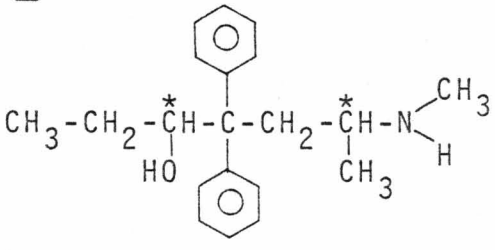
N-demethylation

Deacetylation

N-demethylation



Dinoracetylmethadol



Normethadol

have been reported to possess significant analgesic activity in the rat, while N-acetyldinormethadol has little if any analgesic activity (42).

Henderson and associates (39) recently identified N-acetylnormethadol as a possible metabolite in both urine and plasma of rats dosed with ^{3}H-LAAM. In another study (45), methadone and its metabolites, including 1,5-dimethyl-3,3-diphenyl-2-ethylidene-pyrrolidine, were detected in urine, liver and serum of rats treated with LAAM.

Recently, Misra and associates (46) reported that aromatic hydroxylation of DINORLAAM to p-hydroxydinoracetylmethadol is a significant metabolic pathway for LAAM in the monkey. Peak concentrations of LAAM (73-79 ng/ml) occurred in plasma 2 to 6 hr after 2 mg/Kg oral dose of [2-^{3}H]-LAAM to the monkey, and declined to 5-11 ng/ml 48 hours post dose. Peak concentrations after subcutaneous injection were comparatively higher (117-249 ng/ml) and were sustained between 1-6 hr before declining to 3-10 ng/ml 48 hours after injection. In both acutely and chronically treated monkeys, free LAAM was not detectable in plasma 48 hr after dosing. Plasma half-life values of LAAM were shorter after chronic treatment than after single doses, indicating a faster rate of metabolism of this compound.

Further studies in patients (47), using plasma profiles during a maintenance program, have suggested mean plasma half-lives of LAAM and NORLAAM of 7 and 48 hours,

respectively. Plasma levels of DINORLAAM did not decline during the 48-hour dosage interval. Opiate effects, as manifested by changes in pupil size, appeared to return to the pre-dose value at a slower rate than the rate of LAAM disappearance from plasma, but more rapidly than the rate of disappearance of NORLAAM. This study confirmed that the relatively long duration of opiate effects of LAAM is associated with biotransformation to active and persistent metabolites.

Henderson and associates (48) reported on the pharmacokinetics of LAAM and its nor- and dinor- metabolites after acute and chronic administration of LAAM to narcotic addicts. The plasma decay curve for LAAM was described as biexponential, with average $t_{\frac{1}{2}(\alpha)}$ of 6 hr and $t_{\frac{1}{2}(\beta)}$ of 50 hr following chronic administration. There seemed to be little cumulation of LAAM after repeated doses. In contrast, NORLAAM and DINORLAAM plasma levels were low after acute LAAM dose, but increased five- to ten-fold following chronic administration, with a $t_{\frac{1}{2}}$ of 31 hr for NORLAAM. Plasma levels of DINORLAAM declined so slowly that its $t_{\frac{1}{2}}$ could not be accurately determined. Urinary excretion of LAAM and metabolites was extremely low. Following the first dose of LAAM, only 2% of the total dose appeared in the urine within 72 hours, but this increased to 6% after chronic LAAM administration.

Summarizing the results of the previous studies, it

can be stated with little doubt that the delayed onset and prolonged duration of pharmacologic action of LAAM are caused mainly by *in vivo* formation and cumulation of active metabolites, especially NORLAAM and DINORLAAM. This would account also for the more rapid onset of morphine-like activity observed with oral administration of LAAM than with subcutaneous or intravenous injection (13,15,32)[a]. Another possible explanation for the long duration of action is that both LAAM and its metabolites may be extensively bound to tissue proteins (32,38,47). Tissue binding would delay both biotransformation and excretion and constitute a reservoir of active compound. Data from the recent study by Henderson and associates (48) suggest that methadone, LAAM, NORLAAM and DINORLAAM are indeed bound to tissue proteins, and that these compounds compete for tissue binding sites. A third factor that may contribute to the long duration of LAAM activity is enterohepatic circulation of the parent drug and metabolites (48). Extensive biliary excretion of LAAM has been observed in the rat (39, 50). Although there is no direct evidence of this occurring in man, the very low level of urinary excretion of LAAM and metabolites in patients tends to support this

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[a] An orally administered drug, if absorbed, must pass through the liver where it can be metabolized before reaching the systemic circulation, while less than 30% of the dose passes through the liver in the first circulatory pass after usual intravenous administration (49).

hypothesis.

B. Objectives and Rationale

The primary objective of the present study is to examine and compare the pharmacokinetics of the d- and l-isomers of α-acetylmethadol following acute intravenous and oral administration to the dog, in order to explain the difference in pharmacologic activity between the two isomers. It has been reported (51,52) that the large differences in analgesic potency between d- and l-methadone are due to intrinsic differences in pharmacologic properties, and are not related to differences in disposition or metabolism. Horng and associates (53) recently studied the *in vitro* binding of LAAM and DAAM and their N-demethylated derivatives to the opiate receptors of rat brain, and observed that the N-demethylation of LAAM to NORLAAM and DINORLAAM increased the affinity for opiate receptors, while the N-demethylation of DAAM to NORDAAM had an opposite effect. These authors suggested, therefore, that the shorter duration of activity of DAAM as compared to LAAM is likely due to the difference in affinity of their respective metabolites for the stereoselective receptor sites. On the other hand, there are examples where the distribution characteristics (54) and rate of metabolism (55,56) of a drug are also a function of the stereochemical configuration of the parent compound. This phenomenon of

stereospecificity may be applicable to the isomeric forms of α-acetylmethadol, as postulated by Sung and Way (32), but no studies have been conducted comparing the pharmacokinetics of LAAM and DAAM.

Studies will also be conducted with acute intravenous administration of NORLAAM and DINORLAAM to the dog. Accurate description of the apparent volumes of distribution can then be obtained for these metabolites, thus enabling a more complete pharmacokinetic analysis of the parent drug.

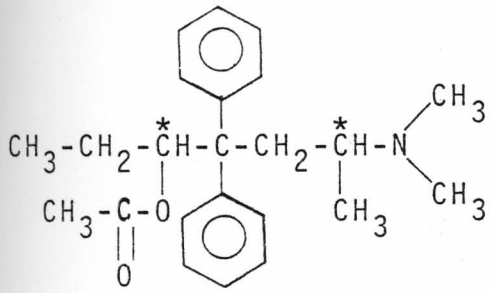
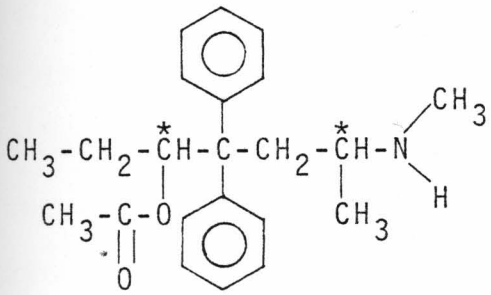
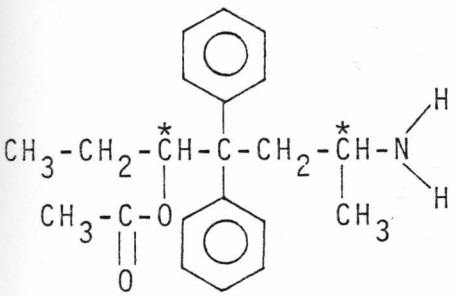
There is also little information on the pharmacokinetics of LAAM and DAAM during multiple doses to individuals or animals not previously exposed to these compounds, and hence on changes in drug and metabolite disposition and metabolism rates due to possible enzyme induction or other factors during repeated doses. Studies in mice have shown that multiple oral administration of methadone caused rapid, sizeable increments in the activity of the N-demethylating enzyme in the liver (57), and increased methadone metabolism resulting from such enzyme induction has been demonstrated in man (58). In the present study, the accumulation characteristics and drug metabolizing enzyme inducing properties of LAAM and DAAM and their respective major metabolites during repeated oral doses of LAAM and DAAM will be examined.

The compounds studied in this thesis, together with

their structural formulae, names and abbreviations, as well as molecular weights, are presented in Table 1.

There have been a number of reports on the untoward effects associated with LAAM in human subjects (13,38,59), and the questions of safety and cumulative toxicity following repeated administration of LAAM still remain to be explored. It is the author's hope that this thesis will provide some of the basic information necessary for the improvement of acetylmethadol maintenance therapy.

Table 1. List of Compounds Studied

Chemical Structure	Name	M.W.
	ACETYLMETHADOL l-α-acetylmethadol = LAAM d-α-acetylmethadol = DAAM	353
	NORACETYLMETHADOL l-α-noracetylmethadol = NORLAAM d-α-noracetylmethadol = NORDAAM	339
	DINORACETYLMETHADOL l-α-dinoracetylmethadol = DINORLAAM d-α-dinoracetylmethadol = DINORDAAM	325

II. EXPERIMENTAL

A. Materials

Pure samples of DAAM, LAAM, NORLAAM, DINORLAAM, α-l-methadol and α-l-normethadol were provided in the hydrochloride salt form by the National Institute on Drug Abuse. Dichloromethane and 1-chlorobutane were reagent grade[a] and were glass distilled prior to use. 1-Octanol[b] was purified by refluxing over NaOH pellets. All other chemicals were either analytical or reagent grade and were used as supplied.

Three male mongrel dogs[c], age 2-4 years and weighing between 14 and 19 Kg, were used in the study. The dogs were individually housed in standard laboratory cages and were fed a regular dry diet with no restrictions on water.

B. Methods

1. Dosing and Dog Plasma Sampling

a. Single Intravenous Dose Study

The drugs, in hydrochloride salt form, were administered to each of the three dogs to deliver the following doses of LAAM, DAAM and molar equivalents of NORLAAM

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[a]Aldrich Chemical Company, Milwaukee, Wisconsin.

[b]Eastman Kodak Company, Rochester, New York.

[c]Animal Care Unit, The University of Wisconsin, Madison, Wisconsin.

and DINORLAAM:

Experiment No.	Drug	Dose
1 (A, B, C)	LAAM	0.6 mg/Kg
2 (A, B, C)	DAAM	0.6 mg/Kg
3 (A, B, C)	NORLAAM	0.575 mg/Kg
4 (A, B, C)	DINORLAAM	0.55 mg/Kg

It was decided, after numerous trials, that the above doses represent the highest that can be given safely to dogs without precipitating serious behavioral problems and other toxic signs. Pure samples of NORDAAM and DINORDAAM were not available for experimentation.

Based on the "Youden Square Plan" (60), the order of treatments were randomized as follows:

Dog	Experiment			
A	1	2	3	4
B	2	1	4	3
C	3	4	2	1

To ensure maximum accuracy of doses, the intravenous solutions were prepared immediately before administration. The hydrochloride salts of DAAM, LAAM and DINORLAAM are

readily soluble in normal saline[a] at room temperature, while that of NORLAAM is not, but is soluble in 10% ethanol in normal saline solution, with vigorous agitation at ca 30°C. Although there is no obvious explanation for the poor aqueous solubility of NORLAAM, even as its hydrochloride salt, this phenomenon has been previously confirmed in other laboratories (61).

In order to maintain uniformity between treatments, all drugs in the intravenous dosing study were dissolved in 10% ethanol in normal saline. For each experiment, the concentration of the solution was adjusted so that the desired dose was contained in an injection volume of 2 ml.

Prior to each experiment, the dogs were fasted for a minimum of 12 hours, but had access to water ad libitum. During the experiment, the dog was placed in a restraining apparatus consisting of a strong sheet which supported its body and through the holes of which its limbs protruded. The limbs were held secure by pediatric limb restraints tied to the shell of the restraining apparatus so that the animal was allowed to stand normally but not to move around and worry indwelling catheters.

Two vein infusion sets[b] were positioned, one in a vein

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[a] 0.9% Sodium Chloride Irrigating Solution, U.S.P., Travenol Laboratories, Deerfield, Illinois.

[b] Miniset® vein infusion set with winged adapter, Travenol Laboratories, Deerfield, Illinois.

in each front leg. Each set consisted of a 19-gauge x 7/8" needle with 30 cm of flexible plastic tubing attached which can hold approximately 0.6 ml of fluid. Clotting of blood in the infusion sets was prevented by infusion of about 2 ml (10 units/ml) of saline-diluted sodium heparin solution[a] into the sets approximately every hour they were in position. Using a 5-ml syringe[b] equipped with a three-way stopcock[c], the designated dose was administered via one infusion set, injection being accomplished in approximately 10 seconds. The infusion set was immediately flushed with 5 ml of normal saline solution and subsequently removed.

Blood samples (8 ml) were collected via the other infusion set shortly before and at 5, 15, 30, 45 and 60 minutes after dosing. Before each sampling, 5 ml of residual fluid was withdrawn to remove any heparin solution in the infusion set. The 8 ml blood sample was then drawn and replaced by 8 ml of normal saline solution. The residual fluid was reinjected, followed by 2 ml of heparin solution. The use of infusion sets in this manner assured intravenous dosing and facilitated rapid collection of blood samples without multiple venipunctures during the first hour of experiment. The infusion set was removed

---

[a] Panheprin®, 5000 U.S.P. units/ml, Abbott Laboratories, North Chicago, Illinois.

[b] Jelco Laboratories, Raritan, New Jersey.

[c] Pharmaseal, Inc., Toa Alta, Puerto Rico.

after taking the 60-minute sample, and the dog released from the restraining apparatus.

Additional blood samples were obtained at 2, 3, 4, 6, 8, 12, 18, 24, 36, 48, 60, 72 and 96 hours after dosing.

Collected blood samples were placed in 10 ml heparinized vacutainers[a] and centrifuged[b] at 2300 rpm for 15 minutes. The plasma was removed and kept deep frozen (-20°C) until analyzed, usually within a week.

Experiments on the same dog were performed at intervals of at least two weeks. This avoided the possibility of changes in pharmacokinetic parameters due to prior exposure to the drug, ensured complete removal of drug from a previous experiment from the body, and allowed complete healing of venipuncture sites.

b. Single Oral Dose Study

The following doses were administered orally, in hydrochloride form, to each of the three dogs:

Experiment No.	Drug	Dose
5 (A, B, C)	LAAM	2 mg/Kg
6 (A, B, C)	DAAM	2 mg/Kg

[a]Green stopper Vacutainer® evacuated glass tubes coated with 143 units of sodium heparin, Becton-Dickinson, Rutherford, New Jersey.

[b]Dynac Centrifuge, Clay Adams, Parsippany, New Jersey.

Food was withheld for 12 hours before each experiment. The drug was dissolved in 25 ml of water and transferred into a Stylex® 2 oz irrigating syringe[a]. A stomach tube, 48" x 1/8" I.D., was used to administer the solution. The dog's mouth was held open and one end of the tube was carefully introduced into its stomach. The solution was injected directly into the stomach tube opening. Twenty-five ml of water was used to flush residual drug from the syringe and the stomach tube into the stomach.

Blood sampling was as described for the intravenous study, except that the 5-minute sample was omitted.

c. Multiple Oral Dose Study

Two weeks following the end of the acute dosing experiments, repeated oral doses of LAAM and DAAM hydrochloride were administered to Dogs A and C in the following pattern:

Experiment No.	Drug	Dose[b]
7 (A, C)	LAAM	1 mg/Kg (x 7)
8 (A, C)	DAAM	1 mg/Kg (x 7)

---

[a] Pharmaseal Laboratories, Glendale, California.

[b] A total of seven doses were given, one dose being administered every 48 hours.

The drugs were administered in a fashion similar to the single oral doses. Blood samples were taken immediately before each dose and at 1, 2, 3 and 4 hours after the first, fourth and seventh (final) doses. Additional blood samples were taken at 15, 30 and 45 minutes and 6, 8, 12, 18, 24 and 36 hours after the first and final doses, and at 48, 60, 72 and 96 hours following the final dose.

2. Assay of Plasma Samples

a. Extraction of Compounds from Plasma

The assay used for the extraction of acetylmethadol and its major metabolites from plasma was adapted from the method described by Kaiko and associates (26), with several modifications.

Three ml of plasma was placed in a 15-ml siliconized centrifuge tube with a teflon-lined screw cap[a]. One ml of 0.2 M phosphate buffer (pH 7.4) was added, followed by one drop of 1-octanol. After thorough mixing on a vortex mixer[b], the sample was extracted with 9 ml of 1-chlorobutane by horizontally shaking at 3 oscillations/sec for 10 min on a flat-bed shaker[c] and then centrifuging at 1000 g (2300 rpm) for 10 min. Eight ml of the upper,

---

[a]Corning Glass Works, Corning, New York.

[b]Super-Mixer, Cole-Parmer, Chicago, Illinois.

[c]Eberbach Corporation, Ann Arbor, Michigan.

organic phase was transferred with a pipet to a second tube, and the contents of the initial tube were discarded. The compounds were then extracted into 5 ml of 0.2 N hydrochloric acid by shaking for 10 min and centrifuging for 10 min. The upper, organic phase was discarded by aspiration. The acid phase was washed by adding 5 ml of n-hexane[a] and shaking for 10 min, followed by centrifuging for 5 min. The hexane was then removed by aspiration and discarded. Seven drops of 50% sodium hydroxide solution were added to the washed acid phase to yield a pH of approximately 13. The compounds were then immediately extracted into 5 ml of dichloromethane by shaking for 10 min followed by centrifuging at 1000 g for 5 min. The upper, aqueous phase was discarded by aspiration. The sample extract was subsequently divided into two equal portions by pipetting 2 ml of the organic phase into each of two 3-ml Reacti-vials[b] and evaporating to dryness under nitrogen[c].

Comments: It has been reported that some spontaneous formation of the amide derivative of noracetylmethadol and dinoracetylmethadol occurs at any pH above neutral (26, 62). The amides result from an intramolecular acyl shift

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[a] n-Hexane, 99+%, Aldrich Chemical Company, Milwaukee, Wisconsin.

[b] Pierce Chemical Company, Rockford, Illinois.

[c] N-Evap, Organomation Assoc., Shrewsbury, Massachusetts.

as shown in Scheme II. However, experiments in our laboratory have shown that this can be minimized by immediately extracting the compounds from the alkaline solution into dichloromethane. It is also important that the final evaporation step be carried out without any heating.

b. Quantitative Determination of Acetylmethadol by Gas-Liquid Chromatography

The residue in one Reacti-vial was dissolved in 20 µl of carbon disulfide which contained 0.3 µg of triacontane[a] as an external standard. Two µl of the sample was injected into the gas chromatograph.

A gas chromatograph[b] equipped with a flame-ionization detector was used. The column was a 6-ft helical glass column with an I.D. of 2 mm and an O.D. of 6 mm, packed with 3% XE-60 on 80/100 mesh Gas-Chrom Q[c], and was conditioned at 220°C initially for 48 hours. The electrometer was set at an amplifier range of 1 and an attenuation of 8. The chart speed on the recorder[d] was 40 cm/hr. The carrier gas was nitrogen with a flow rate of 30 ml/min, while the flame gases were hydrogen and compressed air at flow rates

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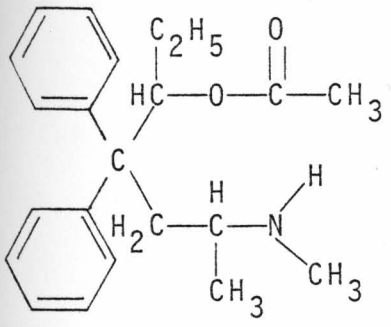
[a]Applied Science Laboratories, State College, Pennsylvania.

[b]Perkin-Elmer 3920B Gas Chromatograph, Norwalk, Connecticut.

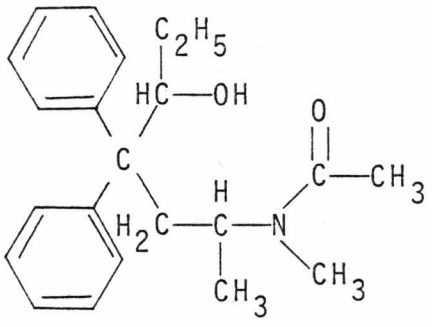
[c]Applied Science Laboratories, State College, Pennsylvania.

[d]Perkin-Elmer 023 Recorder, Norwalk, Connecticut.

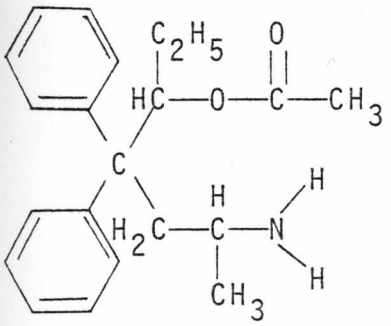
Scheme II. The Alkaline Conversion of Noracetylmethadol and Dinoracetylmethadol to Their Corresponding Amides.



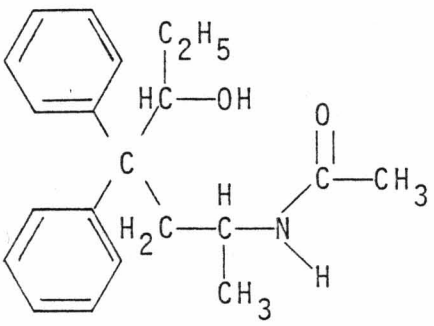
Noracetylmethadol



Noracetylmethadol Amide



Dinoracetylmethadol



Dinoracetylmethadol Amide

of 30 and 360 ml/min, respectively. The temperatures of the detector and injector port were 300°C and 275°C respectively, and a column temperature of 200°C was used for analysis.

Comments: Under these operating conditions, the retention times were 188 sec for acetylmethadol and 609 sec for triacontane.

It was originally hoped to simultaneously measure four major metabolites, i.e., noracetylmethadol, dinoracetylmethadol, methadol and normethadol, as well as the parent drug. In order to achieve optimal peak resolution, a series of different liquid phases of varying polarity and different solid supports and column lengths were tested under various carrier gas flow rates and column temperatures. Unfortunately, because of the close structural resemblance of these compounds, the retention times of at least two of them were so close that peak interference became inevitable, as shown in Table 2. Although the use of a column packed with diethylene glycol succinate yielded some initial success, column bleeding soon became a serious problem even at lower temperatures and this method had to be abandoned.

Since it was not successful in separating dinoracetylmethadol as its unchanged entity, the method of deliberately converting noracetylmethadol and dinoracetylmethadol to their amides for quantitative analysis (26) was

Table 2. Retention Times under Various Column Conditions.

Liquid Phase & Support	Column Length	Column Temp.	Nitrogen Flow Rate (ml/min)	Retention Time (sec)[a] AM	NAM	DNAM	M	NM
3% OV-1 on 100/120 GCQ[b]	6 ft	200	30	326	341	350	304	266
3% QF-1 on 100/120 GCQ[b]	6 ft	200	30	144	175	147	127	87
3% XE-60 on 80/100 GCQ[b]	6 ft	200	30	188	247	204	215	297
3% XE-60 on 80/100 GCQ[b]	8 ft	200	30	286	375	310	323	200
3% XE-60 on 80/100 GCQ[b]	8 ft	195	25	381	505	415	434	263
3% XE-60 on 80/100 GCQ[b]	8 ft	190	25	467	630	515	542	315
3% XE-60 on 80/100 GCQ[b]	12 ft	200	30	487	625	528	539	761
6% GP35C Diethylene Glycol Succinate on Anakrom Abs, 90/100 mesh, NP[c]	6 ft	185	30	321	161	421	507	250

[a] AM, acetylmethadol; NAM, noracetylmethadol; DNAM, dinoracetylmethadol; M, methadol; NM, normethadol. Interfering peaks are underlined.

[b] Applied Science Laboratories, State College, Pennsylvania.

[c] Analabs, Inc., Hamden, Connecticut.

examined. After adjusting the pH of the aqueous phase to 13, the compounds were incubated for 30 min in a heating block[a] at 70°C to effect the conversion of the metabolites to their corresponding amides prior to extraction into dichloromethane. This treatment resulted in good resolution of all five compounds on the 6-ft column packed with 3% XE-60 on 80/100 Gas-Chrom Q by significantly increasing the retention times of noracetylmethadol and dinoracetylmethadol, which were 350 sec and 404 sec respectively at a column temperature of 240°C and nitrogen flow rate of 40 ml/min. However, it was soon noticed that acetylmethadol itself was also affected by this procedure. Tse and Welling (63) reported that part of the acetylmethadol was hydrolyzed to form methadol during incubation at pH 13. Any standard calibration graphs generated from such data will therefore lead to incorrect calculation of acetylmethadol and methadol concentrations in biofluids where the two compounds may be present in variable relative amounts.

Lack of sensitivity of the flame ionization detector imposed an additional problem. Although the minimum detectable plasma concentration of acetylmethadol using the aforementioned assay and operating conditions was only 1 ng/ml, that of the metabolites was quite high, about 50 ng/ml.

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[a]Temp-Blok Module Heater, Scientific Products, McGraw Park, Illinois.

The use of a nitrogen-phosphorus detector[a] on the same gas chromatograph provided little improvement of sensitivity. It was finally decided to prepare electron capture sensitive derivatives of the metabolites for gas chromatography.

c. Quantitative Determination of Noracetylmethadol and Dinoracetylmethadol by Gas-Liquid Chromatography

The method of Walle and Ehrsson (64), with slight modifications, was employed to prepare the fluoroacyl derivatives of the metabolites of acetylmethadol for electron capture detection. To the extracted compounds in the second Reacti-vial (see section a., p. 26) was added 20 µl dichloromethane containing 0.16 µg of α-d-norpropoxyphene hemicitrate[b] as an external standard. The solution was evaporated to dryness under nitrogen. 0.5 ml benzene[c] was added, followed by 0.1 ml of a 0.05 M triethylamine[d] in benzene solution. The contents were thoroughly mixed on a vortex mixer. Ten µl of heptafluorobutyric anhydride[e] was added, and the vial immediately sealed and heated for 15 min

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[a]Perkin-Elmer, Norwalk, Connecticut.

[b]Lilly Laboratories, Indianapolis, Indiana.

[c]Benzene, Sequanal Grade, Pierce Chemical Company, Rockford, Illinois.

[d]Aldrich Chemical Company, Milwaukee, Wisconsin.

[e]Pierce Chemical Company, Rockford, Illinois.

at 70°C in a heating block[a]. It was then allowed to cool, followed by the addition of 1 ml water and shaking for 1 min. One ml of 5% aqueous ammonia solution was added. The vial was shaken for 5 min with subsequent centrifugation[b] for 10 min. Approximately 5 µl of the benzene (upper) phase was injected into the gas chromatograph.

The gas chromatograph[c] was equipped with a Ni^{63} electron capture detector and a 6-ft helical glass column with an I.D. of 2 mm and an O.D. of 6 mm, packed with 3% SE-30 on 100/120 Gas-Chrom Q[d]. The electrometer was set at an attenuation of 8, and the chart speed on the recorder[e] was 40 cm/hr. The carrier gas was 5% methane in argon with a flow rate of 30 ml/min. The column temperature was 200°C, while the temperatures of the detector and injector ports were 300°C and 200°C, respectively.

Comments: Under the above operating conditions, the retention times were 648 sec and 505 sec for the acyl derivatives of noracetylmethadol and dinoracetylmethadol respectively, and 729 sec for that of norpropoxyphene. The

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[a]Temp-Blok Module Heater, Scientific Products, McGraw Park, Illinois.

[b]International Equipment Co., Boston, Massachusetts.

[c]Hewlett Packard 5730A Gas Chromatograph, Palo Alto, California.

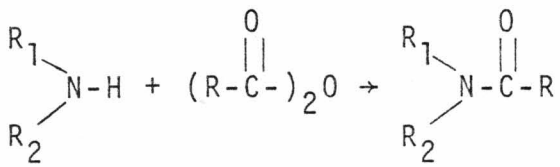
[d]Applied Science Laboratories, State College, Pennsylvania.

[e]Perkin-Elmer 023 Recorder, Norwalk, Connecticut.

method was capable of measuring plasma levels as low as 1 ng/ml of noracetylmethadol and 5 ng/ml of dinoracetylmethadol. Acetylmethadol, being a tertiary amine, did not undergo the acylation process as shown in Scheme III. Nevertheless, its presence was noted as a negative peak at 422 sec.

Methadol and normethadol were also derivatized, but more than one derivative were formed in each case, with retention times of 292 and 371 sec for methadol and 292, 336 and 371 sec for normethadol derivatives. The overlapping of the peaks further complicated the problem, and subsequently led to the decision that methadol and normethadol be excluded from the present study. This may also be rationalized in terms of the reported absence of methadol, and any demethylated biotransformation products of methadol, in the plasma of subjects administered acetylmethadol (26, 38).

Scheme III. A General Acylation Reaction.



3. Stability Test for LAAM, NORLAAM and DINORLAAM in Frozen Plasma (-20°C)

This experiment was carried out to examine whether compounds were subjected to degradation while stored in frozen plasma. Although no blood sample was stored for over three weeks before analysis, this experiment was conducted over a period of one month.

To each of seven test tubes was added 0.4 µg of LAAM, followed by 4 ml of blank dog plasma. After thorough mixing on a vortex mixer, all but one of the tubes were immediately placed in a freezer at -20°C. Three ml of drug-containing plasma (0.1 µg/ml) in the one tube was transferred into a 15-ml siliconized centrifuge tube and analyzed for LAAM as described previously.

The remaining six samples were analyzed at 1, 3, 6, 9, 20 and 30 days following the beginning of the experiment. On each day, one tube was removed from the freezer, the content thawed, and 3 ml of it assayed for LAAM.

The above procedure was used also to study NORLAAM and DINORLAAM stability.

4. Interpretation of Data

a. Standard Calibration Curves and Extraction Efficiency of Assay

Control dog plasma samples (3 ml) to which LAAM has been added at concentrations of 0.005, 0.025, 0.05, 0.1

and 0.2 µg/ml were assayed in quadruplicate. The peak height ratios of LAAM:triacontane were plotted against the known drug concentrations. Data were then processed by analysis of variance (ANOVA) of the linear regression model.

Standard curves for NORLAAM and DINORLAAM were obtained in a similar fashion, but assaying was only done in triplicate. The lower sensitivity of the DINORLAAM assay required the standard curve for this compound to be generated at a slightly higher concentration range, namely, 0.025, 0.05, 0.1, 0.2 and 0.4 µg/ml.

Standard drug samples (0.015, 0.075, 0.15, 0.3 and 0.6 µg of LAAM and NORLAAM, 0.075, 0.15, 0.3, 0.6 and 1.2 µg of DINORLAAM) were also analyzed, and the extraction efficiency for each compound was subsequently calculated as follows:

$$\% \text{ recovery} = \frac{\text{Peak Ht. Ratio(Drug from plasma:External Standard)}}{\text{Peak Ht. Ratio(Drug Standard:External Standard)}} \times \frac{9}{8} \times \frac{5}{2} \times 100\%$$

Both NORLAAM and DINORLAAM and their external standard (norpropoxyphene) were measured as their corresponding heptafluorobutyric anhydride derivatives. The factor $(\frac{9}{8} \times \frac{5}{2})$ accounts for the aliquot sampling in the assay.

b. Plasma Concentration versus Time Data

The plasma concentration versus time data were plotted on a semi-logarithmic graph paper. Based on the results, an appropriate pharmacokinetic model was chosen for analysis of data. Graphic analysis, using the method of residuals (65), was performed to obtain initial estimates of pharmacokinetic parameters. Attempts were then made to fit each individual set of experimental data to the proposed model by the method of least squares, using an iterative nonlinear regression program NREG[a] on a digital computer[b].

Statistical analysis and comparison of the plasma level data and pharmacokinetic parameter values were also performed where appropriate.

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[a]With user supplied subroutine.

[b]Univac 1110 digital computer, Madison Academic Computing Center, The University of Wisconsin, Madison, Wisconsin.

III. RESULTS

A. Assay of Plasma Samples

The GC temperatures and gas flow rates were chosen by experimenting to yield maximum detector sensitivity to the compounds. Under the aforementioned operating conditions, retention times in the XE-60/Gas-Chrom Q column were 188 sec for LAAM and 609 sec for triacontane. A representative chromatogram is shown in Figure 1. Figure 2 is a chromatogram showing the passage of NORLAAM and DINORLAAM through the SE-30/Gas-Chrom Q column. The retention times were 648 sec for NORLAAM, 505 sec for DINORLAAM and 729 sec for norpropoxyphene, all three being detected as their corresponding heptafluorobutyric anhydride derivatives.

Standard calibration curves for LAAM, NORLAAM and DINORLAAM are given in Figures 3, 4 and 5, respectively. The regression equations and ANOVA results are also shown. With all three equations, the intercepts are not significantly different from zero, indicating that peak height ratios are directly proportional to concentrations. It was shown also that both d- and l- enantiomers responded in an identical fashion to GC analysis. Hence, the same standard curves were used to calculate the concentrations of LAAM, NORLAAM, DINORLAAM and their d- counterparts in unknown plasma samples. The reproducibility of the standard curves was examined periodically by analyzing plasma samples

Figure 1. Chromatogram of dog plasma sample containing LAAM (0.2 µg/ml), using the XE-60/Gas-Chrom Q column with a flame ionization detector.

a = LAAM,

b = triacontane.

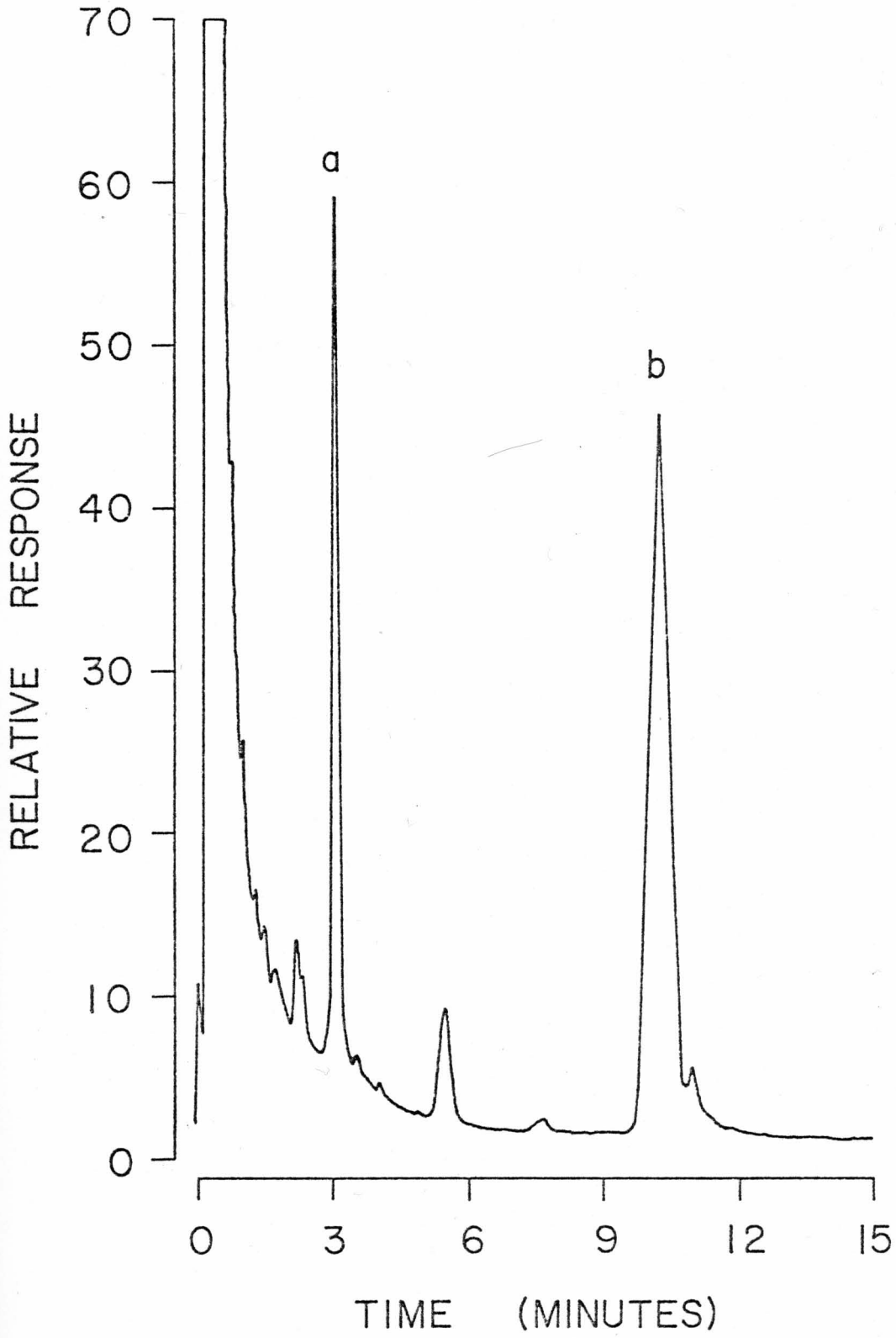

70
60
50
40
30
20
10
0
RELATIVE RESPONSE
a
b
0
3
6
9
12
15
TIME (MINUTES)

Figure 2. Chromatogram of dog plasma sample containing NORLAAM and DINORLAAM (0.2 μg/ml), using the SE-30/ Gas-Chrom Q column with a Ni^{63} electron capture detector.

a = DINORLAAM,

b = NORLAAM,

c = norpropoxyphene,

all three were measured as their corresponding heptafluorobutyric anhydride derivatives.

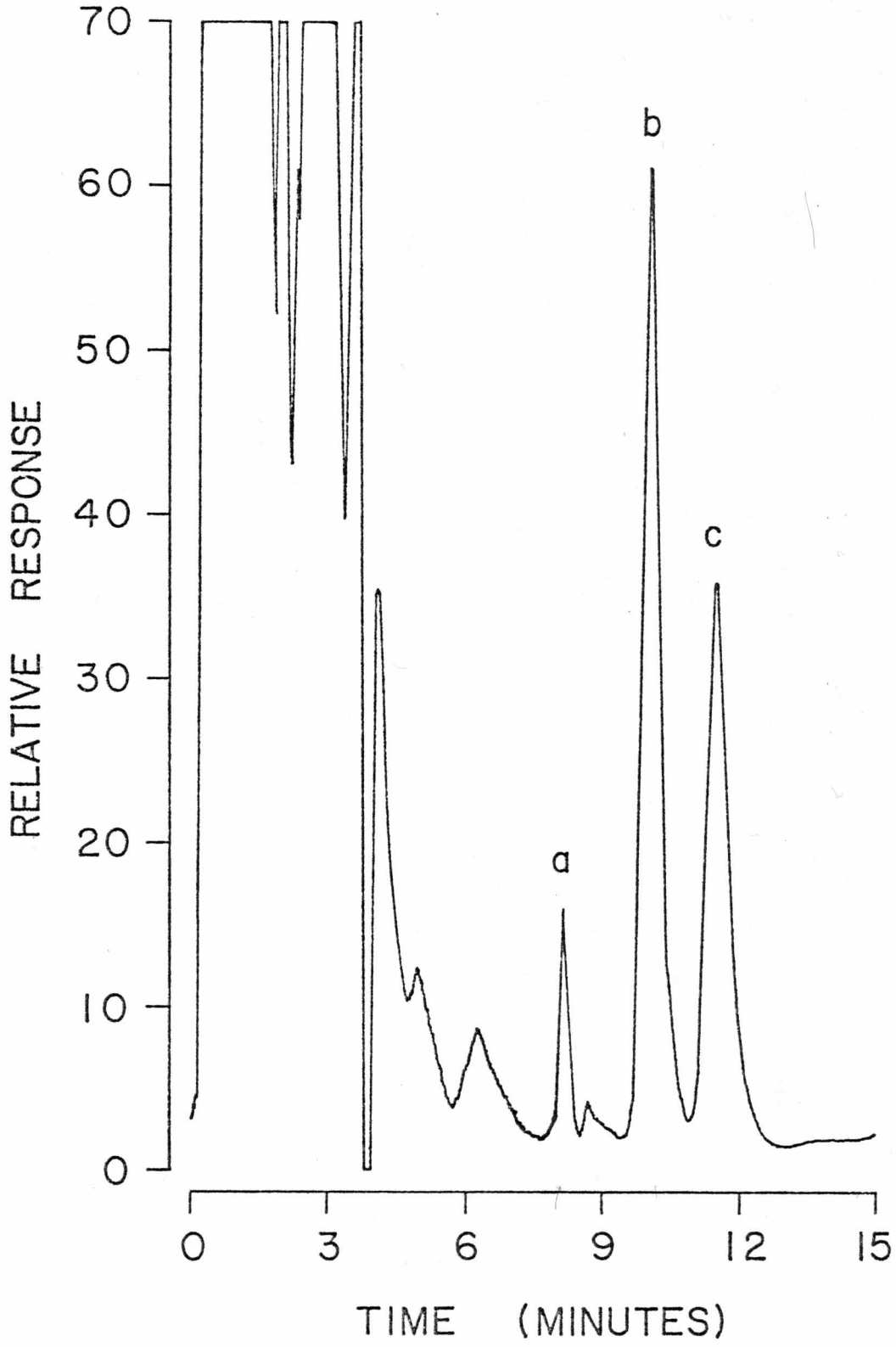

70
60
50
40
30
20
10
0
RELATIVE RESPONSE
a
b
c
0
3
6
9
12
15
TIME (MINUTES)

Figure 3. LAAM standard curve.

Regression equation is

$$y = 6.02(\pm 0.26)x + 0.0079(\pm 0.027)$$

where parenthesis indicates 95% confidence interval.

$$n = 20,\ R^2 = 0.993,\ r = 0.996,\ p < 0.01.$$

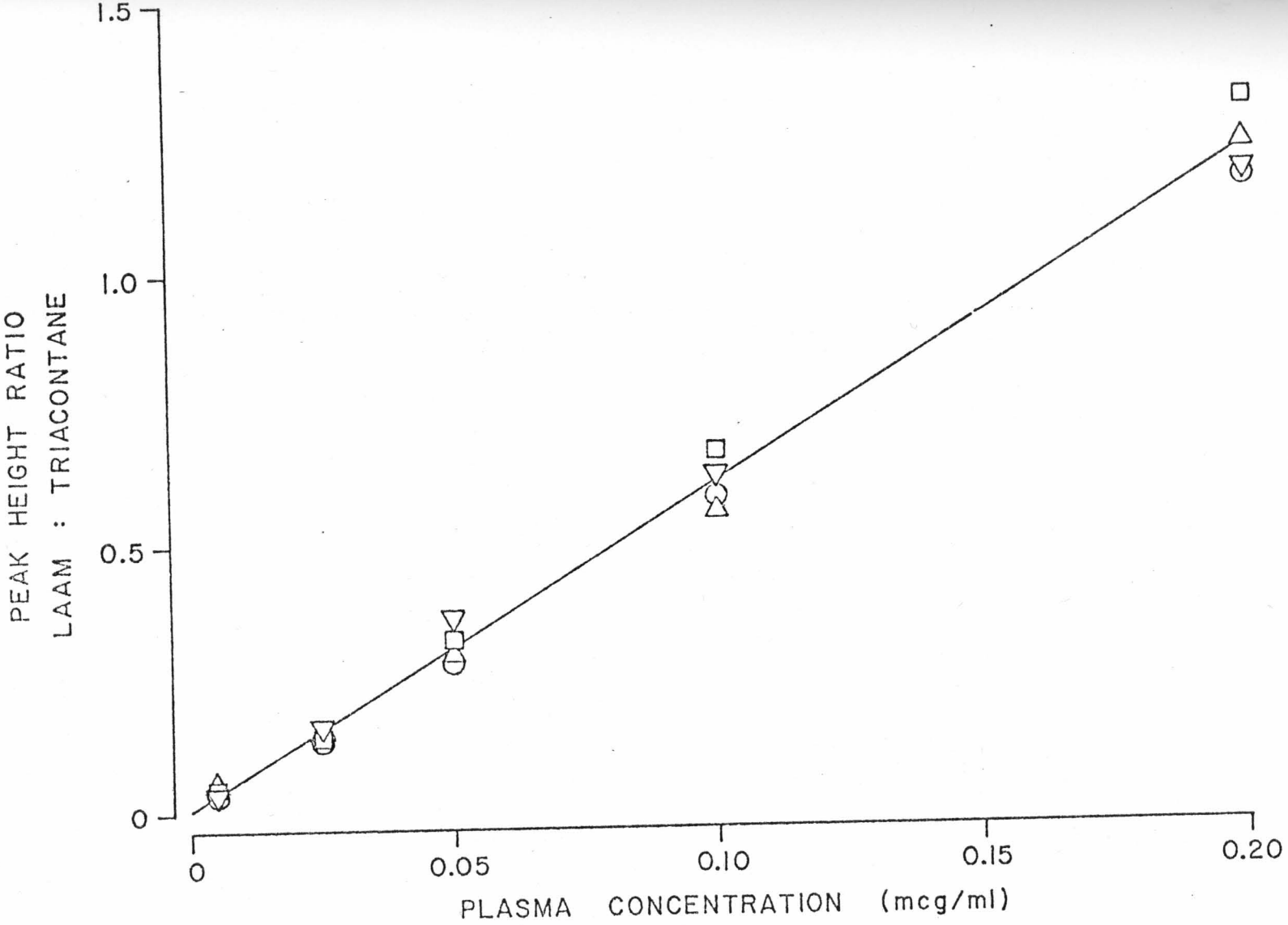

1.5
1.0
0.5
0
PEAK HEIGHT RATIO
LAAM : TRIACONTANE
0
0.05
0.10
0.15
0.20
PLASMA CONCENTRATION (mcg/ml)

Figure 4. NORLAAM standard curve.

Both NORLAAM and external standard were measured as their heptafluorobutyric anhydride derivatives.

Regression equation is

$$y = 7.41(\pm 0.25)x - 0.0001(\pm 0.012)$$

where parenthesis indicates 95% confidence interval.

$$n = 15,\ R^2 = 0.997,\ r = 0.997,\ p < 0.01.$$

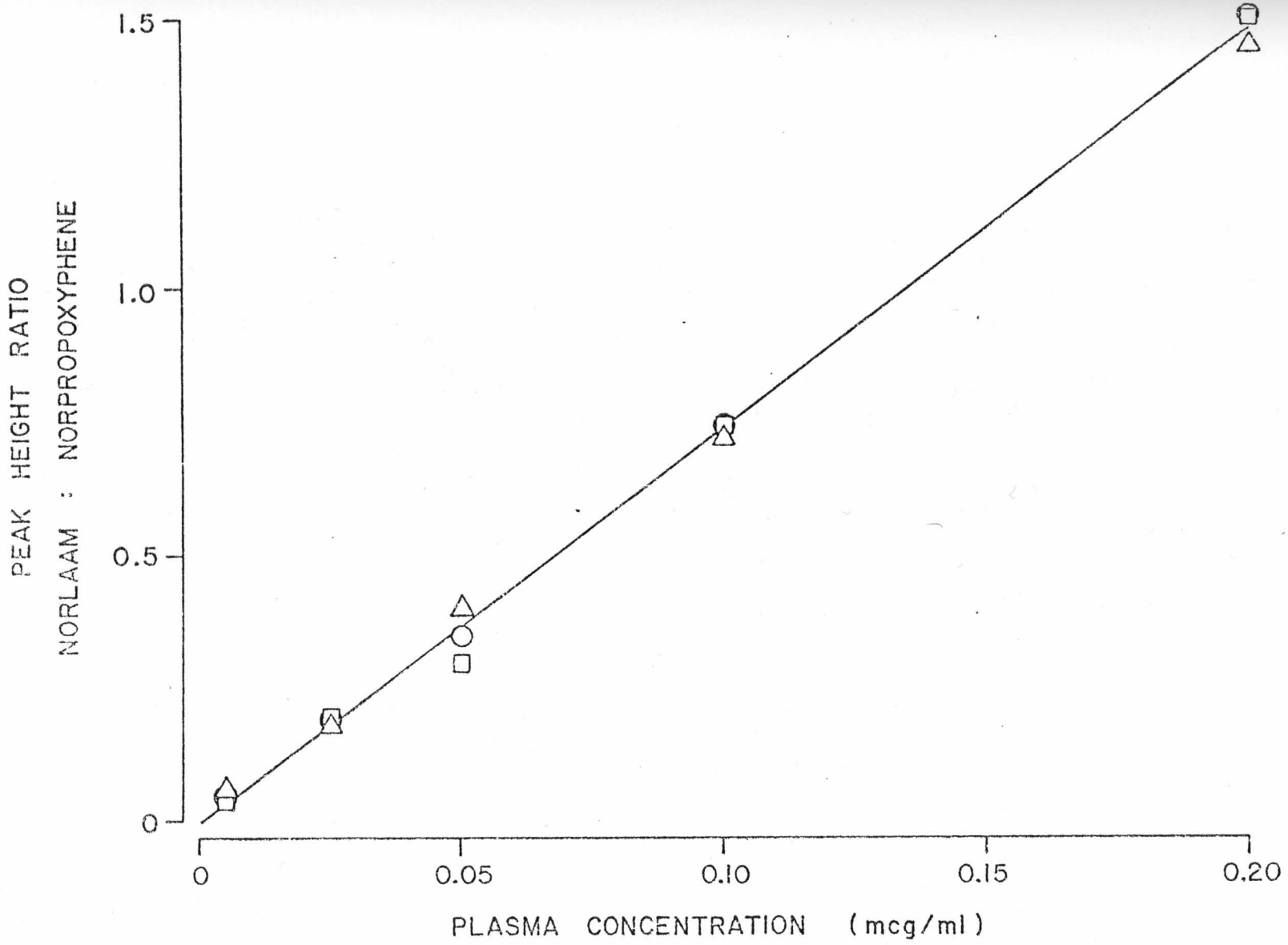

PEAK HEIGHT RATIO
NORLAAM : NORPROPOXYPHENE
1.5
1.0
0.5
0
0
0.05
0.10
0.15
0.20
PLASMA CONCENTRATION (mcg/ml)

Figure 5. DINORLAAM standard curve.

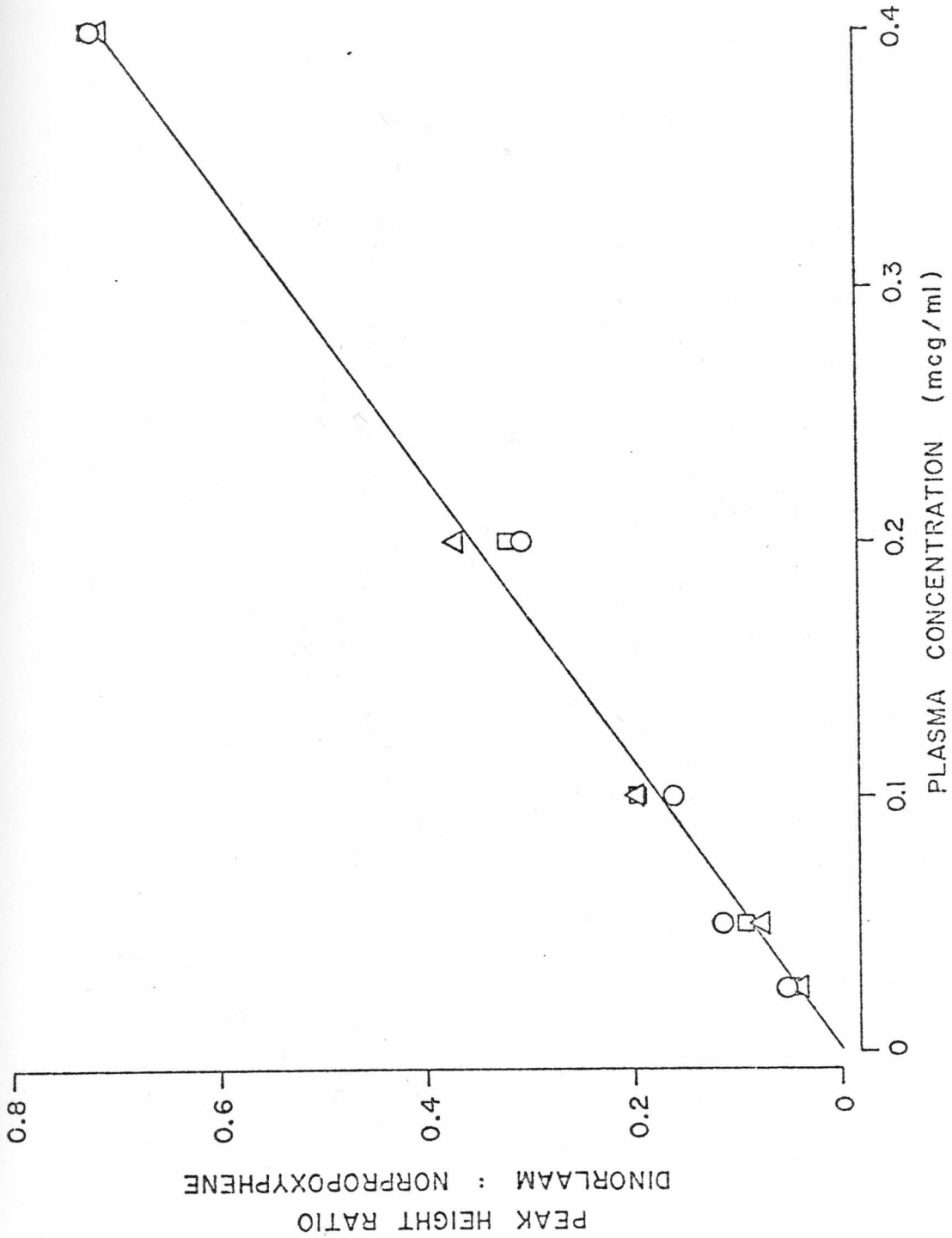
Both DINORLAAM and external standard were measured as their heptafluorobutyric anhydride derivatives.

Regression equation is

$$y = 1.77(\pm 0.089)x - 0.0009(\pm 0.018)$$

where parenthesis indicates 95% confidence interval.

$$n = 15,\ R^2 = 0.993,\ r = 0.997,\ p < 0.01.$$


PEAK HEIGHT RATIO
DINORLAAM : NORPROPOXYPHENE
0.8
0.6
0.4
0.2
0
0
0.1
0.2
0.3
0.4
PLASMA CONCENTRATION (mcg/ml)

containing known concentrations of the drugs.

In the rare instances when unknown plasma levels exceeded the range of the standard curves, the plasma samples were first diluted with distilled water before subsequent assaying. The minimum detectable plasma concentration was ca 1 ng/ml for acetylmethadol and noracetylmethadol, and 5 ng/ml for dinoracetylmethadol.

Results of extraction efficiency calculations are shown in Tables 3, 4 and 5.

B. Stability of LAAM, NORLAAM and DINORLAAM in Frozen Plasma

The observed concentrations of LAAM, NORLAAM and DINORLAAM in plasma samples after being frozen for the designated periods of time are reported in Table 6 and Figure 6.

C. Studies in Dogs

1. Observed Drug-Related Behavioral Changes

Almost immediately following acute intravenous dosing, and about 20 minutes after acute oral dosing of the compounds studied, dogs would either enter a state of depression or fell asleep. This was followed by a period of excitation at about 1 hour after dosing and lasting for 4 or 5 hours, during which time the dogs were restless and irritated. Defecation often occurred shortly after

Table 3. Extraction Efficiency of LAAM Assay.

Equivalent Plasma Conc. (μg/ml)	Peak Height Ratio (LAAM:Triacontane)								
	LAAM Standard	LAAM Recovered from 3 ml Plasma				% Recovery			
0.005	0.13	0.059	0.036	0.045	0.035	124	75.9	94.9	73.8
0.025	0.42	0.15	0.14	0.14	0.17	100	90.3	95.0	112
0.05	0.85	0.29	0.28	0.32	0.37	96.7	91.7	107	121
0.1	1.66	0.55	0.58	0.67	0.63	94.0	98.4	113	106
0.2	3.31	1.22	1.16	1.30	1.17	104	98.2	111	99.7

overall mean = 100 (n = 20)

S.D. = 12.7

C.V.% = 12.7%

Table 4. Extraction Efficiency of NORLAAM Assay.

Equivalent Plasma Conc. (μg/ml)	Peak Height Ratio (NORLAAM:Norpropoxyphene)						
	NORLAAM Standard	NORLAAM Recovered from 3 ml Plasma			% Recovery		
0.005	0.24	0.063	0.047	0.040	74.1	55.3	47.1
0.025	1.14	0.18	0.19	0.20	44.8	47.7	49.2
0.05	2.22	0.40	0.35	0.30	51.1	44.0	37.8
0.1	4.37	0.72	0.75	0.75	46.3	47.9	47.9
0.2	8.75	1.45	1.51	1.51	46.5	48.5	48.4

overall mean = 49.1 (n = 15)

S.D. = 7.86

C.V.% = 16.0%

Table 5. Extraction Efficiency of DINORLAAM Assay.

Equivalent Plasma Conc. (µg/ml)	Peak Height Ratio (DINORLAAM:Norpropoxyphene) DINORLAAM Standard	DINORLAAM Recovered from 3 ml Plasma			% Recovery		
0.025	0.81	0.038	0.052	0.041	13.2	18.1	14.2
0.05	1.56	0.074	0.11	0.092	13.3	20.3	16.6
0.1	3.29	0.20	0.16	0.20	16.8	13.7	16.8
0.2	6.13	0.37	0.31	0.32	16.9	14.0	14.7
0.4	12.2	0.71	0.72	0.72	16.3	16.5	16.6

overall mean = 15.9 (n = 15)

S.D. = 1.98

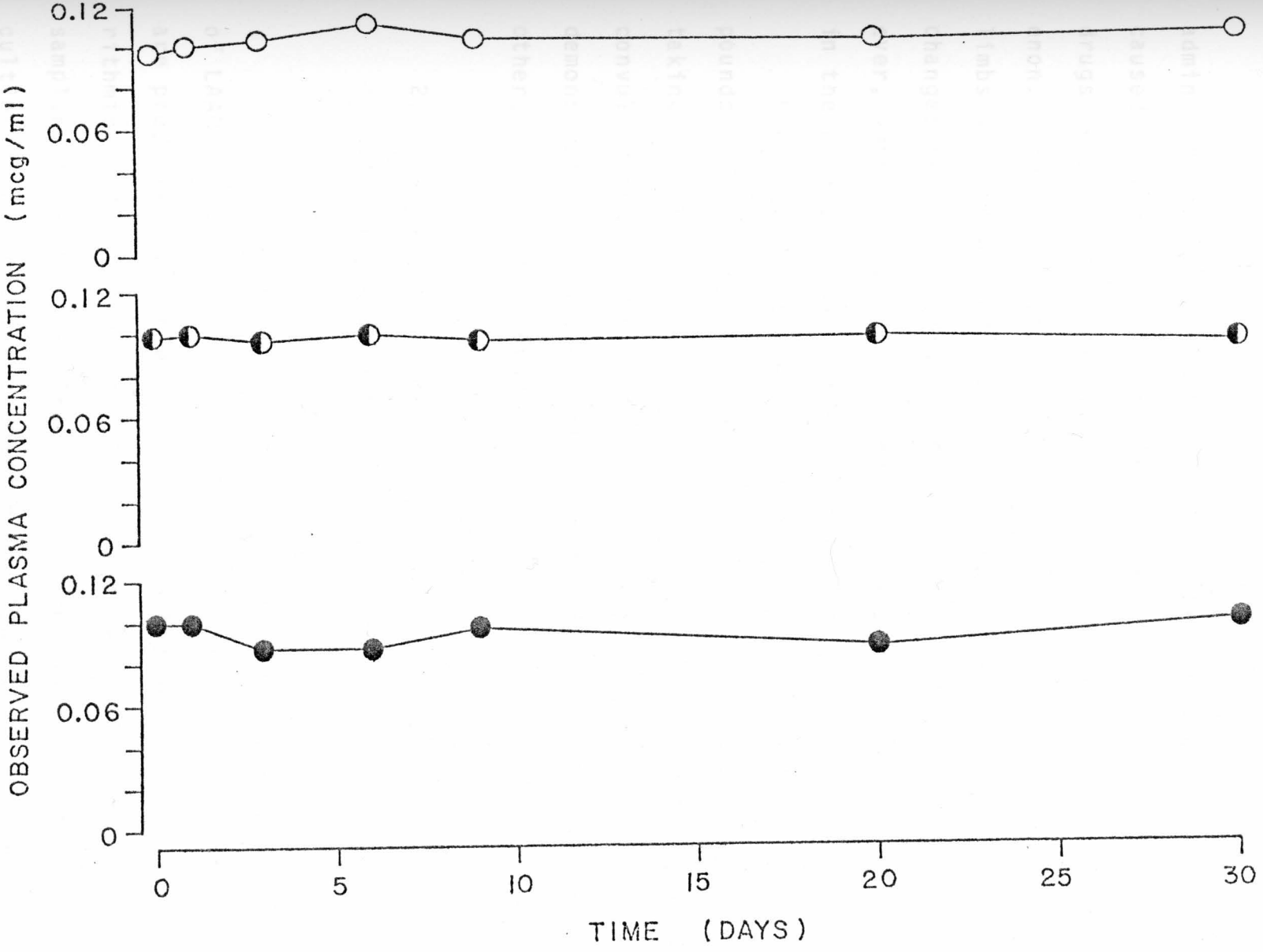
C.V.% = 12.5%

Table 6. Observed Concentrations of LAAM, NORLAAM and DINORLAAM in Plasma[a] Following Periods of Storage at -20°C.

	Concentration (µg/ml)		
Time (Days)	LAAM	NORLAAM	DINORLAAM
0	0.099	0.099	0.097
1	0.10	0.10	0.10
3	0.087	0.097	0.10
6	0.087	0.099	0.11
9	0.097	0.097	0.10
20	0.087	0.097	0.10
30	0.098	0.094	0.10

[a]Initial concentration of each compound in plasma was 0.1 µg/ml.

Figure 6. Observed concentrations of LAAM (●), NORLAAM (◐) and DINORLAAM (○) in plasma after storage at -20°C.


OBSERVED PLASMA CONCENTRATION (mcg/ml)
0.12
0.06
0
0.12
0.06
0
0.12
0.06
0
0
5
10
15
20
25
30
TIME (DAYS)

administration of the drugs. This is believed to have been caused by the contraction of the bowel, induced by the drugs and/or metabolites. Salivation was a common phenomenon. The drugs also caused transient paralysis of hind limbs on several occasions. These drug-related behavioral changes are summarized in Table 7. Toxic responses, however, were not observed after the lower, 1 mg/Kg oral doses in the LAAM and DAAM multiple dosing studies.

Dog B tended to react more violently to dosed compounds than the other two dogs. In fact, two hours after taking a 2 mg/Kg oral dose of LAAM (Experiment 5B), Dog B convulsed and expired. The unfortunate atypical behavior demonstrated by this dog has been previously observed with other morphine type drugs (66).

2. Plasma Concentration versus Time Data

a. Single Dose Studies

i. Plasma Concentrations

The plasma concentration versus time data of LAAM, DAAM and their N-demethylated metabolites studied are presented in Tables 8-25, and plotted on a semilogarithmic scale in Figures 7-23. Slight variations in sampling times between experiments were caused by difficulties occasionally encountered in blood sampling. After dosing LAAM or DAAM, no peaks were observed during gas chromatographic analysis at the retention times of methadol

Table 7. Drug-Related Behavioral Changes in Dogs.

Experiment	Dog	Behavioral Changes			
		Initial Depression	Excitation	Greater-than-Normal Salivation	Paralysis of Hind Limbs
1. 0.6 mg/Kg,	A	D[a]	-[b]	-	-
LAAM, i.v.	B	D	-	-	-
	C	D	-	-	-
2. 0.6 mg/Kg,	A	D	+[c]	+	3-4 hr[d]
DAAM, i.v.	B	-	+	+	2-3 hr
	C	D	-	+	3-4 hr
3. 0.575 mg/Kg,	A	D	+	+	7-8 hr
NORLAAM,	B	S[e]	+	+	7-8 hr
i.v.	C	S	-	+	7-8 hr
4. 0.55 mg/Kg,	A	S	-	+	-
DINORLAAM,	B	D	-	+	-
i.v.	C	S	-	+	-
5. 2 mg/Kg,	A	D	+	+	12-18 hr
LAAM, p.o.	B	S	+	+	2 hr[f]

		C	D	+	+	6-8 hr
6.	2 mg/Kg	A	D	+	+	-
	DAAM, p.o.	B	S	+	+	2-3 hr
		C	D	+	+	2-3 hr

[a]Depressed.

[b]Absence of symptom.

[c]Presence of symptom.

[d]Duration of paralysis after dosing.

[e]Asleep.

[f]Dog B expired at 2 hr after dosing.

Table 8. Experiment 1A. Plasma Concentrations of Drug and Metabolites after Intravenous Administration of 0.6 mg/Kg LAAM to Dog A[a].

	C (μg/ml)		
t (hr)	LAAM	NORLAAM	DINORLAAM
0.083	-[b]	0.080	0.020
0.25	0.17	0.033	0.008_9
0.50	0.11	0.039	0.005_9
0.75	0.096	0.011	0.006_3
1	0.035	0.010	0.005_5
2	0.023	0.007_5	0.005_0
3	0.018	0.006_1	0.005_3
4	0.011	0.006_1	0.005_1
6	0.008_9	0.005_4	0.004_8
8	0.010	0.005_1	0
12	0.005_7	0.004_8	0
18	0.002_5	0.004_6	0
24	0.001_6	0.003_9	0
37	0[c]	0.003_4	0
48	-	0.003_0	0
72	-	0.002_0	0
96	-	0	0

[a]Dog weight: 19 Kg, LAAM dose: 11.4 mg.

[b]Not measured. [c]Below detectable limit.

Figure 7. Experiment 1A. Plasma concentration-time profiles after intravenous administration of 0.6 mg/Kg LAAM to Dog A. Computer determined equation[a] for LAAM (●) is

$$C_1 = 0.26e^{-2.19t} + 0.020e^{-0.11t}$$

Lines for NORLAAM (◐) and DINORLAAM (○) are hand drawn. Note change in time scale after 25 hr.

---

[a]The equation is in the form of $C_1 = Ae^{-\alpha t} + Be^{-\beta t}$, where C_1, A, B, α and β are as defined on pp. 112-113.

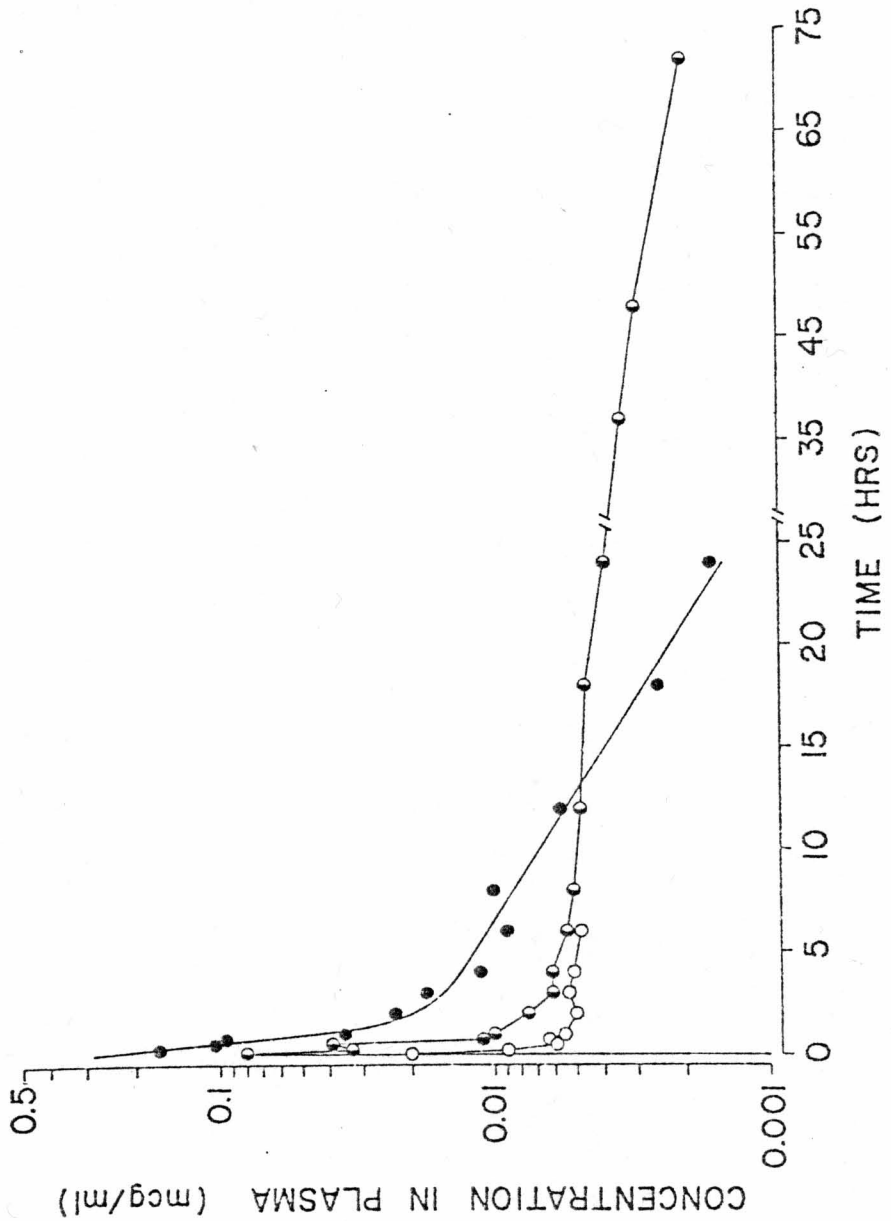

CONCENTRATION IN PLASMA (mcg/ml)
0.5
0.1
0.01
0.001
0
5
10
15
20
25
35
45
55
65
75
TIME (HRS)

Table 9. Experiment 1B. Plasma Concentrations of Drug and Metabolites after Intravenous Administration of 0.6 mg/Kg LAAM to Dog B[a].

	C (μg/ml)		
t (hr)	LAAM	NORLAAM	DINORLAAM
0.083	0.22	0.013	0.010
0.25	0.062	0.004_1	0.007_3
0.50	0.054	0.004_5	0.005_2
0.75	0.031	0.004_3	0.006_5
1	0.028	0.005_3	0.005_4
2	0.025	0.005_3	0.005_2
3	0.022	0.004_4	0.005_1
4	0.019	0.005_3	0
6	0.016	0.004_5	0
8	0.012	0.004_0	0
12	0.010	0.003_7	0
18	0.004_6	0.004_3	0
24	0.002_9	0.003_3	0
36	0	0.003_0	0
48	-	0.002_3	0
60	-	0.001_6	0
72	-	0	0

[a]Dog weight: 18.2 Kg, LAAM dose: 10.92 mg.

Figure 8. Experiment 1B. Plasma concentration-time profiles after intravenous administration of 0.6 mg/Kg LAAM to Dog B. Computer determined equation for LAAM (●) is

$$C_1 = 0.42e^{-10.1t} + 0.034e^{-0.12t}$$

Lines for NORLAAM (◐) and DINORLAAM (○) are hand drawn. Note change in time scale after 25 hr.

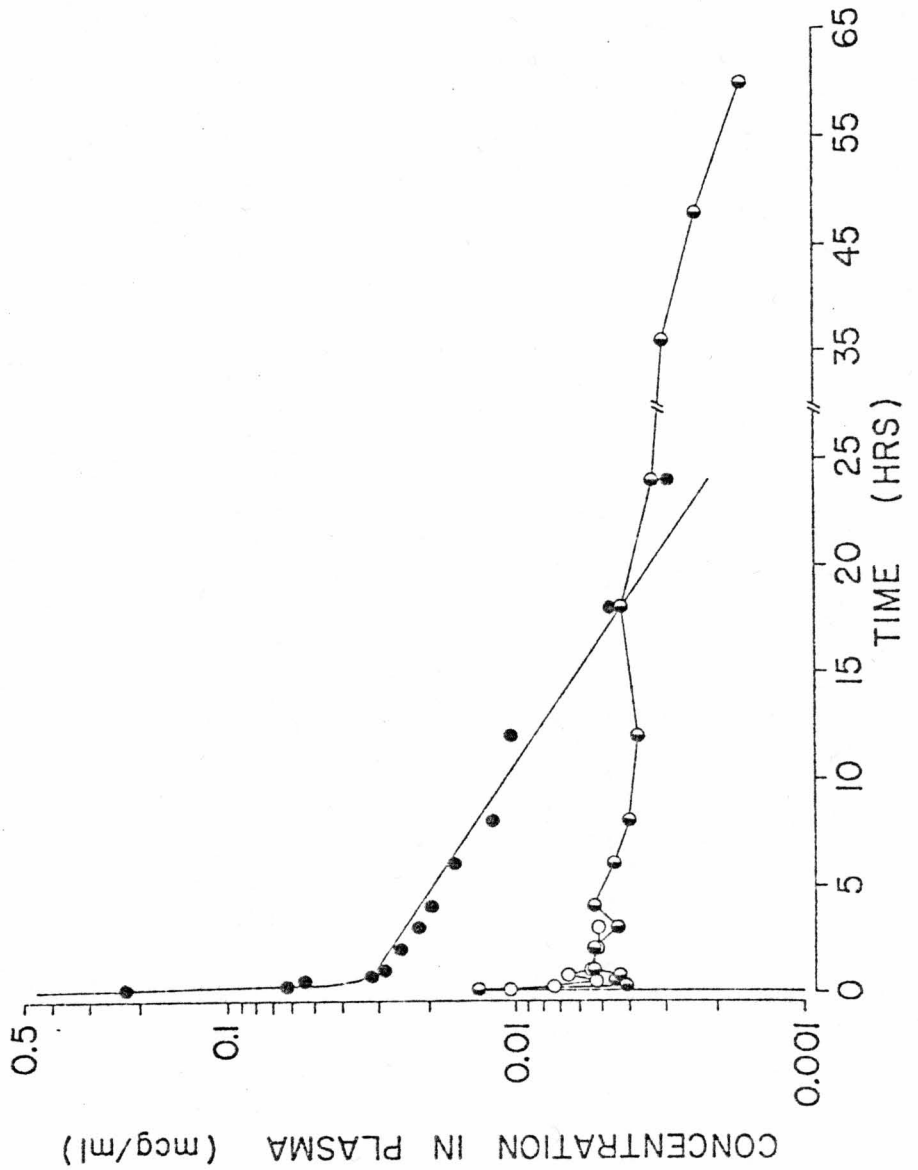

CONCENTRATION IN PLASMA (mcg/ml)
0.5
0.1
0.01
0.001
0
5
10
15
20
25
35
45
55
65
TIME (HRS)

Table 10. Experiment 1C. Plasma Concentrations of Drug and Metabolites after Intravenous Administration of 0.6 mg/Kg LAAM to Dog C[a].

	C (μg/ml)		
t (hr)	LAAM	NORLAAM	DINORLAAM
0.083	0.23	0.013	0.011
0.25	0.073	0.005_7	0.006_5
0.50	0.038	0.005_3	0.006_9
0.75	0.036	0.003_9	0.005_4
1	0.033	0.004_9	0
2	0.034	0.004_7	0
3	0.030	0.004_1	0
4	0.026	0.004_7	0
6	0.021	0.004_7	0
8	0.018	0.004_5	0
12	0.013	0.004_3	0
24	0.003_9	0.003_2	0
36	0	0.002_9	0
48	0	0.002_7	0
72	-	0.001_9	0
96	-	0	0

[a]Dog weight: 14.3 Kg, LAAM dose: 8.58 mg.

Figure 9. Experiment 1C. Plasma concentration-time profiles after intravenous administration of 0.6 mg/Kg LAAM to Dog C. Computer determined equation for LAAM (●) is

$$C_1 = 0.41e^{-9.88t} + 0.038e^{-0.093t}$$

Lines for NORLAAM (◐) and DINORLAAM (○) are hand drawn. Note change in time scale after 25 hr.

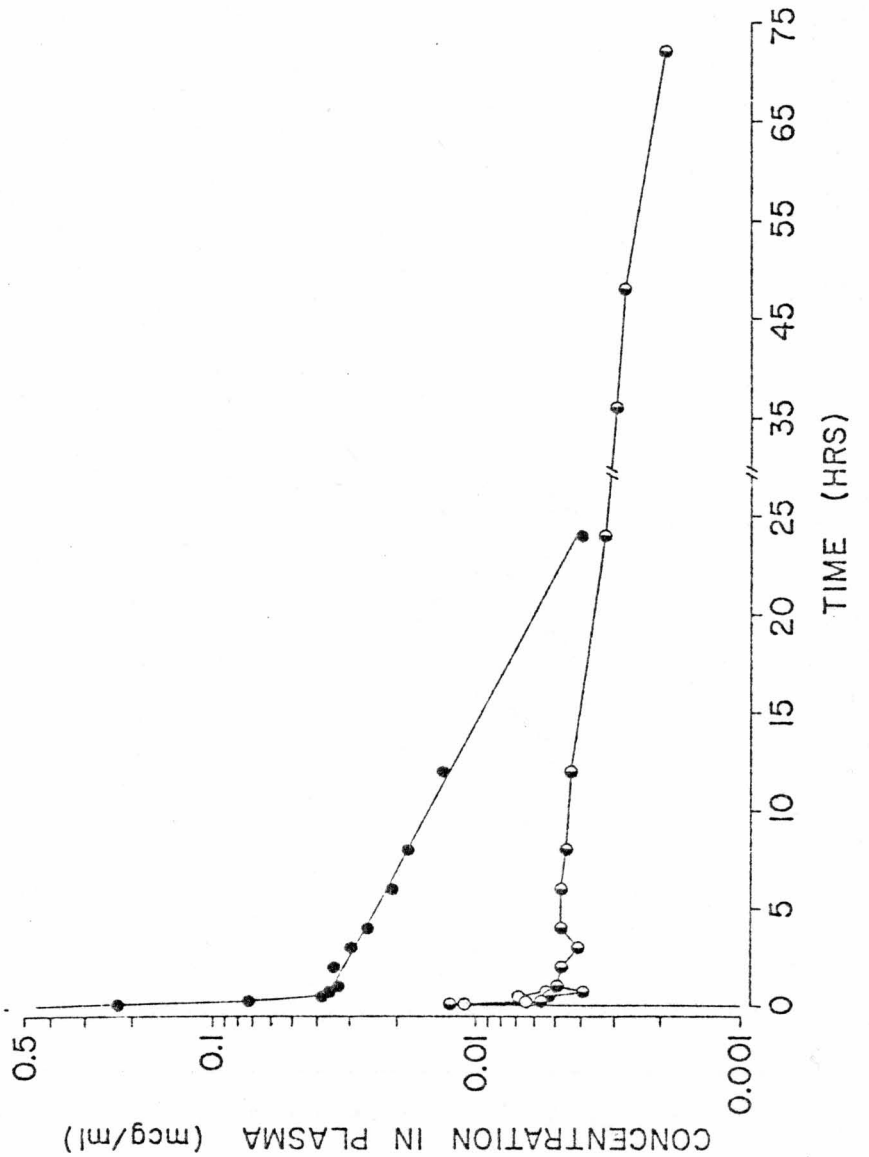

CONCENTRATION IN PLASMA (mcg/ml)
0.5
0.1
0.01
0.001
0
5
10
15
20
25
35
45
55
65
75
TIME (HRS)

Table 11. Experiment 2A. Plasma Concentrations of Drug and Metabolites after Intravenous Administration of 0.6 mg/Kg DAAM to Dog A[a].

	C (μg/ml)		
t (hr)	DAAM	NORDAAM	DINORDAAM
0.083	2.32	0.021	0.007_5
0.25	0.84	0.023	0.005_5
0.50	0.21	0.014	0.005_1
0.75	0.21	0.010	0.005_2
1	0.099	0.011	0
2	0.088	0.008_2	0
3	0.093	0.007_4	0
4	0.086	0.006_9	0
6	0.028	0.005_0	0
8	0.027	0.003_9	0
12	0.011	0.002_3	0
18	0.005_2	0	0
24	0.001_5	0	0
36	0	0	0

[a]Dog weight: 19 Kg, DAAM dose: 11.4 mg.

Figure 10. Experiment 2A. Plasma concentration-time profiles after intravenous administration of 0.6 mg/Kg DAAM to Dog A. Computer determined equation for DAAM (▲) is

$$C_1 = 3.83e^{-6.98t} + 0.15e^{-0.21t}$$

Linear regression line[a] for the β phase (0.75 - 12 hr) of NORDAAM (◭) is

$$\log C_3 = \log 0.011 - 0.058t, \quad r = -0.997$$

The line for DINORDAAM (△) is hand drawn.

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[a] C_3 is the concentration of NORDAAM in the central compartment, as defined on p. 193.

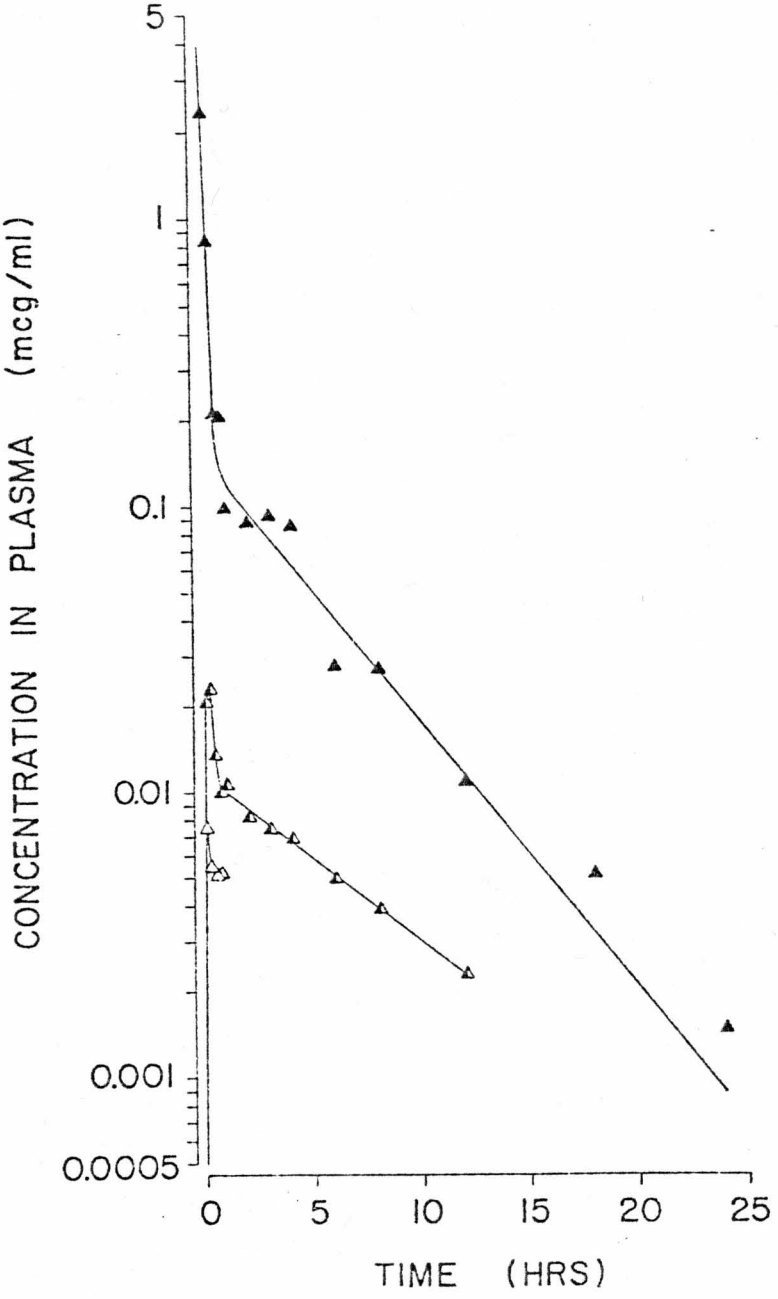
5
1
0.1
0.01
0.001
0.0005
CONCENTRATION IN PLASMA (mcg/ml)
0
5
10
15
20
25
TIME (HRS)

Table 12. Experiment 2B. Plasma Concentrations of Drug and Metabolites after Intravenous Administration of 0.6 mg/Kg DAAM to Dog B[a].

	C (μg/ml)		
t (hr)	DAAM	NORDAAM	DINORDAAM
0.083	0.69	0.059	0.009_7
0.25	0.31	0.061	0.007_5
0.50	0.19	0.067	0.006_2
0.75	0.15	0.036	0.005_0
2	0.16	0.034	0
3	0.13	0.021	0
4	0.098	0.020	0
6	0.065	0.015	0
8	0.053	0.013	0
12	0.029	0.007_7	0
18	0.006_9	0.003_3	0
24.5	0.002_9	0	0
36	0	0	0

[a]Dog weight: 18 Kg, DAAM dose: 10.8 mg.

Figure 11. Experiment 2B. Plasma concentration-time profiles after intravenous administration of 0.6 mg/Kg DAAM to Dog B. Computer determined equation for DAAM (▲) is

$$C_1 = 0.97e^{-8.20t} + 0.19e^{-0.17t}$$

Linear regression line for the β phase (3 - 18 hr) of NORDAAM (◭) is

$$\log C_3 = \log 0.032 - 0.054t, \; r = -0.998$$

The line for DINORDAAM (△) is hand drawn.

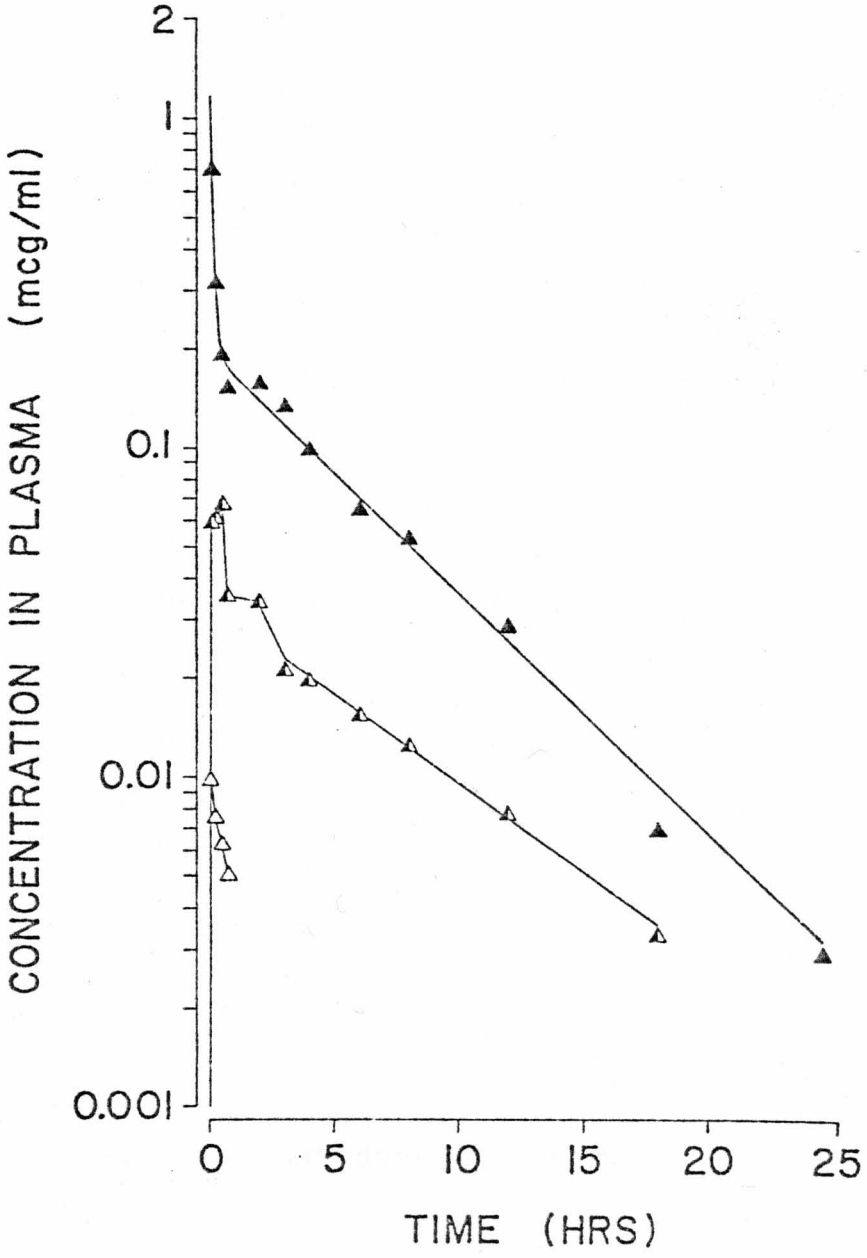
CONCENTRATION IN PLASMA (mcg/ml)
2
1
0.1
0.01
0.001
0
5
10
15
20
25
TIME (HRS)

Table 13. Experiment 2C. Plasma Concentrations of Drug and Metabolites after Intravenous Administration of 0.6 mg/Kg DAAM to Dog C[a].

	C (μg/ml)		
t (hr)	DAAM	NORDAAM	DINORDAAM
0.083	0.36	0.006_1	0
0.25	0.12	0.003_8	0.006_3
0.50	0.10	0.004_6	0.005_3
0.75	0.077	0.003_9	0
1	0.056	0.002_7	0
2	0.058	0.001_8	0
3	0.041	0.002_4	0
4	0.031	0.001_4	0
6	0.022	0	0
8	0.018	0	0
12	0.006_3	0	0
18	0.002_1	0	0
24	0	0	0

[a]Dog weight: 15.1 Kg, DAAM dose: 9.06 mg.

Figure 12. Experiment 2C. Plasma concentration-time profiles after intravenous administration of 0.6 mg/Kg DAAM to Dog C. Computer determined equation for DAAM (▲) is

$$C_1 = 0.67e^{-11.2t} + 0.086e^{-0.22t}$$

Linear regression line for the β phase (1 - 4 hr) of NORDAAM (◭) is

$$\log C_3 = \log 0.003_1 - 0.073t, \; r = -0.736$$

The line for DINORDAAM (△) is hand drawn.

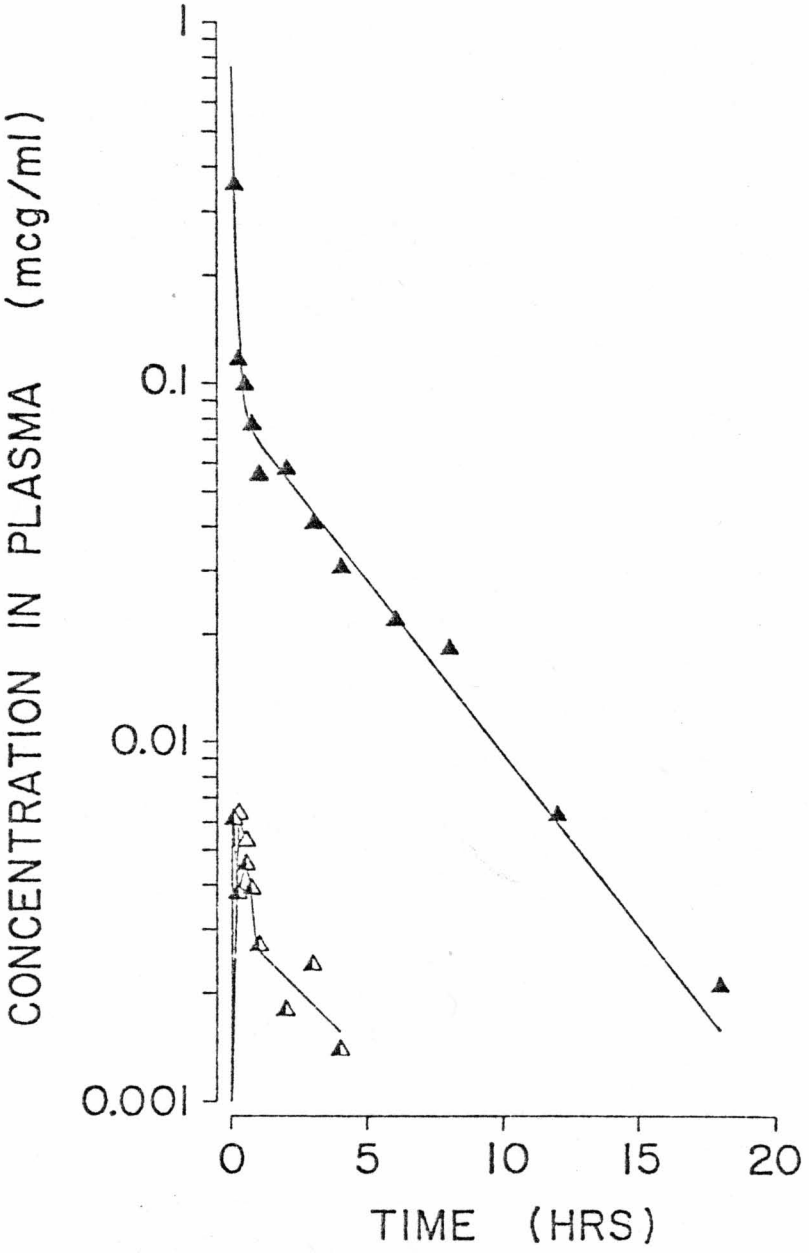
CONCENTRATION IN PLASMA (mcg/ml)
1
0.1
0.01
0.001
0
5
10
15
20
TIME (HRS)

Table 14. Experiment 3A. Plasma Concentrations of Drug and Metabolite after Intravenous Administration of 0.575 mg/Kg NORLAAM to Dog A[a].

	C (μg/ml)	
t (hr)	NORLAAM	DINORLAAM
0.083	-	0.007_7
0.30	0.12	0.009_2
0.50	0.081	0.015
0.75	0.067	0.017
3	0.046	0.049
4	0.046	0.044
6	0.037	0.034
8	0.024	0.033
12	0.024	0.025
24	0.009_5	0.018
36	0.006_3	0.011
48	0.002_2	0.005_7
60	0	0

[a] Dog weight: 18.9 Kg, NORLAAM dose: 10.87 mg.

Figure 13. Experiment 3A. Plasma concentration-time profiles after intravenous administration of 0.575 mg/Kg NORLAAM to Dog A. Computer determined equation for NORLAAM (◐) is

$$C_3 = 0.20e^{-3.44t} + 0.051e^{-0.065t}$$

Linear regression line[a] for the β phase (6 - 48 hr) of DINORLAAM (◯) is

$$\log C_5 = \log 0.044 - 0.018t, \quad r = -0.995$$

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[a] C_5 is the concentration of DINORLAAM in the central compartment, as defined on p. 175.

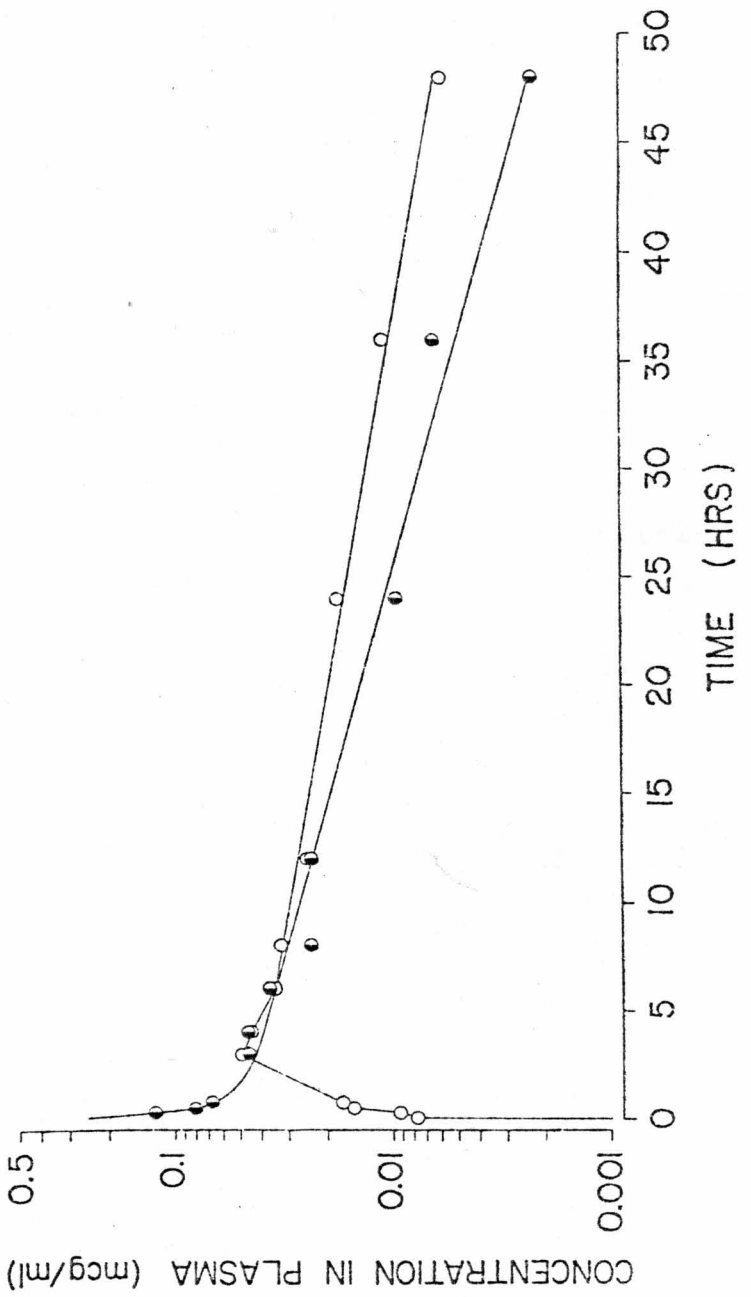

CONCENTRATION IN PLASMA (mcg/ml)
0.5
0.1
0.01
0.001
0
5
10
15
20
25
30
35
40
45
50
TIME (HRS)

Table 15. Experiment 3B. Plasma Concentrations of Drug and Metabolite after Intravenous Administration of 0.575 mg/Kg NORLAAM to Dog B[a].

	C (μg/ml)	
t (hr)	NORLAAM	DINORLAAM
0.083	0.32	0.018
0.25	0.11	0.027
0.50	0.082	0.047
0.75	0.058	0.062
4	0.028	0.077
6	0.015	0.070
8	0.012	0.043
12	0.008_9	0.033
24	0.004_2	0.016
36	0	0.010
48	0	0

[a]Dog weight: 17 Kg, NORLAAM dose: 9.78 mg.

Figure 14. Experiment 3B. Plasma concentration-time profiles after intravenous administration of 0.575 mg/Kg NORLAAM to Dog B. Computer determined equation for NORLAAM (◐) is

$$C_3 = 0.37e^{-4.38t} + 0.026e^{-0.084t}$$

Linear regression line for the β phase (8 - 36 hr) of DINORLAAM (◯) is

$$\log C_5 = \log 0.062 - 0.023t, \; r = -0.993$$

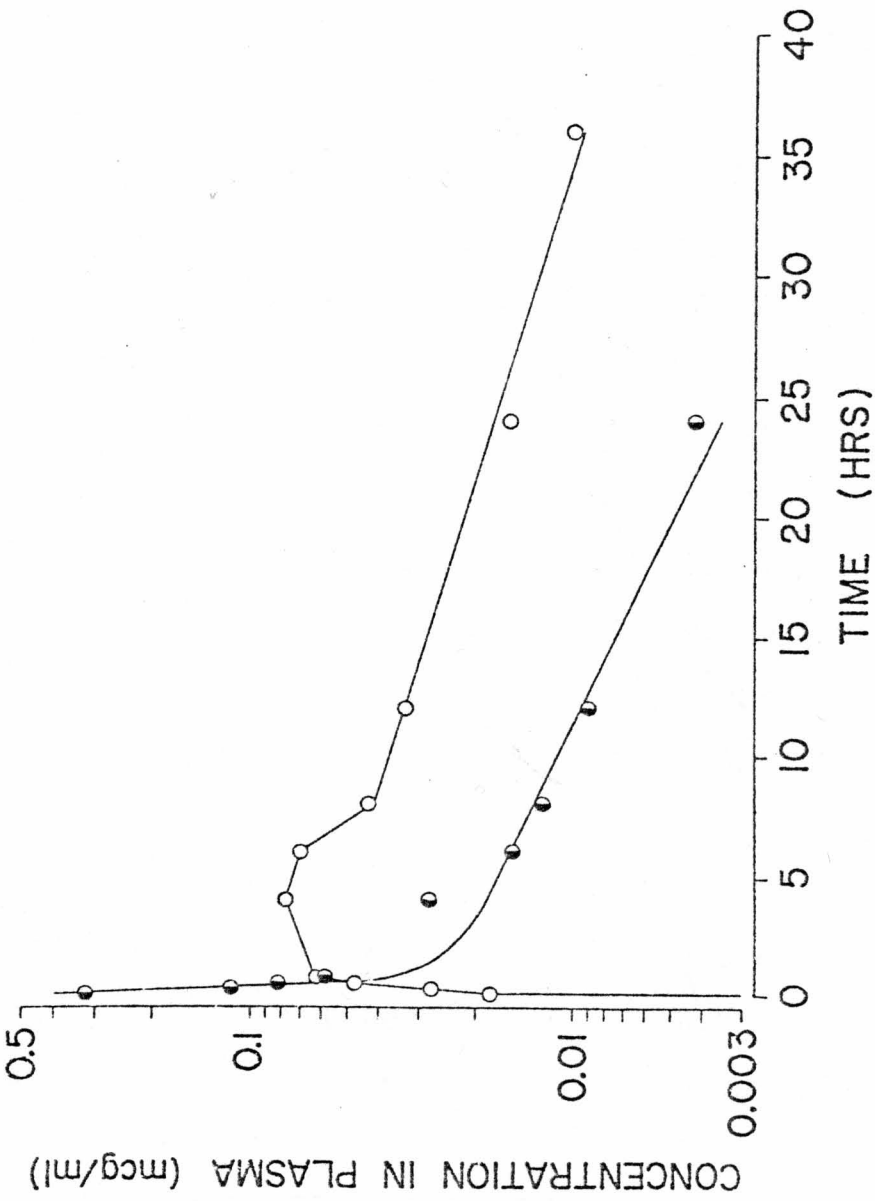

CONCENTRATION IN PLASMA (mcg/ml)
0.5
0.1
0.01
0.003
0
5
10
15
20
25
30
35
40
TIME (HRS)

Table 16. Experiment 3C. Plasma Concentrations of Drug and Metabolite after Intravenous Administration of 0.575 mg/Kg NORLAAM to Dog C[a].

	C (μg/ml)	
t (hr)	NORLAAM	DINORLAAM
0.083	0.18	0.014
0.25	0.091	0.021
0.50	0.067	0.028
0.75	0.049	0.033
1	0.045	0.041
2	0.042	0.049
3	0.037	0.043
4	0.035	0.039
7	0.033	0.034
8	0.031	0.030
12	0.020	0.027
24	0.016	0.016
36	0.007_3	0.011
48	0.005_6	0.007_9
60	0	0

[a]Dog weight: 17.5 Kg, NORLAAM dose: 10.06 mg.

Figure 15. Experiment 3C. Plasma concentration-time profiles after intravenous administration of 0.575 mg/Kg NORLAAM to Dog C. Computer determined equation for NORLAAM (◐) is

$$C_3 = 0.19e^{-4.27t} + 0.039e^{-0.043t}$$

Linear regression line for the β phase (4 - 48 hr) of DINORLAAM (◯) is

$$\log C_5 = \log 0.042 - 0.016t, \; r = -0.993$$

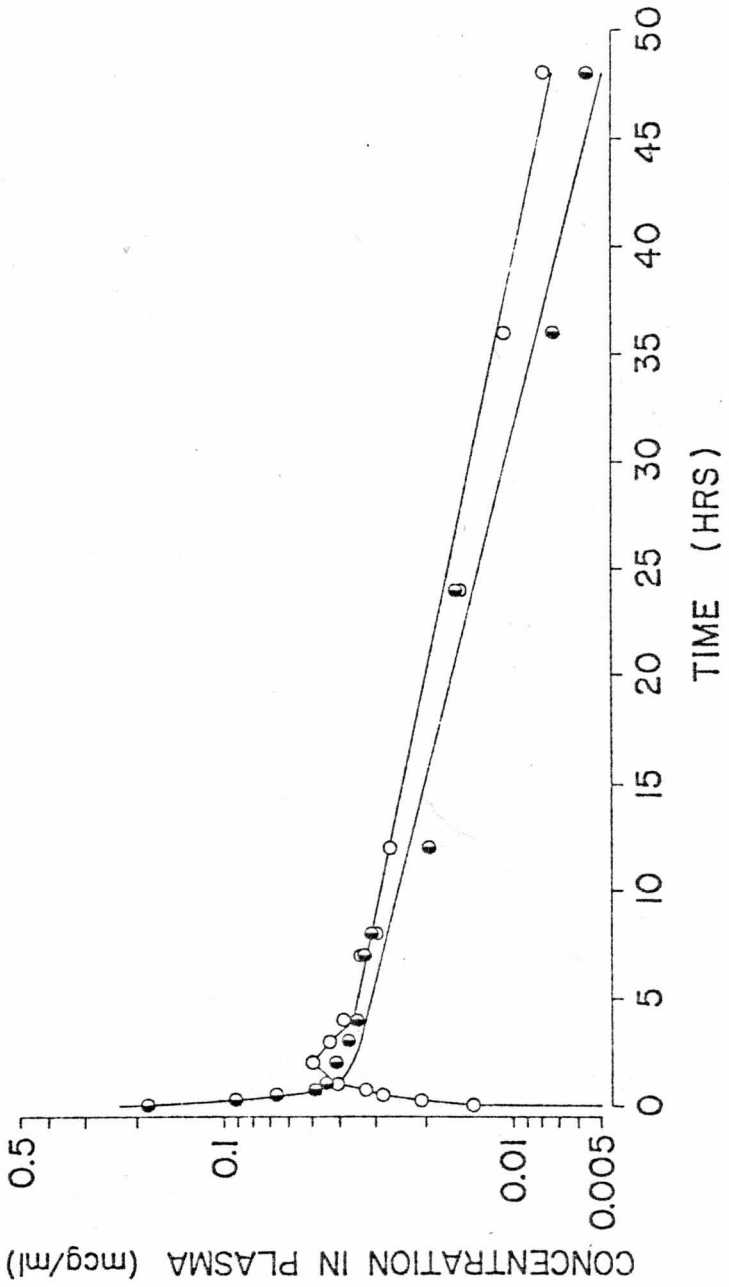

CONCENTRATION IN PLASMA (mcg/ml)
0.5
0.1
0.01
0.005
0
5
10
15
20
25
30
35
40
45
50
TIME (HRS)

Table 17. Experiment 4A. Plasma Concentrations of DINORLAAM after Intravenous Administration of 0.55 mg/Kg DINORLAAM to Dog A[a].

t (hr)	C (μg/ml)
0.083	0.48
0.25	0.32
0.50	0.19
0.75	0.12
1	0.11
2	0.086
3	0.070
4	0.046
6	0.032
8	0.019
12	0.008_1
18	0

[a]Dog weight: 19 Kg, DINORLAAM dose: 10.45 mg.

Figure 16. Experiment 4A. Plasma concentration-time profile after intravenous administration of 0.55 mg/Kg DINORLAAM to Dog A. Computer determined equation for DINORLAAM (◯) is

$$C_5 = 0.50e^{-3.86t} + 0.13e^{-0.23t}$$

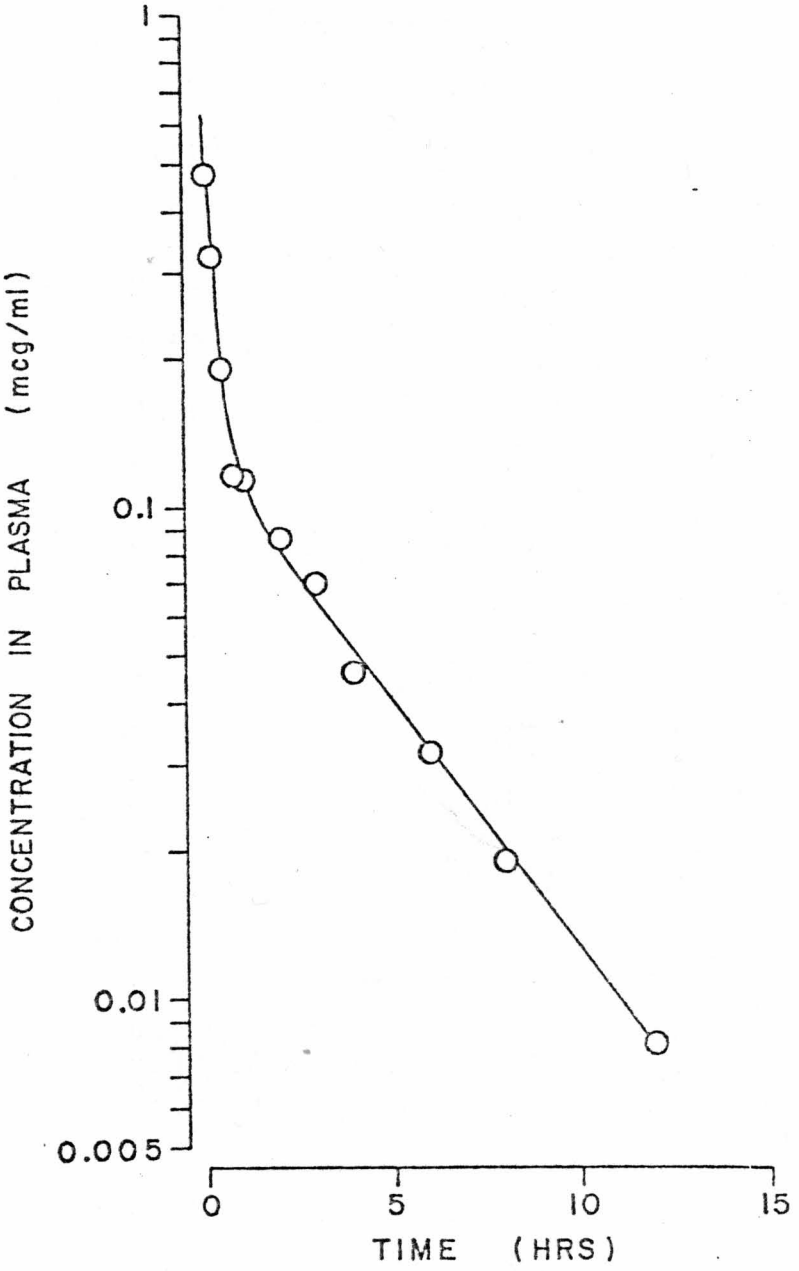

CONCENTRATION IN PLASMA (mcg/ml)
1
0.1
0.01
0.005
0
5
10
15
TIME (HRS)

Table 18. Experiment 4B. Plasma Concentrations of DINORLAAM after Intravenous Administration of 0.55 mg/Kg DINORLAAM to Dog B[a].

t (hr)	C (μg/ml)
0.083	0.21
0.25	0.12
0.50	0.10
0.75	0.057
1	0.040
2	0.035
3	0.030
4	0.027
6	0.017
8	0.010
12	0.004_7
18	0

[a]Dog weight: 17 Kg, DINORLAAM dose: 9.35 mg.

Figure 17. Experiment 4B. Plasma concentration-time profile after intravenous administration of 0.55 mg/Kg DINORLAAM to Dog B. Computer determined equation for DINORLAAM (◯) is

$$C_5 = 0.21e^{-3.47t} + 0.050e^{-0.19t}$$

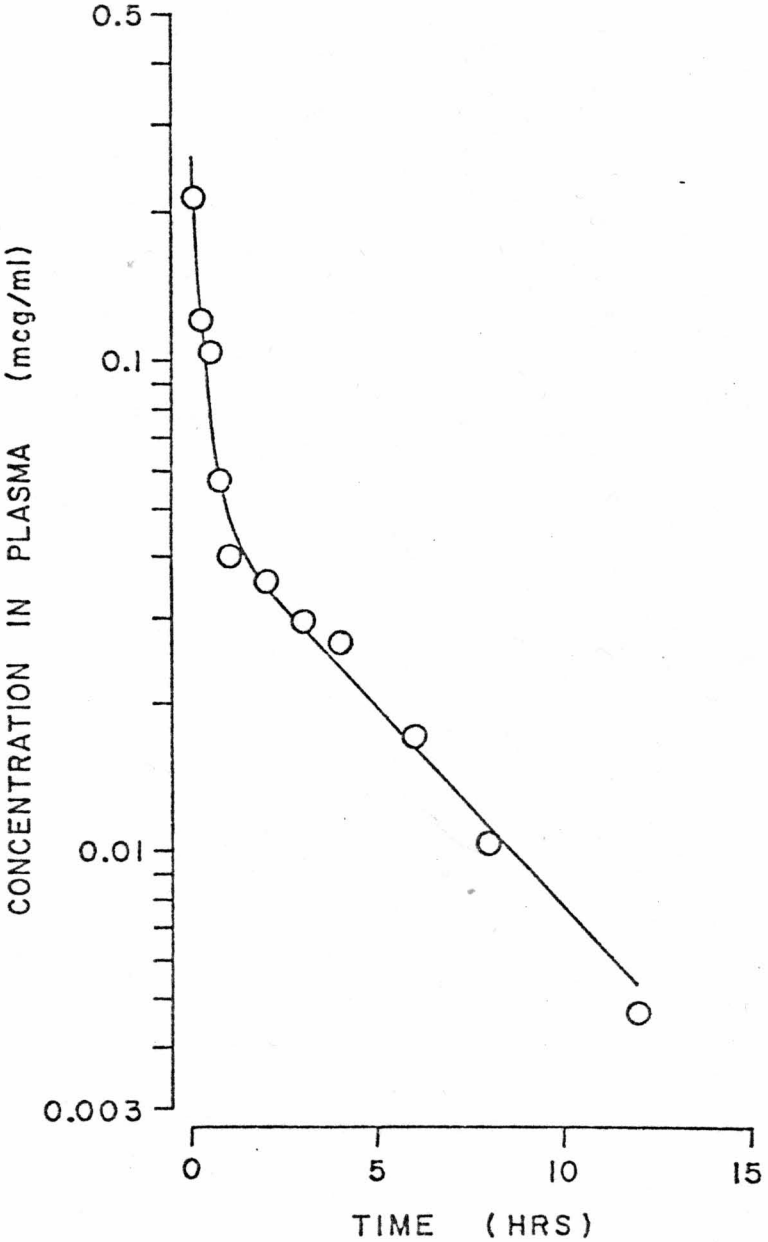

0.5
0.1
0.01
0.003
CONCENTRATION IN PLASMA (mcg/ml)
0
5
10
15
TIME (HRS)

Table 19. Experiment 4C. Plasma Concentrations of DINORLAAM after Intravenous Administration of 0.55 mg/Kg DINORLAAM to Dog C[a].

t (hr)	C (μg/ml)
0.083	0.60
0.25	0.24
0.50	0.099
0.75	0.076
1	0.067
2	0.064
3	0.051
4	0.041
6	0.030
8	0.021
12	0.011
18	0.003_6
24	0

[a] Dog weight: 15.1 Kg, DINORLAAM dose: 8.31 mg.

Figure 18. Experiment 4C. Plasma concentration-time profile after intravenous administration of 0.55 mg/Kg DINORLAAM to Dog C. Computer determined equation for DINORLAAM (◯) is

$$C_5 = 0.91e^{-7.11t} + 0.082e^{-0.17t}$$

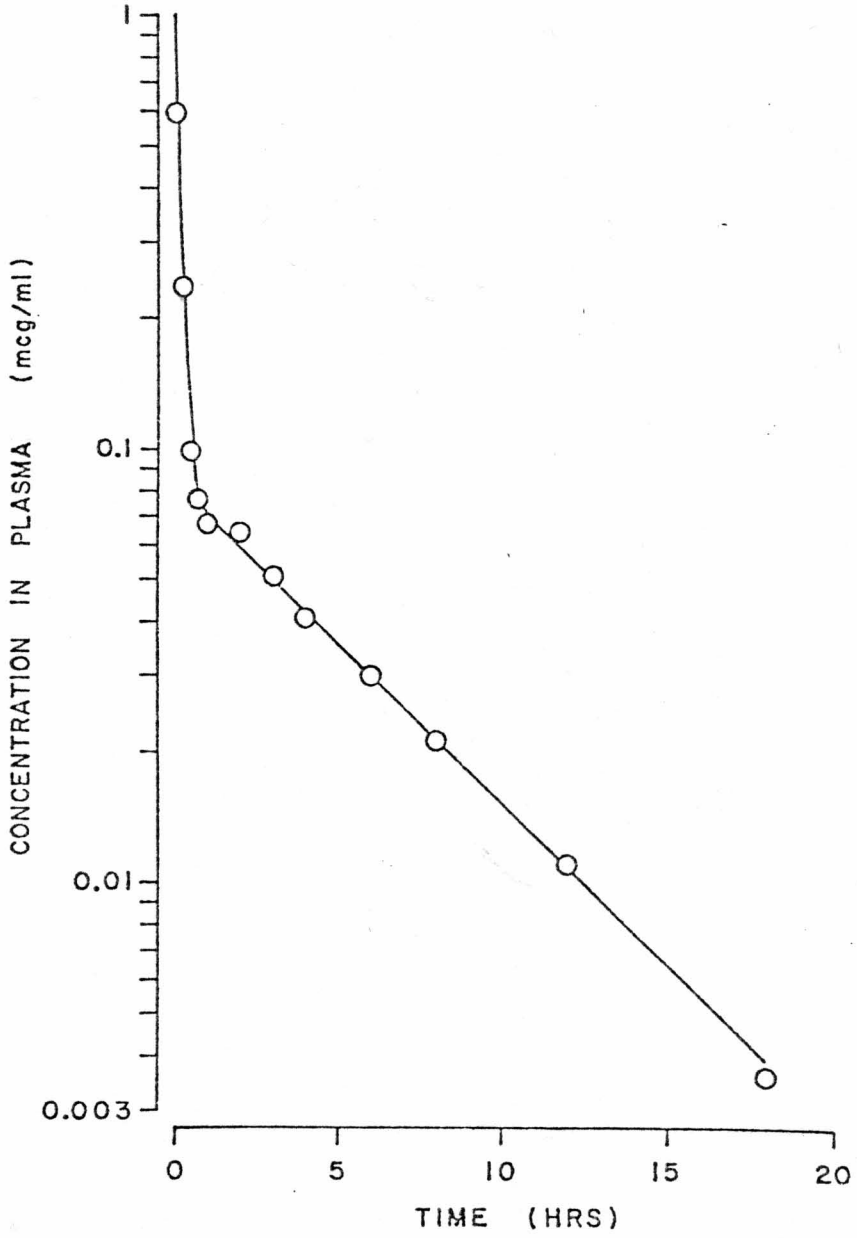

CONCENTRATION IN PLASMA (mcg/ml)
1
0.1
0.01
0.003
0
5
10
15
20
TIME (HRS)

Table 20. Experiment 5A. Plasma Concentrations of Drug and Metabolites after Oral Administration of 2 mg/Kg LAAM to Dog A[a].

	C (μg/ml)		
t (hr)	LAAM	NORLAAM	DINORLAAM
0	0	0	0
0.25	0.020	0.005_0	0.005_7
0.50	0.028	0.016	0.010
0.75	0.042	0.052	0.019
1	0.057	0.064	0.029
2	0.037	0.086	0.042
3	0.034	0.068	0.050
4	0.027	0.051	0.054
6	0.021	0.042	0.041
8	0.019	0.041	0.025
12	0.009_9	0.026	0.017
18	0.005_9	0.020	0.011
24	0.003_8	0.014	0.008_1
36	0.001_5	0.006_4	0
48	0	0.002_9	0
60	-	0	0

[a]Dog weight: 18.7 Kg, LAAM dose: 37.4 mg.

Figure 19. Experiment 5A. Plasma concentration-time profiles after oral administration of 2 mg/Kg LAAM to Dog A. Computer determined equation[a] for LAAM (●) is

$$C_1 = 15.35e^{-1.028t} + 0.034e^{-0.093t} - 15.38e^{-1.032t}$$

Linear regression line for the β phase (4 - 48 hr) of NORLAAM (◐) is

$$\log C_3 = \log 0.063 - 0.028t, \quad r = -0.998$$

and that for the β phase (12 - 24 hr) of DINORLAAM (○) is

$$\log C_5 = \log 0.033 - 0.026t, \quad r = -0.995$$

---

[a] The equation is in the form of $C_1 = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-k_a t}$, where the symbols are as defined on pp. 114-115. Although the close similarity of A and C and also k_a and α might suggest possibility of computer generated artifact (67), the values are in fact realistic and give rise to similar numerical values of microscopic rate constants to those obtained by graphic analysis. This argument applies also to Figures 20-23.

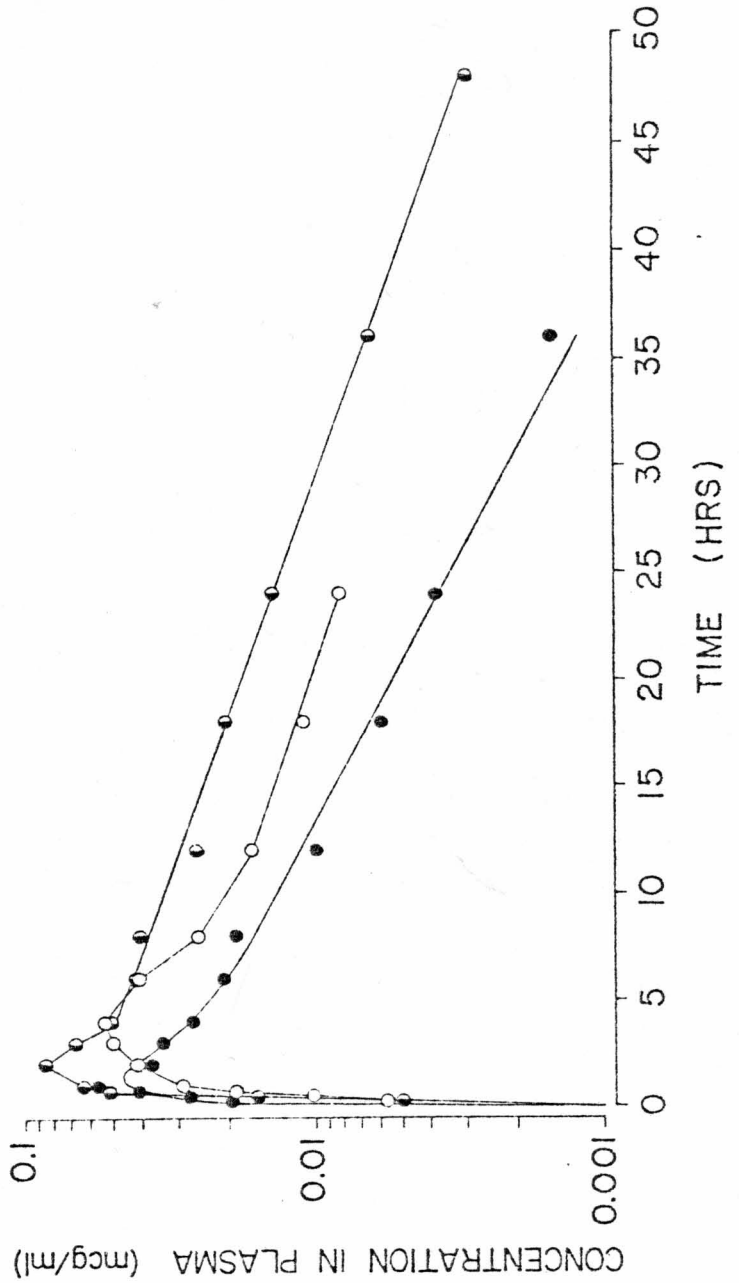

CONCENTRATION IN PLASMA (mcg/ml)
0.1
0.01
0.001
0
5
10
15
20
25
30
35
40
45
50
TIME (HRS)

Table 21. Experiment 5B. Plasma Concentrations of Drug and Metabolites after Oral Administration of 2 mg/Kg LAAM to Dog B[a].

	C (μg/ml)		
t (hr)	LAAM	NORLAAM	DINORLAAM
0	0	0	0
0.25	0.020	0.006_0	0.010
0.50	0.032	0.016	0.019
0.75	0.052	0.037	0.029
1.5	0.056	0.044	0.041
2	Experiment aborted following expiration of dog[b].		

[a]Dog weight: 16.6 Kg, LAAM dose: 33.2 mg.

[b]Due to the limited data obtained in this particular study, graphical illustration of the plasma concentrations is not shown.

Table 22. Experiment 5C. Plasma Concentrations of Drug and Metabolites after Oral Administration of 2 mg/Kg LAAM to Dog C[a].

	C (μg/ml)		
t (hr)	LAAM	NORLAAM	DINORLAAM
0	0	0	0
0.25	0.019	0.004_2	0.015
0.50	0.028	0.009_6	0.021
0.75	0.053	0.019	0.027
1	0.051	0.033	0.033
2	0.040	0.025	0.053
3	0.027	0.022	0.060
4	0.025	0.021	0.057
6	0.017	0.021	0.051
8	0.013	0.018	0.042
12	0.009_2	0.016	0.031
18	0.005_2	0.012	0.024
24	0.002_2	0.008_4	0.019
36	0	0.005_4	0.008_0
48	-	0.002_9	0.004_2
60	-	0	0

[a]Dog weight: 14.5 Kg, LAAM dose: 29.0 mg.

Figure 20. Experiment 5C. Plasma concentration-time profiles after oral administration of 2 mg/Kg LAAM to Dog C. Computer determined equation for LAAM (●) is

$$C_1 = -23.92e^{-1.02t} + 0.027e^{-0.097t} + 23.89e^{-1.016t}$$

Linear regression line for the β phase (2 - 48 hr) of NORLAAM (◐) is

$$\log C_3 = \log 0.026 - 0.020t, \; r = -0.999$$

and that for the β phase (12 - 48 hr) of DINORLAAM (○) is

$$\log C_5 = \log 0.065 - 0.025t, \; r = -0.997$$

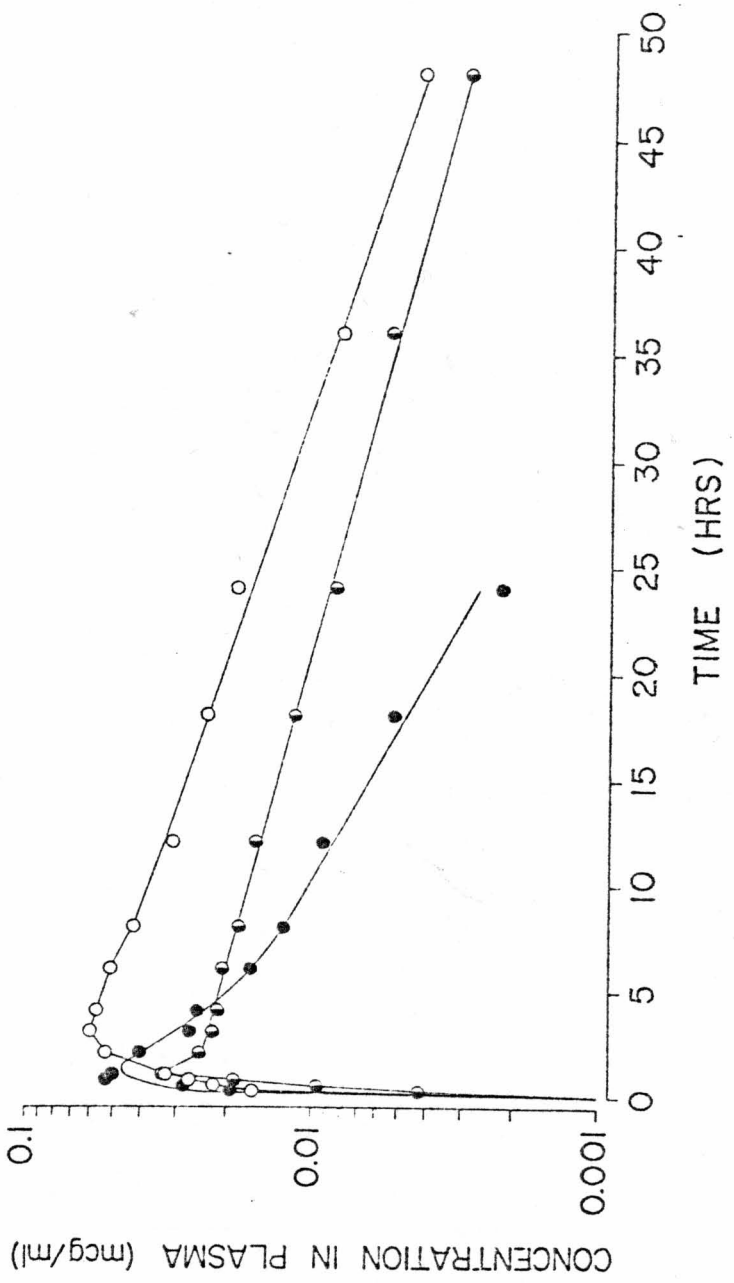

CONCENTRATION IN PLASMA (mcg/ml)
0.1
0.01
0.001
0
5
10
15
20
25
30
35
40
45
50
TIME (HRS)

Table 23. Experiment 6A. Plasma Concentrations of Drug and Metabolites after Oral Administration of 2 mg/Kg DAAM to Dog A[a].

	C (μg/ml)		
t (hr)	DAAM	NORDAAM	DINORDAAM
0	0	0	0
0.25	0.027	0.022	0.022
0.50	0.044	0.059	0.037
0.75	0.054	0.072	0.048
1	0.082	0.092	0.068
2	0.045	0.13	0.097
3	0.039	0.13	0.072
4	0.030	0.093	0.057
6	0.019	0.044	0.053
8	0.017	0.036	0.042
12	0.011	0.020	0.025
24	0.002_5	0.007_7	0.009_3
36	0	0.002_4	0
48	-	0	0

[a]Dog weight: 18.7 Kg, DAAM dose: 37.4 mg.

Figure 21. Experiment 6A. Plasma concentration-time profiles after oral administration of 2 mg/Kg DAAM to Dog A. Computer determined equation for DAAM (▲) is

$$C_1 = 90.67e^{-1.098t} + 0.036e^{-0.11t} - 90.71e^{-1.10t}$$

Linear regression line for the β phase (6 - 36 hr) of NORDAAM (◭) is

$$\log C_3 = \log 0.073 - 0.041t, \; r = -0.997$$

and that for the β phase (4 - 24 hr) of DINORDAAM (△) is

$$\log C_5 = \log 0.086 - 0.041t, \; r = -0.996$$

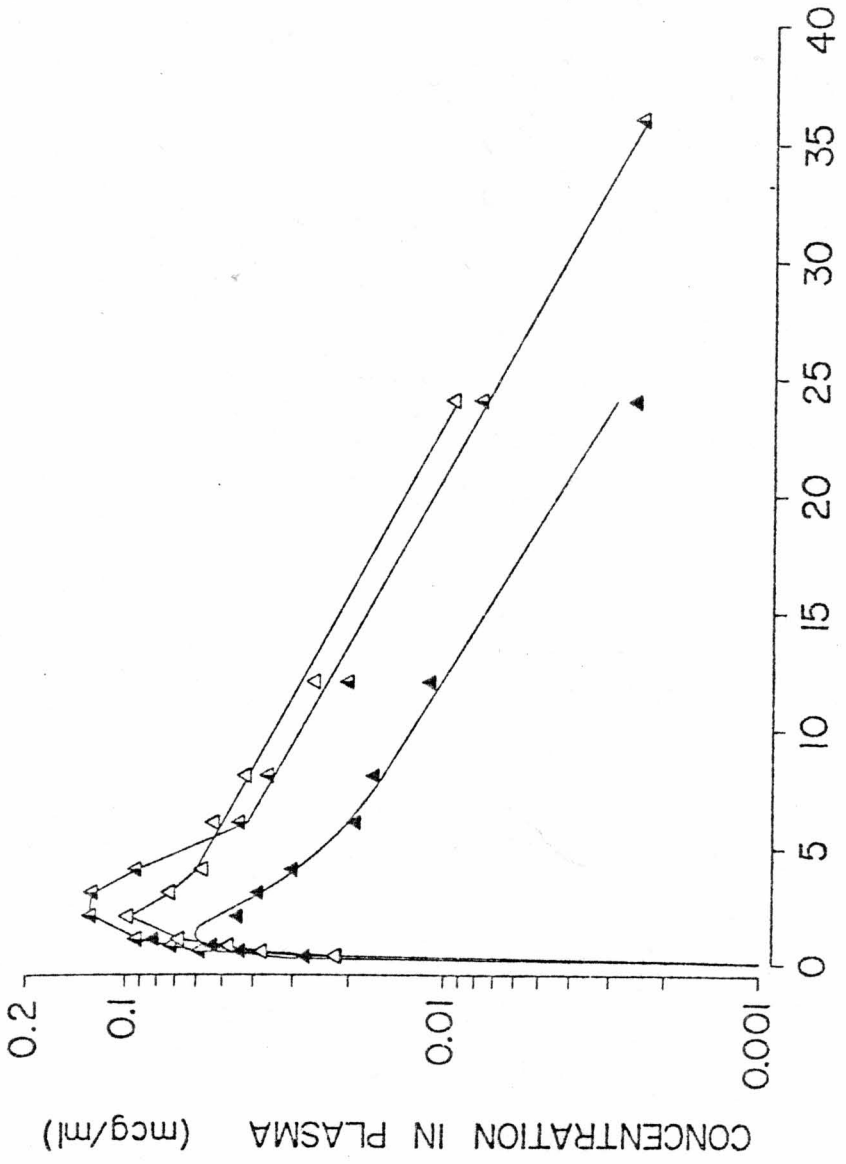

CONCENTRATION IN PLASMA (mcg/ml)
0.2
0.1
0.01
0.001
0
5
10
15
20
25
30
35
40
TIME (HRS)

Table 24. Experiment 6B. Plasma Concentrations of Drug and Metabolites after Oral Administration of 2 mg/Kg DAAM to Dog B[a].

	C (μg/ml)		
t (hr)	DAAM	NORDAAM	DINORDAAM
0	0	0	0
0.25	0.027	0.020	0.010
0.50	0.054	0.037	0.016
0.75	0.070	0.061	0.022
1	0.082	0.077	0.027
2	0.060	0.075	0.031
3	0.050	0.059	0.030
4	0.048	0.052	0.026
6	0.038	0.042	0.018
8	0.029	0.039	0.017
12	0.021	0.023	0.012
18	0.008_7	0.015	0.007_8
24	0.003_7	0.012	0.005_0
36	0	0.002_7	0
48	-	0	0

[a]Dog weight: 16.6 Kg, DAAM dose: 33.2 mg.

Figure 22. Experiment 6B. Plasma concentration-time profiles after oral administration of 2 mg/Kg DAAM to Dog B. Computer determined equation for DAAM (▲) is

$$C_1 = 71.93e^{-1.237t} + 0.070e^{-0.11t} - 72.0e^{-1.239t}$$

Linear regression line for the β phase (3 - 36 hr) of NORDAAM (◭) is

$$\log C_3 = \log 0.075 - 0.039t, \quad r = -0.992$$

and that for the β phase (6 - 24 hr) of DINORDAAM (△) is

$$\log C_5 = \log 0.029 - 0.032t, \quad r = -0.998$$

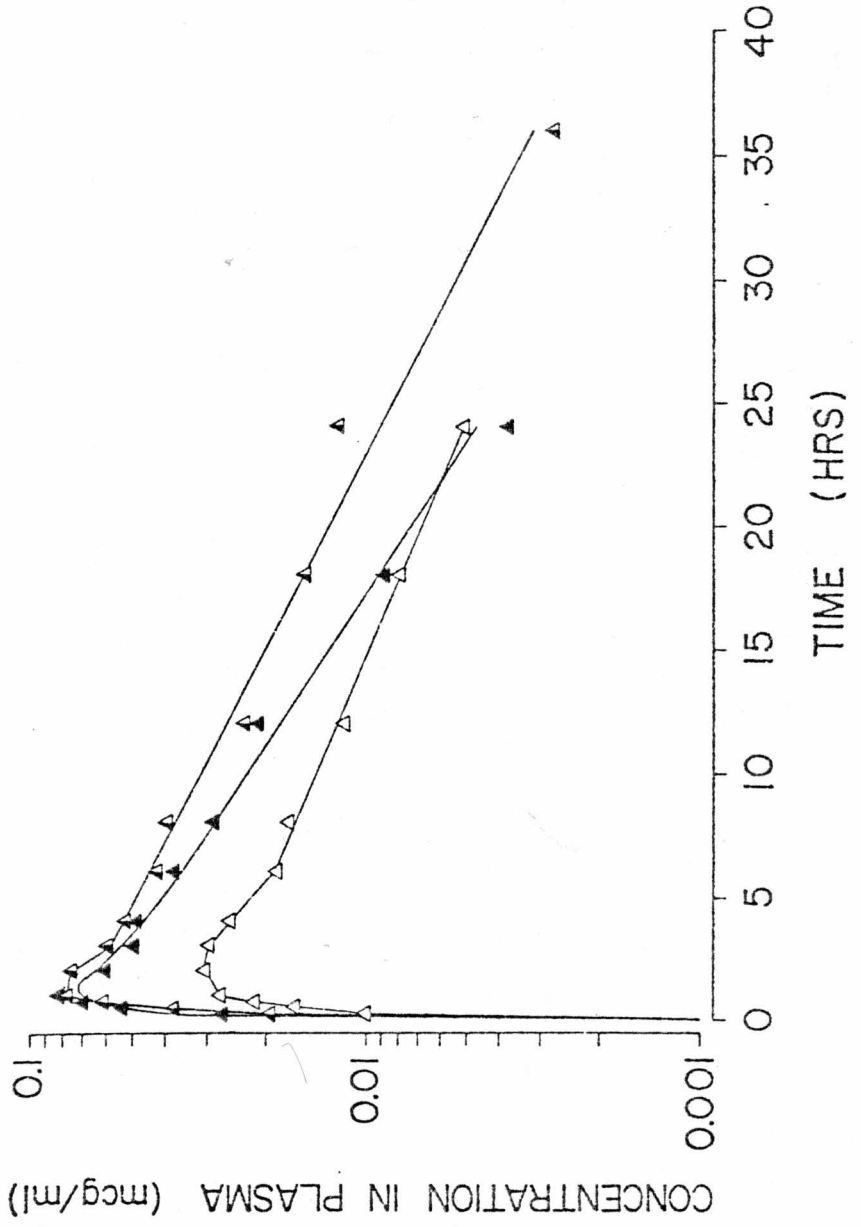

0.1
0.01
0.001
CONCENTRATION IN PLASMA (mcg/ml)
0
5
10
15
20
25
30
35
40
TIME (HRS)

Table 25. Experiment 6C. Plasma Concentrations of Drug and Metabolites after Oral Administration of 2 mg/Kg DAAM to Dog C[a].

	C (μg/ml)		
t (hr)	DAAM	NORDAAM	DINORDAAM
0	0	0	0
0.25	0.017	0.019	0.004_5
0.50	0.034	0.025	0.012
0.75	0.064	0.061	0.015
1	0.047	0.053	0.018
2	0.039	0.040	0.020
3	0.037	0.046	0.020
4	0.032	0.043	0.016
6	0.022	0.037	0.013
8	0.020	0.028	0.011
12	0.012	0.023	0.008_3
24	0.002_4	0.007_9	0
36	0	0.003_4	0
48	0	0	0

[a]Dog weight: 14.3 Kg, DAAM dose: 28.6 mg.

Figure 23. Experiment 6C. Plasma concentration-time profiles after oral administration of 2 mg/Kg DAAM to Dog C. Computer determined equation for DAAM (▲) is

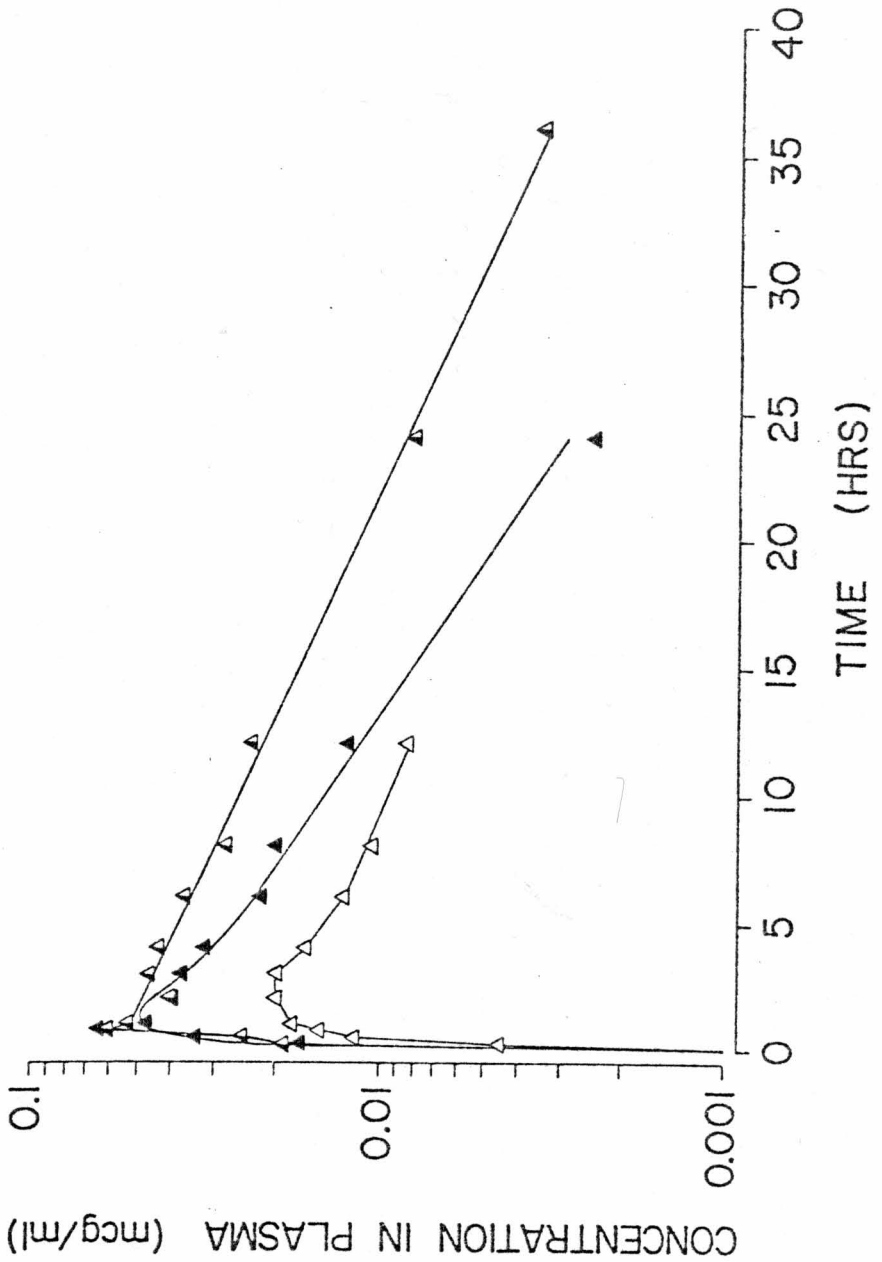
$$C_1 = 46.42e^{-1.146t} + 0.046e^{-0.12t} - 46.46e^{-1.147t}$$

Linear regression line for the β phase (2 - 36 hr) of NORDAAM (◭) is

$$\log C_3 = \log 0.055 - 0.034t, \; r = -0.996$$

and that for the β phase (6 - 12 hr) of DINORDAAM (△) is

$$\log C_5 = \log 0.019 - 0.030t, \; r = -0.993$$


CONCENTRATION IN PLASMA (mcg/ml)
0.1
0.01
0.001
0
5
10
15
20
25
30
35
40
TIME (HRS)

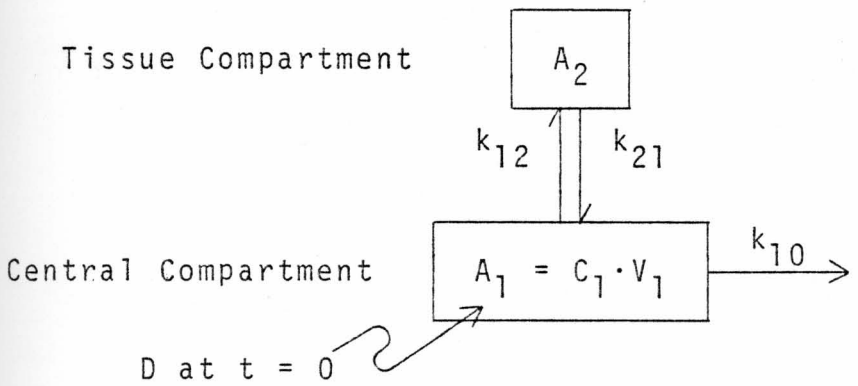
and normethadol, thus confirming the absence of these metabolites, within assay limitations, in the plasma of dogs treated with the parent drugs.

ii. Theoretical

The semilogarithmic plots of plasma concentration versus time, given in Figures 7-18, suggest that the elimination from the body of all compounds studied is biexponential following intravenous doses. It was therefore elected to analyze the data in terms of the pharmacokinetic two compartment open model. Scheme IV describes this model following intravenous administration of a drug.

Scheme IV

Two Compartment Open Model with Rapid Intravenous Injection.



The intravenous dose D is instantaneously introduced into a central compartment of apparent distribution volume V_1, which represents plasma and other tissue and fluid volumes

into which the drug rapidly equilibrates. The tissue compartment consists of other tissues and fluids into which the drug penetrates, but which are not in rapid equilibrium with the central compartment. The quantities A_1 and A_2 refer to amounts of unchanged drug in the central and tissue compartments, respectively. The first-order rate constants k_{12} and k_{21} govern the transfer of drug between the compartments, while k_{10} is the first-order rate constant governing loss of drug from the central compartment by all elimination processes, i.e., excretion and metabolism. Equation 1 describes the concentration of drug in plasma <u>versus</u> time profile appropriate to this model (68).

$$C_1 = \frac{D}{V_1(\beta - \alpha)}[(k_{21} - \alpha)e^{-\alpha t} - (k_{21} - \beta)e^{-\beta t}] \qquad \text{(Eq. 1)}$$

The composite rate constants α and β are defined as

$$\begin{matrix}\alpha \\ \beta\end{matrix} = \frac{1}{2}[(k_{12} + k_{21} + k_{10}) \pm \sqrt{(k_{12} + k_{21} + k_{10})^2 - 4k_{21}k_{10}}] \qquad \text{(Eq. 2)}$$

where β is the slope of the post-distributive log-linear phase of loss of drug from the body, multiplied by -2.303, and α is -2.303 multiplied by the slope of the residual line, i.e., the log-linear phase obtained by subtracting the extrapolated β line from the observed data points during the initial curvilinear distributive phase. The

rate constants k_{12}, k_{21} and k_{10} are calculated using Equations 3-5 (68),

$$k_{21} = \frac{A\beta + B\alpha}{A + B} \qquad \text{(Eq. 3)}$$

$$k_{10} = \frac{\alpha\beta}{k_{21}} \qquad \text{(Eq. 4)}$$

$$k_{12} = \alpha + \beta - k_{21} - k_{10} \qquad \text{(Eq. 5)}$$

where A and B are the intercepts of the α and β extrapolated lines, respectively, on the ordinate. The apparent volume of distribution, V_1, is determined from

$$V_1 = \frac{D}{A + B} \qquad \text{(Eq. 6)}$$

The biological half-life, $t_{1/2,\beta}$, is

$$t_{1/2,\beta} = \frac{0.693}{\beta} \qquad \text{(Eq. 7)}$$

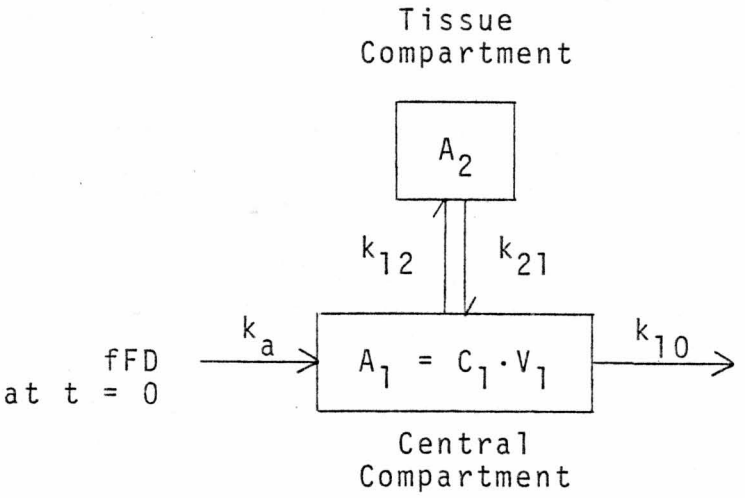
while the distribution half-life, $t_{1/2,\alpha}$, is

$$t_{1/2,\alpha} = \frac{0.693}{\alpha} \qquad \text{(Eq. 8)}$$

The two compartment open pharmacokinetic model as applied to oral administration of drugs is depicted in Scheme V.

Scheme V

Two Compartment Open Model with First-Order Absorption.



F is the fraction of the dose D absorbed from the gastrointestinal tract, while f is the bioavailability factor due to possible first-pass metabolism in the liver. The product, fF, is then the fraction of D appearing in the systemic circulation as unchanged drug. k_a is the first-order rate constant of absorption. The remaining symbols are as defined in Scheme IV. The plasma concentration of drug at time t is defined by Equation 9 (68).

$$C_1 = \frac{k_a fFD}{V_1}\left\{\left[\frac{k_{21} - \alpha}{(k_a - \alpha)(\beta - \alpha)}\right]e^{-\alpha t} + \left[\frac{k_{21} - \beta}{(k_a - \beta)(\alpha - \beta)}\right]e^{-\beta t} + \left[\frac{k_{21} - k_a}{(\alpha - k_a)(\beta - k_a)}\right]e^{-k_a t}\right\} \qquad \text{(Eq. 9)}$$

Graphic estimates of β and α are obtained as described previously, while k_a is -2.303 multiplied by the slope of the

log-linear phase obtained by subtracting the extrapolated α line from the first residual during the absorption phase. The rate constant, k_{21} is calculated using Equation 10 (68),

$$k_{21} = \frac{\beta k_a P + \alpha k_a Q + \alpha\beta R}{P(k_a - \alpha) + Q(k_a - \beta)} \qquad \text{(Eq. 10)}$$

where $P = A/k_a$, $Q = B/k_a$ and $R = C/k_a$. A and B are previously defined, whereas C is the intercept of the k_a extrapolated line on the ordinate, but bearing a negative sign. The rate constants k_{10} and k_{12} are obtained as defined in Equations 4 and 5. The apparent volume of distribution, V_1, is determined by

$$V_1 = \frac{fFD}{P(k_a - \alpha) + Q(k_a - \beta)} \qquad \text{(Eq. 11)}$$

Due to the relatively small number of data points for metabolites in relation to the complexity of the pharmacokinetic models (to be discussed on pp. 172 and 191), complete pharmacokinetic profile was studied only with the administered compound in each experiment.

iii. Results of Least-Squares Fitting

The least-squares estimates of the individual pharmacokinetic parameters are listed in Tables 26-31.

Table 26. Experiment 1 (A, B, C). Computer Estimates of LAAM Pharmacokinetic Parameters after Intravenous Administration of 0.6 mg/Kg LAAM.

Parameter	Dog A	Dog B	Dog C
D (mg)	11.4	10.92	8.58
α (hr^{-1})	2.19	10.1	9.88
$t_{1/2,\alpha}$ (hr)	0.32	0.068	0.070
β (hr^{-1})	0.11	0.12	0.093
$t_{1/2,\beta}$ (hr)	6.27	5.99	7.46
k_{12} (hr^{-1})	1.11 (0.62-1.61)[a]	8.04 (5.41-10.7)	8.06 (7.41-8.71)
k_{21} (hr^{-1})	0.26 (0.10-0.42)	0.87 (0.64-1.09)	0.92 (0.86-0.97)
k_{10} (hr^{-1})	0.92 (0.58-1.27)	1.36 (0.87-1.84)	1.00 (0.91-1.10)
D/V_1 ($\mu g/ml$)	0.28 (0.19-0.37)	0.45 (0.32-0.59)	0.45 (0.42-0.48)
V_1 (l)	40.6	24.1	19.0
V_1 (% body wt)	214	132	133
$V_1 k_{10}$ (ml/min)	626	544	317
V_{dss} (l)	213	248	189

V_{dss} (% body wt)	1122	1360	1300
R^2	0.983	0.993	1.000
r	0.986	0.995	1.000

[a]95% confidence interval, for Tables 26-31.

Table 27. Experiment 2 (A, B, C). Computer Estimates of DAAM Pharmacokinetic Parameters after Intravenous Administration of 0.6 mg/Kg DAAM.

Parameter	Dog A	Dog B	Dog C
D (mg)	11.4	10.8	9.06
α (hr^{-1})	6.98	8.20	11.2
$t_{1/2,\alpha}$ (hr)	0.099	0.085	0.062
β (hr^{-1})	0.21	0.17	0.22
$t_{1/2,\beta}$ (hr)	3.27	4.13	3.13
k_{12} (hr^{-1})	3.51 (2.68-4.34)	5.96 (4.22-7.69)	8.31 (4.90-11.7)
k_{21} (hr^{-1})	0.46 (0.31-0.61)	1.50 (1.20-1.80)	1.48 (1.12-1.84)
k_{10} (hr^{-1})	3.22 (2.61-3.83)	0.92 (0.74-1.10)	1.68 (1.09-2.28)
D/V_1 ($\mu g/ml$)	3.97 (3.36-4.59)	1.17 (0.95-1.38)	0.75 (0.50-1.01)
V_1 (l)	2.87	9.27	12.1
V_1 (% body wt)	15.1	51.5	79.9
$V_1 k_{10}$ (ml/min)	154	142	339
V_{dss} (l)	24.8	46.1	79.9

V_{dss} (% body wt)	130	256	529
R^2	0.999	0.998	0.996
r	0.999	0.999	0.996

Table 28. Experiment 3 (A, B, C). Computer Estimates of NORLAAM Pharmacokinetic Parameters after Intravenous Administration of 0.575 mg/Kg NORLAAM.

Parameter	Dog A	Dog B	Dog C
D (mg)	10.87	9.78	10.06
α (hr^{-1})	3.44	4.38	4.27
$t_{1/2,\alpha}$ (hr)	0.20	0.16	0.16
β (hr^{-1})	0.065	0.084	0.043
$t_{1/2,\beta}$ (hr)	10.7	8.30	16.1
k_{34} (hr^{-1})	2.45 (0.28-4.63)	3.10 (1.43-4.76)	3.31 (1.77-4.86)
k_{43} (hr^{-1})	0.76 (0.48-1.03)	0.36 (0.14-0.59)	0.75 (0.49-1.02)
k_{30} (hr^{-1})	0.29 (0.088-0.50)	1.01 (0.55-1.47)	0.24 (0.17-0.32)
D/V_3 ($\mu g/ml$)	0.25 (0.067-0.43)	0.40 (0.24-0.56)	0.23 (0.17-0.29)
V_3 (l)	47.3	24.6	43.8
V_3 (% body wt)	250	145	250
$V_3 k_{30}$ (ml/min)	232	414	178
V_{dss} (l)	201	235	236

V_{dss} (% body wt)	1062	1381	1350
R^2	0.996	0.976	0.993
r	0.995	0.983	0.993

[a]The parameters k_{34}, k_{43}, k_{30} and V_3 are defined on p. 172.

Table 29. Experiment 4 (A, B, C). Computer Estimates of DINORLAAM Pharmacokinetic Parameters after Intravenous Administration of 0.55 mg/Kg DINORLAAM.

Parameter[a]	Dog A	Dog B	Dog C
D (mg)	10.45	9.35	8.31
α (hr^{-1})	3.86	3.47	7.11
$t_{1/2,\alpha}$ (hr)	0.18	0.20	0.098
β (hr^{-1})	0.23	0.19	0.17
$t_{1/2,\beta}$ (hr)	3.00	3.73	4.10
k_{56} (hr^{-1})	2.20 (1.61-2.79)	2.05 (1.09-3.01)	4.92 (4.60-5.24)
k_{65} (hr^{-1})	0.96 (0.69-1.24)	0.81 (0.38-1.24)	0.74 (0.69-0.79)
k_{50} (hr^{-1})	0.93 (0.80-1.05)	0.79 (0.58-1.01)	1.61 (1.52-1.71)
D/V_5 ($\mu g/ml$)	0.63 (0.56-0.70)	0.26 (0.21-0.31)	1.00 (0.95-1.04)
V_5 (l)	16.7	36.0	8.35
V_5 (% body wt)	88	212	55.3
$V_5 k_{50}$ (ml/min)	258	476	225
V_{dss} (l)	54.9	127	63.6

V_{dss} (% body wt)	289	746	421
R^2	0.998	0.990	1.000
r	0.998	0.990	1.000

[a]The parameters k_{56}, k_{65}, k_{50} and V_5 are defined on p. 166.

Table 30. Experiment 5 (A, C). Computer Estimates of LAAM Pharmacokinetic Parameters after Oral Administration of 2 mg/Kg LAAM.

Parameter	Dog A	Dog C
D (mg)	37.4	29.0
α (hr^{-1})	1.03	1.02
$t_{1/2,\alpha}$ (hr)	0.67	0.68
β (hr^{-1})	0.093	0.097
$t_{1/2,\beta}$ (hr)	7.44	7.13
k_a (hr^{-1})	1.03 (-79.5-81.6)	1.02 (-18.0-20.1)
$t_{1/2,abs}$ (hr)	0.67	0.68
k_{12} (hr^{-1})	0.49 (-60.7-61.7)	0.49 (-12.8-13.8)
k_{21} (hr^{-1})	0.39 (0.052-0.73)	0.31 (0.046-0.57)
k_{10} (hr^{-1})	0.25 (-18.9-19.4)	0.32 (-5.74-6.38)
fFD/V_1 ($\mu g/ml$)	0.098 (-7.56-7.75)	0.11 (-1.92-2.14)
R^2	0.978	0.978
r	0.970	0.973

Table 31. Experiment 6 (A, B, C). Computer Estimates of DAAM Pharmacokinetic Parameters after Oral Administration of 2 mg/Kg DAAM.

Parameter	Dog A	Dog B	Dog C
D (mg)	37.4	33.2	28.6
α (hr^{-1})	1.10	1.24	1.15
$t_{1/2,\alpha}$ (hr)	0.63	0.56	0.60
β (hr^{-1})	0.11	0.11	0.12
$t_{1/2,\beta}$ (hr)	6.55	6.09	6.01
k_a (hr^{-1})	1.10 (-1.53-3.73)	1.24 (-0.34-2.81)	1.15 (-15.6-17.9)
$t_{1/2,abs}$ (hr)	0.63	0.56	0.60
k_{12} (hr^{-1})	0.52 (-1.14-2.18)	0.50 (-1.37-2.36)	0.47 (-13.0-13.9)
k_{21} (hr^{-1})	0.38 (0.0009-0.67)	0.63 (-0.091-1.35)	0.55 (-0.36-1.46)
k_{10} (hr^{-1})	0.34 (-0.43-1.11)	0.22 (-0.12-0.57)	0.24 (-3.32-3.80)
fFD/V_1 ($\mu g/ml$)	0.14 (-0.17-0.45)	0.14 (-0.076-0.35)	0.098 (-1.35-1.54)
R^2	0.968	0.988	0.961
r	0.956	0.982	0.937

Calculated values of plasma clearance[a] and steady state volume of distribution (V_{dss})[b], as well as the coefficients of determination (R^2)[c] and correlation coefficients (r) between observed and predicted values for the computer-fitted data points, are also shown. In order to make all data points contribute equally to the sum of squares, concentrations were individually weighted by the factor 1/C during the computer fitting process for all experiments except Experiment 3 (A, B, C), where the weighting factor was 1/C for concentrations above 0.02 µg/ml, and 10/C for those below this level. Although arbitrary, this decision improved the fit to the terminal phase of the plasma level profiles, resulting in a more accurate definition of the β slope.

iv. Additional Pharmacokinetic Parameters from Graphic Analysis

With the metabolites in Experiments 1, 2, 3, 5 and 6, β was calculated whenever the post-distributive

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[a] Plasma clearance = $V_1 k_{10}$.

[b] $V_{dss} = \frac{V_1(k_{12} + k_{21})}{k_{21}}$

[c] $r^2 = \frac{\Sigma_{observations}^2 - \Sigma_{deviations}^2}{\Sigma_{observations}^2}$

phase (β phase) was well defined (NOR- metabolite in Experiments 2, 5 and 6 and DINOR- metabolite in Experiments 3, 5 and 6). This was achieved by performing linear regressions on the logarithmic plasma level *versus* time data points constituting the β phase. The resulting line has a slope of $-\beta/2.303$.

The areas under plasma level *versus* time curves (AUC) for drugs and metabolites were calculated by trapezoidal rule from t = 0 to t = ∞, or to a certain time following a dose, to facilitate appropriate comparisons between different experiments. In the former case, AUC from t = 0 to t = t', time of the final data point, was determined by trapezoidal rule, while the terminal area, from t = t' to t = ∞, was calculated as C'/β, where C' is the concentration of the final data point. $AUC^{0\rightarrow t'}$ plus $AUC^{t'\rightarrow\infty}$ equals $AUC^{0\rightarrow\infty}$. Peak concentration, C_{max}, was the highest observed drug or metabolite concentration in plasma, and t_{max} was the time after dosing when C_{max} was obtained.

Results from the above calculations are summarized in Tables 32-37.

b. Multiple Dose Studies

i. Plasma Concentrations

The plasma concentration *versus* time data of LAAM, DAAM and their N-demethylated metabolites studied are presented in Tables 38-41 and in Figures 24-27.

Table 32. Experiment 1 (A, B, C). Graphic Estimates of Pharmacokinetic Parameters after Intravenous Administration of 0.6 mg/Kg LAAM.

Parameter	Compound	Dog A	Dog B	Dog C
$AUC^{0\rightarrow\infty}$ (μg hr/ml)	LAAM	0.32	0.34	0.47
$AUC^{0\rightarrow 4\ hr}$ (μg hr/ml)	NORLAAM	0.052	0.020	0.019
$AUC^{0\rightarrow 48\ hr}$ (μg hr/ml)	NORLAAM	0.23	0.15	0.17
C_{max} (μg/ml)	NORLAAM	0.080	0.013	0.013
	DINORLAAM	0.020	0.010	0.011
t_{max} (hr)	NORLAAM	0.083	0.083	0.083
	DINORLAAM	0.083	0.083	0.083

Table 33. Experiment 2 (A, B, C). Graphic Estimates of Pharmacokinetic Parameters after Intravenous Administration of 0.6 mg/Kg DAAM.

Parameter	Compound	Dog A	Dog B	Dog C
β (hr^{-1})	NORDAAM	0.13	0.12	0.17
$t_{1/2,\beta}$ (hr)	NORDAAM	5.21	5.58	4.12
$AUC^{0\rightarrow\infty}$ (μg hr/ml)	DAAM	1.34	1.32	0.47
	NORDAAM	0.090	0.30	0.019
$AUC^{0\rightarrow 4\ hr}$ (μg hr/ml)	NORDAAM	0.039	0.13	0.010
C_{max} (μg/ml)	NORDAAM	0.023	0.067	0.006_1
	DINORDAAM	0.007_5	0.009_7	0.006_3
t_{max} (hr)	NORDAAM	0.25	0.50	0.083
	DINORDAAM	0.083	0.083	0.25

Table 34. Experiment 3 (A, B, C). Graphic Estimates of Pharmacokinetic Parameters after Intravenous Administration of 0.575 mg/Kg NORLAAM.

Parameter	Compound	Dog A	Dog B	Dog C
β (hr^{-1})	DINORLAAM	0.041	0.053	0.036
$t_{1/2,\beta}$ (hr)	DINORLAAM	16.8	13.1	19.1
$AUC^{0\rightarrow\infty}$ (μg hr/ml)	NORLAAM	0.88	0.49	1.00
	DINORLAAM	1.05	1.30	1.15
C_{max} (μg/ml)	DINORLAAM	0.049	0.077	0.049
t_{max} (hr)	DINORLAAM	3.00	4.00	2.00

Table 35. Experiment 4 (A, B, C). Graphic Estimates of DINORLAAM Pharmacokinetic Parameter after Intravenous Administration of 0.55 mg/Kg DINORLAAM.

Parameter	Dog A	Dog B	Dog C
$AUC^{0\rightarrow\infty}$ (μg hr/ml)	0.70	0.33	0.64

Table 36. Experiment 5 (A, B, C). Graphic Estimates of Pharmacokinetic Parameters after Oral Administration of 2 mg/Kg LAAM.

Parameter	Compound	Dog A	Dog B	Dog C
β (hr^{-1})	NORLAAM	0.064	-[a]	0.046
	DINORLAAM	0.060	-	0.057
$t_{1/2,\beta}$ (hr)	NORLAAM	10.8	-	15.2
	DINORLAAM	11.6	-	12.1
$AUC^{0\rightarrow\infty}$ (μg hr/ml)	LAAM	0.41	-	0.34
	NORLAAM	1.01	-	0.57
	DINORLAAM	0.66	-	1.12
$AUC^{0\rightarrow 48\ hr}$ (μg hr/ml)	NORLAAM	0.96	-	0.51
C_{max} (μg/ml)	LAAM	0.057	0.056	0.053
	NORLAAM	0.086	-	0.033
	DINORLAAM	0.054	-	0.060
t_{max} (hr)	LAAM	1.00	1.50	0.75
	NORLAAM	2.00	-	1.00
	DINORLAAM	4.00	-	3.00

[a]Not calculated.

Table 37. Experiment 6 (A, B, C). Graphic Estimates of Pharmacokinetic Parameters after Oral Administration of 2 mg/Kg DAAM.

Parameter	Compound	Dog A	Dog B	Dog C
β (hr^{-1})	NORDAAM	0.095	0.089	0.078
	DINORDAAM	0.094	0.074	0.068
$t_{1/2,\beta}$ (hr)	NORDAAM	7.29	7.82	8.92
	DINORDAAM	7.40	9.39	10.2
$AUC^{0\rightarrow\infty}$ (μg hr/ml)	DAAM	0.42	0.63	0.42
	NORDAAM	0.97	0.85	0.71
	DINORDAAM	0.91	0.40	0.28
C_{max} (μg/ml)	DAAM	0.082	0.082	0.064
	NORDAAM	0.13	0.077	0.061
	DINORDAAM	0.097	0.031	0.020
t_{max} (hr)	DAAM	1.00	1.00	0.75
	NORDAAM	2.00	1.00	0.75
	DINORDAAM	2.00	2.00	2.50

Table 38. Experiment 7A. Plasma Concentrations of Drug and Metabolites after Multiple Oral Doses of LAAM (1 mg/Kg, every 48 hours, x 7 doses) to Dog A[a].

		C (μg/ml)		
Day[b]	t (hr)	LAAM	NORLAAM	DINORLAAM
1	0	0	0	0
	0.25	0.010	0.001_9	0
	0.50	0.015	0.002_3	0.007_1
	0.75	0.018	0.005_4	0.008_9
	1	0.021	0.011	0.011
	2	0.025	0.021	0.016
	3	0.019	0.028	0.023
	4	0.012	0.029	0.026
	6	0.011	0.021	0.018
	8	0.011	0.019	0.015
	12	0.006_4	0.014	0.013
	18	0.003_5	0.009_5	0.008_1
	24	0.002_4	0.008_7	0.005_9
	36	0	0.003_7	0
3	0	0	0.001_7	0
5	0	0	0.003_8	0

Table 38 (continued)

Day[b]	t (hr)	C (μg/ml) LAAM	NORLAAM	DINORLAAM
7	0	0	0.005_9	0
	1	0.016	0.011	0.013
	2	0.020	0.020	0.019
	3	0.017	0.033	0.024
	4	0.011	0.023	0.025
9	0	0	0.006_4	0
11	0	0	0.007_1	0
13	0	0	0.004_2	0
	0.25	0.009_2	0.005_4	0.005_2
	0.50	0.010	0.008_7	0.008_1
	0.75	0.012	0.010	0.009_7
	1	0.017	0.011	0.013
	2	0.023	0.023	0.021
	3	0.016	0.034	0.027
	4	0.011	0.035	0.029
	6	0.008_0	0.025	0.018
	8	0.006_6	0.020	0.017
	12	0.003_2	0.014	0.011
	18	0.001_8	0.011	0.007_7

Table 38 (continued)

		C (μg/ml)		
Day[b]	t (hr)	LAAM	NORLAAM	DINORLAAM
13	24	0	0.006_5	0.005_2
	36	0	0.002_5	0
	48	0	0.001_3	0
	60	0	0	0

[a]Dog weight: 18.5 Kg, LAAM dose: 18.5 mg per dose.

[b]A single dose given on each indicated day.

Figure 24. Experiment 7A. Plasma concentration-time profiles for LAAM (——), NORLAAM (— — —) and DINORLAAM (— · · —) after multiple oral doses of LAAM (7 x 1 mg/Kg) to Dog A. The symbols ◐ (NORLAAM) and ◒ (coincident LAAM and DINORLAAM) indicate pre-dose concentrations in plasma during repeated dosing. Arrows indicate times of dosing. In the inserts, data points constituting the β phase from the first (—▽—) and final (— —▲— —) doses are plotted on a semilogarithmic scale. Equations for linear regression lines are:

a. LAAM, First dose, β phase = 4 - 24 hr

$$\log C_1 = \log 0.018 - 0.038t,\ r = -0.992$$

Final dose, β phase = 4 - 18 hr

$$\log C_1 = \log 0.018 - 0.057t,\ r = -0.993$$

b. NORLAAM, First dose, β phase = 6 - 48 hr

$$\log C_3 = \log 0.030 - 0.025t,\ r = -0.996$$

Final dose, β phase = 6 - 48 hr

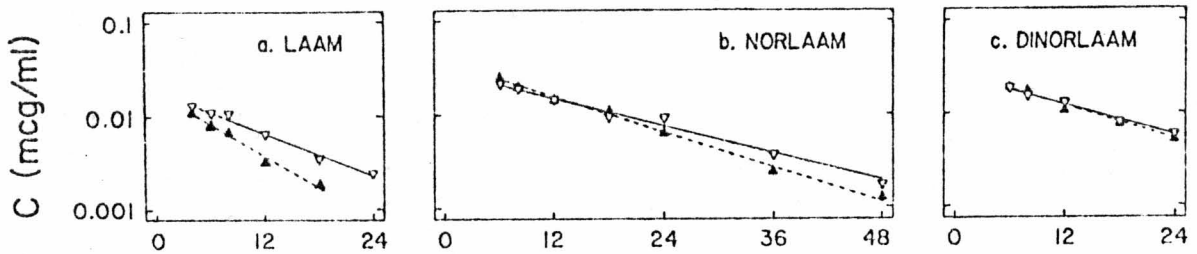
$$\log C_3 = \log 0.036 - 0.031t,\ r = -0.997$$

c. DINORLAAM, First dose, β phase = 6 - 24 hr

$$\log C_5 = \log 0.026 - 0.027t,\ r = -0.996$$

Final dose, β phase = 6 - 24 hr

$$\log C_5 = \log 0.028 - 0.031t,\ r = -0.990$$


C (mcg/ml)
0.1
0.01
0.001
a. LAAM
b. NORLAAM
c. DINORLAAM
0
12
24
36
48
TIME FROM LAST DOSE (HRS)

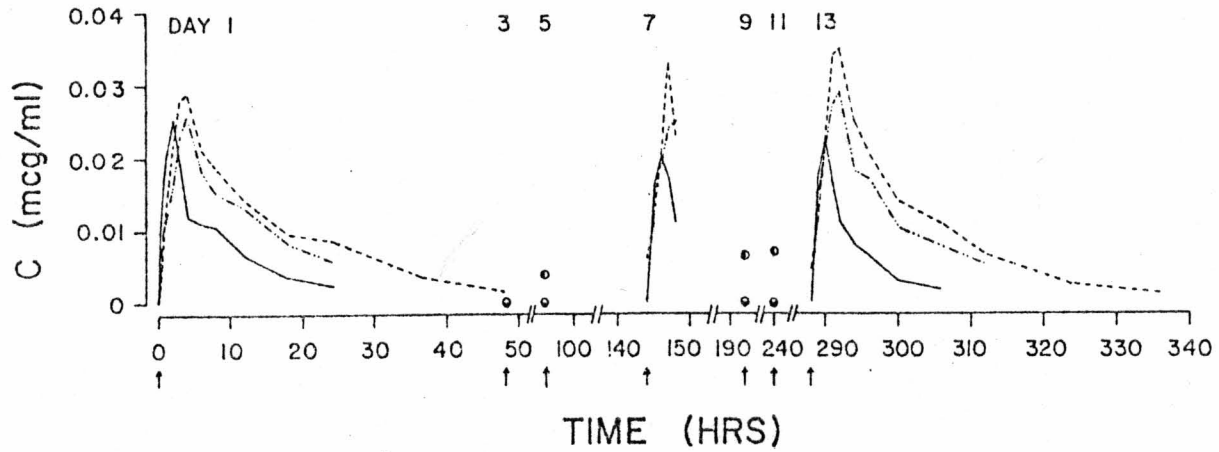

C (mcg/ml)
0.04
0.03
0.02
0.01
0
DAY 1
3
5
7
9
11
13
0
10
20
30
40
50
100
140
150
190
240
290
300
310
320
330
340
TIME (HRS)

Table 39. Experiment 7C. Plasma Concentrations of Drug and Metabolites after Multiple Oral Doses of LAAM (1 mg/Kg, every 48 hours, x 7 doses) to Dog C[a].

		C (μg/ml)		
Day	t (hr)	LAAM	NORLAAM	DINORLAAM
1	0	0	0	0
	0.25	0.007_3	0.008_6	0.005_1
	0.50	0.013	0.021	0.007_9
	0.75	0.018	0.024	0.011
	1	0.021	0.025	0.012
	2	0.022	0.019	0.017
	3	0.019	0.016	0.015
	4	0.011	0.015	0.013
	6	0.007_7	0.014	0.013
	8	0.006_2	0.013	0.011
	12	0.004_7	0.009_8	0.008_6
	18	0.002_6	0.008_4	0.007_0
	24	0.001_1	0.006_6	0.005_2
	36	0	0.003_2	0
3	0	0	0.002_1	0
5	0	0	0.001_7	0

Table 39 (continued)

		C (μg/ml)		
Day	t (hr)	LAAM	NORLAAM	DINORLAAM
7	0	0	0.002_0	0
	1	0.020	0.021	0.012
	2	0.019	0.026	0.016
	3	0.014	0.019	0.016
	4	0.009_9	0.018	0.014
9	0	0	0.001_3	0
11	0	0	0.001_6	0
13	0	0	0.003_1	0
	0.25	0.007_1	0.009_0	0.006_4
	0.50	0.012	0.013	0.011
	0.75	0.014	0.015	0.012
	1	0.019	0.016	0.015
	2	0.018	0.027	0.021
	3	0.013	0.021	0.017
	4	0.009_8	0.019	0.015
	6	0.006_8	0.017	0.016
	8	0.005_1	0.014	0.014
	12	0.003_5	0.015	0.011
	18	0.001_5	0.008_2	0.008_4

Table 39 (continued)

		C (μg/ml)		
Day	t (hr)	LAAM	NORLAAM	DINORLAAM
13	24	0	0.005_2	0.005_1
	36	0	0.003_7	0
	48	-	0.001_5	0
	60	-	0	0

[a]Dog weight: 14.5 Kg, LAAM dose: 14.5 mg per dose.

Figure 25. Experiment 7C. Plasma concentration-time profiles for LAAM (——), NORLAAM (– – –) and DINORLAAM (– · ·–) after multiple oral doses of LAAM (7 x 1 mg/Kg) to Dog C. The symbols ◐ (NORLAAM) and ◒ (coincident LAAM and DINORLAAM) indicate pre-dose concentrations in plasma during repeated dosing. Arrows indicate times of dosing. In the inserts, data points constituting the β phase from the first (–▽–) and final (– –▲– –) doses are plotted on a semilogarithmic scale. Equations for linear regression lines are:

a. LAAM, First dose, β phase = 4 - 24 hr

$$\log C_1 = \log 0.016 - 0.046t, \quad r = -0.994$$

Final dose, β phase = 4 - 18 hr

$$\log C_1 = \log 0.015 - 0.056t, \quad r = -0.996$$

b. NORLAAM, First dose, β phase = 3 - 48 hr

$$\log C_3 = \log 0.018 - 0.020t, \quad r = -0.997$$

Final dose, β phase = 3 - 48 hr

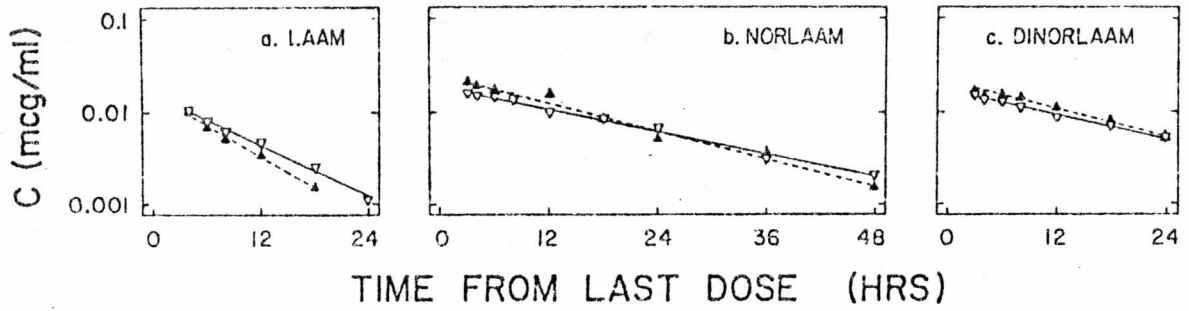
$$\log C_3 = \log 0.024 - 0.025t, \quad r = -0.991$$

c. DINORLAAM, First dose, β phase = 3 - 24 hr

$$\log C_5 = \log 0.017 - 0.021t, \quad r = -0.993$$

Final dose, β phase = 3 - 24 hr

$$\log C_5 = \log 0.021 - 0.024t, \quad r = -0.985$$


a. LAAM
b. NORLAAM
c. DINORLAAM
C (mcg/ml)
0.1
0.01
0.001
0
12
24
36
48
TIME FROM LAST DOSE (HRS)

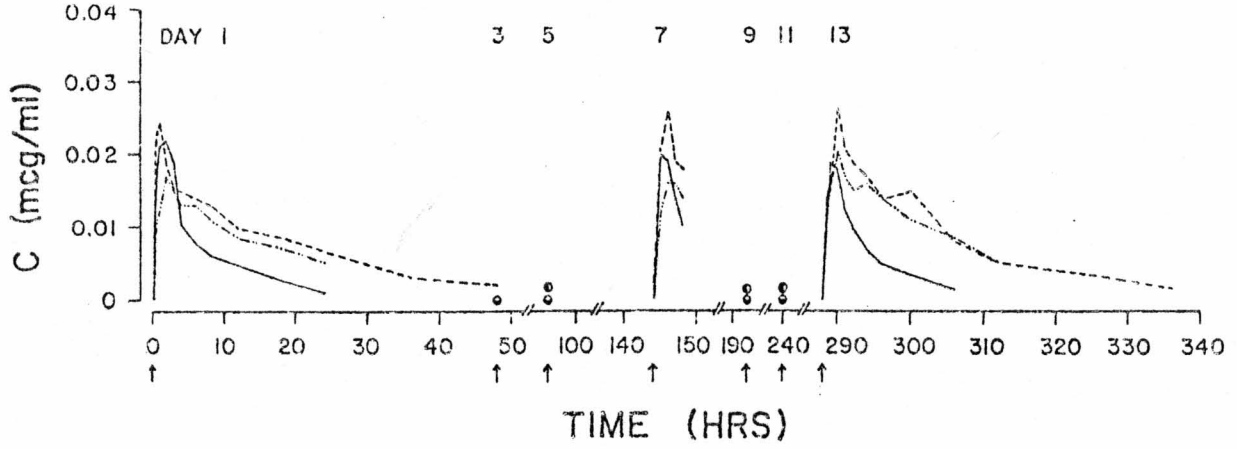

DAY 1
3
5
7
9
11
13
C (mcg/ml)
0.04
0.03
0.02
0.01
0
0
10
20
30
40
50
100
140
150
190
240
290
300
310
320
330
340
TIME (HRS)

Table 40. Experiment 8A. Plasma Concentrations of Drug and Metabolites after Multiple Oral Doses of DAAM (1 mg/Kg, every 48 hours, x 7 doses) to Dog A[a].

		C (μg/ml)		
Day	t (hr)	DAAM	NORDAAM	DINORDAAM
1	0	0	0	0
	0.25	0.005_8	0.007_5	0.009_7
	0.50	0.013	0.013	0.014
	0.75	0.019	0.024	0.019
	1	0.039	0.042	0.031
	2	0.041	0.060	0.049
	3	0.029	0.056	0.039
	4	0.025	0.038	0.031
	6	0.020	0.027	0.027
	8	0.018	0.020	0.023
	12	0.011	0.018	0.012
	18	0.006_0	0.010	0.007_4
	24	0.003_1	0.005_1	0
	36	0	0.001_3	0
3	0	0	0	0
5	0	0	0	0

Table 40 (continued)

		C (μg/ml)		
Day	t (hr)	DAAM	NORDAAM	DINORDAAM
7	0	0	0	0
	1	0.035	0.038	0.036
	2	0.045	0.062	0.048
	3	0.034	0.061	0.042
	4	0.028	0.040	0.035
9	0	0	0	0
11	0	0	0	0
13	0	0	0	0
	0.25	0.005_2	0.011	0.010
	0.50	0.011	0.016	0.017
	0.75	0.013	0.022	0.020
	1	0.023	0.039	0.037
	2	0.037	0.064	0.054
	3	0.034	0.069	0.035
	4	0.023	0.048	0.036
	6	0.021	0.032	0.028
	8	0.015	0.024	0.020
	12	0.010	0.019	0.012
	18	0.004_6	0.008_9	0.008_4

Table 40 (continued)

		C (μg/ml)		
Day	t (hr)	DAAM	NORDAAM	DINORDAAM
13	24	0.002_5	0.004_4	0
	36	0	0.001_4	0
	48	0	0	0

[a]Dog weight: 17.3 Kg, DAAM dose: 17.3 mg per dose.

Figure 26. Experiment 8A. Plasma concentration-time profiles for DAAM (——), NORDAAM (— — —) and DINORDAAM (— · · —) after multiple oral doses of DAAM (7 x 1 mg/Kg) to Dog A. The symbol ▲ (coincident DAAM, NORDAAM and DINORDAAM) indicates pre-dose concentrations in plasma during repeated dosing. Arrows indicate times of dosing. In the inserts, data points constituting the β phase from the first (—▽—) and final (— —▲— —) doses are plotted on a semilogarithmic scale. Equations for linear regression lines are:

a. DAAM, First dose, β phase = 3 - 24 hr

$$\log C_1 = \log 0.039 - 0.046t, \quad r = -0.999$$

Final dose, β phase = 4 - 24 hr

$$\log C_1 = \log 0.038 - 0.050t, \quad r = -0.998$$

b. NORDAAM, First dose, β phase = 4 - 36 hr

$$\log C_3 = \log 0.054 - 0.044t, \quad r = -0.995$$

Final dose, β phase = 4 - 36 hr

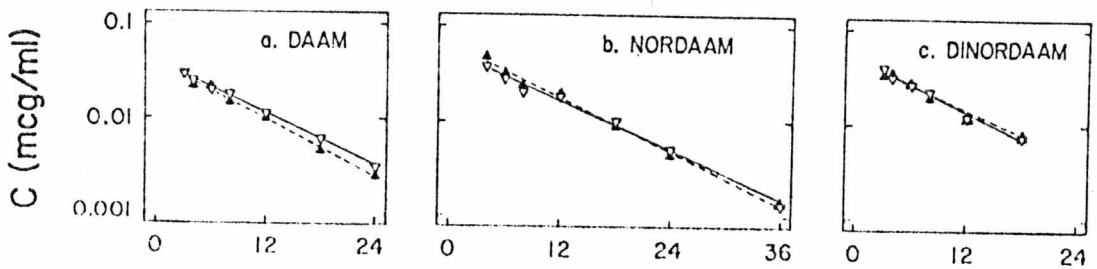
$$\log C_3 = \log 0.064 - 0.047t, \quad r = -0.997$$

c. DINORDAAM, First dose, β phase = 3 - 18 hr

$$\log C_5 = \log 0.052 - 0.048t, \quad r = -0.992$$

Final dose, β phase = 3 - 18 hr

$$\log C_5 = \log 0.049 - 0.045t, \quad r = -0.984$$


C (mcg/ml)
0.1
0.01
0.001
a. DAAM
b. NORDAAM
c. DINORDAAM
0
12
24
36
TIME FROM LAST DOSE (HRS)

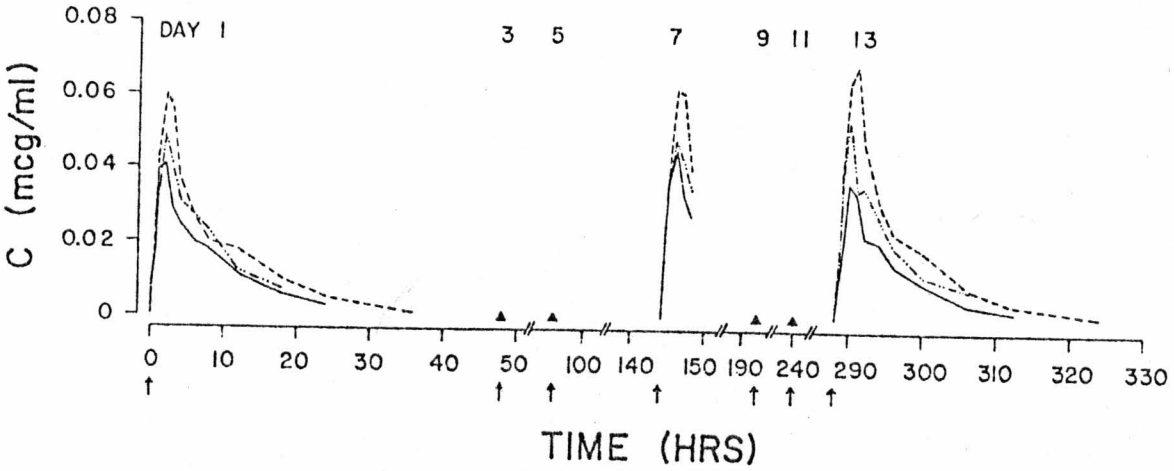

C (mcg/ml)
0.08
0.06
0.04
0.02
0
DAY 1
3
5
7
9
11
13
0
10
20
30
40
50
100
140
150
190
240
290
300
310
320
330
TIME (HRS)

Table 41. Experiment 8C. Plasma Concentrations of Drug and Metabolites after Multiple Oral Doses of DAAM (1 mg/Kg, every 48 hours, x 7 doses) to Dog C[a].

		C (μg/ml)		
Day	t (hr)	DAAM	NORDAAM	DINORDAAM
1	0	0	0	0
	0.25	0.008_2	0.009_8	0.005_2
	0.50	0.020	0.012	0.007_7
	0.75	0.023	0.019	0.011
	1	0.026	0.024	0.013
	2	0.021	0.020	0.016
	3	0.016	0.020	0.015
	4	0.015	0.016	0.014
	6	0.011	0.015	0.011
	8	0.011	0.014	0.012
	12	0.005_4	0.009_2	0.007_2
	18	0.003_1	0.005_6	0.005_6
	24	0.001_8	0.004_2	0
	36	0	0.001_3	0
3	0	0	0	0
5	0	0	0	0

Table 41 (continued)

Day	t (hr)	C (μg/ml) DAAM	NORDAAM	DINORDAAM
7	0	0	0.001_4	0
	1	0.027	0.025	0.013
	2	0.024	0.023	0.017
	3	0.016	0.019	0.016
	4	0.016	0.016	0.014
9	0	0	0	0
11	0	0	0.002_1	0
13	0	0	0.001_9	0
	0.25	0.009_6	0.011	0.008_0
	0.50	0.014	0.020	0.010
	0.75	0.018	0.025	0.013
	1	0.024	0.028	0.015
	2	0.020	0.023	0.018
	3	0.015	0.021	0.019
	4	0.013	0.019	0.016
	6	0.009_6	0.016	0.012
	8	0.008_0	0.014	0.011
	12	0.005_1	0.009_8	0.008_1
	18	0.002_1	0.005_2	0.004_0

Table 41 (continued)

		C (μg/ml)		
Day	t (hr)	DAAM	NORDAAM	DINORDAAM
13	24	0.001_4	0.003_4	0
	36	0	0.001_6	0
	48	0	0	0

[a]Dog weight: 14.3 Kg, DAAM dose: 14.3 mg per dose.

Figure 27. Experiment 8C. Plasma concentration-time profiles for DAAM (——), NORDAAM (— — —) and DINORDAAM (— · · —) after multiple oral doses of DAAM (7 x 1 mg/Kg) to Dog C. The symbols ◭ (NORDAAM), ▲ (coincident DAAM and DINORDAAM) and ▲ (coincident DAAM, NORDAAM and DINORDAAM) indicate pre-dose concentrations in plasma during repeated dosing. Arrows indicate times of dosing. In the inserts, data points constituting the β phase from the first (—▽—) and final (— —▲— —) doses are plotted on a semilogarithmic scale. Equations for linear regression lines are:

a. DAAM, First dose, β phase = 3 - 24 hr

$$\log C_1 = \log 0.022 - 0.047t, \quad r = -0.994$$

Final dose, β phase = 3 - 24 hr

$$\log C_1 = \log 0.020 - 0.050t, \quad r = -0.995$$

b. NORDAAM, First dose, β phase = 2 - 36 hr

$$\log C_3 = \log 0.024 - 0.034t, \quad r = -0.996$$

Final dose, β phase = 2 - 36 hr

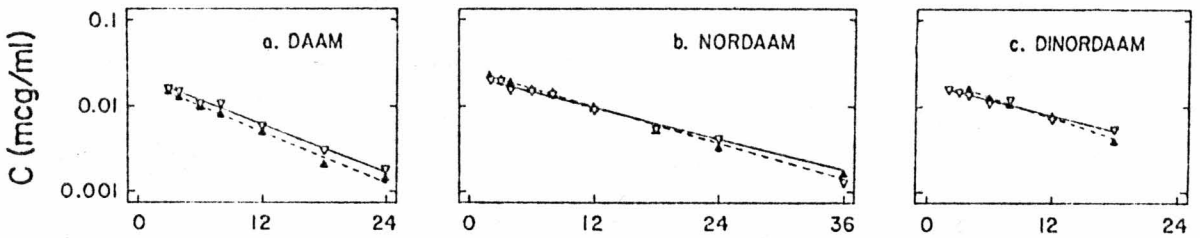
$$\log C_3 = \log 0.026 - 0.035t, \quad r = -0.996$$

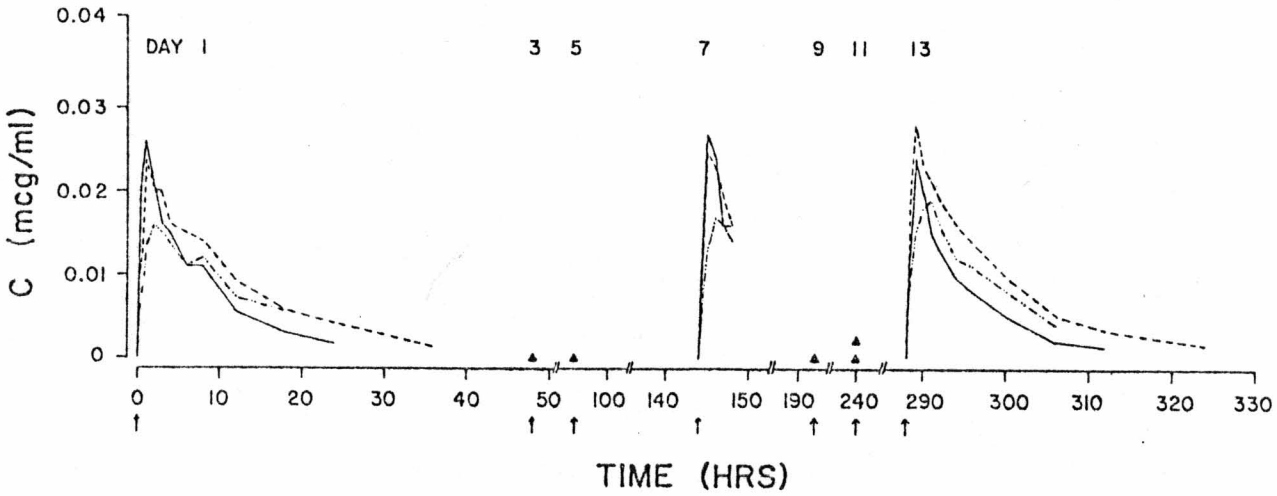
c. DINORDAAM, First dose, β phase = 2 - 18 hr

$$\log C_5 = \log 0.018 - 0.029t, \quad r = -0.978$$

Final dose, β phase = 4 - 18 hr

$$\log C_5 = \log 0.023 - 0.041t, \quad r = -0.992$$


C (mcg/ml)
0.1
0.01
0.001
a. DAAM
b. NORDAAM
c. DINORDAAM
0
12
24
36
TIME FROM LAST DOSE (HRS)


C (mcg/ml)
0.04
0.03
0.02
0.01
0
DAY 1
3
5
7
9
11
13
0
10
20
30
40
50
100
140
150
190
240
290
300
310
320
330
TIME (HRS)

ii. Theoretical

Equation 12 describes the plasma concentration, after multiple doses, of a drug obeying the two compartment open model kinetics with first-order absorption, where elimination occurs only from the central compartment (69). Concentration, $(C_1)_n$, at any time t after the n^{th} dose of drug is

$$(C_1)_n = \frac{k_a fFD}{V_1}\{[\frac{k_{21} - \alpha}{(k_a - \alpha)(\beta - \alpha)}][\frac{1 - e^{-n\alpha\tau}}{1 - e^{-\alpha\tau}}]e^{-\alpha t} + [\frac{k_{21} - \beta}{(K_a - \beta)(\alpha - \beta)}][\frac{1 - e^{-n\beta\tau}}{1 - e^{-\beta\tau}}]e^{-\beta t} + [\frac{k_{21} - k_a}{(\alpha - k_a)(\beta - k_a)}][\frac{1 - e^{-nk_a\tau}}{1 - e^{-k_a\tau}}]e^{-k_a t}\} \qquad \text{(Eq. 12)}$$

where τ is the time interval between two consecutive doses. Other parameters are as defined in Equation 9. Once steady state plasma levels are attained, Equation 12 becomes

$$(C_1)_\infty = \frac{k_a fFD}{V_1}\{[\frac{k_{21} - \alpha}{(k_a - \alpha)(\beta - \alpha)}][\frac{1}{1 - e^{-\alpha\tau}}]e^{-\alpha t} + [\frac{k_{21} - \beta}{(k_a - \beta)(\alpha - \beta)}][\frac{1}{1 - e^{-\beta\tau}}]e^{-\beta t} + [\frac{k_{21} - k_a}{(\alpha - k_a)(\beta - k_a)}][\frac{1}{1 - e^{-k_a\tau}}]e^{-k_a t}\} \qquad \text{(Eq. 13)}$$

where $(C_1)_\infty$ is the plasma concentration of drug during a dosing interval at steady state.

Since the primary objectives of these experiments were to examine the accumulation characteristics and changes in elimination kinetics of the drug and metabolites upon multiple dosing, extensive computer analysis of data was not performed. Comparisons were based primarily on β, AUC, C_{max} and t_{max} values.

The numerical value of β was obtained by linear regression analysis of the plasma levels constituting the β phase. As stated previously, the slope of the line equals $-\beta/2.303$.

Integration of Equation 13 from time zero to τ yields the AUC during a dosage interval after reaching steady state, which is equal to $AUC^{0\rightarrow\infty}$ after the initial single dose, i.e., fFD/V_1k_{10} (65). In the present study, area under curve values were calculated by trapezoidal rule from zero to infinite time ($AUC^{0\rightarrow\infty}$) after the first dose and from 0 to 48 hours ($AUC^{0\rightarrow\tau}$) after the final dose. If the last detectable concentration, C', was at t' where t' < 48 hr, then $AUC^{0\rightarrow\tau} = (AUC^{0\rightarrow t'} + AUC^{t'\rightarrow 48\ hr})$, where $AUC^{t'\rightarrow 48\ hr}$ was calculated using Equation 14.

$$AUC^{t'\rightarrow 48\ hr} = \int_0^{(48\ hr\ -\ t')} C'e^{-\beta t}\cdot dt$$

$$= \frac{C'}{\beta}[1 - e^{-\beta(48\ hr\ -\ t')}] \qquad \text{(Eq. 14)}$$

The accumulation factor, R, is defined as

$$R = \frac{C^{\infty}_{max}}{C^{1}_{max}} = \frac{C^{\infty}_{min}}{C^{1}_{min}} \qquad \text{(Eq. 15)}$$

where C_{max} and C_{min} represent maximum and minimum plasma concentrations, respectively, at steady state or after the first dose, as indicated by the superscripts. It has been shown (65) that if τ is of sufficient length such that the drug is administered in the postabsorptive, postdistributive phase of the preceding dose, as is the case in this study, then

$$R = \frac{1}{1 - e^{-\beta\tau}} \qquad \text{(Eq. 16)}$$

which can also be written as $R = 1/(1 - 2^{-\varepsilon})$, where $\varepsilon = \tau/t_{1/2,\beta}$. After obtaining R from Equation 16, observed C^{1}_{max} values were used to predict C^{∞}_{max}, which can be subsequently compared with the observed C_{max} values after the final (seventh) dose. Values of t_{max} after the first and final doses were also examined.

iii. Pharmacokinetic Parameters from Graphic Analysis

Using the aforementioned methods, pharmacokinetic parameters were calculated for the administered drugs and metabolites. Results are shown in Tables 42 and 43.

Table 42. Experiment 7 (A, C). Pharmacokinetic Parameters after Multiple Oral Doses of LAAM (7 x 1 mg/Kg).

		Dog A		Dog C	
Parameter	Compound	Dose 1	Dose 7	Dose 1	Dose 7
β (hr^{-1})	LAAM	0.087	0.13	0.11	0.13
	NORLAAM	0.059	0.071	0.046	0.057
	DINORLAAM	0.062	0.071	0.049	0.055
$t_{1/2,\beta}$ (hr)	LAAM	8.00	5.27	6.51	5.37
	NORLAAM	11.8	9.77	15.2	12.2
	DINORLAAM	11.1	9.83	14.1	12.6
$AUC^{0\rightarrow\infty}$ (μg hr/ml)	LAAM	0.23	-	0.17	-
	NORLAAM	0.49	-	0.41	-
	DINORLAAM	0.40	-	0.33	-
$AUC^{0\rightarrow\tau}$ (μg hr/ml)	LAAM	-	0.13	-	0.13
	NORLAAM	-	0.47	-	0.39
	DINORLAAM	-	0.37	-	0.34

Predicted R[a]	LAAM	1.02[b]	1.00	1.01[b]	1.00
	NORLAAM	1.06	1.03	1.13	1.07
	DINORLAAM	1.05	1.04	1.10	1.08
C_{max} (μg/ml)	LAAM	0.025	0.023	0.022	0.019
	NORLAAM	0.029	0.035	0.025	0.027
	DINORLAAM	0.026	0.029	0.017	0.021
Predicted C^{∞}_{max} (μg/ml)[a]	LAAM	0.026[b]	0.025	0.022[b]	0.022
	NORLAAM	0.031	0.030	0.028	0.026
	DINORLAAM	0.027	0.027	0.019	0.018
t_{max} (hr)	LAAM	2.00	2.00	2.00	1.00
	NORLAAM	4.00	4.00	1.00	2.00
	DINORLAAM	4.00	4.00	2.00	2.00

[a]Two R values were calculated for each dog, using the slightly different β values from Dose 1 and Dose 7 data. This gave rise to two predicted C^{∞}_{max} values in each case.

[b]The predicted R and C^{∞}_{max} in this column were calculated from pharmacokinetic parameters derived from Dose 1, but as defined, the numerical values apply to the repeated dose case.

Table 43. Experiment 8 (A, C). Pharmacokinetic Parameters after Multiple Oral Doses of DAAM (7 x 1 mg/Kg).

		Dog A		Dog C	
Parameter	Compound	Dose 1	Dose 7	Dose 1	Dose 7
β (hr^{-1})	DAAM	0.11	0.11	0.11	0.12
	NORDAAM	0.10	0.11	0.079	0.081
	DINORDAAM	0.11	0.11	0.067	0.094
$t_{1/2,\beta}$ (hr)	DAAM	6.60	6.05	6.47	5.97
	NORDAAM	6.89	6.40	8.77	8.57
	DINORDAAM	6.24	6.66	10.3	7.34
$AUC^{0\rightarrow\infty}$ (μg hr/ml)	DAAM	0.36	-	0.21	-
	NORDAAM	0.54	-	0.30	-
	DINORDAAM	0.44	-	0.26	-
$AUC^{0\rightarrow\tau}$ (μg hr/ml)	DAAM	-	0.32	-	0.18
	NORDAAM	-	0.58	-	0.31
	DINORDAAM	-	0.45	-	0.23

Predicted R[a]	DAAM	1.01[b]	1.00	1.01[b]	1.00
	NORDAAM	1.01	1.01	1.02	1.02
	DINORDAAM	1.00	1.01	1.04	1.01
C_{max} (μg/ml)	DAAM	0.041	0.037	0.026	0.024
	NORDAAM	0.060	0.069	0.024	0.028
	DINORDAAM	0.049	0.054	0.016	0.019
Predicted C^{∞}_{max} (μg/ml)[a]	DAAM	0.041[b]	0.041	0.026[b]	0.026
	NORDAAM	0.060	0.060	0.025	0.025
	DINORDAAM	0.049	0.049	0.017	0.016
t_{max} (hr)	DAAM	2.00	2.00	1.00	1.00
	NORDAAM	2.00	3.00	1.00	1.00
	DINORDAAM	2.00	2.00	2.00	3.00

[a] Two R values were calculated for each dog, using the slightly different β values from Dose 1 and Dose 7 data. This gave rise to two predicted C^{∞}_{max} values in each case.

[b] The predicted R and C^{∞}_{max} in this column were calculated from pharmacokinetic parameters derived from Dose 1, but as defined, the numerical values apply to the repeated dose case.

IV. DISCUSSION

A. Assay of Plasma Samples

The GC method used for the simultaneous determination of acetylmethadol, noracetylmethadol and dinoracetylmethadol in this study is accurate, selective, and highly sensitive. Although the extraction efficiencies of the metabolites from plasma (ca 49% for NORLAAM and 16% for DINORLAAM) are relatively low compared to that of the parent drug (ca 100%), they are nevertheless consistent. Successful use of triacontane and norpropoxyphene as external standards in the assays for LAAM and metabolites, respectively, has further contributed to the excellent reproducibility of the standard calibration curves.

B. Stability of LAAM, NORLAAM and DINORLAAM

Loss of acetylmethadol via hydrolysis at the 3-C position has been shown to occur at elevated temperatures (63). This and other possible degradation processes, if occurring during the storage period of plasma samples prior to assaying, would lead to incorrect interpretation of drug and metabolite plasma concentrations. Results of the stability studies, as shown in Table 6 and Figure 6, indicated negligible loss of either LAAM, NORLAAM or DINORLAAM from plasma stored at -20°C for up to one month.

C. Studies in Dogs

Although the pharmacologic effects of the compounds were not examined in detail in this study, observed drug-related behavioral changes, as presented in Table 7, suggest that NORLAAM produces more potent and prolonged action after intravenous administration than either the parent drug or the dinor- metabolite. This observation is in good agreement with those reported by earlier investigators (34, 36). Oral doses of LAAM caused more serious and sustained toxic responses than intravenous LAAM, thus confirming previous observations in man (15). This phenomenon is believed to be due in part to first-pass metabolism of LAAM to form NORLAAM prior to its entry into circulation. Comparison of the intravenous and oral doses between LAAM and DAAM supported the general claim that DAAM is more potent than LAAM, while also having a more rapid onset and shorter duration of morphine-like activities (3, 10, 12). Attempts will be made in later sections to explain these differences in terms of the time course of plasma levels of drug and metabolites in the body.

The overall objectives of this study are to examine and compare the pharmacokinetic characteristics of LAAM, DAAM and their N-demethylated metabolites after intravenous and oral administrations of the parent drugs. This cannot be achieved, however, without first gaining knowledge of the distribution volumes in the body of all the compounds

involved. Intravenous dosing of NORLAAM and DINORLAAM was therefore carried out to provide this information. Unfortunately, NORDAAM and DINORDAAM were not available for experimentation, hence limiting to a certain extent the pharmacokinetic analysis of DAAM metabolism.

It was decided to begin the analysis of pharmacokinetic profiles by examining the results of the DINORLAAM intravenous dosing study, i.e., Experiment 4 (A, B, C). A pure system is present here, with no perturbation of kinetics from other compounds.

1. Single Intravenous Administration of DINORLAAM

Average DINORLAAM plasma levels from three dogs are shown in Table 44 and Figure 28. For clarity of presentation, the standard deviation bars have been left out in Figures 28-33.

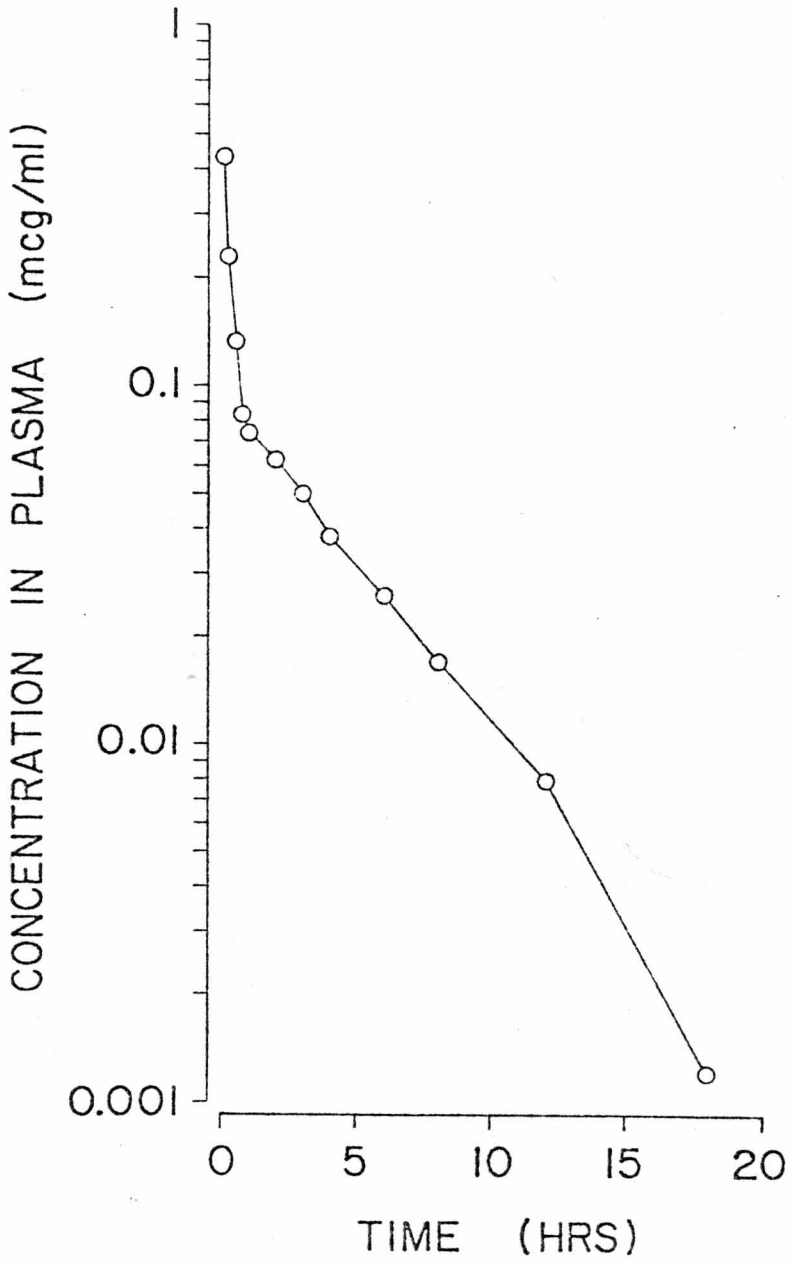
The model used to depict DINORLAAM pharmacokinetics following intravenous administration of this compound to the dog is shown in Scheme VI.

Table 44. Experiment 4 (A, B, C). Average Plasma Concentrations of DINORLAAM after 0.55 mg/Kg Intravenous Doses of DINORLAAM.

t (hr)	Average Plasma DINORLAAM (μg/ml)
0.083	0.43 (0.20)[a]
0.25	0.23 (0.10)
0.50	0.13 (0.052)
0.75	0.083 (0.030)
1	0.074 (0.038)
2	0.062 (0.025)
3	0.050 (0.020)
4	0.038 (0.010)
6	0.026 (0.007_9)
8	0.017 (0.005_7)
12	0.007_9 (0.003_2)
18	0.001_2 (0.002_1)
24	0

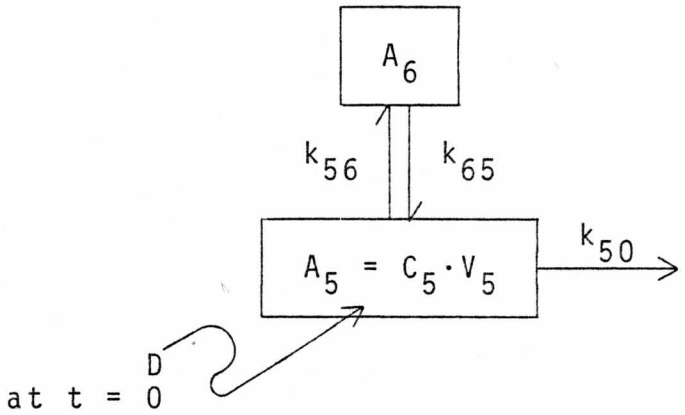
[a]Standard deviation, n = 3, for Tables 44-70.

Figure 28. Experiment 4 (A, B, C). Average plasma concentration-time profile for DINORLAAM (◯) after 0.55 mg/Kg intravenous doses of DINORLAAM.


CONCENTRATION IN PLASMA (mcg/ml)
1
0.1
0.01
0.001
0
5
10
15
20
TIME (HRS)

Scheme VI

Pharmacokinetic Model for Intravenous Administration of DINORLAAM.



Scheme VI is clearly identical to Scheme IV, but with the subscripts of the symbols, 1 and 2, replaced by 5 and 6 to apply specifically to DINORLAAM. Hence, Equation 1, when employed to describe DINORLAAM plasma level *versus* time profiles, becomes

$$C_5 = \frac{D}{V_5(\beta - \alpha)}[(k_{65} - \alpha)e^{-\alpha t} - (k_{65} - \beta)e^{-\beta t}] \qquad \text{(Eq. 17)}$$

and Equation 2 becomes Equation 18.

$$\begin{matrix}\alpha \\ \beta\end{matrix} = \frac{1}{2}[(k_{56} + k_{65} + k_{50}) \pm \sqrt{(k_{56} + k_{65} + k_{50})^2 - 4k_{65}k_{50}}] \qquad \text{(Eq. 18)}$$

Table 45 gives the average of the individual

pharmacokinetic parameter values previously shown in Table 29. Following intravenous administration, DINORLAAM penetrated rapidly into the peripheral compartment, with the distributive phase ending in ca 1 hr. The large apparent volume of distribution, ca 500% body wt at steady state, indicated rapid and extensive tissue uptake of this compound, which may be attributed to its lipophilicity and good membrane penetrating capability. The average biological half-life was 3.6 hr, a value many times smaller than those reported previously (27, 47, 48). This observation will be discussed further in Section 2.b., p. 177.

Plasma clearance of DINORLAAM was calculated to be ca 300 ml/min, although no information was available regarding the relative contributions of metabolism, renal excretion and renal tubular secretion of this drug.

2. Single Intravenous Administration of NORLAAM

Average plasma levels of NORLAAM and its metabolite, DINORLAAM, after dosing NORLAAM intravenously are presented in Table 46 and Figure 29. Scheme VII is the model used to describe the pharmacokinetics of these two compounds.

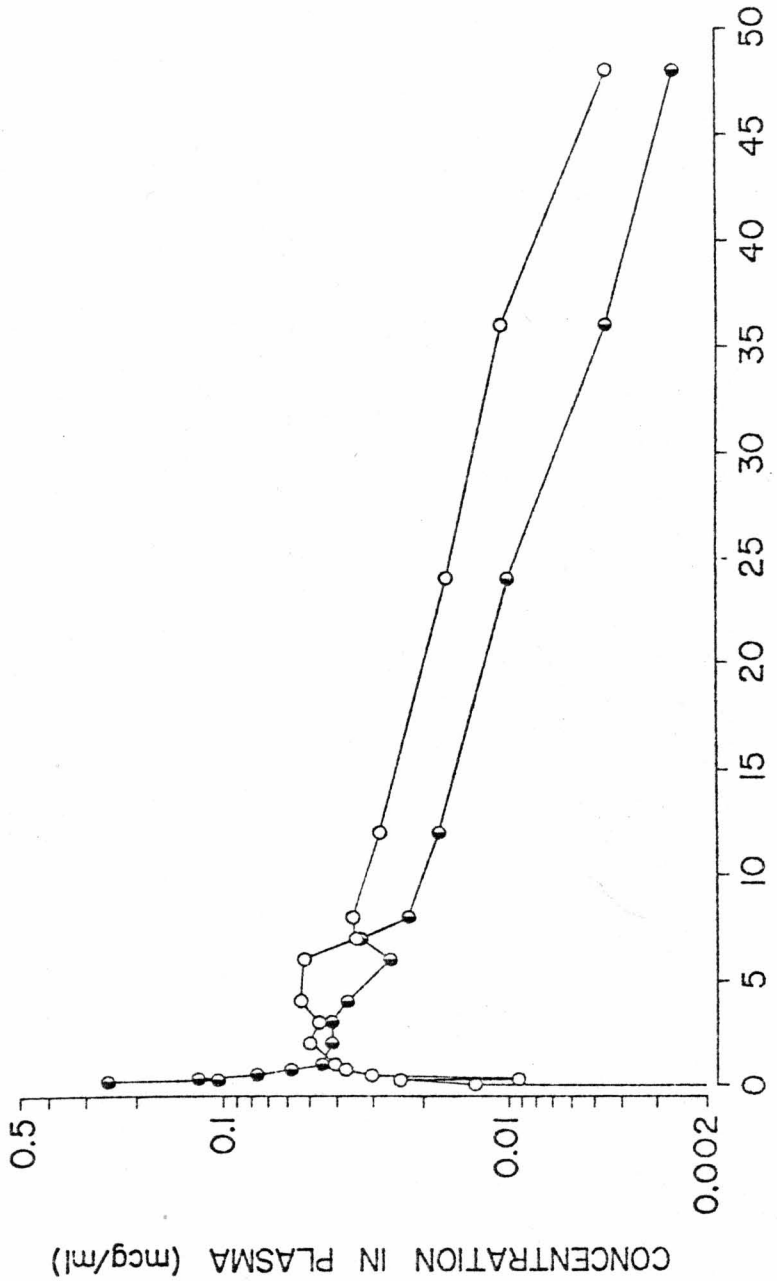
Table 45. Experiment 4 (A, B, C). Average Pharmacokinetic Parameter Values of DINORLAAM after 0.55 mg/Kg Intravenous Doses of DINORLAAM.

Parameter	Average Value
α (hr^{-1})	4.81 (2.00)
$t_{1/2,\alpha}$ (hr)	0.16 (0.054)
β (hr^{-1})	0.20 (0.032)
$t_{1/2,\beta}$ (hr)	3.61 (0.56)
k_{56} (hr^{-1})	3.06 (1.61)
k_{65} (hr^{-1})	0.84 (0.11)
k_{50} (hr^{-1})	1.11 (0.44)
D/V_5 ($\mu g/ml$)	0.63 (0.37)
V_5 (% body wt)	118 (82.4)
$V_5 k_{50}$ (ml/min)	320 (137)
V_{dss} (% body wt)	485 (235)
$AUC^{0\rightarrow\infty}$ (μg hr/ml)	0.56 (0.20)

Table 46. Experiment 3 (A, B, C). Average Plasma Concentrations of NORLAAM and DINORLAAM after 0.575 mg/Kg Intravenous Doses of NORLAAM.

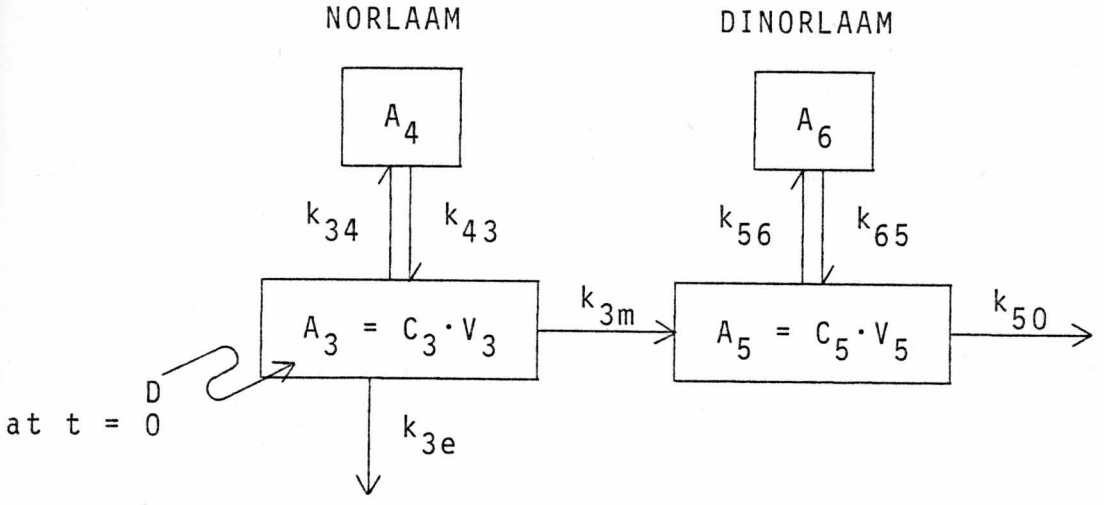
	Average Plasma Concentration (μg/ml)	
t (hr)	NORLAAM	DINORLAAM
0.083	0.25	$0.013\ (0.005_1)$
0.25	0.10	0.024
0.30	0.12	0.009_2
0.50	$0.076\ (0.008_5)$	0.030 (0.016)
0.75	$0.058\ (0.009_1)$	0.037 (0.023)
1	0.045	0.041
2	0.042	0.049
3	0.041	0.046
4	$0.036\ (0.009_3)$	0.054 (0.021)
6	0.026	0.052
7	0.033	0.034
8	$0.022\ (0.009_4)$	$0.035\ (0.006_9)$
12	$0.018\ (0.007_7)$	$0.028\ (0.004_5)$
24	$0.009_9\ (0.005_9)$	$0.016\ (0.001_2)$
36	$0.004_5\ (0.004_0)$	$0.011\ (0.000_4)$
48	$0.002_6\ (0.002_8)$	$0.004_5\ (0.004_1)$
60	0	0

Figure 29. Experiment 3 (A, B, C). Average plasma concentration-time profiles for NORLAAM (◐) and DINORLAAM (◯) after 0.575 mg/Kg intravenous doses of NORLAAM.


CONCENTRATION IN PLASMA (mcg/ml)
0.5
0.1
0.01
0.002
0
5
10
15
20
25
30
35
40
45
50
TIME (HRS)

Scheme VII

Pharmacokinetic Model for Intravenous Administration of NORLAAM.



The left-hand side of this model describes the pharmacokinetics of NORLAAM, and is again identical to Scheme IV, except the subscripts of the parameters have been altered to apply specifically to NORLAAM. The elimination rate constant, k_{30}, is explicitly separated into $k_{3m} + k_{3e}$, where k_{3m} is the rate constant of metabolism to form DINORLAAM and k_{3e} represents other metabolic and excretory processes. The DINORLAAM portion of the model is now analogous to Scheme V, with a first-order rate of appearance of this metabolite.

The rate equations 19-22 describe the rates of change of amounts of drug or metabolite in the various compartments as a function of time.

$$\frac{dA_3}{dt} = -(k_{34} + k_{3e} + k_{3m})A_3 + k_{43}A_4 \qquad \text{(Eq. 19)}$$

$$\frac{dA_4}{dt} = k_{34}A_3 - k_{43}A_4 \qquad \text{(Eq. 20)}$$

$$\frac{dA_5}{dt} = k_{3m}A_3 - (k_{56} + k_{50})A_5 + k_{65}A_6 \qquad \text{(Eq. 21)}$$

$$\frac{dA_6}{dt} = k_{56}A_5 - k_{65}A_6 \qquad \text{(Eq. 22)}$$

Equations 19-22 were solved using Laplace transformations and matrix algebra, as described in Appendix B, to yield Equations 23-26.

$$A_3 = \frac{D}{\beta_n - \alpha_n}[(k_{43} - \alpha_n)e^{-\alpha_n t} - (k_{43} - \beta_n)e^{-\beta_n t}] \qquad \text{(Eq. 23)}$$

$$A_4 = \frac{k_{34}D}{\beta_n - \alpha_n}(e^{-\alpha_n t} - e^{-\beta_n t}) \qquad \text{(Eq. 24)}$$

$$A_5 = \frac{325}{339}\cdot D\cdot k_{3m}\left[\frac{(k_{43} - \alpha_n)(k_{65} - \alpha_n)}{(\beta_n - \alpha_n)(\alpha_d - \alpha_n)(\beta_d - \alpha_n)}\cdot e^{-\alpha_n t} + \right.$$

$$\frac{(k_{43} - \beta_n)(k_{65} - \beta_n)}{(\alpha_n - \beta_n)(\alpha_d - \beta_n)(\beta_d - \beta_n)}\cdot e^{-\beta_n t} +$$

$$\frac{(k_{43} - \alpha_d)(k_{65} - \alpha_d)}{(\alpha_n - \alpha_d)(\beta_n - \alpha_d)(\beta_d - \alpha_d)}\cdot e^{-\alpha_d t} +$$

$$\left.\frac{(k_{43} - \beta_d)(k_{65} - \beta_d)}{(\alpha_n - \beta_d)(\beta_n - \beta_d)(\alpha_d - \beta_d)}\cdot e^{-\beta_d t}\right] \qquad \text{(Eq. 25)}$$

$$A_6 = \frac{325}{339} \cdot D \cdot k_{3m} k_{56} \left[\frac{(k_{43} - \alpha_n)}{(\beta_n - \alpha_n)(\alpha_d - \alpha_n)(\beta_d - \alpha_n)} \cdot e^{-\alpha_n t} + \right.$$

$$\frac{(k_{43} - \beta_n)}{(\alpha_n - \beta_n)(\alpha_d - \beta_n)(\beta_d - \beta_n)} \cdot e^{-\beta_n t} +$$

$$\frac{(k_{43} - \alpha_d)}{(\alpha_n - \alpha_d)(\beta_n - \alpha_d)(\beta_d - \alpha_d)} \cdot e^{-\alpha_d t} +$$

$$\left. \frac{(k_{43} - \beta_d)}{(\alpha_n - \beta_d)(\beta_n - \beta_d)(\alpha_d - \beta_d)} \cdot e^{-\beta_d t} \right] \qquad \text{(Eq. 26)}$$

Since these equations contain the α and β values of both NORLAAM and DINORLAAM, the subscripts 'n' and 'd', respectively, are used to distinguish between them. These composite rate constants have been defined in a general form by Equation 2. The symbol D in these equations is the dose of NORLAAM. Hence, the factor 325/339, representing the ratio of DINORLAAM:NORLAAM molecular weights, is needed in Equations 25 and 26 to yield the equivalent dose of DINORLAAM. Accordingly, the concentrations of NORLAAM (C_3) and DINORLAAM (C_5) in the central compartments are

$$C_3 = \frac{D}{V_3(\beta_n - \alpha_n)} \left[(k_{43} - \alpha_n) e^{-\alpha_n t} - \right.$$

$$\left. (k_{43} - \beta_n) e^{-\beta_n t} \right] \qquad \text{(Eq. 27)}$$

and

$$C_5 = \frac{325}{339}\cdot\frac{Dk_{3m}}{V_5}\left[\frac{(k_{43} - \alpha_n)(k_{65} - \alpha_n)}{(\beta_n - \alpha_n)(\alpha_d - \alpha_n)(\beta_d - \alpha_n)}\cdot e^{-\alpha_n t} + \right.$$

$$\frac{(k_{43} - \beta_n)(k_{65} - \beta_n)}{(\alpha_n - \beta_n)(\alpha_d - \beta_n)(\beta_d - \beta_n)}\cdot e^{-\beta_n t} +$$

$$\frac{(k_{43} - \alpha_d)(k_{65} - \alpha_d)}{(\alpha_n - \alpha_d)(\beta_n - \alpha_d)(\beta_d - \alpha_d)}\cdot e^{-\alpha_d t} +$$

$$\left.\frac{(k_{43} - \beta_d)(k_{65} - \beta_d)}{(\alpha_n - \beta_d)(\beta_n - \beta_d)(\alpha_d - \beta_d)}\cdot e^{-\beta_d t}\right] \qquad \text{(Eq. 28)}$$

a. NORLAAM Pharmacokinetics

Equation 27 is analogous to Equation 1. Table 47 gives the average pharmacokinetic parameter values for this compound.

After intravenous dosing, NORLAAM exhibited similar distribution properties to DINORLAAM. The apparent volume of the central compartment and the distribution volume at steady state were 215 and 1264% body wt, respectively, indicating an extremely large portion of the drug being bound to or otherwise sequestered by tissues. The larger distribution volume of NORLAAM than that of DINORLAAM, probably due to the higher lipophilicity of NORLAAM, results in a lower concentration of NORLAAM in the systemic circulation, and a smaller amount being presented to the site of elimination during each pass. The elimination half-life of NORLAAM (ca 12 hr) is longer than that of DINORLAAM, while plasma clearance values of the two compounds are similar.

Table 47. Experiment 3 (A, B, C). Average Pharmacokinetic Parameter Values of NORLAAM after 0.575 mg/Kg Intravenous Doses of NORLAAM.

Parameter	Average Value
α (hr^{-1})	4.03 (0.51)
$t_{1/2,\alpha}$ (hr)	0.17 (0.024)
β (hr^{-1})	0.064 (0.020)
$t_{1/2,\beta}$ (hr)	11.7 (4.00)
k_{34} (hr^{-1})	2.95 (0.45)
k_{43} (hr^{-1})	0.62 (0.23)
k_{30} (hr^{-1})	0.52 (0.43)
D/V_3 ($\mu g/ml$)	0.29 (0.092)
V_3 (% body wt)	215 (60.8)
$V_3 k_{30}$ (ml/min)	275 (124)
V_{dss} (% body wt)	1264 (176)
$AUC^{0\rightarrow\infty}$ (μg hr/ml)	0.79 (0.27)

b. DINORLAAM Pharmacokinetics

Average pharmacokinetic parameter values of DINORLAAM after intravenous doses of NORLAAM is given in Table 48. As the plasma level of NORLAAM declined, that of DINORLAAM increased and reached a peak at 3 hr, with an average C_{max} of *ca* 0.06 μg/ml.

The average biological half-life of DINORLAAM, calculated from the observed β slopes, was *ca* 16 hr. This figure is approximately four times greater than that obtained from dosing DINORLAAM directly (Table 45). Comparing these half-life values with the half-life of NORLAAM (Table 47) is instructive. It is obvious that here is an example of the so-called 'flip-flop' model (65), where a drug is converted to a metabolite which has a shorter elimination half-life than the parent compound. The rate constant for appearance of metabolite in the body is therefore smaller than the rate constant for its elimination. Under such circumstances, the terminal linear slope of the metabolite concentration *versus* time curve, after dosing the parent drug, is actually controlled by the rate of metabolite formation from the parent drug. The resemblance of the apparent DINORLAAM half-life in Experiment 3 to the NORLAAM half-life supports this reasoning. The values of β and $t_{1/2,\beta}$ in Table 48 are therefore not the true parameter values for DINORLAAM. The interpretation of DINORLAAM half-life in the literature (27, 47, 48) is incorrect for the

Table 48. Experiment 3 (A, B, C). Average Pharmacokinetic Parameter Values of DINORLAAM after 0.575 mg/Kg Intravenous Doses of NORLAAM.

Parameter	Average Value
β (hr^{-1})[a]	0.043 (0.008_4)
$t_{1/2,\beta}$ (hr)[a]	16.3 (2.98)
$AUC^{0\rightarrow\infty}$ (μg hr/ml)	1.17 (0.13)
C_{max} (μg/ml)	0.059 (0.016)
t_{max} (hr)	3.00 (1.00)

[a]Apparent values. See explanation in text.

same reason. This artifact, however, could not have been unveiled without dosing DINORLAAM directly, as was done in this study.

c. Extent of DINORLAAM Formation from NORLAAM

Two methods were used to calculate the fraction of NORLAAM metabolized to DINORLAAM by area[a] analysis.

i. $AUC^{NORLAAM\ (iv)}_{NORLAAM}$ vs. $AUC^{NORLAAM\ (iv)}_{DINORLAAM}$

Integrating Equation 27, followed by substitutions and rearrangements, yields Equation 29, which describes the area under the NORLAAM plasma level curve after an intravenous dose of NORLAAM, D_n.

$$AUC^{NORLAAM\ (iv)}_{NORLAAM} = \int_0^\infty C_3 \cdot dt = \frac{D_n}{V_3(k_{3m} + k_{3e})} = \frac{D_n}{V_3 k_{30}} \qquad \text{(Eq. 29)}$$

The equation for $AUC^{NORLAAM\ (iv)}_{DINORLAAM}$ is derived in Appendix C. It is a difficult and lengthy process to integrate

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[a]Since many different AUC's will be considered, the following nomenclature is adopted: $AUC^{b(c)}_{a}$, which reads the area under the C-t curve of compound 'a' after administering drug 'b' via route 'c'. The abbreviations po and iv indicate oral and intravenous dosing, respectively.

Equation 28, but a rapid method has been introduced (70) and was employed to yield Equation 30,

$$AUC^{NORLAAM\ (iv)}_{DINORLAAM} = \frac{(325/339)D_n f_m}{V_5 k_{50}} \qquad \text{(Eq. 30)}$$

where f_m is the fraction of NORLAAM metabolized to DINORLAAM. From Equations 29 and 30, f_m can be expressed as follows:

$$f_m = \left(\frac{AUC^{NORLAAM\ (iv)}_{DINORLAAM}}{AUC^{NORLAAM\ (iv)}_{NORLAAM}}\right)\left(\frac{339}{325}\right)\left(\frac{V_5 k_{50}}{V_3 k_{30}}\right) \qquad \text{(Eq. 31)}$$

Analysis of data from NORLAAM dosing, Experiment 3 (A, B, C), provided all parameters in Equation 31 except $V_5 k_{50}$, the value of which was obtained from intravenous doses of DINORLAAM, Experiment 4 (A, B, C).

ii. $AUC^{DINORLAAM\ (iv)}_{DINORLAAM}$ vs. $AUC^{NORLAAM\ (iv)}_{DINORLAAM}$

The area under the DINORLAAM plasma concentration-time curve after intravenous DINORLAAM is

$$AUC^{DINORLAAM\ (iv)}_{DINORLAAM} = \frac{D_d}{V_5 k_{50}} \qquad \text{(Eq. 32)}$$

where D_d is the dose of DINORLAAM. Solving Equations 30 and 32 simultaneously gives the equation for f_m,

$$f_m = \left(\frac{AUC^{NORLAAM\ (iv)}_{DINORLAAM}}{AUC^{DINORLAAM\ (iv)}_{DINORLAAM}}\right)\left(\frac{339}{325}\right)\left(\frac{D_d}{D_n}\right) \qquad \text{(Eq. 33)}$$

based on the assumption that $V_5 k_{50}$ retained identical values after dosing NORLAAM and DINORLAAM, and could therefore be cancelled in the equation.

The resulting two sets of f_m values are listed as follows:

	f_m	
Dog	i. Equation 31	ii. Equation 33
A	1.38	1.51
B	3.19	3.90
C	1.43	1.56

The f_m values calculated using the two equations were almost identical for each dog. Although consistent, the percent conversion of over 100% is unrealistic. This is believed to have been caused by the assumed constancy of V_5 and k_{50} values between experiments. Since k_{50} equals $\alpha_d \beta_d / k_{65}$, it is influenced by the microscopic rate constants α_d and β_d. While β_d may be reasonably constant between doses, values of α_d and k_{65}, obtained under the experimental conditions, are quite variable. This appears to be the most likely explanation for the varied and high estimates of f_m values. Observations in this study may serve to indicate the tenuous and dosage-dependent nature

of microscopic rate constants associated with multi-compartment pharmacokinetic modeling, and the difficulties one might expect in using numerical values of such parameters in calculations involving comparisons of individual data.

3. Single Intravenous and Oral Administrations of LAAM and DAAM

Average plasma levels of LAAM, DAAM and their N-demethylated metabolites following intravenous and oral administrations of LAAM and DAAM are shown in Figures 30-33. These data will be tabulated later for comparison purposes.

Scheme VIII describes the pharmacokinetics of LAAM, NORLAAM and DINORLAAM[a] following intravenous administration of LAAM. The drug and metabolites all obey two compartment kinetics.

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[a] DAAM was shown to obey similar pharmacokinetics to LAAM. The same behavior could be assumed for NORDAAM and DINORDAAM, although this was not proved due to the unavailability of these metabolites.

Figure 30. Experiment 1 (A, B, C). Average plasma concentration-time profiles for LAAM (●), NORLAAM (◐) and DINORLAAM (○) after 0.6 mg/Kg intravenous doses of LAAM. Note change in time scale after 25 hr.

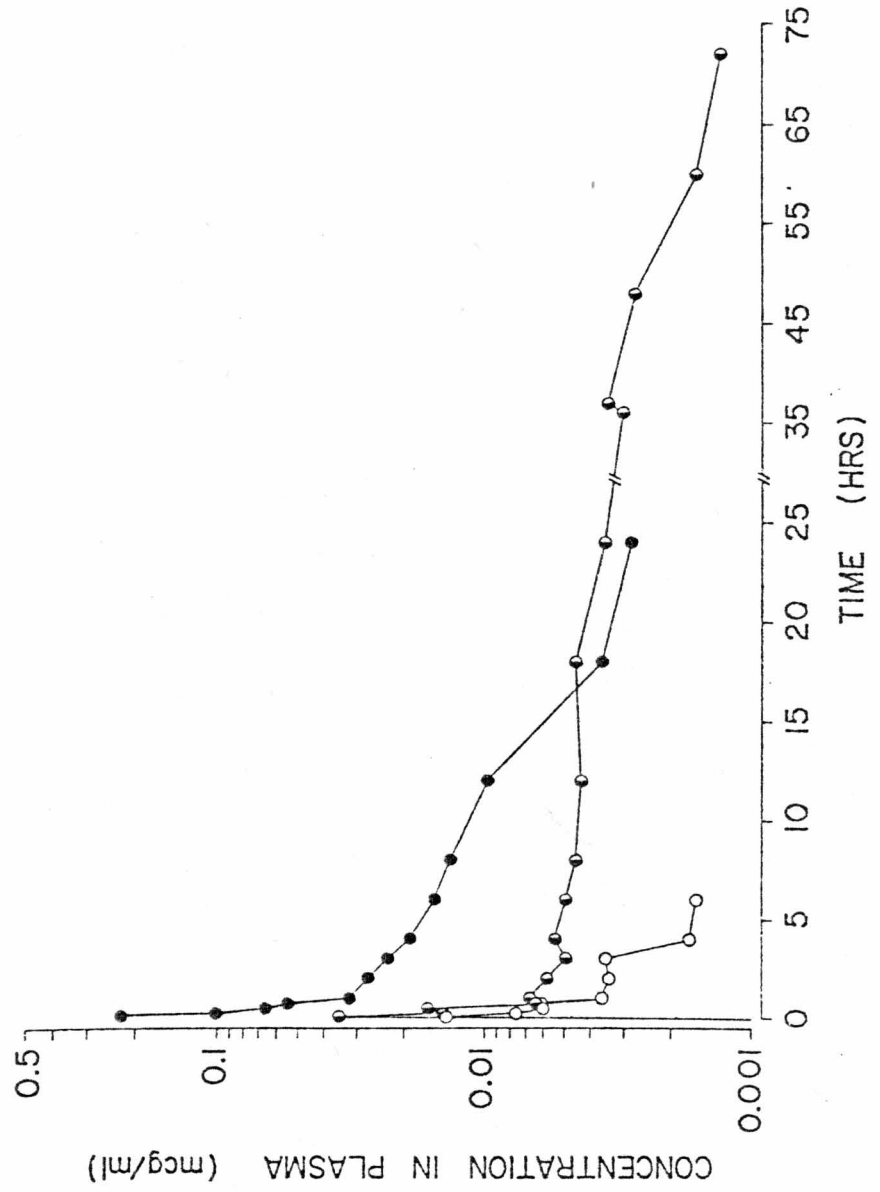

0.5
0.1
0.01
0.001
CONCENTRATION IN PLASMA (mcg/ml)
0
5
10
15
20
25
35
45
55
65
75
TIME (HRS)

Figure 31. Experiment 2 (A, B, C). Average plasma concentration-time profiles for DAAM (▲), NORDAAM (◭) and DINORDAAM (△) after 0.6 mg/Kg intravenous doses of DAAM.

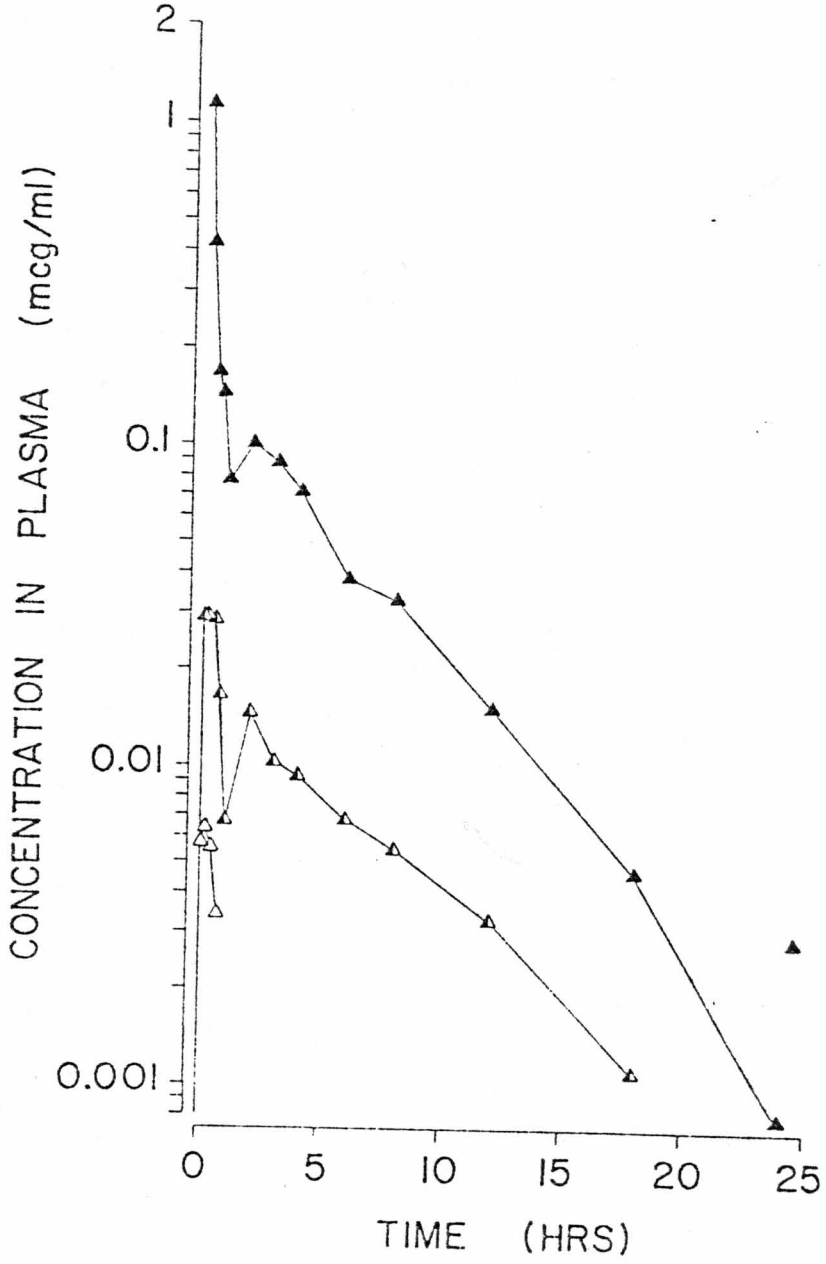

2
1
0.1
0.01
0.001
CONCENTRATION IN PLASMA (mcg/ml)
0
5
10
15
20
25
TIME (HRS)

Figure 32. Experiment 5 (A, B, C). Average plasma concentration-time profiles for LAAM (●), NORLAAM (◐) and DINORLAAM (○) after 2 mg/Kg oral doses of LAAM.

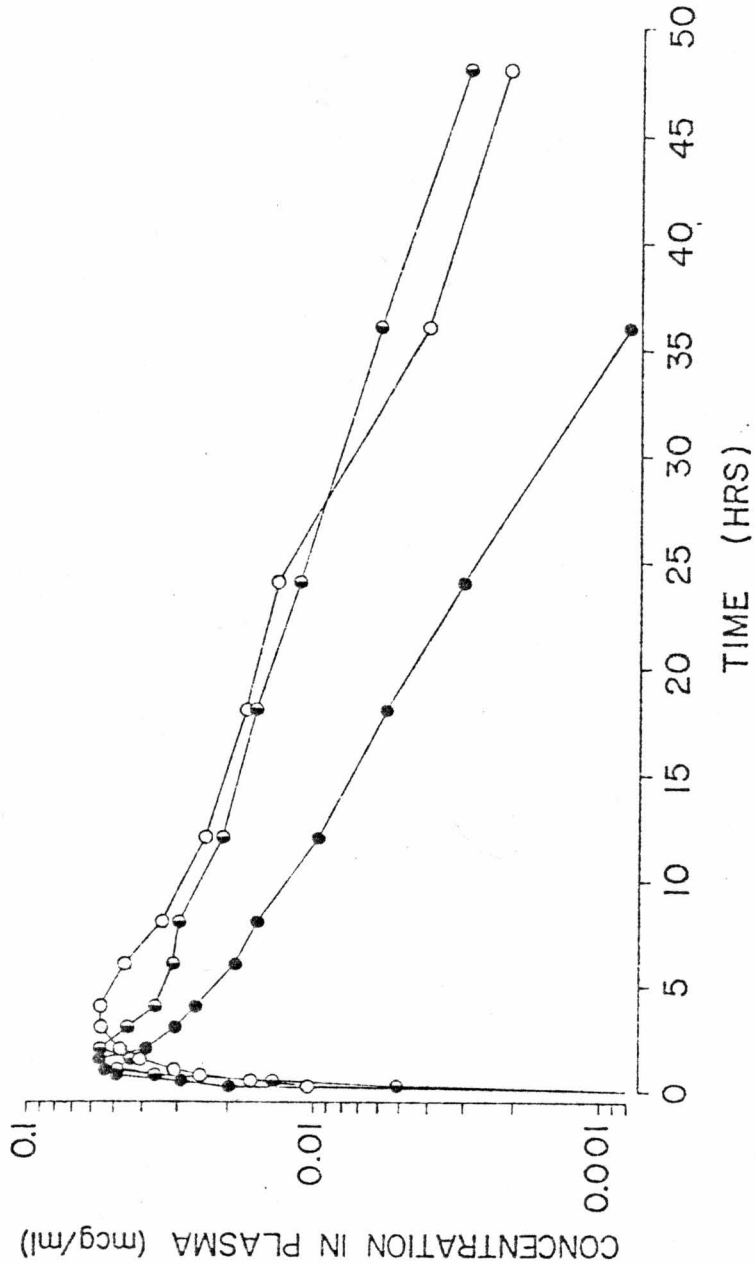
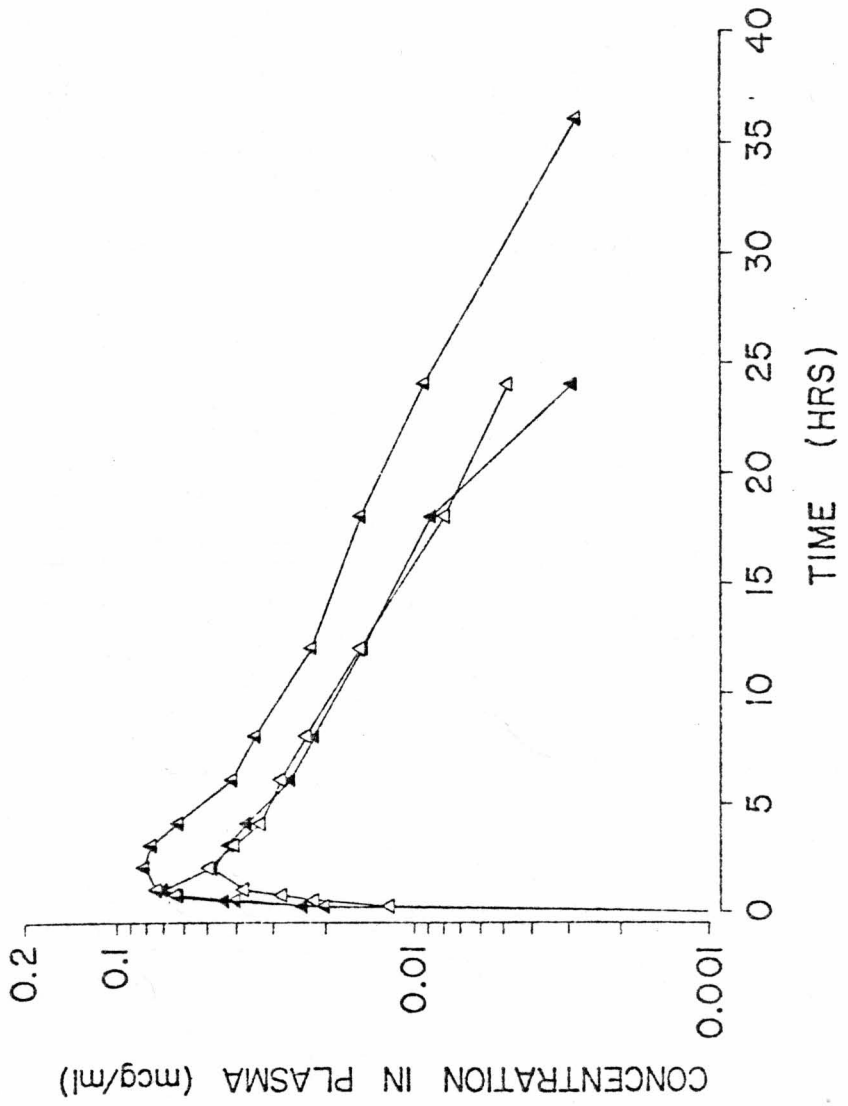
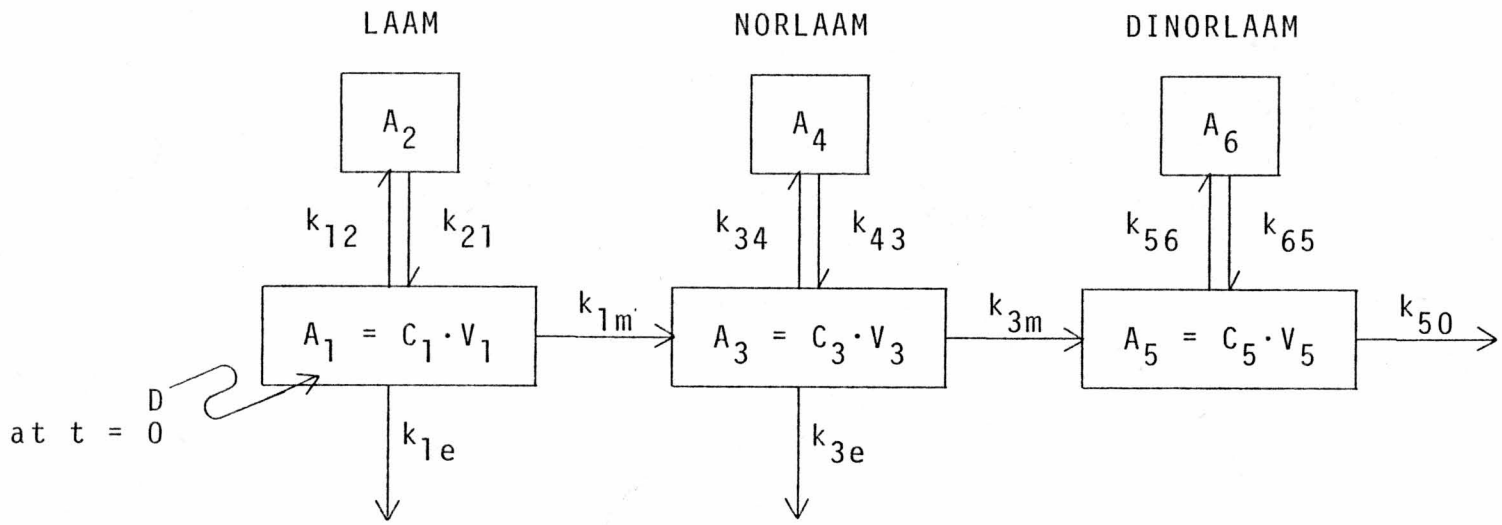

CONCENTRATION IN PLASMA (mcg/ml)
0.1
0.01
0.001
0
5
10
15
20
25
30
35
40
45
50
TIME (HRS)

Figure 33. Experiment 6 (A, B, C). Average plasma concentration-time profiles for DAAM (▲), NORDAAM (◭) and DINORDAAM (△) after 2 mg/Kg oral doses of DAAM.


CONCENTRATION IN PLASMA (mcg/ml)
0.2
0.1
0.01
0.001
0
5
10
15
20
25
30
35
40
TIME (HRS)

Scheme VIII

Pharmacokinetic Model for Intravenous Administration of LAAM.



This model was constructed based on the assumptions that N-demethylation of LAAM is a sequential process (34) and that elimination of LAAM and metabolites occurs only from the central compartment. The LAAM portion of the model is identical to Scheme IV, with the elimination rate constant, k_{10}, explicitly separated into $k_{1m} + k_{1e}$, where k_{1m} is the rate constant of metabolism to form NORLAAM and k_{1e} is that of other metabolic and excretory processes. The remainder of the model is identical to Scheme VII, but with a first-order rate of appearance for NORLAAM.

The equations describing the concentrations of LAAM and NORLAAM in the central compartments can be written analogously to Scheme VII and Equations 27 and 28:

$$C_1 = \frac{D}{V_1(\beta_1 - \alpha_1)}[(k_{21} - \alpha_1)(e^{-\alpha_1 t} - (k_{21} - \beta_1)e^{-\beta_1 t}] \qquad \text{(Eq. 34)}$$

and

$$C_3 = \frac{339}{353} \cdot \frac{Dk_{1m}}{V_3} \left[\frac{(k_{21} - \alpha_1)(k_{43} - \alpha_1)}{(\beta_1 - \alpha_1)(\alpha_n - \alpha_1)(\beta_n - \alpha_1)} \cdot e^{-\alpha_1 t} + \right.$$

$$\frac{(k_{21} - \beta_1)(k_{43} - \beta_1)}{(\alpha_1 - \beta_1)(\alpha_n - \beta_1)(\beta_n - \beta_1)} \cdot e^{-\beta_1 t} +$$

$$\frac{(k_{21} - \alpha_n)(k_{43} - \alpha_n)}{(\alpha_1 - \alpha_n)(\beta_1 - \alpha_n)(\beta_n - \alpha_n)} \cdot e^{-\alpha_n t} +$$

$$\left. \frac{(k_{21} - \beta_n)(k_{43} - \beta_n)}{(\alpha_1 - \beta_n)(\beta_1 - \beta_n)(\alpha_n - \beta_n)} \cdot e^{-\beta_n t} \right] \qquad \text{(Eq. 35)}$$

D is the dose of LAAM, and the factor 339/353, representing the ratio of NORLAAM:LAAM molecular weights, is required in Equation 35 to obtain the equivalent dose of NORLAAM. The symbols α_1 and β_1 are the composite rate constants for LAAM, and have the same definitions as in Equation 2. To obtain the expression for C_5, the plasma concentration of DINORLAAM, a six by six matrix must be solved. The process itself is tedious, and the resulting equation will be hexa-exponential. It would be unrealistic to attempt to fit the limited plasma level data to such an expression. An explicit equation for C_5 is therefore not presented.

The pharmacokinetics of LAAM after oral administration can be described by the general model in Scheme V. To apply specifically to LAAM, the concentration term becomes

$$C_1 = \frac{k_a f F D}{V_1}\{[\frac{k_{21} - \alpha_1}{(k_a - \alpha_1)(\beta_1 - \alpha_1)}]e^{-\alpha_1 t} +$$

$$[\frac{k_{21} - \beta_1}{(k_a - \beta_1)(\alpha_1 - \beta_1)}]e^{-\beta_1 t} +$$

$$[\frac{k_{21} - k_a}{(\alpha_1 - k_a)(\beta_1 - k_a)}]e^{-k_a t}\} \qquad \text{(Eq. 36)}$$

where k_a is the first-order rate constant of absorption of LAAM. Equations for C_3 and C_5 after oral LAAM will not be used for analysis of data and are therefore not included here.

a. Intravenous Administration of LAAM and DAAM

i. LAAM vs. DAAM Pharmacokinetics

Average plasma concentrations are compared when possible in Table 49, whereas average pharmacokinetic parameter values are compared in Table 50. By examining the individual and average values, it is apparent that the plasma concentrations of DAAM during the early phase were higher than those of LAAM. However, due to the limited number of animals used and hence the small number of degrees of freedom, differences were shown significant only at 30 and 45 minutes after dosing. Mean distribution volumes, V_1 and V_{dss}, of LAAM were almost identical to those obtained for NORLAAM, but were approximately four times greater than equivalent values for DAAM, suggesting that the l- isomer distributes into body tissues to a much

Table 49. Experiments 1 (A, B, C) and 2 (A, B, C). Average Plasma Concentrations of LAAM and DAAM after 0.6 mg/Kg Intravenous Doses of LAAM and DAAM, respectively.

	Average Plasma Concentration (μg/ml)		
t (hr)	LAAM	DAAM	p[a]
0.083	0.22	1.12 (1.05)	*[b]
0.25	0.10 (0.06)	0.43 (0.38)	NS[c]
0.50	0.066 (0.035)	0.17 (0.059)	<0.05
0.75	0.054 (0.036)	0.15 (0.066)	<0.1
1	0.032 (0.003_5)	0.078	*
2	0.027 (0.006)	0.10 (0.051)	NS
3	0.023 (0.006)	0.089 (0.046)	NS
4	0.019 (0.007_3)	0.072 (0.036)	NS
6	0.015 (0.006)	0.038 (0.023)	NS
8	0.013 (0.004_1)	0.033 (0.018)	NS
12	0.009_7 (0.003_8)	0.015 (0.012)	NS
18	0.003_6	0.004_7 (0.002_4)	*
24	0.002_8 (0.001_2)	0.000_8	*
24.5	-	0.002_9	*

[a]Paired t-test.

[b]Insufficient data for statistical comparison.

[c]Not significant, $p > 0.1$.

Table 50. Experiments 1 (A, B, C) and 2 (A, B, C). Average Pharmacokinetic Parameter Values of LAAM and DAAM after 0.6 mg/Kg Intravenous Doses of LAAM and DAAM, respectively.

	Average Value		
Parameter	LAAM	DAAM	p
α (hr^{-1})	7.40 (4.52)	8.81 (2.20)	NS
$t_{1/2,\alpha}$ (hr)	0.15 (0.14)	0.082 (0.019)	NS
β (hr^{-1})	0.11 (0.012)	0.20 (0.029)	<0.1
$t_{1/2,\beta}$ (hr)	6.57 (0.78)	3.51 (0.54)	<0.05
k_{12} (hr^{-1})	5.74 (4.00)	5.92 (2.40)	NS
k_{21} (hr^{-1})	0.68 (0.36)	1.14 (0.59)	<0.1
k_{10} (hr^{-1})	1.09 (0.23)	1.94 (1.17)	NS
D/V_1 ($\mu g/ml$)	0.40 (0.10)	1.96 (1.75)	NS
V_1 (% body wt)	159 (47.0)	48.8 (32.5)	NS
$V_1 k_{10}$ (ml/min)	495 (160)	212 (110)	NS
V_{dss} (% body wt)	1261 (124)	305 (204)	<0.02
$AUC^{0\rightarrow\infty}$ (μg hr/ml)	0.38 (0.082)	1.04 (0.50)	NS

greater extent than the d- isomer. Similar differences in distribution properties have been observed in d- and l-methadone (54). It must be realized, however, that the possibility of plasma protein binding exists with LAAM, DAAM and their metabolites (48). A closely related compound, morphine, has been shown to be ca 35% bound to plasma proteins at therapeutic levels (71, 72). Thus, the calculated volumes of distribution must be considered as apparent values, and they need not represent actual distribution spaces. Values of α indicated that the distribution process was extremely rapid for both LAAM and DAAM, with the α phase ending in 1 hr in both cases.

The elimination rate constant, k_{10}, of DAAM was twice that of LAAM. A twofold difference was seen also between the plasma clearance values of LAAM and DAAM. The average biological half-life of LAAM was 6.6 hr, approximately double that of DAAM (3.5 hr). D- and l- isomers of other compounds, including propranolol (55) and warfarin (56), have been shown to differ in a similar fashion. Because of the faster elimination of DAAM, its plasma concentration rapidly declined to a level similar to that of LAAM at ca 20 hr after intravenous dosing.

The average $AUC^{0\rightarrow\infty}$ value, calculated by trapezoidal rule, was smaller for LAAM than for DAAM. This is consistent with the larger plasma clearance of LAAM, since $AUC^{0\rightarrow\infty}$ equals dose/plasma clearance (65).

ii. NORLAAM vs. NORDAAM Pharmacokinetics

Comparisons of average plasma concentrations are shown in Table 51. Comparisons of the average pharmacokinetic parameters are described in Table 52. Plasma levels of both NORLAAM and NORDAAM peaked almost immediately following intravenous dosing of the parent compounds. Average C_{max} values of NORLAAM and NORDAAM were almost identical.

With the exception of the 48 hr sample, differences between NORLAAM and NORDAAM plasma levels were not significant by paired t-tests. Nevertheless, it is clear from Figures 30 and 31 that the NORLAAM plasma C-t curve was more prolonged, with detectable levels occurring at 72 hr, while NORDAAM levels could not be detected after 18 hr. The average half-life of NORDAAM was ca 5 hr, whereas that of NORLAAM could not be calculated due to the sustained nature of the plasma levels. The cause of the low, fluctuating, but persistent plasma levels of NORLAAM is not clear. A possible explanation may be provided by the rapid distribution of LAAM, after intravenous administration, into an extremely large volume (V_1 = 160% body wt). This results instantaneously in a multi-fold dilution of plasma concentrations, hence delivering only a minute portion of the drug to the metabolic site for NORLAAM formation. Henderson and associates (39) reported a similar phenomenon in rats, and attributed the prolonged plasma levels of LAAM

Table 51. Experiments 1 (A, B, C) and 2 (A, B, C). Average Plasma Concentrations of NORLAAM and NORDAAM after 0.6 mg/Kg Intravenous Doses of LAAM and DAAM, respectively.

	Average Plasma Concentration (μg/ml)		
t (hr)	NORLAAM	NORDAAM	p
0.083	0.035 (0.039)	0.029 (0.028)	NS
0.25	0.014 (0.016)	0.029 (0.029)	NS
0.50	0.016 (0.020)	0.028 (0.034)	NS
0.75	0.006_4 (0.004_0)	0.017 (0.017)	NS
1	0.006_7 (0.002_8)	0.006_7	*
2	0.005_8 (0.001_5)	0.015 (0.017)	NS
3	0.004_9 (0.001_1)	0.010 (0.009_7)	NS
4	0.005_4 (0.000_7)	0.009_3 (0.009_3)	NS
6	0.004_9 (0.000_5)	0.006_8 (0.007_9)	NS
8	0.004_5 (0.000_6)	0.005_5 (0.006_5)	NS
12	0.004_3 (0.000_6)	0.003_3 (0.004_0)	NS
18	0.004_5	0.001_1 (0.001_9)	*
24	0.003_5 (0.000_4)	0	*
36	0.003_0	0	*
37	0.003_4	-	*
48	0.002_7 (0.000_4)	0	<0.01
60	0.001_6	0	*
72	0.001_3 (0.001_1)	0	NS

Table 52. Experiments 1 (A, B, C) and 2 (A, B, C). Average Pharmacokinetic Parameter Values of NORLAAM and NORDAAM after 0.6 mg/Kg Intravenous Doses of LAAM and DAAM, respectively.

	Average Value		
Parameter	NORLAAM	NORDAAM	p
C_{max} (μg/ml)	0.035 (0.039)	0.032 (0.032)	NS
t_{max} (hr)	0.083 (0)	0.28 (0.21)	NS
$AUC^{0\rightarrow 4\ hr}$ (μg hr/ml)	0.031 (0.019)	0.061 (0.064)	NS

to its enterohepatic circulation.

iii. DINORLAAM vs. DINORDAAM Pharmacokinetics

Average plasma concentrations and pharmacokinetic values are shown in Tables 53 and 54, respectively. Like the nor- metabolites, both DINORLAAM and DINORDAAM plasma levels reached a maximum almost immediately after intravenous dosing of the parent drugs. No significant differences were observed between the plasma levels of DINORLAAM and DINORDAAM. Detectable levels of DINORDAAM lasted for only 45 min, making it impossible to compare the area under curve of the two compounds. Plasma levels of DINORLAAM, on the other hand, were somewhat more sustained. However, it was not possible to calculate the half-life of either isomer in this case.

b. Oral Administration of LAAM and DAAM

i. LAAM vs. DAAM Pharmacokinetics

Average plasma concentrations and also pharmacokinetic parameters obtained from the two isomers are compared in Tables 55 and 56, respectively.

DAAM achieved higher plasma levels than LAAM during the 24 hours after oral administration. Although t_{max} was ca 1 hr in both cases, average C_{max} of DAAM (0.076 µg/ml) was higher than that of LAAM (0.055 µg/ml). The death of Dog B during the oral LAAM experiment resulted in

Table 53. Experiments 1 (A, B, C) and 2 (A, B, C). Average Plasma Concentrations of DINORLAAM and DINORDAAM after 0.6 mg/Kg Intravenous Doses of LAAM and DAAM, respectively.

	Average Plasma Concentration (μg/ml)		
t (hr)	DINORLAAM	DINORDAAM	p
0.083	$0.014\ (0.005_5)$	$0.005_7\ (0.005_1)$	NS
0.25	$0.007_6\ (0.001_2)$	$0.006_4\ (0.001_0)$	NS
0.50	$0.006_0\ (0.000_9)$	$0.005_5\ (0.000_6)$	NS
0.75	$0.006_1\ (0.000_6)$	$0.003_4\ (0.002_9)$	NS
1	$0.003_6\ (0.003_1)$	0	*
2	$0.003_4\ (0.002_9)$	0	NS
3	$0.003_5\ (0.003_0)$	0	NS
4	$0.001_7\ (0.002_9)$	0	NS
6	$0.001_6\ (0.002_8)$	0	NS

Table 54. Experiments 1 (A, B, C) and 2 (A, B, C). Average Pharmacokinetic Parameter Values of DINORLAAM and DINORDAAM after 0.6 mg/Kg Intravenous Doses of LAAM and DAAM, respectively.

	Average Value		
Parameter	DINORLAAM	DINORDAAM	p
C_{max} (μg/ml)	0.014 (0.005_5)	0.007_8 (0.001_7)	NS
t_{max} (hr)	0.083 (0)	0.14 (0.098)	NS

Table 55. Experiments 5 (A, B, C) and 6 (A, B, C). Average Plasma Concentrations of LAAM and DAAM after 2 mg/Kg Oral Doses of LAAM and DAAM, respectively.

	Average Plasma Concentration (μg/ml)		
t (hr)	LAAM	DAAM	p
0	0	0	-
0.25	0.020 (0.000_3)	0.024 (0.005_9)	NS
0.50	0.029 (0.002_4)	0.044 (0.010)	<0.1
0.75	0.049 (0.006_2)	0.062 (0.008_1)	<0.05
1	0.054	0.070 (0.020)	*
1.5	0.056	-	-
2	0.039	0.048 (0.011)	*
3	0.031	0.042 (0.006_8)	*
4	0.026	0.037 (0.010)	*
6	0.019	0.026 (0.010)	*
8	0.016	0.022 (0.006_1)	*
12	0.009_6	0.015 (0.005_4)	*
18	0.005_6	0.008_7	*
24	0.003_0	0.002_9 (0.000_7)	*
36	0.000_8	0	*

Table 56. Experiments 5 (A, B, C) and 6 (A, B, C). Average Pharmacokinetic Parameter Values of LAAM and DAAM after 2 mg/Kg Oral Doses of LAAM and DAAM, respectively.

	Average Value		
Parameter	LAAM	DAAM	p
α (hr^{-1})	1.02	1.16 (0.071)	*
$t_{1/2,\alpha}$ (hr)	0.68	0.60 (0.036)	*
β (hr^{-1})	0.095	0.11 (0.005_1)	*
$t_{1/2,\beta}$ (hr)	7.28	6.22 (0.29)	*
k_a (hr^{-1})	1.02	1.16 (0.071)	*
$t_{1/2,abs}$ (hr)	0.68	0.60 (0.036)	*
k_{12} (hr^{-1})	0.49	0.50 (0.026)	*
k_{21} (hr^{-1})	0.35	0.50 (0.15)	*
k_{10} (hr^{-1})	0.28	0.27 (0.065)	*
fFD/V_1 ($\mu g/ml$)	0.10	0.13 (0.024)	*
C_{max} ($\mu g/ml$)	0.055 (0.002_0)	0.076 (0.010)	<0.1
t_{max} (hr)	1.08 (0.38)	0.92 (0.14)	NS
$AUC^{0\rightarrow\infty}$ (μg hr/ml)	0.38	0.49 (0.12)	*

insufficient data for statistical comparison of other pharmacokinetic parameters between the two isomers. However, it is noted that, after oral dosing, LAAM and DAAM have almost identical absorption (k_a) and elimination (k_{10}) rate constants. The difference between their half-lives, while significant in the intravenous experiments, is now very slight. This appears to be due mainly to the prolonged half-life of DAAM after oral dosing. This subject will be discussed further in Section d.i., p. 218.

Values of $AUC^{0\rightarrow\infty}$ were slightly greater for DAAM, compared to LAAM. This observation is consistent with the higher plasma levels of DAAM. DAAM also possessed a larger fFD/V_1 than LAAM. However, a more accurate method of assessing the relative bioavailability of these two compounds, upon oral administration, is to compare their fF values, which will be calculated on pp. 214 and 222.

ii. NORLAAM vs. NORDAAM Pharmacokinetics

Average plasma concentrations are compared statistically when possible in Table 57, while average pharmacokinetic parameters are given in Table 58. After oral dosing of parent drugs, both NORLAAM and NORDAAM plasma levels peaked at approximately 1.5 hr. Maximum concentration, C_{max}, was slightly higher for NORDAAM, and the plasma levels of this metabolite were also more sustained, resulting in a slightly larger $AUC^{0\rightarrow\infty}$. Average half-lives

Table 57. Experiments 5 (A, B, C) and 6 (A, B, C). Average Plasma Concentrations of NORLAAM and NORDAAM after 2 mg/Kg Oral Doses of LAAM and DAAM, respectively.

	Average Plasma Concentration (μg/ml)		
t (hr)	NORLAAM	NORDAAM	p
0	0	0	-
0.25	0.005_1 (0.000_9)	0.020 (0.001_6)	<0.005
0.50	0.014 (0.003_7)	0.040 (0.017)	<0.1
0.75	0.036 (0.017)	0.064 (0.006_4)	<0.1
1	0.048	0.074 (0.020)	*
1.5	0.044	-	-
2	0.055	0.081 (0.044)	*
3	0.045	0.077 (0.043)	*
4	0.036	0.063 (0.026)	*
6	0.031	0.041 (0.003_8)	*
8	0.030	0.034 (0.005_8)	*
12	0.021	0.022 (0.001_8)	*
18	0.016	0.015	*
24	0.011	0.009_2 (0.002_4)	*
36	0.005_9	0.002_8 (0.000_5)	*
48	0.002_9	0	*

Table 58. Experiments 5 (A, B, C) and 6 (A, B, C). Average Pharmacokinetic Parameter Values of NORLAAM and NORDAAM after 2 mg/Kg Oral Doses of LAAM and DAAM, respectively.

Parameter	Average Value	
	NORLAAM	NORDAAM
β (hr^{-1})	0.055	0.087 (0.008_8)
$t_{1/2,\beta}$ (hr)	13.0	8.01 (0.83)
$AUC^{0\rightarrow\infty}$ (μg hr/ml)	0.79	0.85 (0.13)
C_{max} (μg/ml)	0.060	0.088 (0.035)
t_{max} (hr)	1.50	1.25 (0.66)

by graphic analysis are 13 hr for NORLAAM and 8 hr for NORDAAM. These are believed to represent true half-life values, since they are longer than those observed for the corresponding parent drugs. Such a postulate has been proven correct for NORLAAM by intravenous administration of the metabolite itself, and is probably also true for NORDAAM. The longer half-lives of both LAAM and NORLAAM than those of their corresponding d- isomers may partially explain the longer duration of morphine-like activity of LAAM as compared to DAAM.

iii. DINORLAAM vs. DINORDAAM Pharmacokinetics

Average plasma concentrations and pharmacokinetic parameter values are presented in Tables 59 and 60, respectively. There were no significant differences between the plasma levels of these metabolites during the absorptive phase, but DINORLAAM levels were more sustained after reaching C_{max}, giving rise to a smaller β slope and a larger $AUC^{0\rightarrow\infty}$ than DINORDAAM. Half-lives calculated from the observed β values are ca 12 hr for DINORLAAM and 9 hr for DINORDAAM, which are almost identical to the half-lives of the corresponding secondary amines, NORLAAM and NORDAAM. Hence, it can be reasonably assumed that DINORDAAM is similar to DINORLAAM in having a shorter biological half-life than the nor- metabolite. In this case the apparent value of observed β actually reflects the rate constant of

Table 59. Experiments 5 (A, B, C) and 6 (A, B, C). Average Plasma Concentrations of DINORLAAM and DINORDAAM after 2 mg/Kg Oral Doses of LAAM and DAAM, respectively.

	Average Plasma Concentration (μg/ml)		
t (hr)	DINORLAAM	DINORDAAM	p
0	0	0	-
0.25	0.010 (0.004_8)	0.012 (0.008_9)	NS
0.50	0.017 (0.005_7)	0.022 (0.014)	NS
0.75	0.025 (0.005_4)	0.028 (0.017)	NS
1	0.031	0.038 (0.027)	*
1.5	0.041	-	-
2	0.047	0.049 (0.042)	*
3	0.055	0.040 (0.028)	*
4	0.055	0.033 (0.021)	*
6	0.046	0.028 (0.022)	*
8	0.034	0.023 (0.017)	*
12	0.024	0.015 (0.009_1)	*
18	0.017	0.007_8	*
24	0.013	0.004_8 (0.004_7)	*
36	0.004_0	0	*
48	0.002_1	0	*

Table 60. Experiments 5 (A, B, C) and 6 (A, B, C). Average Pharmacokinetic Parameter Values of DINORLAAM and DINORDAAM after 2 mg/Kg Oral Doses of LAAM and DAAM, respectively.

Parameter	Average Value	
	DINORLAAM	DINORDAAM
β (hr^{-1})[a]	0.059	0.079 (0.014)
$t_{1/2,\beta}$ (hr)[a]	11.9	8.99 (1.44)
$AUC^{0\rightarrow\infty}$ (μg hr/ml)	0.89	0.53 (0.34)
C_{max} (μg/ml)	0.057	0.049 (0.042)
t_{max} (hr)	3.50	2.17 (0.29)

[a]Apparent values. See explanation in text.

appearance of the dinor- metabolite in the systemic circulation, as discussed previously in Section 2.b., p. 177. Hence, the rate of elimination of the nor- metabolites of both LAAM and DAAM appears to be the limiting factor in the loss of both the nor- and the dinor- metabolites and therefore probably some of the pharmacologic activities.

c. Intravenous and Oral Administration of LAAM

i. LAAM Pharmacokinetics, I.V. vs. P.O.

The average pharmacokinetic parameters of LAAM after intravenous and oral doses are given in Table 61. Paired t-tests could not be performed since only two sets of oral dose data are available.

Relative values of α and k_{12} suggest much faster distribution of LAAM into tissues after intravenous than after oral dosing, perhaps the result of an 'overload effect'. Intravenous dosing also resulted in a larger elimination rate constant (k_{10}) of the drug. However, the half-life of LAAM after intravenous administration (6.6 hr) was only slightly shorter than that after an oral dose (7.3 hr).

Normalized $AUC^{0\rightarrow\infty}$ after intravenous dosing was three times greater than the $AUC^{0\rightarrow\infty}$ value after oral administration. The overall efficiency of appearance of unchanged LAAM in the circulation, fF, after an oral dose, can be estimated as follows (70).

Table 61. Experiments 1 (A, B, C) and 5 (A, B, C). Average Pharmacokinetic Parameter Values of LAAM after 0.6 mg/Kg Intravenous Doses and 2 mg/Kg Oral Doses of LAAM.

	Average Value	
Parameter	I.V.	P.O.
α (hr^{-1})	7.40 (4.52)	1.02
$t_{1/2,\alpha}$ (hr)	0.15 (0.14)	0.68
β (hr^{-1})	0.11 (0.012)	0.095
$t_{1/2,\beta}$ (hr)	6.57 (0.78)	7.28
k_{12} (hr^{-1})	5.74 (4.00)	0.49
k_{21} (hr^{-1})	0.68 (0.36)	0.35
k_{10} (hr^{-1})	1.09 (0.23)	0.28
fFD/V_1 ($\mu g/ml$)[a]	0.40 (0.10)	0.10
$AUC^{0\rightarrow\infty}$ (μg hr/ml)	0.38 (0.082)	0.38

[a] fF = 1 for i.v. doses.

$$AUC_{LAAM}^{LAAM\ (iv)} = \frac{(i.v.\ dose)}{V_1 k_{10}} \qquad \text{(Eq. 37)}$$

$$AUC_{LAAM}^{LAAM\ (po)} = \frac{fF \cdot (p.o.\ dose)}{V_1 k_{10}} \qquad \text{(Eq. 38)}$$

From Equations 37 and 38, fF can be written as:

$$fF = \left(\frac{AUC_{LAAM}^{LAAM\ (po)}}{AUC_{LAAM}^{LAAM\ (iv)}}\right)\left(\frac{i.v.\ dose}{p.o.\ dose}\right) \qquad \text{(Eq. 39)}$$

assuming the product $V_1 k_{10}$ stays constant between intravenous and oral dosing experiments. Using Equation 39, fF values were found to be 0.40 for Dog A and 0.21 for Dog C, but could not be calculated for Dog B because of the lack of AUC data from oral dosing.

ii. NORLAAM Pharmacokinetics, I.V. vs. P.O.

The average plasma levels are compared in Table 62, while the average pharmacokinetic parameters are given in Table 63.

Plasma levels of NORLAAM peaked more rapidly after intravenous doses of LAAM, and were also sustained longer than those after oral dosing. Since intravenous LAAM appears to distribute more extensively into tissues than oral LAAM, small quantities of the parent drug may be continually re-entering into the central compartment and being metabolized to NORLAAM, thereby prolonging the NORLAAM half-life after intravenous LAAM.

Table 62. Experiments 1 (A, B, C) and 5 (A, B, C). Average Plasma Concentrations of NORLAAM after 0.6 mg/Kg Intravenous Doses and 2 mg/Kg Oral Doses of LAAM.

	Average Plasma NORLAAM (μg/ml)		
t (hr)	I.V.	P.O.	p[a]
0	0	0	-
0.083	0.035 (0.039)	-	-
0.25	0.014 (0.016)	0.005_1 (0.000_9)	NS
0.50	0.016 (0.020)	0.014 (0.003_7)	NS
0.75	0.006_4 (0.004_0)	0.036 (0.017)	<0.1
1	0.006_7 (0.002_8)	0.048	*
1.5	-	0.044	-
2	0.005_8 (0.001_5)	0.055	*
3	0.004_9 (0.001_1)	0.045	*
4	0.005_4 (0.000_7)	0.036	*
6	0.004_9 (0.000_5)	0.031	*
8	0.004_5 (0.000_6)	0.030	*
12	0.004_3 (0.000_6)	0.021	*
18	0.004_5	0.016	*
24	0.003_5 (0.000_4)	0.011	*
36	0.003_0	0.005_9	*
37	0.003_4	-	-
48	0.002_7 (0.000_4)	0.002_9	*

Table 62 (continued)

	Average Plasma NORLAAM (μg/ml)		
t (hr)	I.V.	P.O.	p[a]
60	0.001_6	0	*
72	0.001_3 (0.001_1)	-	-

[a] In Tables 62, 64, 67 and 69, average plasma levels after oral doses were normalized to a dose of 0.6 mg/Kg in performing paired t-tests.

Table 63. Experiments 1 (A, B, C) and 5 (A, B, C). Average Pharmacokinetic Parameter Values of NORLAAM after 0.6 mg/Kg Intravenous Doses and 2 mg/Kg Oral Doses of LAAM.

	Average Value	
Parameter	I.V.	P.O.
$AUC^{0\rightarrow 48\ hr}$ (μg hr/ml)	0.18 (0.042)	0.74
C_{max} (μg/ml)	0.035 (0.039)	0.060
t_{max} (hr)	0.083 (0)	1.50

After normalizing for the difference in doses, oral LAAM yielded a somewhat larger $AUC^{0\rightarrow 48\ hr}$ value for NORLAAM than that yielded by intravenous LAAM. This can be explained in terms of the very high concentration of LAAM presented, after oral dosing, to the liver during the first-pass, where it was partially metabolized prior to dilution into the systemic circulation and tissue volumes.

iii. DINORLAAM Pharmacokinetics, I.V. vs. P.O.

Average plasma levels are compared in Table 64, while pharmacokinetic parameters are shown in Table 65. Normalized plasma DINORLAAM levels were higher after oral than intravenous doses of LAAM, again indicating substantial first-pass metabolism of orally administered LAAM before entering the general circulation. DINORLAAM levels after intravenous LAAM peaked almost instantaneously, but rapidly declined and were not detectable after six hours.

d. Intravenous and Oral Administration of DAAM

i. DAAM Pharmacokinetics, I.V. vs. P.O.

The average pharmacokinetic parameters are compared in Table 66. Like LAAM, DAAM appeared to distribute far more rapidly into the tissues after intravenous dosing than after oral dosing. Elimination was also faster after intravenous administration of DAAM, with a

Table 64. Experiments 1 (A, B, C) and 5 (A, B, C). Average Plasma Concentrations of DINORLAAM after 0.6 mg/Kg Intravenous Doses and 2 mg/Kg Oral Doses of LAAM.

	Average Plasma DINORLAAM (μg/ml)		
t (hr)	I.V.	P.O.	p
0	0	0	-
0.083	$0.014\ (0.005_5)$	-	-
0.25	$0.007_6\ (0.001_2)$	$0.010\ (0.004_8)$	<0.1
0.50	$0.006_0\ (0.000_9)$	$0.017\ (0.005_7)$	NS
0.75	$0.006_1\ (0.000_6)$	$0.025\ (0.005_4)$	NS
1	$0.003_6\ (0.003_1)$	0.031	*
1.5	-	0.041	-
2	$0.003_4\ (0.002_9)$	0.047	*
3	$0.003_5\ (0.003_0)$	0.055	*
4	$0.001_7\ (0.002_9)$	0.055	*
6	$0.001_6\ (0.002_8)$	0.046	*
8	0	0.034	*
12	0	0.024	*
18	0	0.017	*
24	0	0.013	*
36	0	0.004_0	*
48	0	0.002_1	*

Table 65. Experiments 1 (A, B, C) and 5 (A, B, C). Average Pharmacokinetic Parameter Values of DINORLAAM after 0.6 mg/Kg Intravenous Doses and 2 mg/Kg Oral Doses of LAAM.

	Average Value	
Parameter	I.V.	P.O.
C_{max} (μg/ml)	0.014 (0.005_5)	0.057
t_{max} (hr)	0.083 (0)	3.50

Table 66. Experiments 2 (A, B, C) and 6 (A, B, C). Average Pharmacokinetic Parameter Values of DAAM after 0.6 mg/Kg Intravenous Doses and 2 mg/Kg Oral Doses of DAAM.

	Average Value		
Parameter	I.V.	P.O.	p[a]
α (hr^{-1})	8.81 (2.20)	1.16 (0.071)	<0.05
$t_{1/2,\alpha}$ (hr)	0.082 (0.019)	0.60 (0.036)	<0.005
β (hr^{-1})	0.20 (0.029)	0.11 (0.005_1)	<0.05
$t_{1/2,\beta}$ (hr)	3.51 (0.54)	6.22 (0.29)	<0.05
k_{12} (hr^{-1})	5.92 (2.40)	0.50 (0.026)	<0.1
k_{21} (hr^{-1})	1.14 (0.59)	0.50 (0.15)	NS
k_{10} (hr^{-1})	1.94 (1.17)	0.27 (0.065)	NS
fFD/V_1 ($\mu g/ml$)[b]	1.96 (1.75)	0.13 (0.024)	NS
$AUC^{0\rightarrow\infty}$ (μg hr/ml)	1.04 (0.50)	0.49 (0.12)	<0.1

[a] In Tables 66, 68 and 70, the values of AUC, fFD/V_1 and C_{max} after oral doses, when calculated, were normalized to a dose of 0.6 mg/Kg in performing paired t-tests.

[b] $fF = 1$ for i.v. doses.

significantly shorter half-life (3.5 hr) than that observed after an oral dose (6.2 hr). The cause of this difference is not clear. Cohen and associates (73) reported a similar case with 5-Fluorouracil and attributed the longer half-life of the oral dose to biliary recycling. This argument, however, does not seem applicable to the present situation, where the overall time span involved is much longer. Continual absorption of DAAM from the gastrointestinal tract is another possible but unlikely explanation.

Normalized $AUC^{0\rightarrow\infty}$ values after intravenous dosing were significantly greater than those from oral doses. Values of fF after oral DAAM are calculated by Equation 40,

$$fF = \left(\frac{AUC^{DAAM\ (po)}_{DAAM}}{AUC^{DAAM\ (iv)}_{DAAM}}\right)\left(\frac{\text{i.v. dose}}{\text{p.o. dose}}\right) \qquad \text{(Eq. 40)}$$

which is analogous to Equation 39. From Equation 40, fF values are 0.10, 0.16 and 0.28 for Dogs A, B and C, respectively. Comparing these values with those for LAAM on p. 214, and assuming that oral doses of both LAAM and DAAM are efficiently absorbed (12), it appears that a greater portion of DAAM was metabolized during the first pass through the liver. This may indicate more efficient hepatic extraction of DAAM than LAAM, hence contributing to the shorter half-life of DAAM.

ii. NORDAAM Pharmacokinetics, I.V. vs. P.O.

Average plasma levels are compared in Table 67, while average pharmacokinetic parameters are compared in Table 68. There were no significant differences between the normalized plasma NORDAAM levels from intravenously and orally administered DAAM. The half-life of NORDAAM increased significantly from 5 hr after intravenous dosing to 8 hr after oral dosing of DAAM, a phenomenon similar to that observed with the parent drug, and discussed in the preceding section.

Differences in C_{max}, t_{max} and $AUC^{0\rightarrow\infty}$ of NORDAAM between the two routes of administration of DAAM are not significant.

iii. DINORDAAM Pharmacokinetics, I.V. vs. P.O.

Average plasma levels are compared in Table 69, whereas average pharmacokinetic parameters are compared in Table 70. After intravenous DAAM, DINORDAAM reached peak plasma levels significantly faster than after oral DAAM, but detectable levels lasted for only ca 1 hr. This made comparisons difficult between the two routes of administration of DAAM.

Table 67. Experiments 2 (A, B, C) and 6 (A, B, C). Average Plasma Concentrations of NORDAAM after 0.6 mg/Kg Intravenous Doses and 2 mg/Kg Oral Doses of DAAM.

	Average Plasma NORDAAM (μg/ml)		
t (hr)	I.V.	P.O.	p
0	0	0	-
0.083	0.029 (0.027)	-	-
0.25	0.029 (0.029)	$0.020\ (0.001_6)$	NS
0.50	0.028 (0.034)	0.040 (0.017)	NS
0.75	0.017 (0.017)	$0.064\ (0.006_4)$	NS
1	0.006_7	0.074 (0.020)	*
2	0.015 (0.017)	0.081 (0.044)	NS
3	$0.010\ (0.009_7)$	0.077 (0.043)	NS
4	$0.009_3\ (0.009_3)$	0.063 (0.026)	NS
6	$0.006_8\ (0.007_9)$	$0.041\ (0.003_8)$	NS
8	$0.005_5\ (0.006_5)$	$0.034\ (0.005_8)$	NS
12	$0.003_3\ (0.004_0)$	$0.022\ (0.001_8)$	NS
18	$0.001_1\ (0.001_9)$	0.015	*
24	0	$0.009_2\ (0.002_4)$	*
36	0	$0.002_8\ (0.000_5)$	<0.02

Table 68. Experiments 2 (A, B, C) and 6 (A, B, C). Average Pharmacokinetic Parameter Values of NORDAAM after 0.6 mg/Kg Intravenous Doses and 2 mg/Kg Oral Doses of DAAM.

	Average Value		
Parameter	I.V.	P.O.	p
β (hr^{-1})	0.14 (0.023)	0.087 (0.008_8)	<0.1
$t_{1/2,\beta}$ (hr)	4.97 (0.76)	8.01 (0.83)	<0.1
$AUC^{0\rightarrow\infty}$ (μg hr/ml)	0.13 (0.14)	0.85 (0.13)	NS
C_{max} (μg/ml)	0.032 (0.032)	0.088 (0.035)	NS
t_{max} (hr)	0.28 (0.21)	1.25 (0.66)	NS

Table 69. Experiments 2 (A, B, C) and 6 (A, B, C). Average Plasma Concentrations of DINORDAAM after 0.6 mg/Kg Intravenous Doses and 2 mg/Kg Oral Doses of DAAM.

	Average Plasma DINORDAAM (μg/ml)		
t (hr)	I.V.	P.O.	p
0	0	0	-
0.083	0.005_7 (0.005_1)	-	-
0.25	0.006_4 (0.001_0)	0.012 (0.008_9)	NS
0.50	0.005_5 (0.000_6)	0.022 (0.014)	NS
0.75	0.003_4 (0.002_9)	0.028 (0.017)	NS
1	0	0.038 (0.027)	*
2	0	0.049 (0.042)	NS
3	0	0.040 (0.028)	NS
4	0	0.033 (0.021)	NS
6	0	0.028 (0.022)	NS
8	0	0.023 (0.017)	NS
12	0	0.015 (0.009_1)	NS
18	0	0.007_8	*
24	0	0.004_8 (0.004_7)	NS

Table 70. Experiments 2 (A, B, C) and 6 (A, B, C). Average Pharmacokinetic Parameter Values of DINORDAAM after 0.6 mg/Kg Intravenous Doses and 2 mg/Kg Oral Doses of DAAM.

	Average Value		
Parameter	I.V.	P.O.	p
C_{max} (µg/ml)	0.007_8 (0.001_7)	0.049 (0.042)	NS
t_{max} (hr)	0.14 (0.098)	2.17 (0.29)	<0.005

e. Extent of NORLAAM Formation from LAAM

With the data from the experiments conducted, the fraction of absorbed LAAM metabolized to NORLAAM, f_m', can be calculated by any one of the four methods described below.

i. $AUC_{LAAM}^{LAAM\ (po)}$ vs. $AUC_{NORLAAM}^{LAAM\ (po)}$

A pharmacokinetic model for orally administered LAAM, specifically designed to include first-pass metabolism in the liver, is presented in Appendix D. Equations were derived to describe the areas under plasma level-time curves of both LAAM and NORLAAM.

$$AUC_{LAAM}^{LAAM\ (po)} = \frac{fFD_1}{V_1 k_{10}} \qquad \text{(Eq. 41)}$$

$$AUC_{NORLAAM}^{LAAM\ (po)} = \frac{(339/353)FD_1 f_m'}{V_3 k_{30}} \qquad \text{(Eq. 42)}$$

D_1 is the dose of LAAM. Equations 41 and 42 are combined to give Equation 43.

$$f_m' = \left(\frac{AUC_{NORLAAM}^{LAAM\ (po)}}{AUC_{LAAM}^{LAAM\ (po)}}\right)\left(\frac{353}{339}\right)\left(\frac{V_3 k_{30}}{V_1 k_{10}}\right) f \qquad \text{(Eq. 43)}$$

The values for areas are available from Experiment 5 (A, C), $V_1 k_{10}$ from Experiment 1 (A, B, C) and $V_3 k_{30}$ from Experiment 3 (A, B, C). Numerical values of f are not known, but approximations can be made by using the fF

values calculated on p. 214, i.e., assuming F = 1. This is rationalized in terms of the reported good absorption of orally dosed LAAM (5, 12, 34, 39).

ii. $\underline{AUC_{LAAM}^{LAAM\ (iv)}}$ vs. $\underline{AUC_{NORLAAM}^{LAAM\ (iv)}}$

By analogy with Equations 29 and 30, the area under the LAAM plasma level curve after an intravenous dose of LAAM is

$$AUC_{LAAM}^{LAAM\ (iv)} = \frac{D_1}{V_1 k_{10}} \qquad \text{(Eq. 44)}$$

and the AUC of NORLAAM after intravenous LAAM is

$$AUC_{NORLAAM}^{LAAM\ (iv)} = \frac{(339/353)D_1 f_m'}{V_3 k_{30}} \qquad \text{(Eq. 45)}$$

Combining Equations 44 and 45 yields the expression for f_m'.

$$f_m' = \left(\frac{AUC_{NORLAAM}^{LAAM\ (iv)}}{AUC_{LAAM}^{LAAM\ (iv)}}\right)\left(\frac{353}{339}\right)\left(\frac{V_3 k_{30}}{V_1 k_{10}}\right) \qquad \text{(Eq. 46)}$$

Numerical values of the parameters were provided by Experiments 1 (A, B, C) and 3 (A, B, C). Since it is impossible to estimate the $AUC^{0\to\infty}$ values of NORLAAM after intravenous LAAM without an accurate definition of β, close approximations were made by using values of $AUC^{0\to 72\ hr}$ in these

calculations[a].

iii. $\frac{AUC_{NORLAAM}^{NORLAAM\ (iv)}}{}$ vs. $\frac{AUC_{NORLAAM}^{LAAM\ (iv)}}{}$

Expressions for these two area terms were given in Equations 29 and 45. Combination of these yields Equation 47.

$$f_m' = \left(\frac{AUC_{NORLAAM}^{LAAM\ (iv)}}{AUC_{NORLAAM}^{NORLAAM\ (iv)}}\right)\left(\frac{353}{339}\right)\left(\frac{D_n}{D_1}\right) \qquad \text{(Eq. 47)}$$

The areas were again determined by trapezoidal rule from zero to 72 hours[b].

iv. $\frac{AUC_{NORLAAM}^{NORLAAM\ (iv)}}{}$ vs. $\frac{AUC_{NORLAAM}^{LAAM\ (po)}}{}$

Equations 29 and 42 described these two areas. Solving them simultaneously resulted in Equation 48.

$$f_m' = \left(\frac{AUC_{NORLAAM}^{LAAM\ (po)}}{AUC_{NORLAAM}^{NORLAAM\ (iv)}}\right)\left(\frac{353}{339}\right)\left(\frac{D_n}{D_1}\right)\left(\frac{1}{F}\right) \qquad \text{(Eq. 48)}$$

By assuming F = 1, values of f_m' can be calculated for Dogs A and C, for which oral LAAM data were available.

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[a] $AUC^{0\rightarrow 72\ hr}$ for LAAM after intravenous LAAM: Dog A = 0.32, Dog B = 0.34, Dog C = 0.47.
$AUC^{0\rightarrow 72\ hr}$ for NORLAAM after intravenous LAAM: Dog A = 0.29, Dog B = 0.20, Dog C = 0.23.

[b] $AUC^{0\rightarrow 72\ hr}$ for NORLAAM after intravenous NORLAAM: Dog A = 0.88, Dog B = 0.49, Dog C = 0.95.

The resulting four sets of f_m' values are listed as follows:

	f_m'			
Dog	i. Eq. 43	ii. Eq. 46	iii. Eq. 47	iv. Eq. 48
A	0.37	0.35	0.33	0.35
B	-	0.48	0.39	-
C	0.21	0.28	0.29	0.21

The four methods yielded almost identical results for each dog, which suggests that the presumption made in methods i and iv, that F = 1, is probably correct. Although the f_m' values from the three dogs are similar and appear to be realistic, it must be realized that the same tenuous assumptions regarding the constancy of distribution volumes and microscopic rate constants as described for f_m (p. 181) have been made here. Nevertheless, the calculated values of f_m and f_m' indicate that the conversion of LAAM to NORLAAM is less efficient than that of NORLAAM to DINORLAAM. On the other hand, LAAM was shown to have a shorter biological half-life than NORLAAM. This can be explained by the existence of other routes of elimination of LAAM, such as renal excretion or formation of metabolites other than NORLAAM.

4. Multiple Dose Studies of LAAM and DAAM

a. Multiple Oral Administration of LAAM

After repeated oral dosing for two weeks, the pharmacokinetics of LAAM, as well as its nor- and dinor-metabolites, were generally unchanged. Although there seemed to be a slight trend toward faster elimination and shorter half-lives of all three compounds, a phenomenon which has been noticed previously in monkeys (46, 74), its significance cannot be tested due to the limited data, and its effect was so small that, intuitively, there appears to be no metabolic enzyme induction or inhibition of any of the compounds during multiple dosing. Unfortunately, these experiments did not provide sufficient information to discuss the possibility of compensating changes. Further evidence of negligible change of kinetics is provided by the similar numerical values of $AUC^{0\rightarrow\infty}$ after the first dose and $AUC^{0\rightarrow\tau}$ after the final dose, as shown in Table 42. There is also an excellent agreement between the C_{max} observed after the final dose and C^{∞}_{max} values predicted from the pharmacokinetic interpretations of both single and repeated dosing data.

According to C^{∞}_{max} values predicted using Equations 15 and 16, and the observed plasma levels in Figures 24 and 25, there is no accumulation of the parent drug and very little accumulation of the metabolites. A lower C_{max} was actually obtained for LAAM after the final dose, which is

consistent with the slightly shorter half-life. Although previous investigators have confirmed the absence of accumulation of LAAM (38, 46, 48, 75), significant accumulation of both NORLAAM and DINORLAAM occurred after repeated oral doses of LAAM (38, 48). In one study (48), the authors reported a five- to tenfold increase in NORLAAM and DINORLAAM plasma levels after chronic administration of LAAM to human subjects. However, the LAAM doses in that study were given three times a week, and reported half-lives were 31 hr for NORLAAM and over 100 hr for DINORLAAM, yielding predicted accumulation factors of ca 1.5 for NORLAAM and 3.4 for DINORLAAM. It is therefore obvious that the degree of accumulation of the drug or metabolites depends heavily on the dosage interval:half-life relations.

b. Multiple Oral Administration of DAAM

Based on the results shown in Figures 26 and 27 and Table 43, almost the same can be said about drug and metabolite pharmacokinetics after repeated doses of DAAM as those of LAAM. Although some differences existed between the parameter values of the two dogs, numerical values within the same animal were consistent between the first and the final doses.

Predicted R values for metabolites were somewhat less than those for the l- isomers, primarily because of the shorter half-lives of the d- forms. Only slight

accumulation of the metabolites was observed. This is similar to the situation with LAAM. There was again excellent agreement between observed and predicted C^{∞}_{max} values of all compounds.

The predictability of C^{∞}_{max} values in the present study provided excellent evidence of the validity of the pharmacokinetic models used to describe the plasma levels in single and repeated dose experiments. If the ε values in this study are applied to man, one may reasonably predict that a longer dosage interval than that currently used would be recommended. From a pharmacokinetic point of view, this is a more appropriate and more desirable dosage regimen since it avoids the possibility of toxic accumulations of drug or metabolites often observed in chronic LAAM therapy. The major objection to this argument, perhaps, is that there may be times between doses when plasma levels of active drug or metabolites are too low to be therapeutically effective, thus risking the occurrence of narcotic withdrawal symptoms. Further studies need to be conducted in patients addicted to narcotics in order to answer such questions and also to achieve optimum LAAM therapy.

V. CONCLUSIONS

An important route of elimination of LAAM, DAAM and their metabolites is via the feces (38, 39, 40), and any pharmacokinetic profiles of these compounds will not be truly complete without inclusion of the fecal data. Nevertheless, sufficient information has been generated here from plasma level data alone to answer the questions outlined in this study. Following is a brief summary of the results and observations:

1. The pharmacokinetics of LAAM, DAAM and their N-demethylated metabolites after intravenous doses to dogs can be adequately described by a two compartment open model.

2. Following intravenous administration, LAAM, NORLAAM and DINORLAAM distributed rapidly into extremely large physiological spaces. Apparent distribution volumes at steady state were 1260, 1260 and 500% body wt for LAAM, NORLAAM and DINORLAAM, respectively, indicating extensive tissue uptake of all three compounds.

3. Average biological half-lives were 6.6 hr for LAAM, 12 hr for NORLAAM and 3.6 hr for DINORLAAM, obtained from separate intravenous administration of each

compound. The interpretation of DINORLAAM half-life in the literature (27, 47, 48), based on DINORLAAM plasma levels following LAAM doses, was shown to be incorrect.

4. After intravenous administration of LAAM, very low, fluctuating but persistent plasma levels of NORLAAM were observed. This appears to be because of rapid distribution of LAAM into a large distribution volume before it can be metabolized. The drug then re-enters slowly into the central compartment and becomes metabolized to sustain the NORLAAM levels. Higher plasma levels of NORLAAM and DINORLAAM were obtained after oral doses of LAAM, probably because of rapid metabolism of LAAM in the liver during first-pass, before entering into the systemic circulation and tissue volumes.

5. Although LAAM had a shorter half-life than NORLAAM, the conversion of LAAM to NORLAAM appeared to be less efficient than that of NORLAAM to DINORLAAM. Hence, other metabolic or excretory processes might be playing an important role in the elimination of the parent drug.

6. After intravenous administration, DAAM had a steady state distribution volume of ca 300% body wt. Its half-life (ca 3.5 hr) was much shorter than that of LAAM, but increased significantly (to ca 6.2 hr) after the drug was dosed orally. The half-life of NORDAAM showed a similar increase from 5 hr after intravenous DAAM to 8 hr after orally administered DAAM. The reason for such changes in half-life values is not clear, but may be associated with biliary recycling or continual absorption of the drug from the gastrointestinal tract.

7. LAAM and DAAM showed no stereoisomeric differences in their absorption characteristics, both compounds being well absorbed from the gastrointestinal tract. Fraction of dose absorbed, F, was shown to be close to unity for LAAM, and is assumed to have a similar numerical value for DAAM. However, the d- and l-isomers differed in the extent of first-pass metabolism. Values of f ranged between 21 and 40% for LAAM and 10 and 28% for DAAM, probably indicating more efficient hepatic extraction of DAAM than LAAM. The more rapid elimination of DAAM may therefore have resulted from differences in the metabolism as well as distribution characteristics of the two isomers.

8. After oral administration of LAAM and DAAM, observed half-life values of NORLAAM and NORDAAM were 13 and 8 hr, respectively, while those of DINORLAAM and DINORDAAM were 12 and 9 hr, respectively. The similarities between these values for nor- and dinor- metabolites suggest that DINORDAAM may resemble DINORLAAM in having a shorter true half-life than the nor- metabolite. Hence, the rate of elimination of the nor- metabolites of both LAAM and DAAM appears to be the limiting factor in the loss of both the nor- and dinor- metabolites. The elimination rate of the nor- metabolite is probably responsible, in part at least, for the time course of observed pharmacologic activity.

9. The longer half-lives of both LAAM and NORLAAM than those of the corresponding d- isomers may partially reflect the different time courses of pharmacologic effects of DAAM and LAAM.

10. The pharmacokinetics of LAAM and DAAM, as well as their nor- and dinor- metabolites, were virtually unchanged by repeated dosing of the parent drugs. Although a slightly shortened half-life was observed after multiple doses in some cases, the difference between first and final dose data was too small to

suggest either metabolic enzyme induction or increased hepatic blood flow. Values of $AUC^{0\rightarrow\infty}$ after initial doses were almost identical to those of $AUC^{0\rightarrow\tau}$ after the final dose. There was also excellent agreement between the observed and predicted C^{∞}_{max} values.

11. The dosage regimen employed for LAAM and DAAM during repeated dosing in dogs resulted in no accumulation of the parent drugs, and very little accumulation of the metabolites. Similar dosage interval:half-life relations may be adapted for use in man, in order to avoid the possibility of toxic accumulation of drug or metabolites often observed in LAAM maintenance therapy.

It should be pointed out, however, that any arguments and conclusions made in this study must be qualified by the small number of dogs tested. Before being applied to any clinical decisions, these data would need to be confirmed in a larger group of animals. The validity of using the dog as a test model for man also needs to be proven.

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VII. APPENDICES

APPENDIX A

Glossary of Terms

A_1 Amount of administered compound in the central compartment.

A_2 Amount of administered compound in the tissue compartment.

A_3 Amount of NORLAAM or NORDAAM in the central compartment.

A_4 Amount of NORLAAM or NORDAAM in the tissue compartment.

A_5 Amount of DINORLAAM or DINORDAAM in the central compartment.

A_6 Amount of DINORLAAM or DINORDAAM in the tissue compartment.

AUC Area under plasma level vs. time curve.

$AUC^{t_1 \rightarrow t_2}$ AUC from time = t_1 to time = t_2.

$AUC_a^{b(c)}$ AUC of compound 'a' after administering drug 'b' via route 'c'.

α Composite first-order rate constant defined by Eq. 2 and represents the distribution phase of a drug obeying two compartment pharmacokinetics.

α_d α of DINORLAAM.

α_l α of LAAM.

α_n α of NORLAAM.

β Composite first-order rate constant defined by Eq. 2 and represents the post-distributive elimination phase of a drug obeying two compartment pharmacokinetics.

β of DINORLAAM.

β of LAAM.

β of NORLAAM.

Observed plasma concentration of drug or metabolite.

Concentration of administered compound in the central or plasma compartment.

Concentration of NORLAAM or NORDAAM in the central or plasma compartment.

Concentration of DINORLAAM or DINORDAAM in the central or plasma compartment.

Concentration of the final data point on a plasma level vs. time profile.

$C_1)_n$ Concentration of administered compound in the central compartment after the n^{th} dose during repeated dosing.

$C_1)_\infty$ Concentration of administered compound in the central compartment during a dosing interval at steady state.

max Highest observed drug or metabolite concentration in plasma.

1 max Highest observed drug or metabolite concentration in plasma after the first dose during repeated dosing.

C^{∞}_{max} Highest observed drug or metabolite concentration in plasma at steady state during repeated dosing.

C^{1}_{min} Minimum concentration of drug or metabolite in plasma after the first dose during repeated dosing.

C^{∞}_{min} Minimum concentration of drug or metabolite in plasma at steady state during repeated dosing.

D Dose of administered compound.

D_d Dose of DINORLAAM.

D_l Dose of LAAM.

D_n Dose of NORLAAM.

ε Dosage interval (τ):half-life ratio of drug or metabolite.

F Fraction of dose D of an orally administered compound absorbed from the gastrointestinal tract.

f Fraction of absorbed oral dose of a compound which reaches the systemic circulation in unchanged form.

fF Fraction of dose D of an orally administered compound appearing unchanged in the systemic circulation.

f_m Fraction of intravenous dose of NORLAAM metabolized to DINORLAAM.

f_m' Fraction of absorbed LAAM metabolized to NORLAAM.

k_a Apparent first-order rate constant of absorption of an orally administered compound obeying two compartment pharmacokinetics.

k_{12} Apparent first-order rate constant governing the transfer of an administered compound from the central compartment into the tissue compartment.

k_{21} Apparent first-order rate constant governing the transfer of an administered compound from the tissue compartment into the central compartment.

k_{1m} Apparent first-order rate constant governing metabolism of LAAM or DAAM to the nor-metabolite.

k_{1e} Apparent first-order rate constant governing all excretory and metabolic processes of LAAM or DAAM except metabolism to form the nor-metabolite.

k_{10} Apparent first-order rate constant governing the loss of an administered compound from the central compartment by all elimination processes.

k_{34} Apparent first-order rate constant governing the transfer of NORLAAM or NORDAAM from the central compartment into the tissue compartment.

k_{43} Apparent first-order rate constant governing the transfer of NORLAAM or NORDAAM from the tissue compartment into the central compartment.

k_{3m} Apparent first-order rate constant governing metabolism of NORLAAM or NORDAAM to the dinor-metabolite.

k_{3e} Apparent first-order rate constant governing all excretory and metabolic processes of NORLAAM or NORDAAM except metabolism to form the dinor-metabolite.

k_{30} Apparent first-order rate constant governing the loss of NORLAAM or NORDAAM from the central compartment by all elimination processes, i.e., $k_{3m} + k_{3e}$.

k_{56} Apparent first-order rate constant governing the transfer of DINORLAAM or DINORDAAM from the central compartment into the tissue compartment.

k_{65} Apparent first-order rate constant governing the transfer of DINORLAAM or DINORDAAM from the tissue compartment into the central compartment.

k_{50} Apparent first-order rate constant governing the loss of DINORLAAM or DINORDAAM from the central compartment by all elimination processes.

R Accumulation factor of drug or metabolite during repeated dosing.

t Time after administration of a compound.

t'	Time when the final data point on a plasma level *vs*. time profile was taken.
$t_{1/2,abs}$	Absorption half-life of an orally administered compound obeying two compartment pharmacokinetics.
$t_{1/2,\alpha}$	Half-life of the distribution phase of a compound obeying two compartment pharmacokinetics.
$t_{1/2,\beta}$	Biological half-life of a compound obeying two compartment pharmacokinetics.
t_{max}	Time after dosing when C_{max} of drug or metabolite was obtained.
τ	Time interval between consecutive doses of a compound in a multiple dose regimen.
V_1	Apparent volume of the central compartment occupied by an administered compound obeying two compartment pharmacokinetics.
V_3	Apparent volume of the central compartment occupied by NORLAAM or NORDAAM.
V_5	Apparent volume of the central compartment occupied by DINORLAAM or DINORDAAM.
V_1k_{10}	Plasma clearance of an administered compound obeying two compartment pharmacokinetics.
V_3k_{30}	Plasma clearance of NORLAAM or NORDAAM.
V_5k_{50}	Plasma clearance of DINORLAAM or DINORDAAM.

V_{dss} Apparent volume of distribution at steady state of an administered compound obeying two compartment pharmacokinetics.

APPENDIX B

Equations for Scheme VII Describing The Amount of NORLAAM and DINORLAAM in Plasma and Tissue Compartments as a Function of Time After Intravenous Administration of NORLAAM

The differential equations B1-B4 describe the rates of change of amounts of NORLAAM or DINORLAAM in the various compartments as a function of time.

$$\frac{dA_3}{dt} = -(k_{34} + k_{3e} + k_{3m})A_3 + k_{43}A_4 \qquad \text{(Eq. B1)}$$

$$\frac{dA_4}{dt} = k_{34}A_3 - k_{43}A_4 \qquad \text{(Eq. B2)}$$

$$\frac{dA_5}{dt} = k_{3m}A_3 - (k_{56} + k_{50})A_5 + k_{65}A_6 \qquad \text{(Eq. B3)}$$

$$\frac{dA_6}{dt} = k_{56}A_5 - k_{65}A_6 \qquad \text{(Eq. B4)}$$

Application of the Laplace transform to these linear differential equations converts them into linear algebraic equations. The Laplace transform of a time dependent function f(t) is L[f(t)], or F(s), as defined by Equation B5,

$$F(s) = \int_0^\infty e^{-st} f(t)dt \qquad \text{(Eq. B5)}$$

where s is a real number such that the integral converges for some finite value of s and all greater values. The Laplace transforms of common functions in pharmacokinetics do not have to be calculated with Equation B5 since they are tabulated in Laplace transform tables (76).

The Laplace transforms of Equations B1-B4 are

$$(s + k_{34} + k_{3e} + k_{3m})\bar{A}_3 + (-k_{43})\bar{A}_4 = D \qquad \text{(Eq. B6)}$$

$$(-k_{34})\bar{A}_3 + (k_{43} + s)\bar{A}_4 = 0 \qquad \text{(Eq. B7)}$$

$$(-k_{3m})\bar{A}_3 + (s + k_{56} + k_{50})\bar{A}_5 + (-k_{65})\bar{A}_6 = 0 \qquad \text{(Eq. B8)}$$

$$(-k_{56})\bar{A}_5 + (s + k_{65})\bar{A}_6 = 0 \qquad \text{(Eq. B9)}$$

where the bar represents the Laplace transform of that quantity and D is the dose of NORLAAM. Equations B6-B9 can be solved for $\bar{A}_3$, $\bar{A}_4$, $\bar{A}_5$ and $\bar{A}_6$ by matrix algebra utilizing Cramer's rule. The matrix of coefficients is

$$\Delta = \begin{vmatrix} (s + k_{34} + k_{3e} + k_{3m}) & -k_{43} & 0 & 0 \\ -k_{34} & (s + k_{43}) & 0 & 0 \\ -k_{3m} & 0 & (s + k_{56} + k_{50}) & -k_{65} \\ 0 & 0 & -k_{56} & (s + k_{65}) \end{vmatrix} \qquad \text{(Eq. B10)}$$

which upon expansion becomes

$$\Delta = [s^2 + (k_{34} + k_{43} + k_{3e} + k_{3m})s + k_{43}(k_{3e} + k_{3m})][s^2 + (k_{56} + k_{65} + k_{50})s + k_{65}k_{50}] \qquad \text{(Eq. B11)}$$

If α_n and β_n are defined as

$$\begin{matrix}\alpha_n \\ \beta_n\end{matrix} = \frac{1}{2}[(k_{34} + k_{43} + k_{3e} + k_{3m}) \pm$$

$$\sqrt{(k_{34} + k_{43} + k_{3e} + k_{3m})^2 - 4k_{43}(k_{3e} + k_{3m})}] \quad \text{(Eq. B12)}$$

and α_d and β_d as

$$\begin{matrix}\alpha_d \\ \beta_d\end{matrix} = \frac{1}{2}[(k_{56} + k_{65} + k_{50}) \pm$$

$$\sqrt{(k_{56} + k_{65} + k_{50})^2 - 4k_{65}k_{50}}] \quad \text{(Eq. B13)}$$

Then Equation B11 reduces to Equation B14.

$$\Delta = (s + \alpha_n)(s + \beta_n)(s + \alpha_d)(s + \beta_d) \quad \text{(Eq. B14)}$$

The $\overline{A}_3$ matrix is

$$\Delta\overline{A}_3 = \begin{vmatrix} D & -k_{43} & 0 & 0 \\ 0 & (k_{43} + s) & 0 & 0 \\ 0 & 0 & (s + k_{56} + k_{50}) & -k_{65} \\ 0 & 0 & -k_{56} & (s + k_{65}) \end{vmatrix} \quad \text{(Eq. B15)}$$

which can be expanded to

$$\Delta\overline{A}_3 = D(k_{43} + s)(s + \alpha_d)(s + \beta_d) \quad \text{(Eq. B16)}$$

By Cramer's Rule,

$$\bar{A}_3 = \frac{\Delta \bar{A}_3}{\Delta} \qquad \text{(Eq. B17)}$$

or

$$\bar{A}_3 = \frac{D(k_{43} + s)}{(s + \alpha_n)(s + \beta_n)} \qquad \text{(Eq. B18)}$$

The anti-Laplace of Equation B18, found in tables (76), is

$$A_3 = \frac{(Dk_{43} - D\alpha_n)e^{-\alpha_n t} - (Dk_{43} - D\beta_n)e^{-\beta_n t}}{\beta_n - \alpha_n} \qquad \text{(Eq. B19)}$$

which rearranges to Equation B20, i.e., text Equation 23, which describes the amount of NORLAAM in the plasma or central compartment as a function of time.

$$A_3 = \frac{D}{\beta_n - \alpha_n}[(k_{43} - \alpha_n)e^{-\alpha_n t} - (k_{43} - \beta_n)e^{-\beta_n t}] \qquad \text{(Eq. B20)}$$

Utilizing a similar development (Equations B21-B23) yields Equation B24 which describes the amount of NORLAAM in the tissue compartment as a function of time, i.e., text Equation 24.

$$\Delta \bar{A}_4 = \begin{vmatrix} (s + k_{34} + k_{3e} + k_{3m}) & D & 0 & 0 \\ -k_{34} & 0 & 0 & 0 \\ -k_{3m} & 0 & (s + k_{56} + k_{50}) & -k_{65} \\ 0 & 0 & -k_{56} & (s + k_{65}) \end{vmatrix} \qquad \text{(Eq. B21)}$$

$$\Delta\bar{A}_4 = Dk_{34}(s + \alpha_d)(s + \beta_d) \qquad \text{(Eq. B22)}$$

$$\bar{A}_4 = \frac{\Delta\bar{A}_4}{\Delta} = \frac{Dk_{34}}{(s + \alpha_n)(s + \beta_n)} \qquad \text{(Eq. B23)}$$

$$A_4 = \frac{k_{34}D}{\beta_n - \alpha_n}(e^{-\alpha_n t} - e^{-\beta_n t}) \qquad \text{(Eq. B24)}$$

Similarly,

$$\Delta\bar{A}_5 = \begin{vmatrix} (s + k_{34} + k_{3e} + k_{3m}) & -k_{43} & D & 0 \\ -k_{34} & (k_{43} + s) & 0 & 0 \\ -k_{3m} & 0 & 0 & -k_{65} \\ 0 & 0 & 0 & (s + k_{65}) \end{vmatrix} \qquad \text{(Eq. B25)}$$

$$= Dk_{3m}(k_{43} + s)(s + k_{65}) \qquad \text{(Eq. B26)}$$

$$\bar{A}_5 = \frac{\Delta\bar{A}_5}{\Delta} = \frac{Dk_{3m}(k_{43} + s)(s + k_{65})}{(s + \alpha_n)(s + \beta_n)(s + \alpha_d)(s + \beta_d)} \qquad \text{(Eq. B27)}$$

$$= Dk_{3m}\left[\frac{(k_{43} - \alpha_n)(k_{65} - \alpha_n)}{(\beta_n - \alpha_n)(\alpha_d - \alpha_n)(\beta_d - \alpha_n)(s + \alpha_n)} + \frac{(k_{43} - \beta_n)(k_{65} - \beta_n)}{(\alpha_n - \beta_n)(\alpha_d - \beta_n)(\beta_d - \beta_n)(s + \beta_n)} + \frac{(k_{43} - \alpha_d)(k_{65} - \alpha_d)}{(\alpha_n - \alpha_d)(\beta_n - \alpha_d)(\beta_d - \alpha_d)(s + \alpha_d)} + \frac{(k_{43} - \beta_d)(k_{65} - \beta_d)}{(\alpha_n - \beta_d)(\beta_n - \beta_d)(\alpha_d - \beta_d)(s + \beta_d)}\right] \qquad \text{(Eq. B28)}$$

By taking anti-Laplace, and inserting the ratio of molecular weights of DINORLAAM:NORLAAM (325/339) in order to convert D into its equivalent dose of DINORLAAM, the amount of DINORLAAM in the central compartment as a function of time is described by Equation B29, i.e., text Equation 25.

$$A_5 = \frac{325}{339}\cdot D\cdot k_{3m}\left[\frac{(k_{43}-\alpha_n)(k_{65}-\alpha_n)}{(\beta_n-\alpha_n)(\alpha_d-\alpha_n)(\beta_d-\alpha_n)}\cdot e^{-\alpha_n t} + \frac{(k_{43}-\beta_n)(k_{65}-\beta_n)}{(\alpha_n-\beta_n)(\alpha_d-\beta_n)(\beta_d-\beta_n)}\cdot e^{-\beta_n t} + \frac{(k_{43}-\alpha_d)(k_{65}-\alpha_d)}{(\alpha_n-\alpha_d)(\beta_n-\alpha_d)(\beta_d-\alpha_d)}\cdot e^{-\alpha_d t} + \frac{(k_{43}-\beta_d)(k_{65}-\beta_d)}{(\alpha_n-\beta_d)(\beta_n-\beta_d)(\alpha_d-\beta_d)}\cdot e^{-\beta_d t}\right] \qquad \text{(Eq. B29)}$$

Following the same procedure, the amount of DINORLAAM in the tissue compartment as a function of time is described by Equation B33, i.e., text Equation 26.

$$\Delta\bar{A}_6 = \begin{vmatrix} (s + k_{34} + k_{3e} + k_{3m}) & -k_{43} & 0 & D \\ -k_{34} & (k_{43} + s) & 0 & 0 \\ -k_{3m} & 0 & (s + k_{56} + k_{50}) & 0 \\ 0 & 0 & -k_{56} & 0 \end{vmatrix} \qquad \text{(Eq. B30)}$$

$$= Dk_{3m}k_{56}(s + k_{43}) \qquad \text{(Eq. B31)}$$

$$\overline{A}_6 = Dk_{3m}k_{56}\left[\frac{(k_{43} - \alpha_n)}{(\beta_n - \alpha_n)(\alpha_d - \alpha_n)(\beta_d - \alpha_n)(s + \alpha_n)} + \right.$$

$$\frac{(k_{43} - \beta_n)}{(\alpha_n - \beta_n)(\alpha_d - \beta_n)(\beta_d - \beta_n)(s + \beta_n)} +$$

$$\frac{(k_{43} - \alpha_d)}{(\alpha_n - \alpha_d)(\beta_n - \alpha_d)(\beta_d - \alpha_d)(s + \alpha_d)} +$$

$$\left.\frac{(k_{43} - \beta_d)}{(\alpha_n - \beta_d)(\beta_n - \beta_d)(\alpha_d - \beta_d)(s + \beta_d)}\right] \qquad \text{(Eq. B32)}$$

$$A_6 = \frac{325}{339} \cdot Dk_{3m}k_{56}\left[\frac{(k_{43} - \alpha_n)}{(\beta_n - \alpha_n)(\alpha_d - \alpha_n)(\beta_d - \alpha_n)} \cdot e^{-\alpha_n t} + \right.$$

$$\frac{(k_{43} - \beta_n)}{(\alpha_n - \beta_n)(\alpha_d - \beta_n)(\beta_d - \beta_n)} \cdot e^{-\beta_n t} +$$

$$\frac{(k_{43} - \alpha_d)}{(\alpha_n - \alpha_d)(\beta_n - \alpha_d)(\beta_d - \alpha_d)} \cdot e^{-\alpha_d t} +$$

$$\left.\frac{(k_{43} - \beta_d)}{(\alpha_n - \beta_d)(\beta_n - \beta_d)(\alpha_d - \beta_d)} \cdot e^{-\beta_d t}\right] \qquad \text{(Eq. B33)}$$

APPENDIX C

Area Under the Concentration-Time Curve of DINORLAAM after Intravenous Administration of NORLAAM

The area under the plasma level-time curve of DINORLAAM after intravenous dosing of NORLAAM may be obtained by integrating text Equation 28 from zero to infinite time. This is a tedious task, requiring 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 Laplace transforms as recently described by Wagner (70). From the definition of Laplace transform,

$$L[f(t)] = \int_0^\infty e^{-st} f(t)dt \qquad \text{(Eq. C1)}$$

it is noticed that a time dependent function can be integrated between the limits of zero and infinite time simply by setting s equal to zero in the Laplace transformed equation, i.e.,

$$L[f(t)]\Big|_{s=0} = \int_0^\infty f(t)dt \qquad \text{(Eq. C2)}$$

By first converting Equation B27 to the concentration form, i.e., dividing it by V_5, the AUC for DINORLAAM can be written as follows,

$$AUC^{NORLAAM\ (iv)}_{DINORLAAM} = \left.\frac{Dk_{3m}(k_{43} + s)(k_{65} + s)}{V_5(s + \alpha_n)(s + \beta_n)(s + \alpha_d)(s + \beta_d)}\right|_{s=0} \qquad \text{(Eq. C3)}$$

which, after correction for DINORLAAM dose, yields Equation C4.

$$AUC^{NORLAAM\ (iv)}_{DINORLAAM} = \frac{(325/339)Dk_{3m}k_{43}k_{65}}{V_5\alpha_n\beta_n\alpha_d\beta_d} \qquad \text{(Eq. C4)}$$

By analogy to text Equation 4, it is obvious that $k_{30} = \alpha_n\beta_n/k_{43}$ and $k_{50} = \alpha_d\beta_d/k_{65}$, which, when substituted into Equation C4, gives Equation C5

$$AUC^{NORLAAM\ (iv)}_{DINORLAAM} = \frac{(325/339)Dk_{3m}}{V_5k_{30}k_{50}} \qquad \text{(Eq. C5)}$$

Since k_{3m}/k_{30} equals the fraction of NORLAAM dose metabolized to DINORLAAM, f_m, Equation C5 then becomes Equation C6, or text Equation 30.

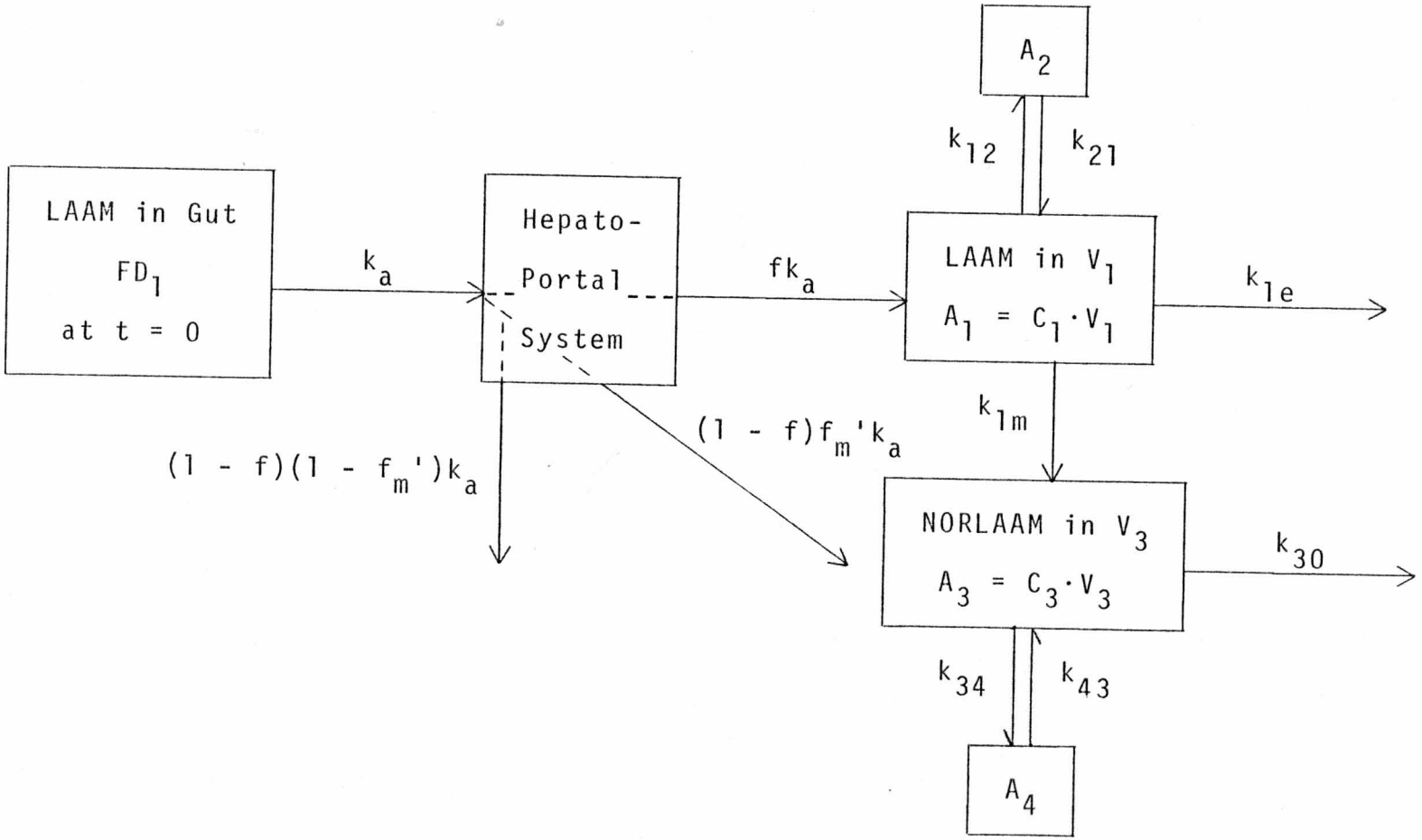
$$AUC^{NORLAAM\ (iv)}_{DINORLAAM} = \frac{(325/339)Df_m}{V_5k_{50}} \qquad \text{(Eq. C6)}$$

APPENDIX D

Pharmacokinetic Model for Oral Administration of LAAM, with Equations to Describe the Area Under the Concentration-Time Curves of LAAM and NORLAAM

Scheme DI

Pharmacokinetic Model for Oral Administration of LAAM.



The portion of this model dealing with LAAM is actually similar to text Scheme V, except it includes the hepato-portal system as an integral part of the central compartments of LAAM and NORLAAM. D_1 is the dose of LAAM administered, and F is the fraction of D_1 absorbed from the GI tract. LAAM is absorbed into the hepato-portal system with an apparent first-order rate constant k_a. A fraction, f, of the absorbed dose reaches the systemic circulation as unchanged LAAM, while the remaining fraction, (1 - f), is metabolized to NORLAAM, $(1 - f)f_m'$, and other metabolites, $(1 - f)(1 - f_m')$, prior to entering the systemic circulation. Other symbols are as defined in text Scheme VIII.

It is assumed in this model that $f_m' = k_{1m}/(k_{1m} + k_{1e})$, i.e., the fraction of absorbed LAAM metabolized to NORLAAM during the first-pass is the same as that after the drug reaches the systemic circulation. Thus, it is assumed that the liver is the only metabolic organ, and metabolism during the first-pass is not saturated (77).

The equation for the AUC of LAAM in this model has been derived (65):

$$AUC_{LAAM}^{LAAM\ (po)} = \frac{fFD_1}{V_1 k_{10}} \qquad \text{(Eq. D1)}$$

where $k_{10} = k_{1m} + k_{1e}$

The appearance of NORLAAM in the systemic circulation is via two routes. First, NORLAAM is being formed as a

biotransformation product of the portion of LAAM in the systemic circulation. The yield from this process is $(fFD_1)(f_m')(339/353)$, where (339/353) is the ratio of NORLAAM:LAAM molecular weights, and is needed since metabolism of LAAM to NORLAAM occurs on a mole to mole basis. In addition, NORLAAM is also formed during the first-pass, the amount being equal to $(FD_1)(1 - f)(f_m')(339/353)$. Since both LAAM and NORLAAM obey two compartment pharmacokinetics, the equation for the AUC of NORLAAM can be written by analogy to Equation D1. Substituting the numerator in Equation D1 with the total amount of NORLAAM appearing in the systemic circulation, and the denominator with plasma clearance of NORLAAM, yields Equation D2,

$$AUC_{NORLAAM}^{LAAM\ (po)} = \frac{fFD_1 f_m'(339/353) + FD_1(1 - f)f_m'(339/353)}{V_3 k_{30}} \qquad \text{(Eq. D2)}$$

which can be simplified to give Equation D3, i.e., text Equation 42.

$$AUC_{NORLAAM}^{LAAM\ (po)} = \frac{(339/353)FD_1 f_m'}{V_3 k_{30}} \qquad \text{(Eq. D3)}$$

APPENDIX E

Typical Computer Output Using the Program NREG

```
*  *  *  *  *  *  UNIVAC 1110 TIME/SHARING EXEC --- MULTI-PROCESSOR SYSTEM --- VER,  MACC 33.25  SITE *   MACC    *  *  *  *  *  *
RUNID: C00192        PROJECT: 05994       USER: 3965817681         CREATED ON FEB  6, 1978 AT 15:23:13    INPUT DEVICE: NCCR
          FILE NAME: PR@000C00192         PART NUMBER: 00          PRINTED ON FEB  6, 1978 AT 15:25:10   OUTPUT DEVICE: NCPR1


#RUN TSE,5994,3965817681,SM,700/500
#FOR,SI      ,MAIN
FORTRAN-MACC 1.17S-02/06/78-15:23:16            MAIN
00101       1.            PARAMETER
00102       2.           1NEXPD = 50,
00103       3.           2NVAR = 1,
00104       4.           3NPD = 7,
00105       5.           4NROBS = NEXPD,
00106       6.           5NRPRD = NEXPD,
00107       7.           6NS=(17+NEXPD*NVAR)*NPD+2*NRPRD*NVAR+2*(NPD+1)*(NPD+2)+1
00108       8.            REAL THINT(NPD),THFIN(NPD),YOBS(NROBS,NVAR),YPRD(NROBS,NVAR),S(NS)
00109       9.           1,TOL(4),T(NEXPD),BNDLO(NPD),BNDHI(NPD),NAMES(NPD),DIFF(NPD),FIX(NP
00109      10.           2D),WTS(NROBS,NVAR)
00113      11.            INTEGER ITEMS(12),INFO(7),IDENT(13),NEXP,NP
00118      12.            COMMON T
00119      13.            COMMON/LIM/NEXP1,NEXP2,K,AL,BE
00126      14.            DATA FIX/7*1.0/
00117      15.            DATA DIFF/7*0.001/
00118      16.            DATA BNDLO/7*1.0E-4/
00119      17.            DATA BNDHI/7*1.0E4/
00116      18.            DATA NAMES/6HK1    ,6HK2    ,6HKEL   ,6HDV    ,3*6H      /
00120      19.            DATA TOL/1.5E-6,1.0E-9,0.0,1.0E-16/
00122      20.            DATA INFO/1,10,5*1/
00124      21.            DATA ITEMS/
00124      22.           11,NVAR,1,NROBS,NRPRD,0,'MARQ','ALL','NREG','CENT','REL',50/
00124      23.            READ(5,110) (IDENT(I), I=1,13)
00130      24.       110  FORMAT(13A6)
00135      25.            READ (5,120) NEXP1,NEXP2,NP
00142      26.       120  FORMAT(2X,I3,2X,I3,3X,I2)
00143      27.            NEXP = NEXP1 + NEXP2
00148      28.            ITEMS(1) = NEXP
00145      29.            ITEMS(3)=NP
00146      30.            READ(5,100)( THINT(I), I=1,NP )
00150      31.            READ (5,100) (YOBS(I,1),I=1,NEXP1)
00162      32.            READ (5,100) (WTS(I,1),I=1,NEXP1)
00174      33.            READ (5,100) (T(I), I = 1,NEXP1)
00176      34.            K = NEXP1+ 1
00177      35.            IF (K.GE.NEXP) GO TO 66
00201      36.            READ (5,100) (YOBS(I,1),I=K,NEXP)
00207      37.            READ (5,100) (WTS(I,1),I=K,NEXP)
00215      38.            READ (5,100) (T(I),I=K,NEXP)
00223      39.         66 CONTINUE
00224      40.       100  FORMAT(8F10.5)
00225      41.            EXTERNAL F,DERIV
00226      42.            CALL NREG(THINT,NAMES,BNDLO,BNDHI,FIX,DIFF,THFIN,YOBS,YPRD,
00226      43.           1WTS,F,'NONE',ITEMS,TOL,INFO,IDENT,S)
00227      44.            CALL OUTPUT(NEXP,NEXPD,THFIN,NVAR,NP,NRPRD,YPRD,YOBS)
00230      45.            END

      END OF COMPILATION:        NO  DIAGNOSTICS.
#FOR,SI    , SUBF
FORTRAN-MACC 1.17S-02/06/78-15:23:21            SUBF
00101       1.            SUBROUTINE F(TH,Y,NEXP,NVAR,NP,NRPRD)
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00102       2.          DIMENSION TH(1),Y(NRPRD,1),T(1)
00103       3.          COMMON T
00104       4.          COMMON/LIM/NEXP1,NEXP2,K,AL,BE
00105       5.          TH1 = TH(1)
00106       6.          TH2 = TH(2)
00107       7.          TH3 = TH(3)
00110       8.          TH4 = TH(4)
00111       9.          AL = 0.5*((TH1+TH2+TH3)+SQRT((TH1+TH2+TH3)**2-4*TH2*TH3))
00112      10.          BE = 0.5*((TH1+TH2+TH3)-SQRT((TH1+TH2+TH3)**2-4*TH2*TH3))
00113      11.          DO 10 I = 1,NEXP1
00114      12.       10 Y(I,1) = (TH4/(AL-BE))*((TH2-BE)*EXP(-BE*T(I))-(TH2-AL)*EXP(-AL*T(
                        1I)))
00120      14.          IF( K.EQ.NEXP ) GO TO 30
00122      15.       30 CONTINUE
00123      16.          RETURN
00124      17.          END

      END OF COMPILATION:        NO  DIAGNOSTICS.
#FOR,SI      ,OTP
FORTRAN-MACC 1.175-02/06/78-15:23:26          OTP
00101       1.          SUBROUTINE OUTPUT(NEXP,NEXPD,THFIN,NVAR,NP,NRPRD,YPRD,YOBS)
00102       2.          DIMENSION T(1),YOBS(1),YPRD(1)
00103       3.          COMMON T
00104       4.          COMMON/LIM/NEXP1,NEXP2,K,AL,BE
00105       5.          K = 0
00106       6.       200 CONTINUE
00107       7.          SO2 = 0.0
00110       8.          SP2 = 0.0
00111       9.          SC = 0.0
00112      10.          SYOBS = 0.0
00113      11.          SDEV = 0.0
00114      12.          COR = 0.0
00115      13.          RS = 0.0
00116      14.          YOM = 0.0
00117      15.          YPM = 0.0
00120      16.          K = K + 1
00121      17.          IF (K.EQ.1) GO TO 210
00123      18.          LL = NEXP1 + 1
00124      19.          LU = NEXP
00125      20.          GO TO 250
00126      21.       210 LL = 1
00127      22.          LU = NEXP1
00130      23.       250 DO 10 I = LL,LU
00131      24.          SYOBS = SYOBS + YOBS(I)**2
00132      25.          SDEV = SDEV + (YOBS(I)-YPRD(I))**2
00133      26.          YOM=YOM+YOBS(I)
00134      27.          YPM=YPM+YPRD(I)
00137      28.       10 CONTINUE
00141      29.          RS = (SYOBS-SDEV)/SYOBS
00142      30.          YOM=YOM/NEXP
00143      31.          YPM=YPM/NEXP
00144      32.          DO 20 I=1,NEXP
00147      33.          SC = SC+(YOBS(I)-YOM)*(YPRD(I)-YPM)
00150      34.          SO2 = SO2 + (YOBS(I)-YOM)**2
00151      35.          SP2 = SP2 + (YPRD(I)-YPM)**2
00152      36.       20 CONTINUE
00156      37.          COR = SC/(SQRT(SO2)*SQRT(SP2))
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00155     38.            WRITE(6,100) K,RS,K,COR
00163     39.        100 FORMAT(1H0,'R-SQUARED FOR FUNCTION',I1,' =',F6.4/1X,'COR FOR FUNCT
                      1ION',I1,' =',F6.4)
00168     41.            IF (NEXP2.EQ.0) GO TO 66
0016A     42.            IF(K.GT.1) GO TO 300
00170     43.            SYOBS1 = SYOBS
00171     44.            SDEV1 = SDEV
00172     45.            GO TO 200
00173     46.        300 SYOBS = SYOBS + SYOBS1
00174     47.            SDEV = SDEV + SDEV1
00175     48.            RS = (SYOBS - SDEV)/SYOBS
00176     49.            WRITE(6,111) RS
00201     50.        111 FORMAT(1X,'OVER ALL R-SQUARED=',F6.4)
00202     51.         66 CONTINUE
00203     52.            WRITE (6,101) AL,BE
00207     53.        101 FORMAT(1X,'ALPHA = ',F10.5,10X,'BETA = ',F10.5)
00210     54.            CALL PRPL (NEXP,NEXPD,THFIN,NVAR,NP,NRPRD,YPRD,YOBS)
00211     55.            RETURN
00212     56.            END

      END OF COMPILATION:        NO  DIAGNOSTICS.
*FOR,SI      ,PRPL
FORTRAN-MACC 1.178-02/06/78-15:23:32              PRPL
00101      1.            SUBROUTINE PRPL (NEXP,NEXPD,THFIN,NVAR,NP,NRPRD,YPRD,YOBS)
00102      2.            DIMENSION T(1),YOBS(NRPRD,1),YPRD(NRPRD,1)
00103      3.            COMMON T
00104      4.            COMMON/LIM/NEXP1,NEXP2,K
00105      5.            MM = 0
00106      6.            CALL DEVSET(6HPRNTER)
00107      7.        200 CONTINUE
00110      8.            MM = MM + 1
00111      9.            IF(MM.EQ.1) GO TO 50
00113     10.            DO 140 I = 1,NEXP2
00116     11.            MN = NEXP1 + I
00117     12.            YOBS(I,1) = YOBS(MN,1)
00120     13.        140 T(I) = T(MN)
00122     14.            LU = NEXP2
00123     15.            GO TO 60
00124     16.         50 CONTINUE
00125     17.            LU = NEXP1
0012A     18.         60 CONTINUE
00127     19.            YMAX = YOBS(1,1)
00130     20.            DO 10 I = 2,LU
00131     21.         10 YMAX = AMAX1(YMAX,YOBS(I,1))
00135     22.            IF( MM.EQ.2 ) GO TO 170
00137     23.            TMAX = T(1)
00140     24.            DO 15 I = 2,NEXP
00143     25.         15 TMAX = AMAX1(TMAX,T(I))
00145     26.            BMAX = TMAX/(NEXPD-NEXP1-NEXP2)
0014A     27.            NM1 = NEXP1 + NEXP2 + 1
00147     28.            NM2 = NEXP1 + NEXP2 + 2
00150     29.            T(NM1) = 0.0
00151     30.            DO 30 I = NM2,NEXPD
00154     31.            J = I - 1
00155     32.         30 T(I) = T(J) + BMAX
00157     33.            IF ( MM.EQ.1 ) GO TO 160
00161     34.            GO TO 170
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00162      35.       160 NEX1 = NEXP1
00163      36.           NEXP1 = NEXPD
00164      37.           K = NEXPD
00165      38.           GO TO 180
00166      39.       170 CONTINUE
00167      40.           NEXP1 = 1
00170      41.           K = 1
00171      42.           NEXP = NEXPD
00172      43.       180 CONTINUE
00173      44.           CALL F(THETIN,YPRD,NEXPD,NVAR,NP,NRPRD)
00174      45.           NEXP1 = NEX1
00175      46.           DO 20 I = 1,NEXPD
00200      47.        20 YMAX = AMAX1(YMAX,YPRD(I,1))
00201      48.           YSCLE = YMAX/8.8
00202      49.           TSCLE = TMAX/8.8
00203      50.           CALL GRAPH(T,6HLINEAR,YPRD,6HLINEAR,4HEXPD,15,5HBLANK,'TIMES$','FU
00204      51.          1NCTION VALUES$',4HAXES,'PLOT OF OBSERVED,O'S AND PREDICTED,P'S
00205      52.          2VALUES$',12,0,12,0)
00206      53.           CALL GRAPH(T,'SCAL1',YOBS,'SCAL1',LU,14,5HBLANK)
00207      54.           IF (NEXP2,EQ,0) GO TO 66
00210      55.           IF(MM,EQ,1) GO TO 200
00212      56.        66 CONTINUE
00213      57.           RETURN
00214      58.           END

      END OF COMPILATION:        NO  DIAGNOSTICS.
$MAP
MAP2R-1 RLI807 02/06-15:23:39


ADDRESS LIMITS     001000 111077      36928 IBANK WORDS DECIMAL
                   112000 142461      12594 DBANK WORDS DECIMAL
STARTING ADDRESS   110655



            SEGMENT $MAIN$          001000 111077      112000 142461

NEXPIS/NAGFORFUN10          $(1)    001000 001047      $(0)    112000 112000
UR3SBH/UWCC                 $(1)    001050 001126
UR2MSK/UWCC                 $(1)    001127 001260      $(0)    112001 112026
                                                       $(2)    BLANK$COMMON
URITBI/UWCC01               $(1)    001261 001446
UESCBT/NAGUTILITY02         $(1)    001447 002266      $(0)    112027 112166
CHARSCN/PSPEL10             $(1)    002267 002373      $(0)    112167 112171
ATANS/NAGFORFUN10           $(1)    002374 002634      $(2)    112172 112231
NEXPBS/NAGFORFUN12          $(1)    002635 003064      $(2)    112232 112311
OBJTBN/GSPV03               $(1)    003065 003410      $(0)    112312 112401
                                                       $(2)    GSPCTL
GSPCBS/V74                  $(1)    003411 003444      $(0)    112402 112666
STPOGEN/GSPW87              $(1)    003445 005115      $(0)    112667 113041
                            $(3)    000000             $(2)    GSPCTL
OTCTCG/GSPW07               $(1)    005116 006475      $(0)    113042 113173
                            $(3)    GSPCTL             $(2)    TCGCTL
RSTRPG/GSPW27               $(1)    006476 007205      $(0)    113174 113311
                            $(3)    GSPCTL             $(2)    000000
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NR1FMK/MAGNLINREG00	$(1)	007206	007447	$(0)	113312 113372
				$(2)	BLANK$COMMON
NR1PO/MAGNLINREG00	$(1)	007450	007551	$(0)	113373 113446
				$(2)	BLANK$COMMON
NR1RRT/MAGNLINREG00	$(1)	007552	007672	$(0)	113447 113524
				$(2)	BLANK$COMMON
NR1DRN/MAGNLINREG00	$(1)	007673	007752	$(0)	113525 113542
ASIN&COS$/MAGFORFUN10	$(1)	007753	010176	$(0)	113543 113570
NR1LIT/MAGNLINREG01	$(1)	010177	010347	$(0)	113571 113622
	$(3)	NR1BLK		$(2)	BLANK$COMMON
NR1SKP/MAGNLINREG00	$(1)	010350	010773	$(0)	113623 113670
				$(2)	BLANK$COMMON
NR1WSC/MAGNLINREG00	$(1)	010774	011262	$(0)	113671 113741
				$(2)	BLANK$COMMON
NR1PTV/MAGNLINREG01	$(1)	011263	011645	$(0)	113742 114067
NR1SRT/MAGNLINREG00	$(1)	011646	012075	$(0)	114070 114121
				$(2)	BLANK$COMMON
NR1GVD/MAGNLINREG00	$(1)	012076	014215	$(0)	114122 114333
				$(2)	BLANK$COMMON
NR1TRS/MAGNLINREG00	$(1)	014216	015052	$(0)	114334 114470
				$(2)	BLANK$COMMON
NR1DIF/MAGNLINREG00	$(1)	015053	015647	$(0)	114471 114565
NR1CW/MAGNLINREG00	$(1)	015650	015665	$(0)	114566 114566
UPDATE/MAGUTILITY00	$(1)	015666	015704	$(0)	114567 114570
UPTIME/MAGUTILITY00	$(1)	015705	015713	$(0)	114571 114571
SYMBOL/GSPV35	$(1)	015714	016102	$(0)	114572 114611
				$(2)	GSPCTL
INTSCT/GSPV53	$(1)	016103	016266	$(0)	114612 114624
LOCATE/GSPV76	$(1)	016267	017055	$(0)	114625 114770
				$(2)	GSPCTL
NEXPR$/MAGFORFUN10	$(1)	017056	017216	$(2)	114771 115012
SIN&COS$/MAGFORFUN10	$(1)	017217	017364	$(2)	115013 115042
DSCPT$/MAGFORFUN10	$(1)	017365	017446	$(2)	115043 115062
PLOG06/CMNGR10	$(1)	017447	017517	$(0)	115063 115074
PLOG07/PSPPE10	$(1)	017520	017571	$(0)	115075 115116
PLOG08-TXTDM/GSPW47	$(1)	017572	020733	$(0)	115117 115331
				$(2)	000000
DPAGE/GSPV04	$(1)	020734	021023	$(0)	115332 115336
				$(2)	000000
AXDIST/GSPV95	$(1)	021024	021063	$(0)	115337 115347
DUB	$(1)	021064	021104	$(0)	115350 115361
				$(2)	BLANK$COMMON
POSITN/GSPV54	$(1)	021105	021234	$(0)	115362 115400
				$(2)	GSPCTL
PLOG0L/PSPRL10	$(1)	021235	021331	$(0)	115401 115404
PLOG03/GSPV55	$(1)	021332	021651	$(0)	115405 115445
BASIS1/PSPRL00	$(1)	021652	022422	$(0)	115446 115651
DEXPR/MAGFORFUN11	$(1)	022423	022603	$(2)	115652 115710
DLOG$/MAGFORFUN10	$(1)	022604	022736	$(2)	115711 116016
NEXPR$/MAGFORFUN11	$(1)	022737	023126	$(2)	116017 116040
PLOG02/GSPV35	$(1)	023127	023326	$(0)	116041 116127
PLOG01/GSPV35	$(1)	023327	023607	$(0)	116130 116225
PLOG05/PSPRL00	$(1)	023610	023736	$(0)	116226 116255
				$(2)	BLANK$COMMON
ADDHOL/GSPV03	$(1)	023737	024042	$(0)	116256 116272
				$(2)	GSPCTL
LITEM/GSPV03	$(1)	024043	024135	$(0)	116273 116325

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                                                    $(2)    GSPCTL
SHOIWG/GSPW67           $(1)    024136 030052       $(0)    116326 116625
                        $(3)    TCGCTL              $(2)    GSPCTL
TCG/GSPW57              $(1)    030053 031402       $(0)    116626 116761
                        $(3)    TCGCTL              $(2)    GSPCTL
PCOOOO/GSPV04           $(1)    031403 031547       $(0)    116762 117007
                        $(3)    GSPCTL              $(2)    000000
PAGOFF/GSPV95           $(1)    031550 031756       $(0)    117010 117070
                        $(3)    GSPCTL              $(2)    000000
MCORE/GSPV94            $(1)    031757 032001
NR1HO/NAGNLINREG04      $(1)    032002 032545       $(0)    117071 117613
NR1HC/NAGNLINREG07      $(1)    032546 035314       $(0)    117614 120507
NR1NST/NAGNLINREG01     $(1)    035315 037017       $(0)    120510 121324
                        $(3)    NR1BLK              $(2)    BLANK$COMMON
NR1FPV/NAGNLINREG00     $(1)    037020 037225       $(0)    121325 121432
                                                    $(2)    BLANK$COMMON
NR1BLK(COMMONBLOCK)                                         121433 121450
NR1NR/NAGNLINREG02      $(1)    037226 041671       $(0)    121451 121717
                        $(3)    NR1BLK              $(2)    BLANK$COMMON
NR1HV/NAGNLINREG02      $(1)    041672 042635       $(0)    121720 122075
NR1PUT/NAGNLINREG00     $(1)    042636 042771       $(0)    122076 122121
                                                    $(2)    BLANK$COMMON
NR1BOS/NAGNLINREG01     $(1)    042772 043055       $(0)    122122 122153
NR1BOF/NAGNLINREG00     $(1)    043056 043133       $(0)    122154 122167
                                                    $(2)    BLANK$COMMON
NR1LTN/NAGNLINREG00     $(1)    043134 043231       $(0)    122170 122220
                                                    $(2)    BLANK$COMMON
NR1BOA/NAGNLINREG00     $(1)    043232 043303       $(0)    122221 122246
                                                    $(2)    BLANK$COMMON
NR1LTZ/NAGNLINREG00     $(1)    043304 043370       $(0)    122247 122271
                                                    $(2)    BLANK$COMMON
NR1GOD/NAGNLINREG01     $(1)    043371 043531       $(0)    122272 122325
                                                    $(2)    BLANK$COMMON
NR1PST/NAGNLINREG00     $(1)    043532 044156       $(0)    122326 122462
                                                    $(2)    BLANK$COMMON
NR1TWS/NAGNLINREG01     $(1)    044157 044243       $(0)    122463 122476
                                                    $(2)    BLANK$COMMON
ALOG$/NAGFORFUN10       $(1)    044244 044361       $(2)    122477 122552
GSTXT/V36               $(1)    044362 044715       $(0)    122553 123046
                                                    $(4)    GSPCTL
TRWPIT/GSPW87           $(1)    044716 045102       $(0)    123047 123134
                                                    $(4)    TCGCTL
                                                    $(6)    GSPCTL
LINE/GSPVN6             $(1)    045103 046420       $(0)    123135 123367
                                                    $(2)    GSPCTL
DSHOFF/PSPRL00          $(1)    046421 046655       $(0)    123370 123455
                        $(3)    000000              $(2)    BLANK$COMMON
AXLIN/GSPW47            $(1)    046656 053535       $(0)    123456 124335
                                                    $(2)    GSPCTL
PLOOOP/PSPRL00          $(1)    053536 054010       $(0)    124336 124407
GROLIN/CMNGR10          $(1)    054011 054522       $(0)    124410 124600
PLOOO4/PSPRL00          $(1)    054523 054561       $(0)    124601 124611
                                                    $(2)    BLANK$COMMON
BASIS2=3/PSPRL00        $(1)    054562 054704       $(0)    124612 124616
SCALE/GSPV77            $(1)    054705 057244       $(0)    124617 125375
GRFUNC/GSPV76           $(1)    057245 057307       $(0)    125376 125405
ARGSET/GSPV76           $(1)    057310 057432       $(0)    125406 125430
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PLOGGH/PSPRL01          $(1)    057433 057522    $(0)    125431 125471
IHGHDLR/GSPWN6          $(1)    057523 060750    $(0)    125472 125610
                        $(3)    TCGCTL           $(2)    GSPCTL
PAGE/GSPV05             $(1)    060751 061422    $(0)    125611 125733
                        $(3)    GSPCTL           $(2)    000000
ROWVEC/PSPRL00          $(1)    061423 061703    $(0)    125734 125773
                                                 $(2)    BLANK$COMMON
DSHCLR/PSPRL00          $(1)    061704 061742    $(0)    125774 126002
                        $(3)    000000           $(2)    BLANK$COMMON
COSSW/PSPRL00           $(1)    061743 062022    $(0)    126003 126007
                        $(3)    000000           $(2)    BLANK$COMMON
VECTOR/GSPWN6           $(1)    062023 062552    $(0)    126010 126046
                                                 $(2)    GSPCTL
WRTEXT/GSPW67           $(1)    062553 063343    $(0)    126047 126154
                                                 $(2)    GSPCTL
NOSYM$/FCRID            $(1)    063344 063453    $(2)    126155 126160
GSPRNT/W57              $(1)    063454 064532    $(0)    126161 126332
                        $(3)    TCGCTL           $(2)    GSPCTL
STORGT/GSPW97           $(1)    064533 064733    $(0)    126333 127044
                                                 $(4)    GSPCTL
                                                 $(6)    000000
PLOGGE/GSPV86           $(1)    064734 065304    $(0)    127045 127150
                                                 $(2)    GSPCTL
UERLTO/UWCC             $(1)    065305 065313    $(0)    127151 127151
UWERR1/WAGFORFUN02      $(1)    065314 066124    $(2)    127152 127611
ERR$
WTSR$/FCRID                                      $(2)    127612 127656
NETCW$/FCRID            $(1)    066125 066425    $(2)    127657 127714
FCRID$2/FCRID           $(1)    066426 070755    $(2)    127715 132405
NEETI$/FCRID            $(1)    070756 072021    $(2)    132406 132623
NEETO$/FCRID            $(1)    072022 072412    $(2)    132624 132665
NEIEOS/WAGLINREG10      $(1)    072413 076160    $(0)    132666 134255
UPXITP$/W/GUTILITY01    $(1)    076161 076240    $(2)    134256 134314
GRECW/GSPV18            $(1)    076241 102615    $(0)    134315 135636
                        $(3)    000000           $(2)    GSPCTL
000000(COMMONBLOCK)                              135637 135720
TCGCTL(COMMONBLOCK)                              135721 135726
GSPCTL(COMMONBLOCK)                              135727 136343
GSPSET/GSPWN7           $(1)    102616 103275    $(0)    136344 137026
                        $(3)    TCGCTL           $(2)    GSPCTL
                                                 $(4)    000000
FCRID$3/FCRID           $(1)    103276 103662    $(2)    137027 137033
EXPE/WAGFORFUN10        $(1)    103663 103765    $(2)    137034 137057
SCPT$/WAGFORFUN10       $(1)    103766 104035    $(2)    137060 137071
FCRID$4/FCRID           $(1)    104036 104332    $(2)    137072 137113
FCRID$1/FCRID           $(1)    104333 107524    $(2)    137114 140210
NREG/WAGLINREG02        $(1)    107525 107650    $(0)    140211 140223
LIM(COMMONBLOCK)                                 140224 140230
BLANK$COMMON(COMMONBLOCK)                        140231 140312
PRPL                    $(1)    107651 110214    $(0)    140313 140416
                        $(3)    LIM              $(2)    BLANK$COMMON
OTP                     $(1)    110215 110521    $(0)    140417 140515
                        $(3)    LIM              $(2)    BLANK$COMMON
SUBF                    $(1)    110522 110654    $(0)    140516 140551
                        $(3)    LIM              $(2)    BLANK$COMMON
MAIN                    $(1)    110655 111077    $(0)    140552 142461
                        $(3)    LIM              $(2)    BLANK$COMMON
```

```
SYS$.RLIBS. LEVEL 25
END MAP
@XQT
```

SUMMARY OF THE DATA

ITEMS

ITEMS(1): NEXP	= 12	NUMBER OF EXPERIMENTS	
ITEMS(2): NVAR	= 1	NUMBER OF VARIABLES	
ITEMS(3): NP	= 4	NUMBER OF PARAMETERS	
ITEMS(4): NROBS	= 12	ROW DIMENSION OF ARRAY YOBS	
ITEMS(5): NRPRD	= 12	ROW DIMENSION OF ARRAY YPRD	
ITEMS(6): NRWTS	= 12	ROW DIMENSION OF ARRAY WTS	
ITEMS(7): METHOD	= 'MARQ'	MARQUARDT'S METHOD	
ITEMS(8): KWTS	= 'ALL'	WEIGHTING BY OBSERVATION	
ITEMS(9): KDERIV	= 'NREG'	NREG CALCULATED DERIVATIVES	
ITEMS(10): KSYM	= 'CENT'	CENTRAL DIFFERENCES	
ITEMS(11): KDIFF	= 'REL'	RELATIVE DIFFERENCES	
ITEMS(12): MAXIT	= 50	LIMIT ON NUMBER OF ITERATIONS	

TOLERANCES

TOL(1) =	1.5000000-06	REL. CHANGE IN A PARAMETER
TOL(2) =	1.0000000-09	REL. CHANGE IN SUM OF SQUARES
TOL(3) =	0.0000000	RATIO TO INITIAL SUM OF SQUARES
TOL(4) =	1.0000000-16	PIVOT TOLERANCE

SUNDRIES

NUMBER OF ACTIVE PARAMETERS = 4
NUMBER OF CELLS OF SCRATCH REQUIRED = 201

PARAMETERS

NO.	NAME	INITIAL VALUE	PROPORTION FOR DERIV. ESTIMATE	LOWER BOUND	UPPER BOUND
1	K1	8.7262000+00	1.0000000-03	1.0000000-04	1.0000000+04
2	K2	9.4430000-01	1.0000000-03	1.0000000-04	1.0000000+04
3	KEL	1.0873000+00	1.0000000-03	1.0000000-04	1.0000000+04
4	DV	4.7840000-01	1.0000000-03	1.0000000-04	1.0000000+04

SINGULAR VALUES AND VECTORS AT INITIAL POINT

SING. VALUES		1.5730335+00	2.7736185-01	1.9939391-01	1.9809371-02
PAR.	1 K1	.0361295	.0261760	-.1033964	-.9936391
	2 K2	-.1783784	-.9827820	-.0389695	-.0283209
	3 KEL	.1242457	-.0587584	.9854920	-.0995788
	4 DV	-.9754172	.1732103	.1288257	-.0443094

SETUP TIME = .146 SECONDS

THE ITERATIONS

ITERATION NO, 1	BASE POINT		TEST POINT
SUM OF SQUARES	7.2679271-04		4.4515677-04
L	0		0
LAMBDA	0.0000000		0.0000000
GAMMA	1.0000000+00		1.0000000+00
ANGLE IN DEGREES	.0000		88.0690
MAX. PIVOT REDUCTION	1.0000000+00		6.9134096-02
PAR. 1 K1	8.7262000+00		7.9920374+00
2 K2	9.4430000-01		9.1585684-01
3 KEL	1.0873000+00		9.9363741-01
4 DV	4.7840000-01	S	4.4927654-01

CUMULATIVE NO. OF FUNCTION CALLS = 2 ITERATION TIME = .080 SECONDS CUMULATIVE TIME = .225 SECONDS

THE LAST ITERATION

ITERATION NO, 4	BASE POINT		TEST POINT
SUM OF SQUARES	4.4101815-04		4.4101819-04
L	0		0
LAMBDA	0.0000000		0.0000000
GAMMA	1.0000000+00		1.0000000+00
ANGLE IN DEGREES	82.0865		85.3239
MAX. PIVOT REDUCTION	2.0972256-01		2.1672292-01
PAR. 1 K1	8.0590262+00	S	8.0590343+00
2 K2	9.1509552-01		9.1509575-01
3 KEL	1.0034319+00		1.0034329+00
4 DV S	4.5280175-01		4.5280214-01

CUMULATIVE NO. OF FUNCTION CALLS = 5 ITERATION TIME = .027 SECONDS CUMULATIVE TIME = .365 SECONDS

ITERATION TERMINATES:
MAX. RELATIVE CHANGE IN A PARAMETER .LT. TOL(1) = 1.5000000-06

FINAL PARAMETER VALUES

SUM OF SQUARES = 4.4101815-04

			FINAL VALUE
AR.	1	K1	8.0590262+00
	2	K2	9.1509552-01
	3	KEL	1.0034319+00
	4	DV	4.5280175-01

ITERATION SUMMARY

ITER NO.	SUM OF SQUARES	L	LAMBDA	GAMMA	ANGLE	NO. PIVOT FAILURES	NO. BOUNDS FAILURES	MAX. PIVOT REDUCTION	TIME IN SECONDS
0	7.2679271-04	0	0.0000000	1.0000000+00	.0000	0	0	1.0000000+00	.000
1	4.4515577-04	0	0.0000000	1.0000000+00	88.0690	0	0	6.9134096-02	.060
2	4.4101921-04	0	0.0000000	1.0000000+00	86.7596	0	0	1.0854786-01	.085
3	4.4101815-04	0	0.0000000	1.0000000+00	82.0865	0	0	2.0972256-01	.027
4	4.4101819-04	0	0.0000000	1.0000000+00	85.3239	0	0	2.1672292-01	.027

SINGULAR VALUES AND VECTORS AT TERMINAL POINT

SING. VALUES			1.6768348+00	2.8603747-01	2.1755048-01	2.2665580-02
PAR.	1	K1	.0365924	.0312971	.0988515	.9939366
	2	K2	-.1703403	-.9844239	.0203240	.0346507
	3	KEL	.1250747	-.0446830	-.9865847	.0949227
	4	DV	-.9767300	.1671328	-.1272241	.0433492

NORMALIZING ELEMENTS AND CORRELATION MATRIX

PARAMETER NO,		1	2	3	4
NORM, ELTS,		4,3854741+01	3,7691739+00	6,1753510+00	2,1634633+00
PAR,	1 K1	1,0000000			
	2 K2	,4036185	1,0000000		
	3 KEL	,6704698	,2742647	1,0000000	
	4 DV	,8817152	,1105349	,7879456	1,0000000

CONFIDENCE LIMITS ON LINEAR HYPOTHESIS

		LOWER LIMIT	FINAL VALUE	UPPER LIMIT
PAR,	1 K1	7,4078032+00	8,0590262+00	8,7102493+00
	2 K2	8,5912499-01	9,1509552-01	9,7106604-01
	3 KEL	9,1173078-01	1,0034319+00	1,0951331+00
	4 DV	4,2067530-01	4,5280175-01	4,8492819-01

EXPLORATION

		LOWER TEST	SUM OF SQUARES	LINEAR EST, OF SUM OF SQUARES	UPPER TEST	SUM OF SQUARES	LINEAR EST, OF SUM OF SQUARES
PAR,	1 K1	7,8962206+00	5,7274546-04	5,6864570-04	8,2218319+00	5,6541004-04	5,6864570-04
	2 K2	9,0110289-01	4,7282975-04	4,7252288-04	9,2908815-01	4,7239051-04	4,7252288-04
	3 KEL	9,8050666-01	4,8997992-04	4,8843569-04	1,0263572+00	4,8734907-04	4,8843569-04
	4 DV	4,4477013-01	6,1425069-04	6,1425041-04	4,6083336-01	6,1424964-04	6,1425041-04

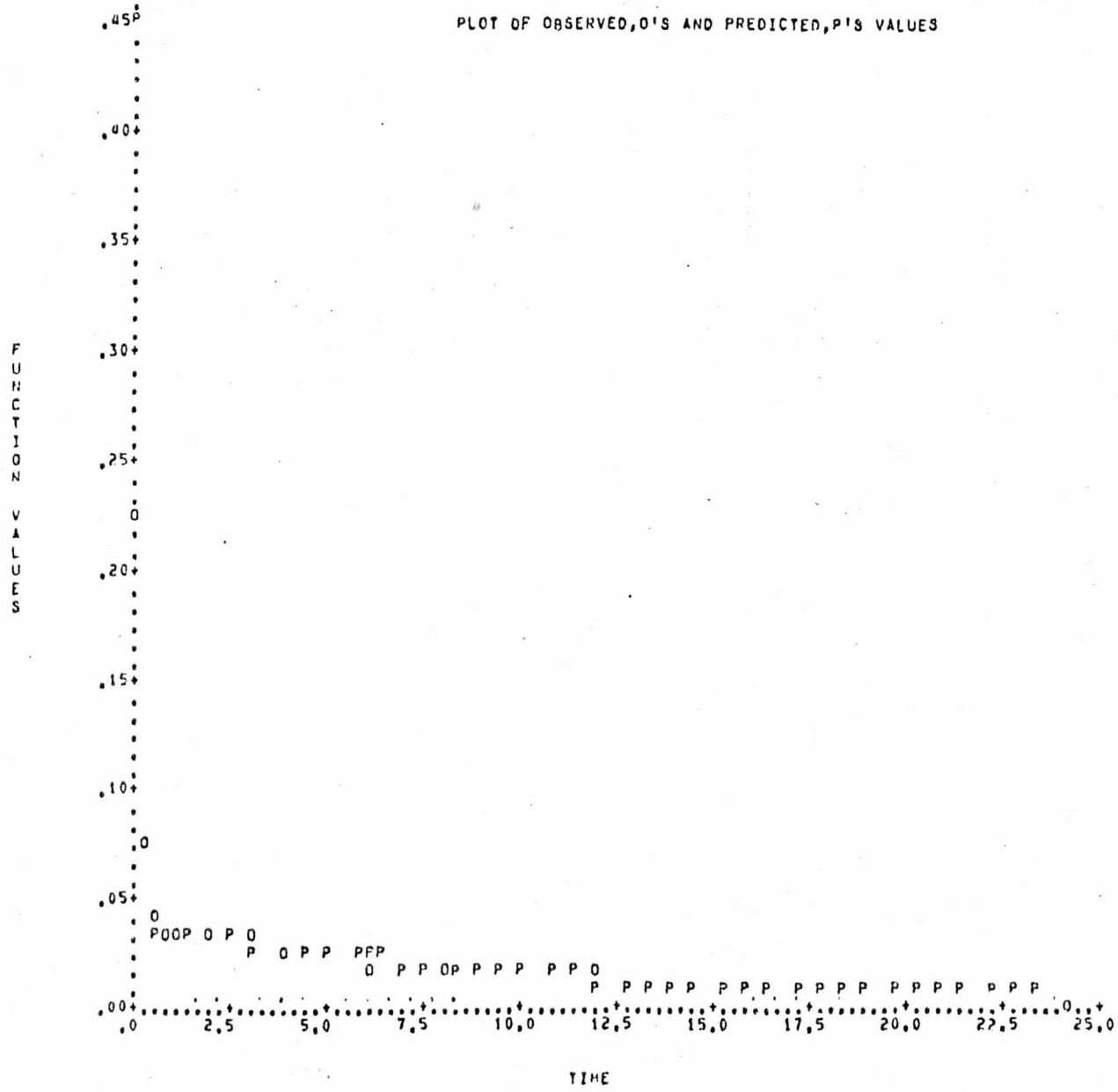
F I N A L F U N C T I O N V A L U E S A N D R E S I D U A L S

WEIGHTED ROOT MEAN SQUARE RESIDUAL = 7.4247739-03 NO, DEGREES OF FREEDOM = 8
THIS IS THE SCALE UNIT IN THE GRAPH OF THE WEIGHTED RESIDUALS,

EXPT, NO,	VAR, NO,	PREDICTION	OBSERVATION	RESIDUAL	WEIGHTED RES,	GRAPH OF WEIGHTED RESIDUAL -2 -1 0 +1 +2
1	1	2.2594024-01	2.2570000-01	-1.4023669-04	-2.9516409-04	*
2	1	7.2191621-02	7.2600000-02	4.0837843-04	1.5154087-03	***
3	1	3.9256199-02	3.8200000-02	-1.0561990-03	-5.4041893-03	********
4	1	3.5712406-02	3.6100000-02	3.8755906-04	2.0397276-03	****
5	1	3.4669362-02	3.3100000-02	-1.5693624-03	-8.6257842-03	************
6	1	3.1574555-02	3.4200000-02	2.6254449-03	1.4196837-02	**********
7	1	2.8773546-02	2.9600000-02	8.2645332-04	4.8033933-03	*******
8	1	2.6221019-02	2.5700000-02	-5.2101933-04	-3.2500082-03	*****
9	1	2.1775188-02	2.0800000-02	-9.7518833-04	-6.7619308-03	**********
10	1	1.8083157-02	1.8000000-02	-8.3157560-05	-6.1984464-04	**
11	1	1.2470933-02	1.3200000-02	7.2906644-04	6.3458103-03	**********
12	1	4.0904686-03	3.9000000-03	-1.9046862-04	-3.0499373-03	*****

TIME SINCE END OF THE LAST ITERATION = .429 SECONDS TOTAL TIME = .794 SECONDS

R-SQUARED FOR FUNCTION1 = .9998
COR FOR FUNCTION1 = .9998
ALPHA = 9.88466 BETA = .09290


PLOT OF OBSERVED,O'S AND PREDICTED,P'S VALUES
FUNCTION VALUES
TIME
.45
.40
.35
.30
.25
.20
.15
.10
.05
.00
0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
25.0

```
#FIN

    RUNID: C00192   PROJECT: 05994              USER: 3965817681

  ITEM                                AMOUNT       COST

CPU TIME                        00:00:11.712        $0.44
FILE I/O REQUESTS                       1435        $0.65
FILE I/O WORDS                       1228708        $0.56
SWAP REQUESTS                              1        $0.00
SWAP WORDS                              3584        $0.00
MEMORY USAGE                           0.735        $0.46
CARDS IN                                 193        $0.24
PAGES PRINTED                             14        $0.56
ER + CC                                   18        $0.18
JOB CHARGE                                 1        $0.20

TOTAL COST                                          $3.29

    THE ABOVE DOLLAR AMOUNTS ARE APPROXIMATE AND ARE BASED ON RATES FOR STANDARD RUNS

    INITIATION  TIME:   15:23:13 FEB  6,1978

    TERMINATION TIME:   15:25:07 FEB  6,1978

   PREVIOUS RUN TIME:   15:18:38 FEB  6,1978
```

APPENDIX F

Simultaneous Determination of Acetylmethadol and its Major Metabolites by Gas-Liquid Chromatography

by

Francis L. S. Tse and Peter G. Welling

Reprinted from

Journal of Chromatography, 135 (1977) 205–207

CHROM. 9879

Note

Simultaneous determination of acetylmethadol and its major metabolites by gas–liquid chromatography

FRANCIS L. S. TSE and PETER G. WELLING*

Center for Health Sciences, School of Pharmacy, University of Wisconsin, Madison, Wisc. (U.S.A.)

(Received December 9th, 1976)

Kaiko *et al.*[1] recently described a method utilizing solvent extraction and gas–liquid chromatography for the quantitative determination of acetylmethadol simultaneously with its two major metabolites, noracetylmethadol and dinoracetylmethadol, in human biofluids. The metabolites were measured as their corresponding amides, which were formed by adding four drops of 50% sodium hydroxide solution to the final extract (resultant pH = 13) and incubating at 70° for 30 min. These authors reported that acetylmethadol itself was unaffected by this procedure.

We have adapted this method to determine acetylmethadol, noracetylmethadol, dinoracetylmethadol, as well as another major metabolite, methadol, quantitatively in biological fluids. During the course of generating standard curves for these four compounds, however, it was repeatedly observed that the detector response to acetylmethadol was low while that to methadol was high. It was therefore decided to investigate the possibility of loss of acetylmethadol via alkaline hydrolysis, hence the *in situ* synthesis of methadol during the assay procedure.

MATERIALS AND METHODS

α-*l*-Acetylmethadol hydrochloride, α-*l*-noracetylmethadol hydrochloride, α-*l*-dinoracetylmethadol hydrochloride and α-*l*-methadol hydrochloride were provided by the National Institutes of Health (Bethesda, Md., U.S.A.).

A Perkin-Elmer Model 3920B gas chromatograph equipped with a flame-ionization detector was used. The column was a 6-ft. helical glass column with an I.D. of 2 mm and an O.D. of 6 mm, packed with 3% XE-60 on 80–100-mesh Gas-Chrom Q. The electrometer was set at an amplifier range of 1 and an attenuation of 8. The carrier gas was nitrogen with a flow-rate of 30 ml·min^{-1} while the flame gases were hydrogen and compressed air at flow-rates of 30 and 360 ml·min^{-1} respectively. The temperatures of the detector and injector port were 300° and 275° respectively, and a column temperature of 200° was used for analysis.

Acetylmethadol hydrochloride (0.4 μg) was placed in each of two 15-ml siliconized centrifuge tubes with Teflon-lined screw-caps. Five milliliters of 0.2 *N* hydrochloric acid were added to each tube, followed by four drops of 50% sodium

* To whom inquiries should be directed.

hydroxide solution to yield a pH of approximately 13. After thorough mixing, the contents of one tube were immediately extracted into 8 ml of chloroform by shaking for 10 min followed by centrifuging at 1000 *g* for 5 min. The second tube was incubated in a heating block at 70° for 30 min prior to extraction with chloroform. The upper, aqueous phase was removed by aspiration and discarded, while the organic phase was concentrated by evaporation under nitrogen, transferred into a 1-ml Reacti-vial and subsequently evaporated to dryness. The residue was dissolved in 20 μl of carbon disulfide which contained 0.8 μg of triacontane as external standard. One microliter of the sample was injected into the gas chromatograph. The drug: triacontane peak-height ratios were calculated and compared. The experiment was repeated four times.

RESULTS AND DISCUSSION

Under the aforementioned operating conditions, the retention times were 190 sec for acetylmethadol, 213 sec for methadol and 549 sec for triacontane.

The samples which were not incubated yielded only the acetylmethadol and the triacontane peaks. Incubation at pH 13 resulted in a significantly lower acetylmethadol peak and the appearance of a sizable peak at 213 sec, the retention time of methadol. Fig. 1 shows the relative peak heights of acetylmethadol and methadol from acetylmethadol samples with and without incubation.

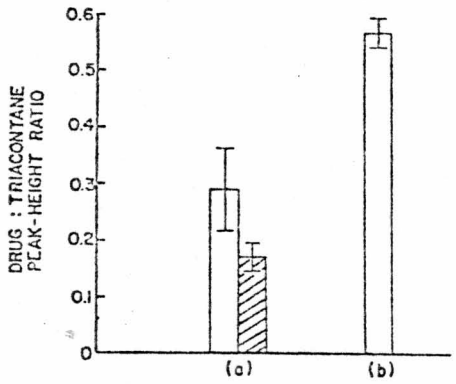


Fig. 1. Relative peak heights of acetylmethadol (□) and methadol (▨) from acetylmethadol samples (a) with and (b) without incubation at 70° for 30 min. Values represent the average from four experiments. The vertical bars indicate 1 standard deviation.

The results show that part of the acetylmethadol was hydrolyzed to form methadol during incubation at pH 13, a step which is necessary in order to convert noracetylmethadol and dinoracetylmethadol into their corresponding amides for better peak resolution. Any standard calibration graphs generated from such data will inevitably lead to incorrect calculation of acetylmethadol and methadol concentrations in biofluids where acetylmethadol and methadol may be present in variable relative amounts.

Hence, in order to measure simultaneously acetylmethadol, noracetylmetha-

dol, dinoracetylmethadol and methadol, it seems necessary, after the addition of strong base in the last step in the extraction procedure[1], to divide the sample extract into two equal portions. One portion is immediately extracted into chloroform and subsequently analyzed for acetylmethadol and methadol, while the other is incubated and then analyzed for noracetylmethadol and dinoracetylmethadol as their corresponding amides.

REFERENCES

1 R. F. Kaiko, N. Chatterjie and C. E. Inturrisi, *J. Chromatogr.*, 109 (1975) 247.