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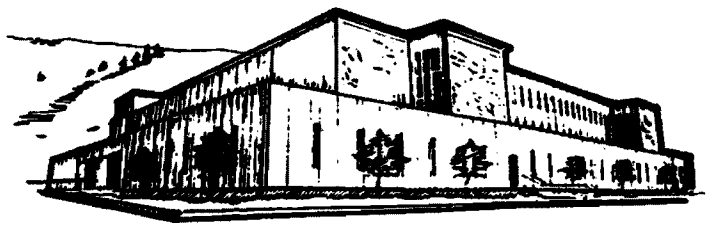

COMPARISON OF TETRACYCLINE HYDROCHLORIDE CONCENTRATIONS IN VENOUS AND CAPILLARY BLOOD OF DOMESTIC RABBITS

By

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Comparison of tetracycline hydrochloride concentrations in venous and capillary blood of domestic rabbits (56 pp.)

Director: Todd G. Cochran, Ph.D

The purpose of this study was to investigate the feasibility of using capillary blood sampling as a method for therapeutic drug monitoring and pharmacokinetic analysis. Tetracycline hydrochloride was used as a model drug because a convenient and sensitive fluorometric analysis was available.

Tetracycline hydrochloride concentrations in serum were compared to drug concentrations in whole blood of domestic rabbits. Results of this study indicated that there existed no significant difference between the two concentrations. The original analytical procedure was modified to use a small sample volume of 15.7 μL.

The drug concentrations in venous blood and capillary blood of domestic rabbits were compared. Results of this study demonstrated that tetracycline hydrochloride concentrations in capillary blood samples are accurate and unbiased estimators of concentrations in venous blood samples.

This study serves as a model for drug analysis using small volumes of capillary blood. The finding that there is no significant difference in tetracycline hydrochloride concentration between venous and capillary blood shows that drug monitoring of tetracycline can be performed accurately by measuring capillary blood tetracycline concentrations. The collection of excess sample or the need to inflict a painful procedure on that population where venous blood collection is difficult is unnecessary.

ACKNOWLEDGEMENTS

The author wishes to express his appreciation to Dr. Todd G. Cochran for his guidance and direction throughout the course of this research. My special thanks to Dr. Tomoo Itoh for his constant encouragement and to Mr. Hemant N. Joshi for his help with statistical analysis.

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CHAPTER I

INTRODUCTION

The analysis of drug content in plasma or serum typically requires venesection and several milliliters of venous blood. However, blood sampling may pose a problem in pediatric and geriatric patients. The patient's age, weight, disease state, and acceptance of procedure, as well as variations in availability of the veins for puncture, are all factors in obtaining an adequate blood sample. The decrease in subcutaneous and supportive connective tissues associated with aging process makes the veins more difficult to penetrate (21). As a result of such age related changes, the incidence of trauma related to venipuncture is higher among geriatric patients. In any form of repetitive study, the determination of drug concentration in saliva might alleviate most of these difficulties, but unfortunately the effects, side effects and serum drug concentrations have not been shown to be correlated with salivary concentrations in many cases.

An ideal method for drug determination should be rapid, incorporate a simple sampling technique, and require a small sample size. The ability of a method to utilize a capillary sampling technique without significantly affecting its precision is a great advantage. With the advent of micro-volume assay methods, capillary blood sampling using a finger lancet puncture technique furnishing a whole blood sample volume of 100-800 μL (28), has been used as an alternative to traditional venous methods of obtaining blood samples

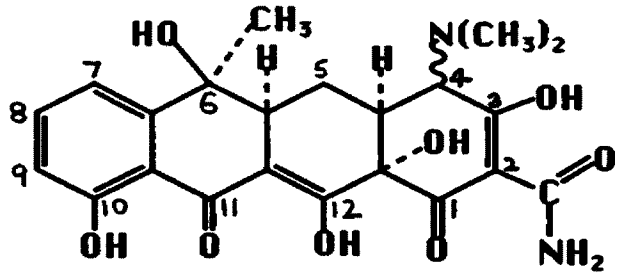
for clinical determination of blood levels for a number of drugs and physiological substances. The advantages of using capillary samples are particularly evident in geriatric and pediatric patients. Capillary blood sampling provides a less invasive, less traumatic, and often less painful method of obtaining blood samples for serum level determinations in these patients. This method is being increasingly accepted, being a simple procedure easily undertaken by nonskilled personnel and by patients themselves. Capillary sampling has considerable promise in large-scale screening studies, in pediatric studies, in neonatal studies, or in any form of repetitive study because capillary blood sampling has substantial practical and economic advantages over venous blood drawing.

Previous studies have evaluated capillary and venous blood drug concentrations. Plasma cholesterol was measured in 181 subjects (12) using two methods: the automated ferric chloride method on venous blood and a micro-GLC (gas-liquid chromatography) method on capillary blood (20 μL). Mean ± s.d. cholesterol levels were essentially identical for the two methods with a correlation coefficient of 0.96. A small collector bottle, with a precision-molded top, allowed capillary blood from a finger-prick to be automatically metered into a diluent for subsequent laboratory analysis (10). This device has been sucessfully used by patients to obtain full blood counts, glycosylated haemoglobin values and blood glucose measurements. For the blood glucose determination a very small sample size (capillary blood) of 15 μL showed a good correlation (R=0.99) when compared with venous samples of a larger volume. Immunoassays of gentamicin using 500-800 μL blood samples from

gentamicin treated children demonstrated no significant difference between clinically relevant concentrations of gentamicin in venous and capillary blood serum samples (8). Tobramycin serum concentrations in capillary samples (100-200 μL) were found to be highly correlated (R=0.996, n=73) with those determined in venous samples (19). A good correlation was found between capillary and venous sample concentrations of theophylline (27) and phenytoin (28) with sample sizes of 500-800 μL using the standard finger lancet method.

Tetracycline was used as the model drug in the present study because a convenient and sensitive fluorometric analysis is available. Tetracycline is a broad spectrum antibiotic, effective against gram positive and gram negative bacteria, fungi, etc. Though tetracycline may be bactericidal to some microbes at high concentration, its activity is primarily bacteriostatic. Therefore, it is important that the circulating levels of tetracycline be maintained above the minimum inhibitory concentration for pathogenic organisms during a course of a treatment.

Tetracycline (I) is amphoteric, forming salts with acids or bases. The drug is commonly marketed as the hydrochloride salt, in which the tetracycline molecule is protonated at the C-4 dimethyl amino group.



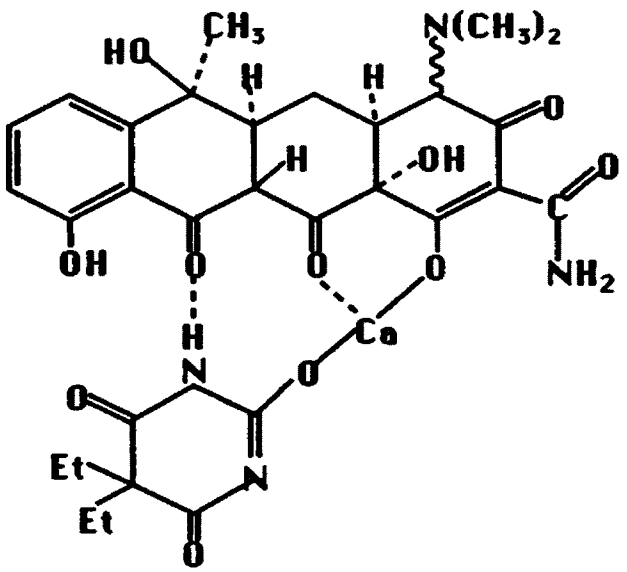
(I)

The pH of the medium has a great effect on the stability of tetracycline and its interaction with other molecules. Most tetracyclines are unstable in basic solutions (7). Tetracycline undergoes oxidation with atmospheric oxygen (11), which may be catalyzed by riboflavin under light (17). Epimerization occurs at the C-4 position and leads to the formation of anhydrotetracycline, a biologically inactive compound often found in aged tetracycline products (24). Dehydration and aromitization of the C-ring lead to the formation of anhydro derivatives, a process occuring predominantly at low pH.

Tetracycline in biological fluids has been assayed primarily by microbiological (7) and fluorometric (13, 14, 18, 29) techniques. The disadvantages inherent in microbiological methods are that long sample incubation times are required (4), the results may be subject to large errors (1), and the assay is limited in specificity since several tetracycline like compounds have similar biological activity (20). Spectrophotometric methods (2) are insensitive, and interference from other material cannot always be excluded. High-pressure liquid chromatography (16) and polarographic analysis (5) of tetracyclines

also have been described. Fluorometric analysis is relatively simple and specific for the tetracyclines (14, 23).

The fluorometric analysis is based on the fluorescence quantification of tetracycline extracted from biological fluids. Tetracycline forms complexes with many divalent and trivalent cations and some of these compounds are strongly fluorescent when compared to nonchelated tetracycline. A method combining both the extraction and fluorescence was reported by Kohn (14) in which tetracycline was extracted with ethyl acetate from solutions containing 0.16 M calcium chloride, 1.8 N trichloroacetic acid and 0.9 M barbital sodium at pH 9. The function of barbital sodium in Kohn's method was to enhance both extraction and fluorescence by forming a neutral ternary complex with calcium and tetracycline. A structure of this complex (II) was proposed by Kohn based on his solvent distribution data.



II

Comparison between microbiologic and fluorometric methods for the determination of tetracycline in dog plasma showed a reasonable agreement (14).

Pharmacokinetic studies of tetracycline in the domestic rabbit following intravenous or oral administration have been reported (22). Three male and three female rabbits were injected intravenously with tetracycline hydrochloride at 10mg/kg body weight. Mean serum pharmacokinetic parameters were elimination half-life = 120 minutes and time zero intercept of the log-linear elimination phase = 7.5 mcg/mL. The serum concentration vs time curve obtained in these studies indicated that a biexponential decay model best described the elimination of tetracycline after intravenous administration. The tetracycline assay was carried out using the plate disk method (microbiological method) in these studies. The correlation coefficient comparing the microbiological analysis with fluorometric analysis of serum samples for tetracycline from five dogs was found to be 0.988 (13).

Several investigators have demonstrated that no clinically significant differences exist in the values for hemocrit (6), erythrocyte count, hemoglobin, leukocyte count, platelet count (26), lipoproteins (9), plasma cholesterol (12), phenytoin (28), gentamicin (8), tobramycin (19), and theophylline (3, 27) when venous and capillary blood samples are compared. Despite differences in methodology and locale of blood sampling, in all instances a good correlation has been established between capillary and venous sample concentrations.

The objective of this study was to determine if there exists any significant difference between conventional venous samples and capillary samples for determination of tetracycline hydrochloride concentrations. Use of whole blood samples rather than serum for analysis would allow smaller sample sizes to be used, facilitating the procedure and causing less trauma to the animal.

It was not feasible to draw enough blood from capillaries of the rabbit's ear at regular intervals to obtain serum for the determination of tetracycline hydrochloride concentrations. Therefore, whole blood drug concentrations were compared to serum drug concentrations to establish if they could be correlated. If a good correlation exists, whole blood could be used for the determination of tetracycline hydrochloride concentrations in capillary and venous samples. A good correlation had been found between plasma and whole blood theophylline concentrations in neonates (15).

CHAPTER II

EXPERIMENTAL

Materials

Tetracycline Hydrochloride, Nutritional Biochemicals Corp.
Trichloroacetic Acid, Analyzed Reagent, J.T. Baker Co.
Calcium Chloride Dihydrate, ACS Reagent Grade, Allied Chemical Co.
Ethyl Acetate, Analytical Reagent, Mallinkrodt Chemical Works.
Sodium Barbital, Purified, Fisher Scientific Co.
Pre-cal micro-hematocrit tubes/heparinized (ammonium heparin), Becton, Dickinson and Company, Parsippany, N.J. 07054

Procedures

Method of analysis:

Tetracycline was analyzed by Kohn's method (14) which is based on the solvent extraction of a highly fluorescent tetracycline complex. In Kohn's procedure an aliquot of sample (serum or urine) was diluted with distilled water to a total volume of 4.5 mL. To this was added 1 mL of a solution of 1.8 N trichloroacetic acid and 0.16 M calcium chloride (for the precipitation of proteins). The resulting solution was centrifuged. A 4 mL portion of supernatant was pipetted into a glass stoppered centrifuge tube containing 3.0 mL of ethyl acetate and 4 mL of 0.9 M sodium barbital. The tube was shaken vigorously for about two minutes and the phases were

allowed to separate (barbital precipitates, but then dissolves in the ethyl acetate phase). The fluorescence of the ethyl acetate layer was measured using a spectrophotofluorometer. Kohn's assay procedure was modified for this research work to use a smaller sample size.

The following reagents were prepared in distilled water:

18.56 g sodium barbital diluted to 100 mL. . . . . 0.9 M

29.41 g trichloroacetic acid diluted to 100 mL. . . . . 1.8 N

2.35 g calcium chloride dihydrate diluted to 100 mL. . . . . 0.16 M

Extraction procedure:

An aliquot of sample (serum, blood or aqueous solution for standard curves) was added to 2 mL of distilled water in a glass stoppered centrifuge tube of 15 mL capacity and vortexed. To this was added 0.5 mL of a solution of 1.8 N trichloroacetic acid and 0.16 M calcium chloride. The resulting solution was then centrifuged for 15 minutes at 3000 r.p.m. Two mL of the supernatant was transferred to a stoppered glass tube of 15 mL capacity containing 2 mL of ethyl acetate and 2 mL of 0.9 M sodium barbital. The resulting solution was vortexed at a high speed for 2 minutes and the phases allowed to separate.

Measurement of fluorescence:

The fluorescence of the ethyl acetate layer was measured using a spectrophotofluorometer.[a] Maximum sensitivity for tetracycline

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[a] Aminco-Bowman spectrophotofluorometer

hydrochloride was obtained when the excitation wavelength was set at 390 nm and emission wavelength at 515 nm.

Standard curves for tetracycline hydrochloride:

Standard curves were prepared for tetracycline hydrochloride concentrations in the range of 0.1 mcg/mL to 100 mcg/mL. All drug dilutions were made in distilled water.

100 mg (tetracycline hydrochloride) diluted to 100 mL. . . . . 1000 mcg/mL

10 mL (1000 mcg/mL) diluted to 100 mL. . . . . 100 mcg/mL

7.5 mL (1000 mcg/mL) diluted to 100 mL. . . . . 75 mcg/mL

5.0 mL (1000 mcg/mL) diluted to 100 mL. . . . . 50 mcg/mL

2.5 mL (1000 mcg/mL) diluted to 100 mL. . . . . 25 mcg/mL

1.0 mL (1000 mcg/mL) diluted to 100 mL. . . . . 10 mcg/mL

5.0 mL (100 mcg/mL) diluted to 100 mL. . . . . 5 mcg/mL

2.5 mL (100 mcg/mL) diluted to 100 mL. . . . . 2.5 mcg/mL

1.0 mL (100 mcg/mL) diluted to 100 mL. . . . . 1 mcg/mL

1.0 mL (50 mcg/mL) diluted to 100 mL. . . . . 0.5 mcg/mL

1.0 mL (10 mcg/mL) diluted to 100 mL. . . . . 0.1 mcg/mL

Standard curves were then prepared for tetracycline hydrochloride concentrations in the range of 1 mcg/mL to 25 mcg/mL. All dilutions were freshly prepared in duplicate in distilled water at the beginning of each experiment.

100 mg (tetracycline hydrochloride) diluted to 100 mL. . . . . 1000 mcg/mL

2.5 mL (1000 mcg/mL) diluted to 100 mL. . . . . 25 mcg/mL

2.0 mL (1000 mcg/mL) diluted to 100 mL. . . . . 20 mcg/mL

1.5 mL (1000 mcg/mL) diluted to 100 mL. . . . . 15 mcg/mL

1.0 mL (1000 mcg/mL) diluted to 100 mL. . . . . 10 mcg/mL
5.0 mL (100 mcg/mL) diluted to 100 mL. . . . . 5 mcg/mL
1.0 mL (100 mcg/mL) diluted to 100 mL. . . . . 1 mcg/mL

Preliminary spectrofluorometry studies:

The purpose of this study was to attain maximum sensitivity using the spectrophotofluorometer for a small sample size, while maintaining adequate peak/trough resolution. A variety of slit settings and cells were evaluated. Initially, round glass sample tubes of size 0.8 cm x 7 cm were utilized. However, 1.0 cm rectangular quartz cells were used for all analysis procedures. Reflecting mirrors were placed in the mirror holder of the cell compartments to increase the sensitivity. The quartz cells were rinsed three or four times with ethyl acetate between measurements. For routine analysis, cuvettes and pipettes were washed in 20% nitric acid. All other glassware was washed with laboratory detergent solution, rinsed at least three times with distilled, de-ionized water and allowed to dry in a dust free area in order to prevent fluorescent impurities.

Sample size selection:

Initially a sample size of 100 µL was chosen for analysis. This was followed by a reduction in sample size to 50 µL obtained with calibrated glass capillary tubes. Heparinized capillary tubes were used for collection of blood samples. It was not feasible to draw 50 µL blood samples from the rabbit ear capillaries at regular intervals. Therefore, it was necessary to further reduce the sample

size by using heparinized microhematocrit capillary tubes. The volume of these tubes was determined to be 15.7 µL by measurement of the weight increase when filled to mark with distilled water, as shown in Table I.

Table I. Determination of volume of heparinized micro-hematocrit capillary tubes used for collecting blood samples.

WEIGHT OF FILLED TUBES (grams)	WEIGHT OF EMPTY TUBES (grams)	DIFFERENCE IN WEIGHT (grams)
0.235	0.219	0.016
0.234	0.219	0.015
0.234	0.218	0.016
0.232	0.217	0.015
0.235	0.219	0.016
0.237	0.220	0.017
0.235	0.220	0.015
0.235	0.219	0.016
0.235	0.219	0.016
0.232	0.217	0.015

Average weight difference = 0.0157 g, std. dev. = 0.0006.
Volume of capillary tubes = 15.7 µL.

In vivo evaluation of tetracycline hydrochloride concentrations:

Three domestic white rabbits, weighing 1.5 to 2.0 kg were housed single in stainless steel cages in a separate animal room at an environmental temperature of 19-20° C. Rabbits were fed rabbit chow, and had free access to drinking water.

Surgical procedures:

The rabbit was restrained in a special holding box as shown in Figure 1. The upper portion of the ear lobe was cleanly shaven. Hot water was applied to the outer portion of the ear and rubbed briskly with hand to cause vasodilation.

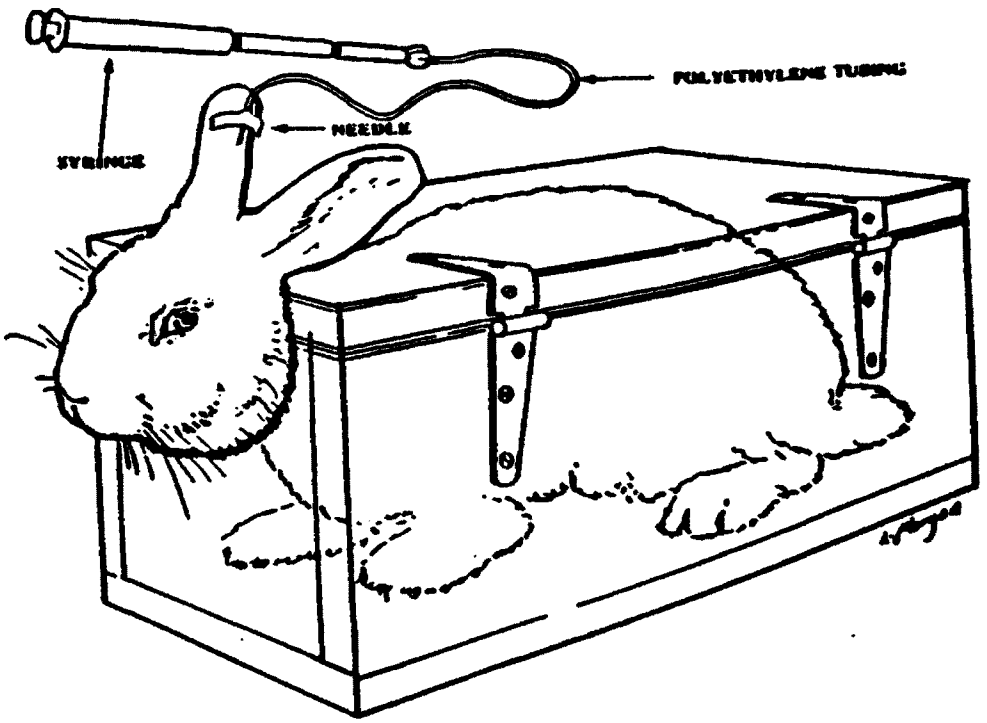

Figure 1. Holding box for rabbits prior to intravenous drug administration.

Intravenous tetracycline study:

The rabbits were injected with the antibiotic at 10 mg per kg of bodyweight. The volume of drug solution to be injected was kept at a constant value of 0.5 mL, as it is not feasible to inject a larger volume. The drug concentration to be injected was calculated as follows.

$$\text{Concentration of drug} = \frac{\text{Animal weight x Dose of the drug}}{\text{Volume of drug solution}}$$

The solution was freshly prepared and immediately injected in order to prevent turbidity caused by decomposition of the drug. The drug solution was injected into the marginal ear vein using a standard butterfly intravenous injection needle and syringe (Figure 2).

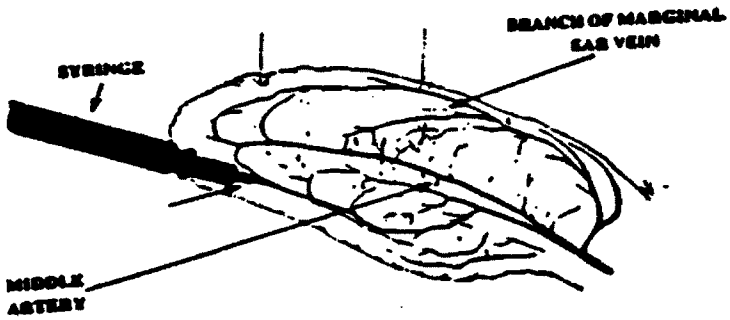

Figure 2. Rabbit marginal ear veins and arteries. (Syringe and needle in blood vessel).

Rabbits were allowed to move freely in a room after injection to aid in better blood circulation. The marginal ear vein was poked or raptured with a needle to collect venous blood samples. Capillary blood samples were collected by pricking the ear lobe tip with a lancet, taking care to avoid the branches of the marginal ear vein. Both samples were collected in heparinized capillary tubes (capillary action) to the calibrated mark. The blood samples were collected in duplicate for tetracycline assay at 0.5, 1, 2, 3, 4, 5 and 6 hours.

Samples were refrigerated until analysis. Analysis was completed within 12 hours of sample collection.

A blank (blood sample obtained prior to injection; extracted by Kohn's procedure) was used for zero adjustment. A tetracycline hydrochloride concentration of 25 mcg/mL in distilled water was used to set the relative intensity at 90 on the meter of spectrophotofluorometer. The relative intensity values of all the other standard solutions were recorded in the descending order. The relative intensity values of capillary and venous blood samples were recorded consecutively starting with the 0.5 hour sample to the 6 hour sample. Intermittant measurements of the blank settings and the highest standard (25 mcg/mL) were checked to ensure that they gave relative intensities of 0 and 90 respectively.

Comparison of whole blood and serum drug concentrations:

The purpose of this experiment was to determine whether the tetracycline concentrations in whole blood and serum can be correlated. In order to carry out capillary blood analysis, it is necessary to collect a sufficient volume of blood to allow it to clot, so that serum can be obtained. This is not feasible with a small time intervals between capillary blood samples.

Two rabbits weighing 2.4 kg and 1.9 kg (rabbits 1 and 2) were injected with antibiotic at 10 mg per kg body weight. A time interval of 30 minutes was placed between these two injections to allow sufficient time for sample collection. For the analysis of whole blood, samples were collected directly from the marginal ear vein in heparinized capillary tubes. In case of serum, a sample volume of

1-2 mL was collected in blood collecting tubes, allowed to clot, centrifuged and serum separated. The serum was drawn into heparinized capillary tubes for further analysis. Blood samples were collected in duplicate for tetracycline assay at 1, 2, 3, 4, 5 and 6 hours. Samples were refrigerated until analysis.

Comparison of drug concentrations in capillary and venous blood:

Three rabbits (A, B and C) weighing 1.92 kg, 1.82 kg, and 1.48 kg respectively were injected with the antibiotic at 10 mg per kg body weight. Whole blood samples were used in this study. Capillary and venous blood samples were collected in duplicate in heparinized capillary tubes at 0.5, 1, 2, 3, 4, 5 and 6 hours. Samples were refrigerated until analysis.

The experiment was performed in triplicate on each rabbit, with a "washout" period of 3 days between runs. Samples were collected in duplicate for each set of experiments for the three rabbits. Standards were extracted and analyzed in duplicate for each experiment. Average relative intensity values were used in the preparation of standard curves. The unknown concentration was calculated using the following equation.

$$Y = a + bX$$

Where: Y = relative intensity of the sample.

a = intercept.

b = slope of the straight line.

X = unknown concentration.

Data analysis:

The average values of the capillary blood concentrations for the three rabbits in each experiment were plotted against the average values of the venous blood concentrations. A linear regression analysis was carried out on this data. The results were further analyzed by analysis of covariance (ANCOVA), since there are more than two variables involved (25). An analysis of covariance tests a dependant variable Y for homogeneity among group means in a design similar to that of the closely related analysis of variance. Analysis of covariance of the data (capillary and venous blood concentrations with respect to time) was performed using the "Biometry" statistical program software (25) on a Zenith data systems computer.

CHAPTER III

RESULTS AND DISCUSSION

Standard curves for tetracycline hydrochloride:

In prelimnary experiments, an aliquot of 100 μL of each standard was used with Kohn's extraction procedure in the preparation of the standard curves. Standard curves for drug concentrations in the range of 0.1 mcg/mL to 100 mcg/mL were linear with correlation coefficients of 0.991 or better.

When the in-vivo study was carried out it was observed that the drug concentration in the 0.5 hour blood sample (first blood sample after the injection) did not exceed 20 mcg/mL. When an aliquot of 15.7 microliters (volume of the capillary tubes utilized in drawing samples) of each standard was used, the minimum tetracycline hydrochloride concentration analyzable with confidence was found to be 1 mcg/mL. Therefore, standard curves were prepared for tetracycline hydrochloride concentrations in the range of 1 mcg/mL to 25 mcg/mL.

The extraction procedure for determination of tetracycline hydrochloride for the standard curves was the same as the analysis procedure used for whole blood and serum samples. Aqueous solutions of tetracycline hydrochloride were used for the standard curve. It was not practical to prepare standard curves by spiking standard whole blood and serum samples. The reasons are: (1) The volumes of serum and blood necessary would require sacrificing many rabbits. (2) It is difficult to handle non-heparinized

whole blood samples. (3) Since a very small sample size (15.7 μL) is diluted to 2 mL for the analysis, there should be little or no difference in using serum, whole blood or aqueous samples in the extraction procedure. When whole blood samples were used for analysis, the addition of these samples to water resulted in hemolysis. The diluted samples were stored in the refrigerator for at least two hours before analysis.

Preliminary spectrofluorometry study:

Maximum sensitivity for the analysis of tetracycline hydrochloride was determined to be at an excitation wavelength of 390 nm and an emission wavelength of 515 nm. When glass sample tubes of 0.8 cm x 7 cm were used the best slit setting was obtained with the excitation monochromator exit slit (Ex) at 2 mm and the emission monochromator entrance slit (Em) and the exit slit to the photomultiplier tube (PM) at 5 mm. Use of 1 cm rectangular quartz cells along with reflecting mirrors in the mirror holders of the cell compartment and the same slit setting gave a fourfold increase in sensitivity. The mirrors reflect the fluorescence of the sample solution, thus increasing the relative intensity measurement. By increasing the excitation monochromator exit slit to 5 mm, a ninefold increase in sensitivity was realized with very little loss of resolution. In this case the excitation slit is opened wide to excite as many molecules as possible and thus increase sensitivity.

Comparison of whole blood and serum drug concentrations:

The spectrophotofluorometer parameters for this part of the study are shown in Table II. Consistent relative intensity values were obtained for tetracycline hydrochloride concentrations in the range of 1 mcg/mL to 25 mcg/mL (Table III). The standard curve for these drug concentrations was highly linear with a correlation coefficient (R^2) of 0.999 (Fig 3).

Table II. Spectrofluorometer parameters.

PARAMETER	SETTING
Voltage	800 Ψ
Slits	Ex: 5, Em: 5, PM: 5 mm
Blank	3.34 x 100 (subtracted)
Excitation wavelength	390 nm
Emission wavelength	515 nm
Sensitivity	79

Table III. Concentration vs. relative fluorescence intensity for tetracycline hydrochloride in water: (Kohn's extraction procedure using 15.7 μL of sample). Standard for comparison of whole blood and serum concentrations.

CONCENTRATION (mcg/mL)	RELATIVE INTENSITY Sample 1	RELATIVE INTENSITY Sample 2	AVERAGE RELATIVE INTENSITY
25	90	90	90
20	73	68	70.5
15	55.5	50.5	53
10	36	38	37
5	16.5	20.5	18.5
1	3.4	5.2	4.3
0	0	0	0

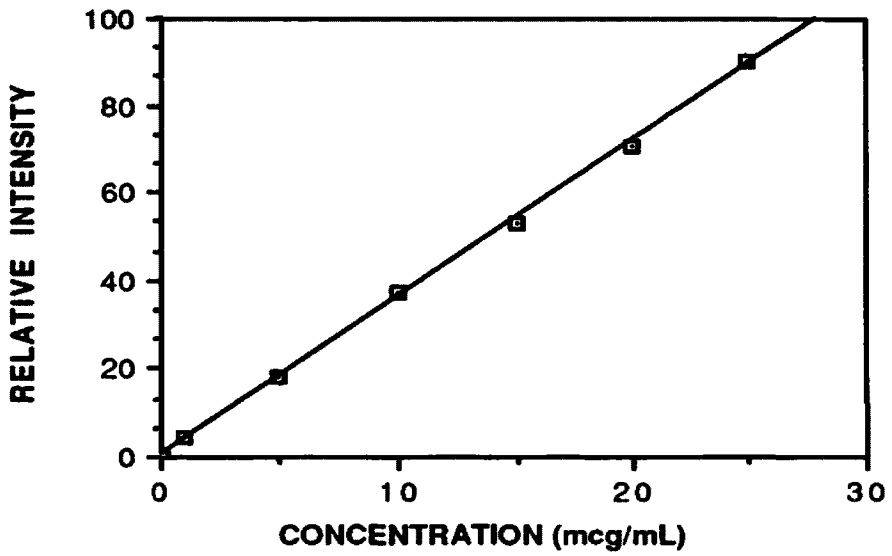

Figure 3. Concentration vs. relative fluorescence intensity of tetracycline hydrochloride in water: (Kohn's extraction procedure using 15.7 μL of sample). Standard curve for comparison of whole blood and serum drug concentrations.

$Y = 0.520 + 3.548X \qquad R^2 = 0.999$

The data obtained from sampling of whole blood and serum of rabbits 1 and 2 are shown in Tables IV and V respectively. The average concentration values obtained from whole blood and serum samples of both the rabbits are shown in Table VI.

Linear regression analysis of the average whole-blood versus serum drug concentrations (data in Table VI) showed a good linear correlation (Figure 4). Exact agreement between tetracycline hydrochloride concentrations in blood and serum would result in an equation with a slope of 1.00, an intercept of 0.0 and an R^2 of 1.00. The actual slope was 0.907 with an intercept of 0.339 and an R^2 of 0.985, thus showing only a small difference between whole blood and serum drug concentrations. A whole blood tetracycline hydrochloride concentration of 10.0 μg/mL corresponds to a serum concentration of 9.41 μg/mL using the regression equation. Therefore, it was concluded that use of whole blood samples for tetracycline hydrochloride analysis was valid for this study.

Table IV. Tetracycline hydrochloride concentrations in whole blood.

TIME (Hours)	RELATIVE INTENSITY Sample 1	CONC. (mcg/mL)	RELATIVE INTENSITY Sample 2	CONC. (mcg/mL)	AVERAGE CONC. (mcg/mL)
Rabbit 1					
1.0	33.0	9.154	39.0	10.846	10.000
2.0	20.0	5.490	25.0	6.900	6.195
3.0	12.0	3.236	18.5	5.068	4.152
4.0	10.5	2.813	12.5	3.377	3.095
5.0	6.0	1.545	9.4	2.503	2.024
6.0	4.2	1.037	7.6	1.995	1.516
Rabbit 2					
1.0	30.0	8.318	25.0	6.900	7.605
2.0	16.5	4.504	13.5	3.658	4.081
3.0	13.0	3.517	10.0	2.672	3.095
4.0	9.5	2.531	8.1	2.136	2.334
5.0	8.0	2.108	4.6	1.150	1.629
6.0	5.4	1.375	3.9	0.953	1.164

Table V. Tetracycline hydrochloride concentrations in serum.

TIME (Hours)	RELATIVE INTENSITY Sample 1	CONC. (mcg/mL)	RELATIVE INTENSITY Sample 2	CONC. (mcg/mL)	AVERAGE CONC. (mcg/mL)
Rabbit 1					
1.0	36.0	10.000	30.5	8.450	9.225
2.0	22.5	6.195	19.0	5.209	5.702
3.0	16.0	4.363	12.0	3.236	3.800
4,0	13.0	3.517	9.1	2.418	2.968
5.0	9.4	2.503	6.4	1.657	2.080
6.0	6.3	1.629	4.5	1.122	1.376
Rabbit 2					
1.0	24.5	6.759	29.5	8.168	7.464
2.0	13.5	3.658	18.0	4.927	4.293
3.0	12.0	3.236	16.0	4.363	3.800
4.0	9.2	2.446	12.0	3.236	2.841
5.0	6.4	1.657	9.4	2.503	2.080
6.0	3.3	0.784	7.1	1.855	1.320

Table VI. Average tetracycline hydrochloride concentrations obtained from whole blood and serum samples of Rabbit 1 and Rabbit 2.

AVERAGE WHOLE BLOOD CONCENTRATION (mcg/mL)	AVERAGE SERUM CONCENTRATION (mcg/mL)
Rabbit 1	
10.000	9.225
6.195	5.702
4.152	3.800
3.095	2.968
2.024	2.080
1.516	1.376
Rabbit 2	
7.605	7.464
4.081	4.293
3.095	3.800
2.334	2.841
1.629	2.080
1.164	1.320

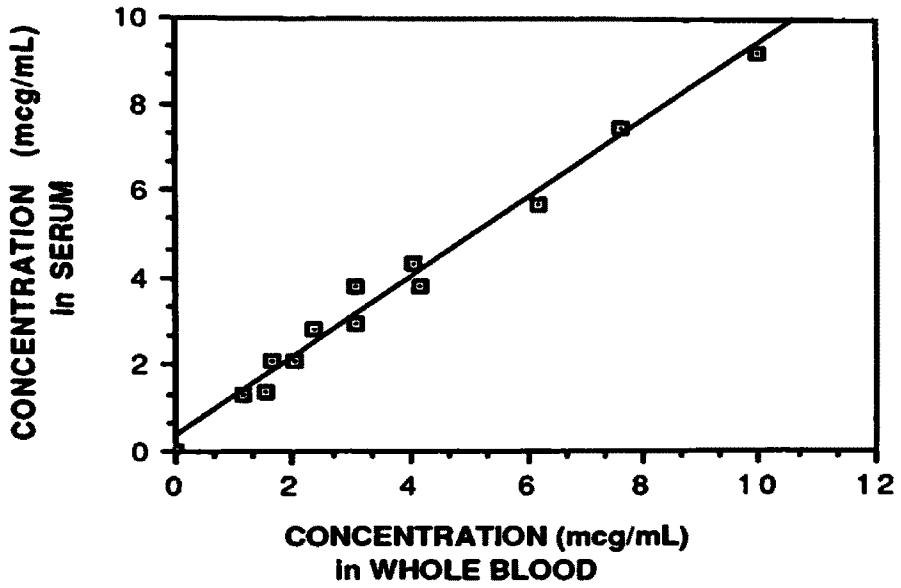

Figure 4. Relationship of whole blood and serum tetracycline hydrochloride concentrations.
$Y = 0.339 + 0.907X$ $R^2 = 0.985$

Comparison of drug concentrations in capillary and venous blood:

The spectrophotofluorometer parameters for study on rabbit A are shown in Table VII. In preparation of the standard curve, consistent relative intensity values were obtained for tetracycline hydrochloride in the concentration of 1 mcg/mL to 25 mcg/mL (Table VIII). A plot of average relative fluorescence intensity versus concentration was highly linear with a correlation coefficient (R^2) of 0.999 (Figure 5). Similar standard curves were obtained for studies on Rabbits B and C. The data are shown in Tables XIV-XVII and Figures 11 and 12 in the supplemental data section.

The capillary and venous blood concentrations of tetracycline hydrochloride in Rabbits A, B and C are shown in Tables IX and X. Plots of concentration vs. time and log concentration vs. time for the capillary and venous blood samples for Rabbit A are shown in

Figures 6-9. Similar plots of the data from Rabbits B and C are shown in Figures 13-20 in the supplemental data section. These graphs clearly indicate that tetracycline hydrochloride follows a biexponential elimination in rabbits after intravenous injection. The terminal portion of the data (after 2 hours) is log-linear, consistent with a first order elimination of the drug following an initial distribution phase. The mean elimination half life was 2.141 hours for the three rabbits. These results are consistent with previous reports on the pharmacokinetics of tetracycline in rabbits in which the elimination was found to be biexponential with a terminal half-life of 2.0 hours (22).

Table VII. Spectrofluorometer parameters.

PARAMETER	SETTING
Voltage	800 Ψ
Slits	Ex: 5, Em: 5, PM: 5 mm
Blank	Set 1: 2.96 x 100 (subtracted)
	Set 2: 2.33 x 100 (subtracted)
	Set 3: 1.37 x 100 (subtracted)
Excitation wavelength	390 nm
Emission wavelength	515 nm
Sensitivity	Set 1: 68
	Set 2: 71
	Set 3: 76

Table VIII. Concentration vs. relative fluorescence intensity for tetracycline hydrochloride in water: (Kohn's extraction procedure using 15.7 µl of sample). Standard for comparison of capillary and venous blood drug concentrations for Rabbit A.

CONCENTRATION (mcg/mL)	RELATIVE INTENSITY						RELATIVE INTENSITY (Average)	STD. DEV.
	SET 1		SET 2		SET 3			
25	90	89	90	88	90	92	89.83	1.33
20	70	67.5	70.5	65.5	71	73	69.58	2.67
15	56.5	52.5	54.5	52.5	54.5	51.5	53.67	1.83
10	37	35	37.5	32.5	37	38	36.17	2.07
5	18.5	16	18	19	18	19	18.08	1.11
1	5.0	6.0	4.4	3.4	4.2	3.8	4.47	0.93
0	0	0	0	0	0	0	0	0

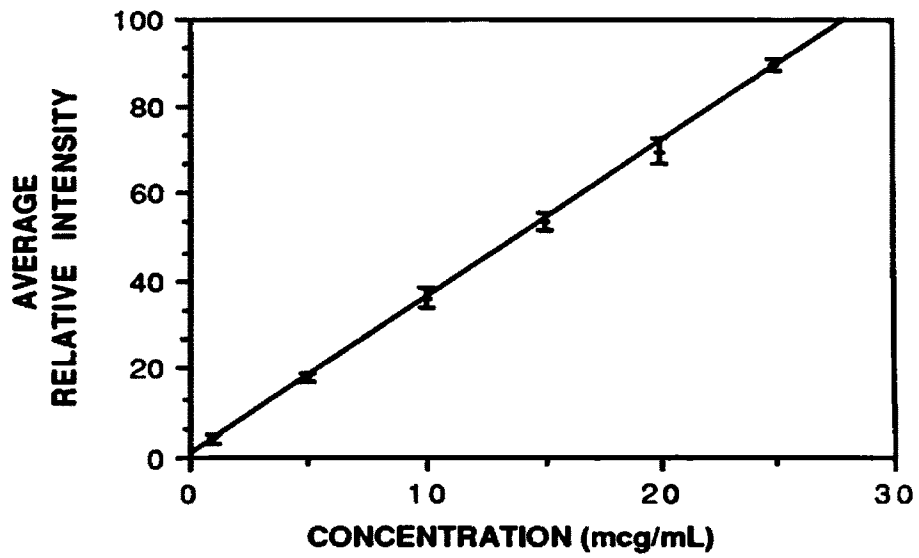

Fig 5. Concentration vs. relative fluorescence intensity for tetracycline hydrochloride in water: (Kohn's extraction procedure using 15.7 µL of sample). Standard curve for comparison of capillary and venous blood drug concentration for Rabbit A. $Y = 0.434 + 3.536X$ $R^2 = 0.999$

TableIX. Capillary blood concentrations of tetracycline hydrochloride for Rabbits A, B and C.

TIME (Hours)	CONCENTRATION (mcg/mL)						AVERAGE CONC. (mcg/mL)	STD. DEV.	LOG CONC.
	SET 1		SET 2		SET 3				
	a	b	a	b	a	b	Rabbit A		
0.5	16.330	13.481	16.788	14.213	14.903	13.515	14.872	1.415	1.171
1.0	10.633	10.063	11.781	9.635	11.015	9.629	10.459	0.850	1.019
2.0	5.079	6.788	6.917	5.629	5.743	5.050	5.868	0.814	0.765
3.0	3.513	5.222	4.343	3.627	3.662	2.968	3.889	0.786	0.583
4.0	2.373	3.513	3.770	3.197	3.246	2.690	3.313	0.518	0.491
5.0	1.519	2.658	1.767	1.480	2.135	1.775	1.889	0.444	0.267
6.0	1.234	1.519	1.195	1.052	1.469	1.247	1.286	0.176	0.106
	SET 1		SET 2		SET 3				
	a	b	a	b	a	b	Rabbit B		
0.5	13.589	11.767	17.080	18.947	13.411	15.231	15.004	2.643	1.171
1.0	9.384	8.543	12.482	13.488	9.491	10.611	10.667	1.939	1.022
2.0	5.599	5.179	7.884	8.603	5.992	6.832	6.682	1.347	0.818
3.0	4.758	3.917	5.585	6.447	4.172	4.732	4.935	0.938	0.687
4.0	3.216	2.796	3.718	4.292	3.192	4.032	3.541	0.569	0.545
5.0	2.319	1.590	2.712	3.718	2.297	2.633	2.545	0.698	0.392
6.0	1.871	1.422	1.821	2.827	1.429	2.129	1.917	0.523	0.270
	SET 1		SET 2		SET 3				
	a	b	a	b	a	b	Rabbit C		
0.5	9.772	11.436	9.817	11.618	10.969	12.670	11.047	1.119	1.041
1.0	7.294	8.723	8.848	7.186	8.417	9.976	8.407	1.047	0.922
2.0	3.724	4.724	4.139	4.970	4.448	5.582	4.598	0.651	0.659
3.0	3.153	4.010	3.723	2.892	3.455	4.022	3.543	0.461	0.546
4.0	2.010	2.724	3.030	2.089	2.463	3.314	2.605	0.517	0.408
5.0	1.582	2.010	2.172	1.479	1.953	2.350	1.924	0.337	0.279
6.0	1.211	1.524	1.618	1.258	1.074	1.612	1.383	0.232	0.136

Table X. Venous blood concentrations of tetracycline hydrochloride for Rabbits A, B and C.

TIME (Hours)	CONCENTRATION (mcg/mL)						AVERAGE CONC. (mcg/mL)	STD. DEV.	LOG CONC.
	SET 1		SET 2		SET 3				
	a	b	a	b	a	b	Rabbit A		
0.5	16.899	15.617	15.215	13.069	14.903	16.290	15.332	1.325	1.184
1.0	8.639	9.779	11.066	8.491	10.323	11.849	10.025	1.329	0.998
2.0	5.649	4.652	6.059	4.628	5.604	6.576	5.528	0.772	0.739
3.0	4.367	3.085	3.913	3.626	3.384	4.633	3.835	0.589	0.579
4.0	2.943	1.946	3.627	2.625	3.246	2.968	2.893	0.573	0.453
5.0	1.661	1.519	1.767	1.910	2.135	2.968	1.993	0.522	0.289
6.0	0.949	1.234	1.481	1.052	1.108	2.052	1.313	0.406	0.111
	SET 1		SET 2		SET 3				
	a	b	a	b	a	b	Rabbit B		
0.5	14.710	13.448	16.936	15.355	10.751	13.411	14.102	2.103	1.145
1.0	9.804	11.066	11.620	10.327	7.812	8.932	9.927	1.400	0.993
2.0	5.319	4.338	7.309	6.447	4.872	5.852	5.690	1.082	0.749
3.0	3.777	3.356	5.154	4.436	3.612	4.452	4.131	0.669	0.612
4.0	2.375	2.795	4.149	3.430	2.493	3.192	3.072	0.663	0.479
5.0	1.534	2.179	2.855	2.396	1.597	2.353	2.152	0.507	0.322
6.0	1.057	1.646	2.195	2.022	1.149	1.961	1.672	0.476	0.207
	SET 1		SET 2		SET 3				
	a	b	a	b	a	b	Rabbit C		
0.5	11.293	12.864	8.155	9.263	9.409	10.969	10.325	1.701	1.009
1.0	8.865	9.722	6.078	7.324	7.283	8.275	7.925	1.298	0.894
2.0	4.153	5.152	3.030	3.723	3.739	4.873	4.112	0.790	0.607
3.0	3.724	4.295	2.632	2.116	2.633	3.455	3.143	0.817	0.485
4.0	2.582	2.867	2.199	1.812	2.009	2.747	2.369	0.425	0.369
5.0	1.810	2.125	1.756	1.452	1.783	2.123	1.842	0.254	0.261
6.0	1.125	1.982	1.341	0.981	0.960	1.726	1.353	0.419	0.123

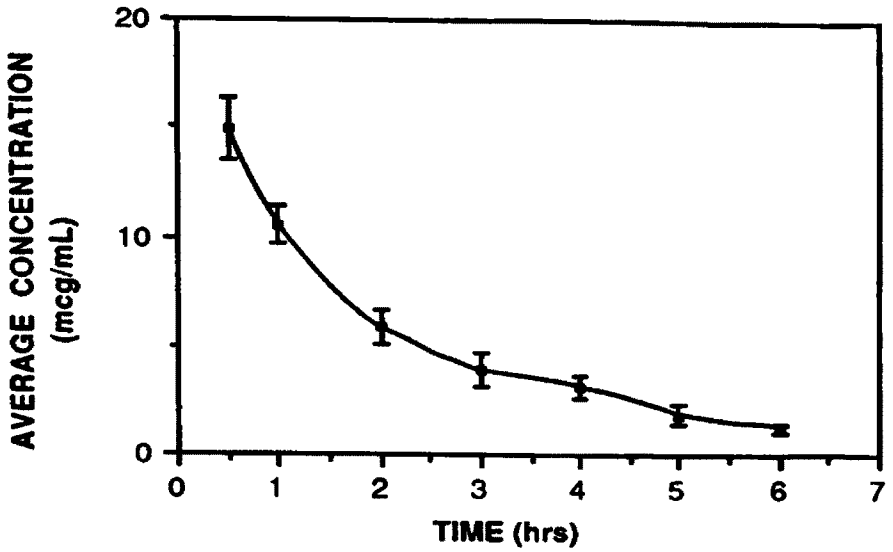

Figure 6. Plot of average capillary blood concentrations of tetracycline hydrochloride vs. time: Rabbit A.

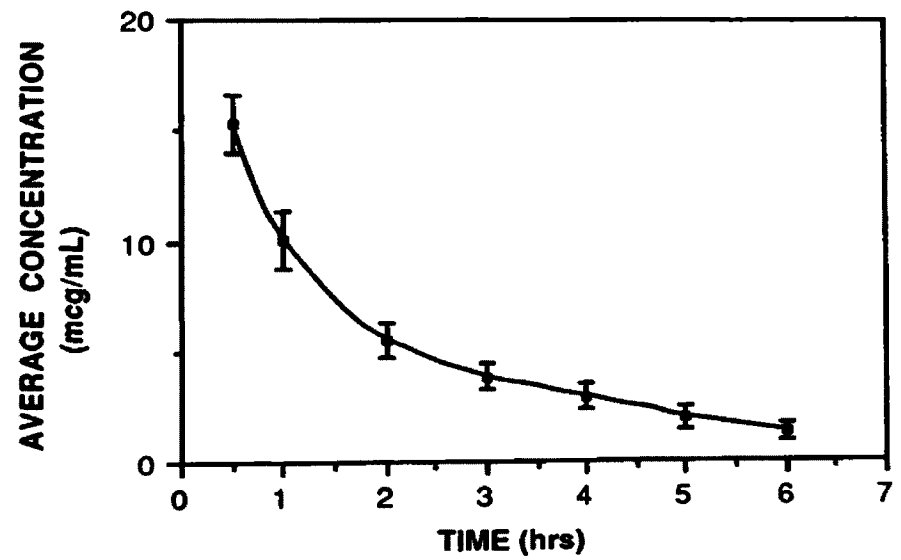

Figure 7. Plot of average venous blood concentrations of tetracycline hydrochloride vs. time: Rabbit A.

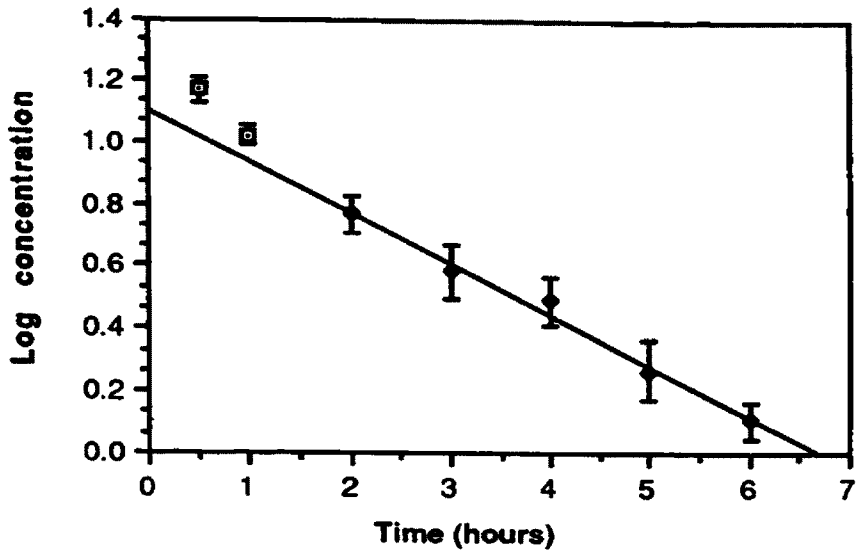

Figure 8. Plot of log capillary blood concentrations of tetracycline hydrochloride versus time: Rabbit A.

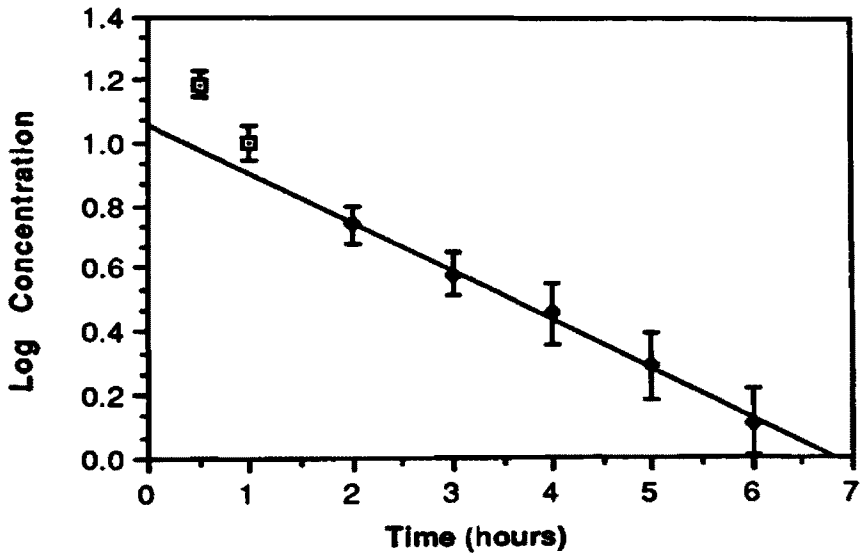

Figure 9. Plot of log venous blood concentrations of tetracycline hydrochloride versus time: Rabbit A.

The relationship between capillary and venous blood concentrations of tetracycline hydrochloride was analyzed by least squares regression. The average capillary and venous blood concentrations from the studies on rabbits A, B and C are shown in Table XI. The concentrations in the table are the average values from the two determinations (a and b) of each set of experiments shown in Tables IX and X. The data in this table corresponds to the blood samples collected at the 0.5, 1, 2, 3, 4, 5 and 6 hours, respectively.

Table XI. Average capillary and venous concentrations of tetracycline hydrochloride for Rabbits A, B and C.

AVERAGE CAPILLARY CONCENTRATION (mcg/mL)	AVERAGE VENOUS CONCENTRATION (mcg/mL)
RABBIT A Set 1	
14.906	16.258
10.348	9.209
5.934	5.151
4.938	3.726
2.943	2.445
2.089	1.614
1.377	1.092
RABBIT A Set 2	
15.501	14.142
10.708	9.779
6.273	5.344
3.985	3.770
3.484	3.126
1.624	1.839
1.124	1.267
RABBIT A Set 3	
14.209	15.596
10.323	11.086
5.397	6.090
3.315	4.009
2.698	3.107
1.955	2.552
1.358	1.580
RABBIT B Set 1	
12.678	14.079
8.964	10.435
5.389	4.809
4.338	3.576
3.006	2.585
1.955	1.857
1.647	1.352

AVERAGE CAPILLARY CONCENTRATION (mcg/mL)		AVERAGE VENOUS CONCENTRATION (mcg/mL)
	RABBIT B Set 2	
18.014		16.146
12.985		10.974
8.244		6.878
6.016		4.795
4.005		3.790
3.215		2.626
2.324		2.109
	RABBIT B Set 3	
14.321		12.081
10.051		8.372
6.412		5.362
4.452		4.032
3.612		2.843
2.465		1.975
1.779		1.555
	RABBIT C Set 1	
10.579		12.079
8.009		9.294
4.224		4.653
3.582		4.010
2.367		2.725
1.796		1.968
1.368		1.554
	RABBIT C Set 2	
10.718		8.708
8.017		6.701
4.555		3.377
3.308		2.374
2.560		2.006
1.826		1.604
1.438		1.161
	RABBIT C Set 3	
11.820		10.189
9.197		7.779
5.015		4.306
3.739		3.044
2.889		2.378
2.152		1.953
1.343		1.343

The results of linear regression analysis of the data in Table XI are shown in Figure 10. Exact correlation between tetracycline hydrochloride concentrations in capillary and venous blood samples would result in a slope of 1.00, an intercept of 0.0 and a correlation coefficient (R) of 1.00. The slope of the regression line for the 63 data points is 1.00 with an intercept of 0.354 (positive capillary concentration bias) and a correlation coefficient of 0.978. These results indicate a high degree of correlation between the sampling methods (capillary vs venous blood). For the 63 paired samples the drug concentration in the capillary samples was not substantially different from those in venous blood samples.

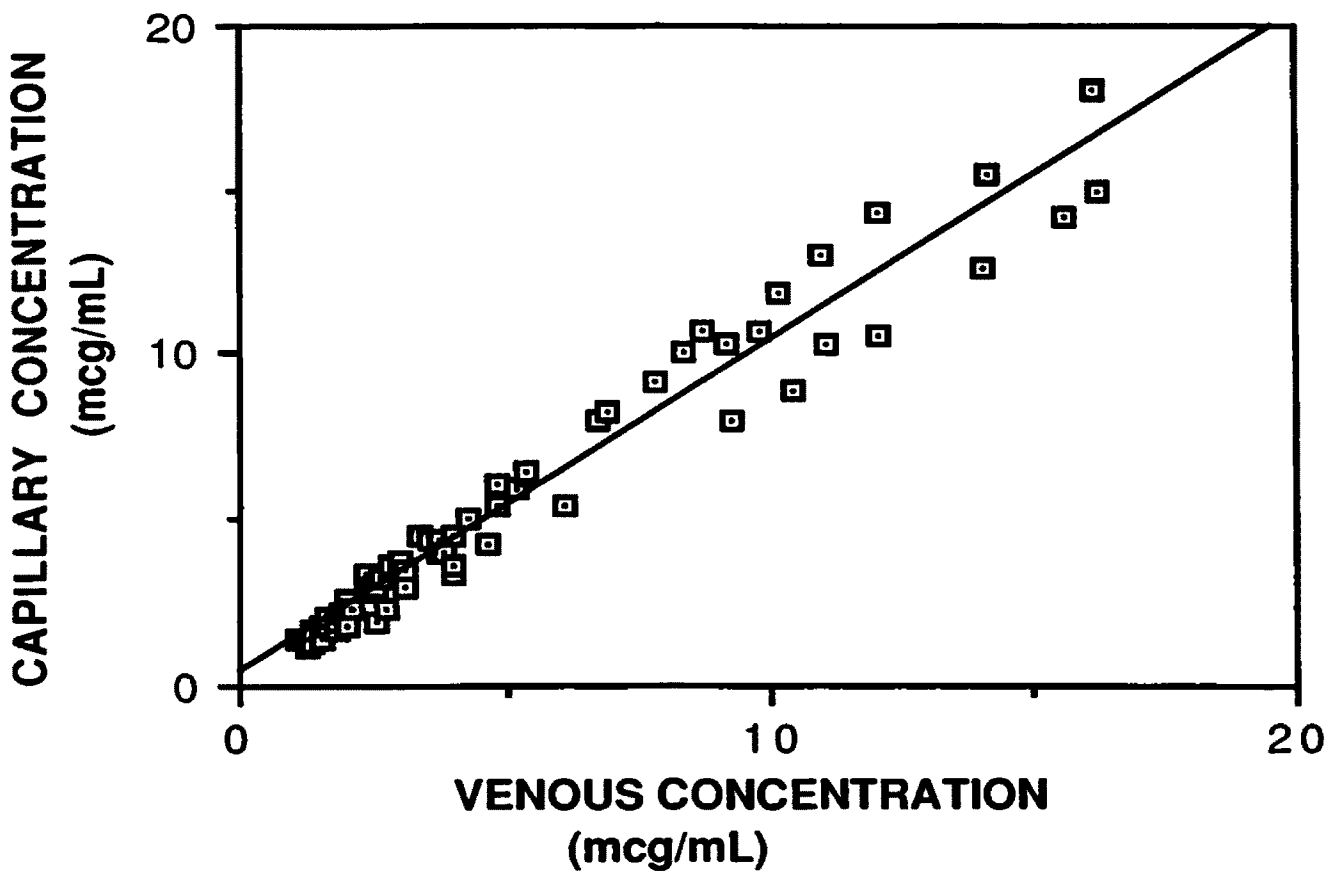

Figure 10. Relationship between capillary and venous blood concentrations of tetracycline hydrochloride.
Y = 0.354 + 1.000X R= 0.978

The results of linear regression analysis on the nine individual sets of capillary vs venous blood tetracycline hydrochloride concentrations (from Table XI) are shown in Table XII. The slopes of the regression lines for the nine data sets ranged between 0.830 and 1.207, intercepts ranged between - 0.280 and 1.003, and all correlation coefficients (R) were greater than 0.991.

Table XII. Linear regression analysis of capillary vs. venous tetracycline hydrochloride concentrations. Equation: Y = a + bX. Y = capillary drug concentration, a = intercept, b = slope, X = venous drug concentration.

		Set 1	Set 2	Set 3
Rabbit A	Intercept (a) =	1.003	- 0.159	- 0.280
	Slope (b) =	0.899	1.116	0.936
	Correlation coefficient (R) =	0.991	0.999	0.999
Rabbit B	Intercept (a) =	0.835	0.223	0.019
	Slope (b) =	0.830	1.125	1.186
	Correlation coefficient (R) =	0.994	0.998	0.999
Rabbit C	Intercept (a) =	0.070	0.162	- 0.036
	Slope (b) =	0.866	1.207	1.175
	Correlation coefficient (R) =	0.999	0.997	0.999

The small positive bias in the capillary concentration of tetracycline hydrochloride seen in Figure 10 (intercept = 0.354) does not appear to be systematic. From the data in Table XI it can be seen that the drug concentrations in capillary blood samples exceeded that found in the venous blood samples in five of the nine data sets. In

the other four data sets the venous blood tetracycline hydrochloride concentrations were greater than those in the capillary blood samples collected at the same time. The individual regression data shown in Table XII further indicates this bias is not systematic. In the five data sets where the capillary drug concentration was greater than the venous drug concentration (slopes greater than 1.00), the intercept values were positive in three of the five cases. In the data sets where the slopes were less than 1.00, the intercepts were positive in three of the four cases. These results indicate that the small positive bias of the Y intercept observed in linear regression of the combined data sets (63 pairs) is not systematic. Therefore, analysis of tetracycline hydrochloride concentrations in capillary blood samples provides an accurate and unbiased estimate of the concentrations in the venous blood.

It can be seen fom Table XI that the largest variation between capillary and venous concentrations of tetracycline hydrochloride values occurred during the distribution phase (before two hours). The difference here may be real because during the distribution phase the concentration of the drug in the central compartment (tissues and fluids with which a drug rapidly equilibrates following administration) rapidly decreases while the concentration of the drug in the peripheral compartment (tissues and fluids with which a drug slowly equilibrates from the central compartment) increases. After the distribution is complete, the drug will be lost (eliminated) from both compartments at a proportional rate.

Statistical analysis:

From the results discussed above it was concluded that a strong correlation exists between capillary and venous blood concentrations of tetracycline hydrochloride and that measurement of capillary concentrations of the drug provides an accurate and unbiased estimate of the venous concentrations. The statistical validity of these conclusions was evaluated by analysis of covariance (ANCOVA). Analysis of covariance is based on a linear model However, the drug concentration vs time data obtained in this study was not linear over the entire time period. As clearly seen from Figures 8 and 9, the data is not linear during the distribution phase (before two hours). Therefore, it was not possible to carry out analysis of covariance on the entire data set (during the entire time period) simultaneously. The data shown in Figures 8 and 9 indicate that tetracycline blood concentrations can be described by a two compartment (biexponential) model following intravenous administration. In this case, the distribution and elimination phase can be described, mathematically, by linear models. However, since it was not practical to collect capillary blood samples more frequently than at the stated time periods, only two samples were collected during the distribution phase (at 0.5 and 1.0 hours). With only two data points in the distribution phase of each data set, statistical analysis of the linearity and homogeneity of the data sets would not be valid.

Analysis of covariance was used to statistically evaluate the elimination phase (β phase) data obtained between 2 and 6 hours. The concentration values were converted into the natural logarithms by the ANCOVA computer program and plotted versus time. These

plots were linear. Regression analysis was carried out on these data points. The slopes of the regression lines were then compared. The ANCOVA program used in the data analysis also computes the common slope for all the data points (β phase) for all the three experiments on each rabbit. This was used to calculate the elimination half-life of tetracycline hydrochloride in each rabbit.

Three sets of experiments were performed on each animal, with capillary and venous concentration values in duplicate for each set. The total number of groups were 12 (6 capillary and 6 venous). The concentration versus time values for each set was considered as one group. The statistic for computing regression was entered separately for each group. The ANCOVA program gives differences among adjusted means, testing for homogeneity of the Y intercepts and differences among the slopes, testing for homogeneity of slopes. In this study, only the difference in the slopes of the regression lines of each group was compared. The test statistic used in ancova is the 'F' ratio.

$$F = \frac{\text{Mean square value of difference among separate slopes}}{\text{Mean square value of deviation of each group from its separate slope}}$$

The computed 'F' ratio is then compared with the critical 'F' value. The critical 'F' value, which is a function of degrees of freedom for the denominator and significance level, is given in the table of 'F' distribution (25). At a critical α level (selected significance level), if the computed 'F' ratio is greater than or equal to the critical 'F' value, then there is heterogeneity of the slopes of the regression lines. However if the 'F' ratio is smaller than the critical 'F' value then the

difference among the slopes is non significant, that is; the slopes are homogeneous.

The elimination phase (ß phase) values were obtained from Tables IX and X (concentration values from 2 to 6 hours for rabbits A and C and 3 to 6 hours for rabbit B). Results obtained by comparison of slopes using the ANCOVA program can be seen in Table XIII. The degrees of freedom of the denominator of rabbit B is different from that of rabbits A and C because the concentration values at the two hour data point was not used for rabbit B. During initial ANCOVA analysis of the data from rabbit B, considerable variance was observed when the two hour data point was included. Therefore, the concentration values from 3 to 6 hours were used for the statistical analysis of rabbit B.

The critical 'F' value from the table of 'F' distribution at the 0.05 α level for 11 degrees of freedom in the numerator and 36 degrees of freedom in the denominator is 2.11. This is greater than the computed 'F' ratios of 1.627 for rabbit A and 2.093 for rabbit C. This indicates homogeneity of slopes of regression lines at the 0.05 α level ($P>0.05$).

The critical 'F' values at the 0.001 α level and at the 0.05 α level with 11 degrees of freedom in the numerator and 24 degrees of freedom in the denominator are 4.50 and 2.22 respectively. The computed 'F' ratio for rabbit B is 3.890 which is smaller than the critical 'F' value at the 0.001 α level but greater than the critical 'F' value at the 0.05 level. This indicates homogeneity of regression slopes at the 0.001 α level ($p > 0.001$, $p < 0.05$).

An α level of 0.05 was selected for the comparison of the slopes as it is the most commonly used level to show statistical significance. However, if the comparison of the slopes is carried out at the less stringent 0.001 α level, the computed 'F' ratio for each of the three rabbits would be less than the critical 'F' value, indicating that the difference in slopes is not significant. Therefore, there is homogeneity of slopes of the twelve regression lines for the elimination phase data of each rabbit. Thus, it can be concluded that there is a good correlation statistically between the slopes of the elimination phase data of each rabbit when capillary and venous blood drug concentrations are compared.

Table XIII. Differences among elimination phase slopes from ANCOVA.

	SOURCE OF VARIATION	df	SS	MS	Significance level (α)	F Value (Critical)	F Ratio (Computed)	prob-value
Rabbit A	Among slopes	11	0.283	0.026	0.05	2.11	1.627 (NS)	p > 0.05
	Sum of group deviation	36	0.569	0.016	0.001	3.95		
	Common slope = - 0.368							
Rabbit B	Among slopes	11	0.183	0.017	0.05	2.22	3.890 (NS)	0.001 < p < 0.05
	Sum of group deviation	24	0.102	0.004	0.001	4.50		
	Common slope = - 0.321							
Rabbit C	Among slopes	11	0.198	0.018	0.05	2.11	2.093 (NS)	p > 0.05
	Sum of group deviation	36	0.309	0.009	0.001	3.95		
	Common slope = - 0.291							

df = degrees of freedom, ss = sum of squares, ms = mean squares
(NS) = Not statistically significant.

Half-life of tetracycline:

The ANCOVA program used in this data analysis computes the common slope for all data points of all the groups under study for each rabbit. The data points (elimination phase concentration values) were converted into natural logarithmic form and plotted versus time. These plots were linear. The slopes of all the regression lines were taken into account and a common slope value was computed for the entire data for each rabbit. This was used to

calculate the elimination half-life (ke) of the drug for each rabbit as shown.

Rabbit A: Slope = - 0.368.

ke = - slope = - (- 0.368) = 0.368 hr^{-1}.

$t_{1/2}$ = 0.693/ke = 0.693/0.368 = 1.883 hours.

Rabbit B: Slope = - 0.321.

ke = 0.321 hr^{-1}.

$t_{1/2}$ = 2.159 hours.

Rabbit C: Slope = - 0.291.

ke = 0.291 hr^{-1}.

$t_{1/2}$ = 2.381 hours

The average half life of tetracycline hydrochloride was determined to be equal to 2.141 hours with a standard deviation of 0.249.

Significance of study:

The present study was a model using a very small sample size. Two of the studies reported in the literature (10 and 12) have used sample sizes of 15-20 µL, while the remaining studies (8, 19, 27 and 28) have used sample sizes in the range of 500-800 µL. The larger volumes are possible using the standard finger lancet method when human beings are chosen as subjects; however collection of this volume does require significant milking of the finger wound. Results of the present study using 15.7 µl sample compare well to those reported in the literature.

The establishment of an easier, more convenient blood collection method that involves a substantially smaller sample has clinically relevant significance. The finding that there is no significant difference in tetracycline hydrochloride concentration between venous and capillary blood shows that the collection of excess sample or the need to inflict a painful procedure on that population where venous collection is difficult is unwarranted. Thus drug monitoring of tetracycline concentrations can be performed accurately by measuring capillary blood tetracycline concentrations. Certainly the risks of capillary collection are less than with venipuncture.

An ideal method for drug determination should be rapid, incorporate a simple sampling technique and require a small sample size. Capillary sampling has considerable promise in large-scale screening studies, in pediatric studies, in neonatal studies or in any form of repetitive study because capillary blood sampling has substantial practical and economic advantages over venous blood sampling.

This study shows that rabbits can be used as animal models for evaluation of the correlation between capillary and venous blood drug concentrations for various drugs for which therapeutic drug monitoring and pharmacokinetic analysis is done on a regular basis.

This study consisted of development of a model for use of small volume, capillary whole blood samples for the analysis of tetracycline hydrochloride concentrations. There were some limitations to this model. The use of rabbit ear blood samples restricted the volumes and sample times that could be used. The extraction and fluorescence analytical procedure, while sensitive, had

a lower limit of 1.0 μg/mL. With extension of this model to those in humans and with improved analytical techniques such as HPLC analysis with flourescence detection, some of these limitations may be overcome.

CHAPTER IV

CONCLUSIONS

The aim of this study was to investigate if concentrations of tetracycline hydrochloride in capillary blood can be correlated to those in venous blood.

The conclusions resulting from this study are:

1. There is no significant difference between tetracycline hydrochloride concentrations in capillary and venous blood. Therefore, capillary sampling for determination of tetracycline hydrochloride offers a convenient and viable alternative to venous sampling.

2. A good correlation exists between whole blood and serum tetracycline hydrochloride concentrations. Therefore, whole blood can be used as an alternative to serum or plasma in determining tetracycline hydrochloride concentrations.

3. A very small sample volume can be used for assay of tetracycline hydrochloride in whole blood samples.

4. The study can be used as a model for use of a small sample volume in drug concentration studies. The collection of excess sample or the need to inflict a painful procedure on that population where venous blood collection is difficult is not necessary.

CHAPTER V

SUPPLEMENTAL DATA

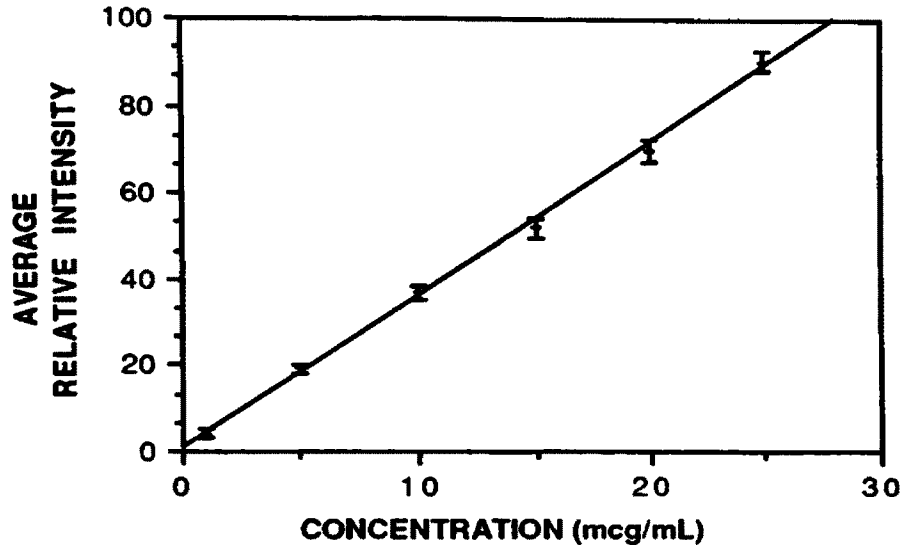

Figure 11. Concentration vs. relative fluorescence intensity for tetracycline hydrochloride in water: (Kohn's extraction procedure using 15.7 µL of sample). Standard curve for comparison of capillary and venous blood drug concentrations for Rabbit B. $Y = 0.572 + 3.539X$ $R^2 = 0.999$

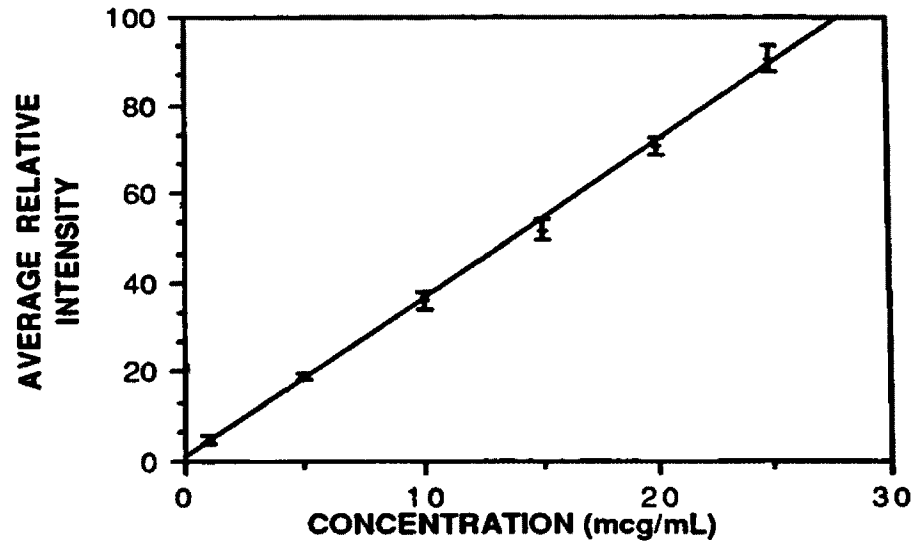

Figure 12. Concentration vs. relative fluorescence intensity for tetracycline hydrochloride in water: (Kohn's extraction procedure using 15.7 µL of sample). Standard curve for comparison of capillary and venous blood drug concentrations for Rabbit C. $Y = 0.452 + 3.546X$ $R^2 = 0.999$

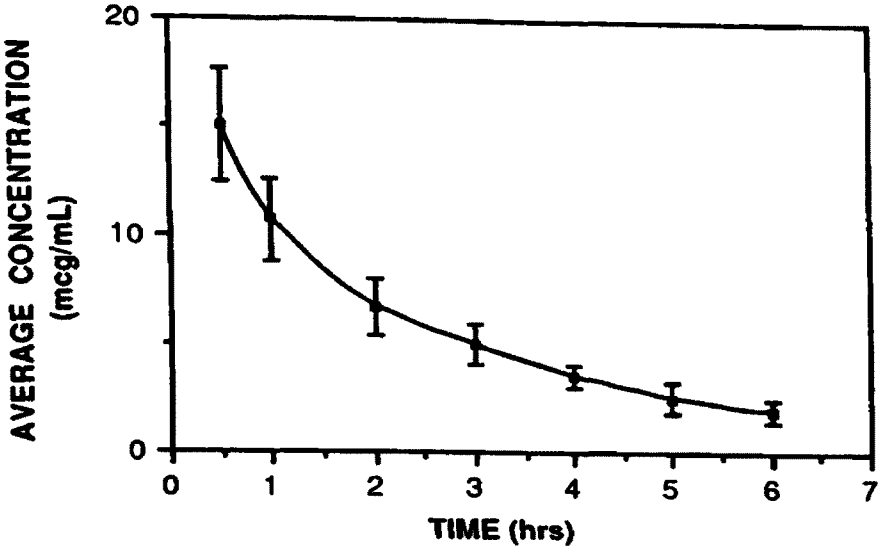

Figure 13. Plot of average capillary blood concentrations of tetracycline hydrochloride versus time for Rabbit B.

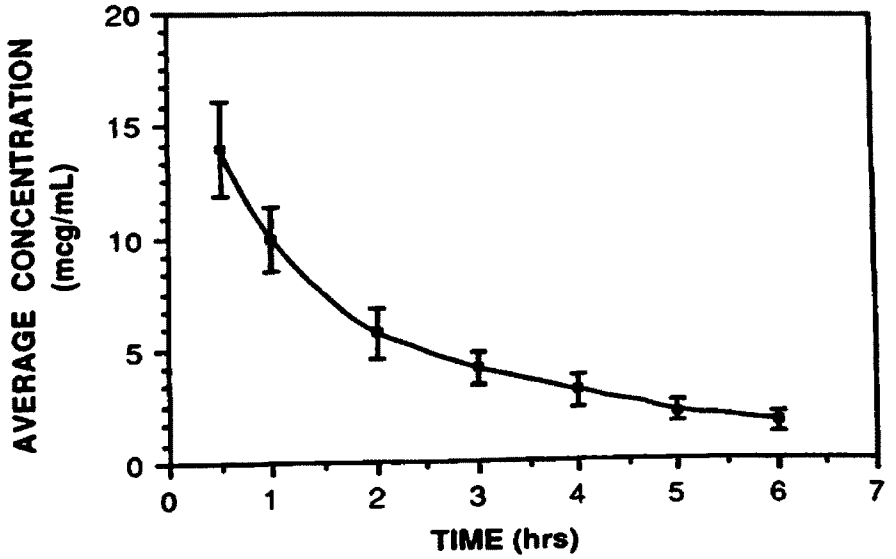

Figure 14. Plot of average venous blood concentrations of tetracycline hydrochloride versus time for Rabbit B.

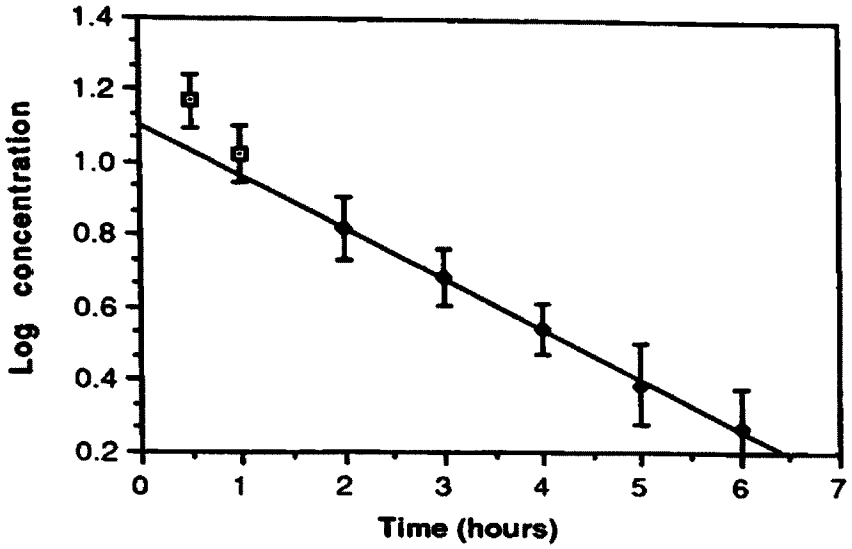

Figure 15. Plot of log capillary blood concentrations of tetracycline hydrochloride versus time for Rabbit B.

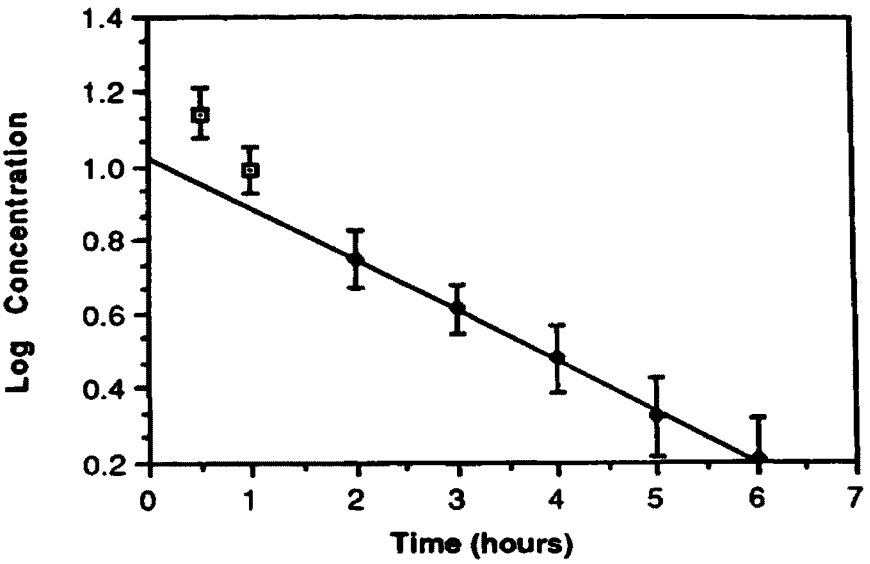

Figure 16. Plot of log venous blood concentrations of tetracycline hydrochloride versus time for Rabbit B.

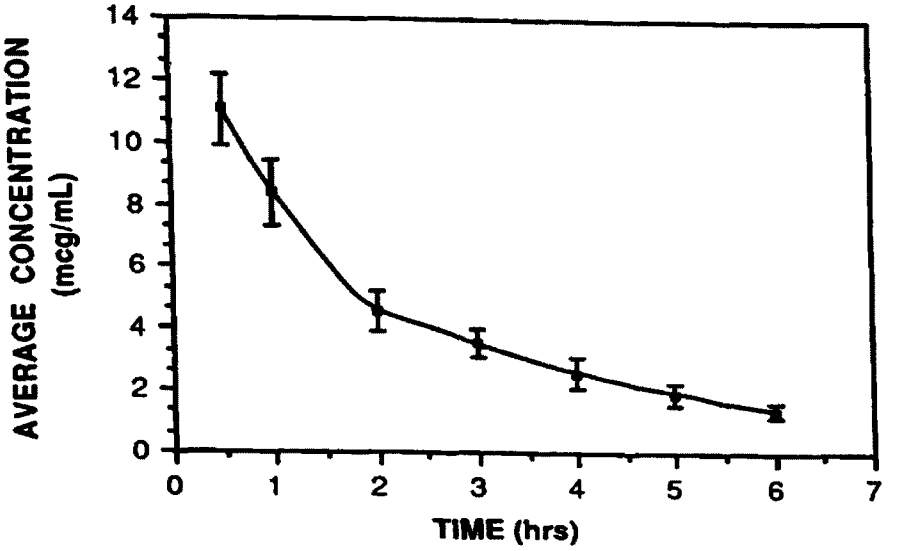

Figure 17. Plot of average capillary blood concentrations of tetracycline hydrochloride versus time for Rabbit C.

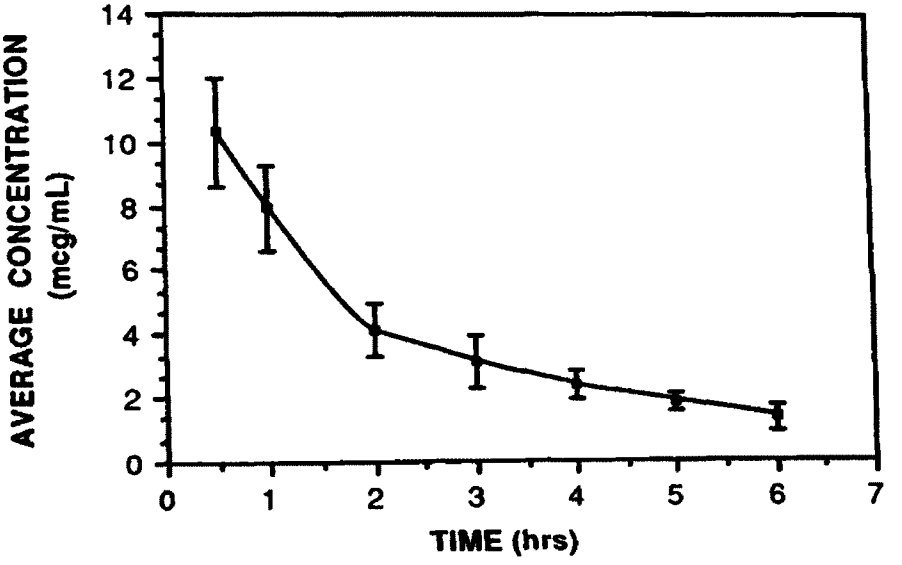

Figure 18. Plot of average venous blood concentrations of tetracycline hydrochloride versus time for Rabbit C.

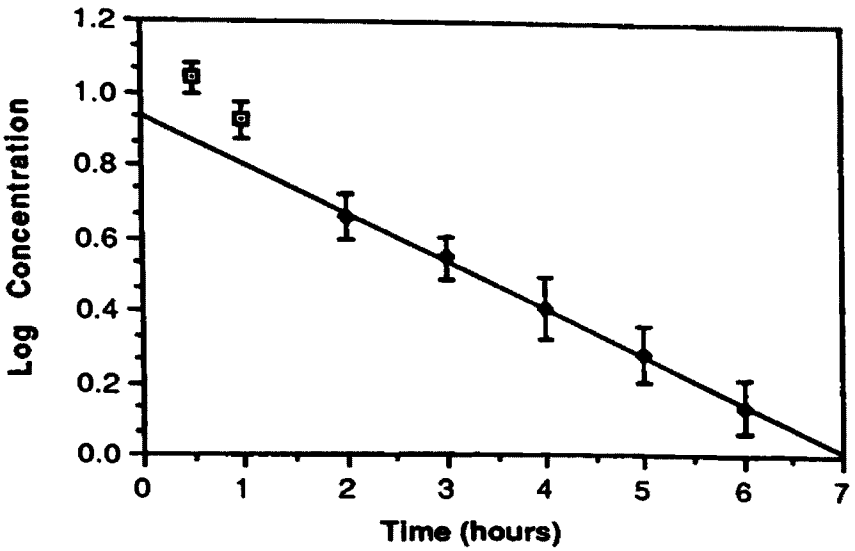

Figure 19. Plot of log capillary blood concentrations of tetracycline hydrochloride versus time for Rabbit C.

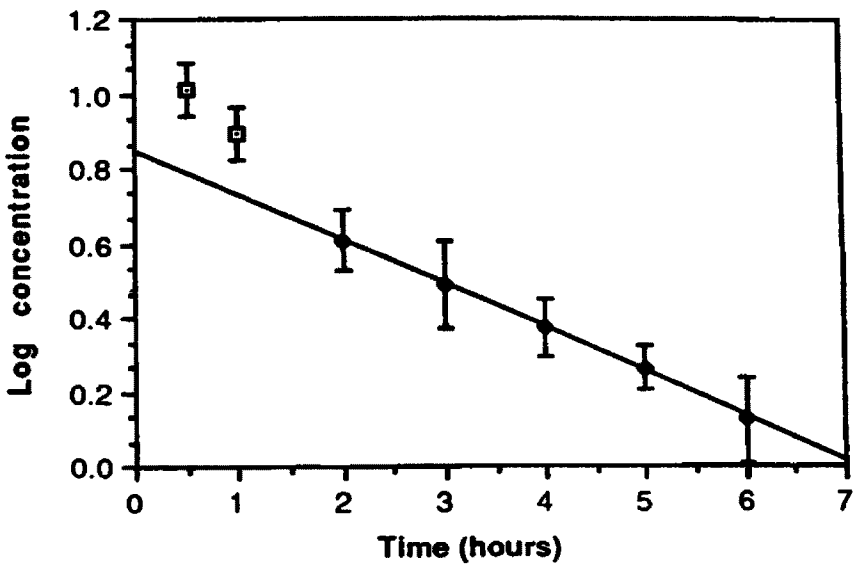

Figure 20. Plot of log venous blood concentrations of tetracycline hydrochloride versus time for Rabbit C.

Table XIV. Spectrofluorometer parameters.

PARAMETER	SETTING
Voltage	800 Ψ
Slits	Ex: 5; Em: 5, PM: 5 mm
Blank	Set 1: 3.16 x 100 (subtracted)
	Set 2: 2.74 x 100 (subtracted)
	Set 3: 3.63 x 100 (subtracted)
Excitation wavelength	390 nm
Emission wavelength	515 nm
Sensitivity	Set 1: 74
	Set 2: 75
	Set 3: 77

Table XV. Concentration vs. relative fluorescence intensity for tetracycline hydrochloride in water: (Kohn's extraction procedure using 15.7 μL of sample). Standard for comparison of capillary and venous blood drug concentrations for Rabbit B.

CONCENTRATION (mcg/mL)	RELATIVE INTENSITY						RELATIVE INTENSITY (Average)	STD. DEV.
	SET 1		SET 2		SET 3			
25	90	92	90	87	90	94	90.5	2.34
20	68	71.5	71	67	68.5	73.5	69.92	2.48
15	52.5	54.5	50.5	53.5	49	52	52.0	2.0
10	36.5	39	34.5	36.5	37.5	38.5	37.08	1.63
5	18.5	19	19	21	18.5	20.5	19.42	1.07
1	4.4	3.6	3.8	3.0	3.5	5.9	4.03	1.02
0	0	0	0	0	0	0	0	0

Table XVI. Spectrofluorometer parameters.

PARAMETER	SETTING
Voltage	800 Ψ
Slits	Ex: 5; Em: 5; PM: 5 mm
Blank	Set 1: 1.28 x 100 (subtracted)
	Set 2: 3.36 x 100 (subtracted)
	Set 3: 2.43 x 100 (subtracted)
Excitation wavelength	390 nm
Emission wavelength	515 nm
Sensitivity	Set 1: 82
	Set 2: 73
	Set 3: 83

Table XVII. Concentration vs. relative fluorescence intensity for tetracycline hydrochloride in water: (Kohn's extraction procedure using 15.7 μL of sample). Standard for comparison of capillary and venous blood drug concentrations for Rabbit C.

CONCENTRATION (mcg/mL)	RELATIVE INTENSITY						RELATIVE INTENSITY (Average)	STD. DEV.
	SET 1		SET 2		SET 3			
25	90	89	90	96	90	88	90.5	2.81
20	68.5	71.5	70	73	69	73	70.83	1.96
15	49	52	49.5	54.5	51	54	51.67	2.27
10	35	37	37	38	33	36	36.0	1.79
5	18	19	19	19	20	19	19.0	0.63
1	3.7	5.9	4.6	6.0	4.2	3.6	4.67	1.05
0	0	0	0	0	0	0	0	0

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