



HAL
open science

Pharmacokinetics of penicillin G in plasma and interstitial fluid collected with dialysis fiber bundles in sheep

Im Cooke, Rf Bevill, Dr Nelson, Gd Koritz

► **To cite this version:**

Im Cooke, Rf Bevill, Dr Nelson, Gd Koritz. Pharmacokinetics of penicillin G in plasma and interstitial fluid collected with dialysis fiber bundles in sheep. *Veterinary Research*, 1996, 27 (2), pp.147-160. hal-00902408

HAL Id: hal-00902408

<https://hal.science/hal-00902408>

Submitted on 11 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Pharmacokinetics of penicillin G in plasma and interstitial fluid collected with dialysis fiber bundles in sheep

IM Cooke¹, RF Bevill², DR Nelson³, GD Koritz^{2*}

¹ Department of Environmental Health and Safety;

² Department of Veterinary Biosciences;

³ Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL 61801, USA

(Received 19 July 1995; accepted 16 November 1995)

Summary — A new method of collection of interstitial fluid (ISF) (the site of most bacterial infections) was developed for the determination of free (unbound) penicillin G concentrations in sheep. Dialysis fiber bundles for the collection of ISF were first characterized in vitro and subsequently implanted in the subcutaneous fascia of the dorsal thorax parallel to the vertebral column in sheep. The sheep were then dosed intravenously with 26.4 and 52.9 mg/kg of sodium penicillin G using a crossover experimental design. Plasma and ISF dialysate were collected after dosing for determination of penicillin G concentrations using high pressure liquid chromatography (HPLC). The concentration of penicillin G in the ISF dialysate was calculated with the recovery ratio determined for each fiber bundle. The decline of penicillin G concentrations in ISF dialysate paralleled the disappearance of the drug from plasma providing evidence for the rapid diffusion of penicillin G into the fiber bundles. Pharmacokinetic analysis determined that the disposition of penicillin G was best described by a two-compartment open model with penicillin concentrations in plasma (C_p) defined by two biexponential equations, $C_p = 170.64e^{-7.16t} + 31.04e^{-1.56t}$ for the low dose and $C_p = 418.19e^{-6.15t} + 59.85e^{-1.56t}$ for the high dose.

penicillin G / pharmacokinetics / interstitial fluid / sheep / dialysis fiber bundle

Résumé — Pharmacocinétique de la pénicilline G prélevée à l'aide de faisceaux de fibres de dialyse dans le plasma et le liquide interstitiel chez le mouton. Une nouvelle méthode de détermination de la concentration de pénicilline G libre (non-liée) dans le liquide interstitiel – site de la plupart des infections bactériennes – a été développée. Des faisceaux de fibres de dialyse destinées aux prélèvements du liquide interstitiel ont été initialement calibrés in vitro. Les fibres de dialyse ont ensuite été implantées chez le mouton dans la fascia sous-cutané du thorax dorsal parallèlement à la colonne ver-

* Correspondence and reprints

tébrale. Les moutons ont ensuite reçu par voie intraveineuse 26 et 52 mg/kg de pénicilline G sodique en utilisant un plan expérimental croisé. Du plasma et des dialysats de liquide interstitiel ont été collectés et les concentrations de pénicilline G ont été déterminées par chromatographie liquide à haute performance. La concentration de pénicilline G dans les dialysats de liquide interstitiel a été calculée en utilisant le rapport de récupération déterminé pour chaque faisceau de fibres. La diminution des concentrations de pénicilline G dans le dialysat interstitiel était parallèle à celle du médicament dans le plasma, suggérant une diffusion rapide de la pénicilline G dans les fibres de dialyse. L'analyse pharmacocinétique a conduit à décrire la disposition de la pénicilline G selon un modèle bicompartimental ouvert. Les concentrations de pénicilline dans le plasma ont été décrites par deux équations biexponentielles, $C_p = 170,64e^{-7,16t} + 31,04e^{-1,56t}$ pour la dose inférieure et $CP = 418,19e^{-6,15t} + 59,85e^{-1,56t}$ pour la dose supérieure.

pénicilline G / pharmacocinétique / liquide interstitiel / mouton / faisceau de fibres de dialyse

INTRODUCTION

Pharmacokinetic investigations to optimize antibacterial dosage regimens are often based on plasma or serum drug concentration versus time profiles. However, such studies disregard the free (unbound) and therefore active drug concentrations achieved at the site of bacterial infection which is of fundamental importance to the optimization of antibiotic dosage regimens. This need to understand the distribution of antibacterial agents in tissues was recognized shortly after the introduction of penicillin when antibiotic concentrations were found to persist in infected wound exudates (Eagle et al, 1953).

Since most bacterial infections occur in interstitial fluid (ISF), many techniques were developed to sample the fluid for the determination of antibiotic concentrations. The implanted fibrin clot was one of the earliest approaches (Weinstein et al, 1951). Skin windows and skin blisters techniques were also developed to sample tissue fluid (Raeburn, 1971; Harding and Eilon, 1977; Gillette and Wise, 1978; Schreiner et al, 1978; Wise et al, 1980; Findlay et al, 1981). However, the prolonged penetration of drug into the blister fluid and the small fluid volumes obtained limited sampling frequency. Paper discs implanted subcutaneously (Bergeron et al, 1977) were limited by small sample

volume and the inability to obtain multiple samples. Techniques were also developed in both humans (Engeset et al, 1973; Bergan and Engeset, 1987) and other animals (Smith et al, 1970; Staub et al, 1975; Cohen et al, 1984) to obtain peripheral lymph samples as a representative of ISF (Courtice, 1971). A variety of tissue chambers for ISF collection were subsequently developed (Guyton, 1963; Burke, 1964; Calnan et al, 1972; Chisholm et al, 1973; Ryan, 1978; Bergan, 1981; Guyton, 1981; Beadle et al, 1989), which varied considerably in size, shape and diameter (Gardner et al, 1973; Carbon et al, 1977; Bengtsson et al, 1984; Clarke, 1989). High chamber surface area to internal volume ratios and long diffusional paths, however, resulted in unacceptably long equilibration times between drug concentrations in ISF and those in chamber fluid. Dialysis tubes that filter either ISF or serum through a semipermeable membrane have also been used for fluid collection (Peterson and Gerding, 1978; Gerding et al, 1982). Although several units may be implanted subcutaneously to facilitate multiple sampling, this increases the variability in the data (Dan et al, 1981). Lastly, a microdialysis fiber system incorporating small hollow dialysis fibers was introduced to overcome limitations of the larger dialysis tubes (Bunnnett et al, 1983, 1984; Ruckebusch et al, 1985; Pairet et al, 1986; Ruckebusch and Malbert, 1987). Rapid equilibration was

achieved as a result of the short diffusional distances and multiple samples could be obtained from the same fiber bundle.

The purpose of this study was to develop a simple and reliable technique using dialysis fiber bundles to sample ISF without the limitations of previous methods and to use the technique to determine the disposition of an antibacterial drug in ISF *in vivo*. Penicillin G and the sheep were chosen as the model antibacterial drug and test species, respectively.

MATERIALS AND METHODS

Fiber construction

Spectra/Por[®] hollow fiber bundles (Spectrum Medical Industries Inc, Los Angeles, CA, USA) with a molecular weight cut off of 9 000 Da and working volumes of 654 μL were used. This internal volume plus a flush volume of 1 mL provided a final sample volume sufficient to permit analysis of penicillin G concentrations by high pressure liquid chromatography (HPLC). Each bundle consisted of 176 fibers with an exposed length of 21 cm and an internal diameter of 150 μm . A 6.25 cm piece of heat shrink tubing was applied around the injection end of the bundle cap. A stopcock was then fitted into the free end of the tubing. The tip of the stopcock was inserted as close to the fiber ends within the tubing as possible to minimize dead space. A 3.75 cm piece of heat shrink tubing was also applied to the opposite collection end of the bundle and plugged with a removable stylet. Two surgical clips were gently applied at one third and two thirds of the length of the fiber bundle to spread the fibers. The controlled force applied to the clips did not result in occlusion of the fibers.

Fiber characterization

The permeability of the fibers to penicillin G was first characterized *in vitro*. The equilibration time and percent recovery for penicillin G concentrations within the fibers in relation to those concentrations in fluid bathing the fibers were deter-

mined for six fiber bundles. The equilibration time was defined as the middle collection interval of the consecutive collection intervals for which there was no significant difference in sequential ratios of penicillin G concentrations in fiber dialysate and bath fluid. The percent recovery was calculated as the ratio of penicillin G concentrations in fiber dialysate and bath fluid when equilibrium was attained. The experiment was designed as a three-way factorial design. The order in which each fiber bundle, collection interval and concentration was used was completely randomized.

The dialysis fibers were filled with isotonic saline and immersed in a beaker containing a bath of isotonic saline with 0.2% bovine serum albumin (Sigma, Saint Louis, MO, USA) to simulate the drug binding phenomena occurring in ISF (Bunnett et al, 1983) and fortified with 5, 25 or 50 $\mu\text{g/mL}$ of penicillin G. The solution was continuously stirred with a magnetic stirrer (Corning Hot Plate Stirrer PC-351, Corning Lab Products, Corning, NY, USA) to ensure an even distribution of penicillin G. The 9 000 Da molecular exclusion limit of the fibers excluded protein-bound drug from the dialysate. Samples of dialysate were collected at intervals of 0, 1, 2, 5 and 10 min by flushing the fiber bundle with saline to obtain 1 mL of saline and dialysate in the collection syringe. The sampling procedure was completed within 15 s. Subsequently, the fiber bundle was flushed with 5 mL of saline, the stopcock was closed, and the stylet plugs replaced at the injection and collection ends of the fiber bundle. The dialysate and beaker fluid were then analyzed for penicillin G by HPLC. The concentration of penicillin G in the dialysate was divided by the concentration of penicillin G in the beaker fluid to obtain ratios of penicillin G inside and outside the fibers at the different collection times.

Pharmacokinetic study

Six male Suffolk-cross sheep (44–64 kg) were determined to be healthy by clinical examination prior to the start of the experiment. The animals were acclimated to their surroundings for a week, and then each was surgically implanted with a dialysis fiber bundle which had been sterilized with ethylene oxide. The animals were not provided food or water during the 24 h interval prior to surgery. Anesthesia was induced with a 1.1 mL/kg *iv* bolus injection of 500 mg ketamine (Ketaset, Fort Dodge Laboratories Inc, Fort

Dodge, IA, USA) and 50 mg xylazine (Rompun, Mobay Corporation, Shawnee, KS, USA) in 500 mL of 5% glycerol guaiacolate (Gecolate, Summit Hill Laboratories, Navesink, NJ, USA). Endotracheal tubes were placed in the sheep and anesthesia was maintained with halothane during the surgical procedure. The skin over the dorsal thorax was clipped and scrubbed in preparation for surgery. Two small skin incisions were made 22 cm apart in the dorsal thorax parallel to the vertebral column on the left side. A hollow stainless steel trocar equipped with a cutting edge was tunneled through the subcutaneous fascia between the incisions. The fiber bundle was inserted in the trocar which was then gently pulled out leaving the fiber bundles in position subcutaneously. The ends of the fiber bundles were sutured to the skin and flushed daily with saline.

Sixteen hours prior to dosing, food was withheld and a 16 g, 8.3 cm catheter (Angiocath, Deseret Medical Inc, Sandy, UT, USA) with an internal volume of 0.2 mL was implanted in the jugular vein for both dosing and blood collection. The sheep were dosed with sodium penicillin G (Sigma, Saint Louis, MO, USA) 48 h after surgery. Three sheep were dosed with the low dose of penicillin G (26.4 mg/kg or 44 000 IU/kg) followed by the high dose (52.9 mg/kg or 88 000 IU/kg), 2 days after the first. The other three sheep received the high dose of penicillin G followed by the low dose. The order in which each sheep was dosed with the low or high dose was randomized. The dose was injected during a 30 s interval via the jugular catheter followed by a 10 mL flush with saline which had been shown to prevent carryover of penicillin to subsequent blood samples. Blood and ISF dialysate samples were collected before dosing (control) and at 10, 20, 30, 45 min and 1, 1.5, 2, 3, 4 and 5 h post-dosing. Blood samples (5 mL) were collected in 1% heparin after withdrawing and discarding 2 mL of blood from the catheter. Saline (5 mL) was injected to replace the blood volume followed by 1.5 mL of heparinized saline to maintain catheter patency between collections. Blood samples were centrifuged and plasma collected. ISF dialysate was collected by flushing the fiber bundle with saline to obtain 1 mL of the ISF dialysate within 15 s. The fiber bundle was then flushed with 5 mL saline and refilled. All samples were analyzed for penicillin G concentrations by HPLC.

The fiber bundle was removed from the sheep at the end of the experiment, 7 days after implantation. To remove the bundle, the sheep were

tranquilized with xylazine (0.11 mg/kg) intravenously. The fiber bundles were cut off at one end and easily withdrawn. The open wound was then sutured.

Penicillin G analysis

A HPLC assay developed by Tyckowska and Aronson (1988) was modified to improve the detection and quantitation of penicillin G. A HPLC system from Waters Associates (Milford, MA, USA), consisting of a pump (Model 6000 A), an automated injector (Model 712) and a variable wavelength detector (Model 484), was used for the isocratic separation, detection and quantitation of penicillin G in all samples. A Brownlee Spheri-5 phenyl column (250 x 4.6 mm) equipped with a phenyl Newguard column (Brownlee Laboratories Inc, Santa Clara, CA, USA) was used to separate penicillin G from plasma components. The column was heated and maintained at 50 °C with a water jacket (All Tech Associates, Arlington Heights, IL, USA) and a Haake constant temperature water bath equipped with an external circulation pump (Cole Parmer Instrument Company, Chicago, IL, USA). A Hewlett Packard (Rolling Meadows, IL, USA) recording integrator (Model 3396 A) and disc drive (Model 9114 B) were used to record and store the chromatograms and print reports of each analysis.

The standard, penicillin G sodium salt, was from Sigma (Saint Louis, MO, USA). Acetonitrile and orthophosphoric acid (85%) were from Fisher Scientific (Fairlawn, NJ, USA); triethylamine and octanesulfonate were from Regis Chemical Company (Morton Grove, IL, USA); methanol was from American Burdick and Jackson (Muskegon, MI, USA). All solvents were HPLC grade. The reagents were analytical grade. Deionized glass-distilled water was used for all preparations. The mobile phase was prepared by dilution of 300 mL acetonitrile with 680 mL water, 5 mL triethylamine, 5 mL phosphoric acid and 10 mL of a 0.5 M octanesulfonate solution. The eluant used for extraction and concentration of the sample was prepared by dilution of 90 mL acetonitrile with 10 mL of modified mobile phase prepared as described above but without acetonitrile.

Plasma and ISF dialysate samples were extracted and concentrated with prepacked resin columns (Bond-Elut, Analytichem International, Harbor City, CA, USA). The columns were pre-

conditioned with two 1 mL methanol washes followed by two 1 mL phosphate buffer washes. A vacuum (17.5 cm of mercury) was used to increase the flow of solvents and reduce elution time. The sample (1 mL) was added to the conditioned column followed by two 1 mL buffer washes. A vacuum was applied to the column outlet to remove free liquid from the resin. Penicillin G was extracted from the resin with 200 μ L of the eluant. Centrifugation (2.5 min at 140 *g*) was used to increase movement of the fluids through the column. The extraction was repeated with 100 μ L of eluant to obtain a 3.33-fold concentration of penicillin G in the sample. The combined eluates were then transferred to an injection vial equipped with a 0.3 mL limited volume insert (Crom Tech, Inc, Apple Valley, MN, USA). Complete recovery (100%) of penicillin G was obtained with this method.

The processed sample (25 μ L) was placed in the automated injector and introduced into the mobile phase being delivered to the reverse phase HPLC column. The mobile phase flow was 1.2 mL/min. The ultraviolet absorption of the mobile phase as it emerged from the column was monitored at 210 nm. Changes in absorption were integrated, recorded and stored on the disc drive.

Standards containing 1, 2, 4, 8 and 16 μ g/mL of drug in sheep plasma were analyzed for penicillin G concentrations, and standard curves correlating penicillin G concentrations to peak integral areas under UV absorption response curves were established. This correlation was defined by a regression equation which was subsequently used to calculate penicillin G concentrations in an unknown sample. Samples with penicillin G concentrations greater than 16 μ g/mL were diluted with eluant after extraction to achieve a concentration within the range of the standard curve. The standard curves were linear within the range of 0.5 to 16 μ g/mL and passed through the origin. The coefficients of correlation obtained when the areas of the eluted peaks were compared to the penicillin G concentrations were always greater than 0.995. The level of quantitation of the assay was found to be 0.5 μ g/mL. The limit of detection of the assay was 1 μ g/mL. Absence of other interfering peaks confirmed the specificity of the assay. Reliability of the assay was determined to be 0.9906. Reproducibility of the assay was also excellent as indicated by coefficient of variation values which were all less than 10% for duplicate samples.

Data analysis

Fiber characterization

A multiple regression analysis (with dummy coding used for fiber identity) was used to determine if there were any significant differences in the ratios obtained between the collection intervals, fibers and penicillin G concentrations. If a significant difference was found, the least significant difference between collection intervals was determined (Snedecor and Cochran, 1980). $P \leq 0.05$ was required for statistical significance.

Pharmacokinetic study

The Autoan computer program (Sedman and Wagner, 1976) was used to determine which exponential equation best fit the plasma and ISF dialysate data and to obtain initial estimates of the pharmacokinetic parameters. An *F*-test in the computer program determined which exponential equation best fitted each animal's data. The SAAM27 curve-fitting computer program based on nonlinear iterative least-squares regression analysis (Berman and Weiss, 1977) was subsequently used to obtain final values of the rate constants and volumes using the initial estimates obtained with the Autoan program. Drug concentrations were weighted by the reciprocal of each observation for the Autoan and SAAM27 fitting procedures. Pharmacokinetic parameters (*A*, *B*, α , β , $t_{1/2\alpha}$, $t_{1/2\beta}$, V_{Darea} , V_c , V_{ss} , *CL* and *AUC*) were calculated using standard formulas (Gibaldi and Perrier, 1982). These pharmacokinetic parameters are defined according to Baggot (1977) (see table II below). Free drug concentrations in plasma were calculated after correction of the plasma penicillin G concentrations for 30% protein binding (Ziv and Sulman, 1972). A paired *t*-test was used to determine whether there were any significant differences in the *CL*, V_{Darea} , V_{ss} and after the administration of the low and high dose of penicillin G. $P \leq 0.05$ was required for statistical significance.

RESULTS

Fiber characterization

The ratios of the concentrations of penicillin G in the dialysate to the beaker bath solution

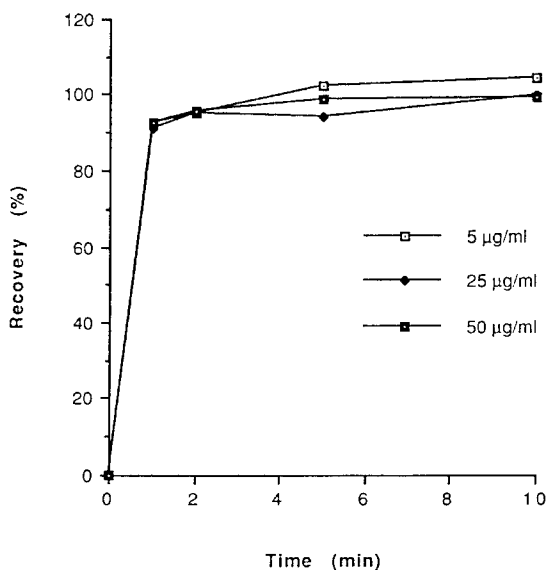
for each fiber bundle at each concentration and collection interval are reported in table I. The percent recovery of penicillin G from a representative fiber bundle immersed in saline containing penicillin G for different

collection intervals is shown in figure 1. The percent recovery in figure 1 was corrected for the dilution of 654 μL of saline and ISF dialysate contained within the fibers to the total collection volume of 1 mL. The analy-

Table I. Ratios of the concentrations of penicillin G in the fiber dialysate/beaker bath solution at specified times after immersion of fiber bundles in saline solution containing three concentrations of penicillin G.

Collection interval (min)	Concentration			Mean \pm SD
	5 $\mu\text{g/mL}$	25 $\mu\text{g/mL}$	50 $\mu\text{g/mL}$	
<i>Fiber 1</i>				
1	0.555	0.667	0.728	0.650 \pm 0.09
2	0.624	0.634	0.691	0.650 \pm 0.04
5	0.607	0.697	0.713	0.672 \pm 0.06
10	0.620	0.668	0.733	0.674 \pm 0.06
<i>Fiber 2</i>				
1	0.672	0.660	0.672	0.668 \pm 0.01
2	0.691	0.689	0.694	0.691 \pm 0.00
5	0.741	0.683	0.715	0.713 \pm 0.03
10	0.757	0.721	0.719	0.732 \pm 0.02
<i>Fiber 3</i>				
1	0.618	0.559	0.636	0.604 \pm 0.04
2	0.659	0.625	0.649	0.644 \pm 0.02
5	0.712	0.647	0.598	0.652 \pm 0.06
10	0.729	0.653	0.618	0.667 \pm 0.06
<i>Fiber 4</i>				
1	0.762	0.741	0.680	0.728 \pm 0.04
2	0.816	0.750	0.703	0.756 \pm 0.06
5	0.818	0.768	0.740	0.775 \pm 0.04
10	0.799	0.747	0.745	0.764 \pm 0.03
<i>Fiber 5</i>				
1	0.569	0.500	0.537	0.535 \pm 0.03
2	0.630	0.571	0.553	0.585 \pm 0.04
5	0.617	0.640	0.598	0.618 \pm 0.02
10	0.654	0.619	0.634	0.636 \pm 0.02
<i>Fiber 6</i>				
1	0.667	0.704	0.651	0.674 \pm 0.03
2	0.695	0.688	0.701	0.695 \pm 0.01
5	0.765	0.725	0.767	0.752 \pm 0.02
10	0.794	0.740	0.743	0.759 \pm 0.03

Fig 1. Calculated percent recovery of penicillin G from the dialysate collected from a representative fiber bundle at specified times after immersion in saline solutions containing three concentrations of the drug.



sis of variance showed that there was no significant difference in the ratios between the varying penicillin G concentrations used ($P = 0.2183$). There was, however, a significant difference in the ratios between the different fiber bundles ($P = 0.0001$) and collection intervals ($P = 0.0001$) used. Since there was a significant difference in the ratios between the collection intervals used, the least significant difference between collection intervals for each fiber bundle was determined ($P = 0.05$). There was a significant difference between the 1 and 10 min collection intervals for fiber bundles 2, 4, 5 and 6. There was no significant difference between any of the collection intervals for fiber bundles 1 and 3. The mean (\pm SD) values of the ratios obtained at the 5 and 10 min collection intervals were 0.697 ± 0.066 and 0.705 ± 0.060 , respectively. Since there was no significant difference in the mean ratios between the 2 through 10 min collection intervals for all of the fiber bundles in vitro, equilibration time for the dialysis fibers was determined to be 5 min, ie, the middle collection interval. As a result, the mean recovery ratio for each fiber bundle was

determined at 5 min. These values were subsequently used to calculate corrected penicillin G concentrations in the ISF dialysate. These corrected values were used as predictors of penicillin G concentrations within the ISF. The 5 min collection interval also defined the minimum time interval between sample collections in vivo. The percent recovery was calculated as the ratio of the concentrations of penicillin G in the fiber dialysate and beaker solution when equilibrium was attained (5 min). As can be seen from figure 1, recoveries were approximately 100%. Pilot studies showed that there were no changes in the equilibration time or percent recovery of the fiber bundles after ethylene oxide sterilization.

Pharmacokinetic study

The concentration of penicillin G in the ISF dialysate was calculated with the recovery ratio determined at the 5 min collection interval for each fiber bundle obtained in the characterization study (table I). The mean concentrations of penicillin G in plasma and

ISF dialysate following intravenous administration of the low and high dose are shown in figures 2 and 3, respectively. The decline of penicillin G concentrations in the ISF paralleled the disappearance of the drug from plasma. The data for each animal was best described by a two-compartment open model (fig 4) which was defined by the biexponential equation $C_p = Ae^{-\alpha t} + Be^{-\beta t}$, where C_p is the plasma concentration of the drug at time t . The individual and mean values of the pharmacokinetic parameters for the plasma and ISF dialysate are shown in tables II and III, respectively. The paired t -test determined that there were no significant differences in CL ($P = 0.1715$), V_{Darea} ($P = 0.4024$), V_{ss} ($P = 0.2307$) or β ($P = 0.9943$) after the administration of the low and high dose of penicillin G.

DISCUSSION

As there was a significant difference between the penicillin G concentrations among fiber bundles, each bundle was iden-

tified, characterized, maintained and used as a separate system in the pharmacokinetic studies. The absence of a significant difference in the ratios between the various penicillin G concentrations used indicated that the permeability characteristics of the fiber bundles were similar.

The profiles in figures 2 and 3 demonstrate that the decline of penicillin G concentration in the ISF dialysate paralleled the disappearance of the drug from plasma. This provides further evidence that the diffusion of drug to and from the fiber bundles was very rapid. The rapid diffusion of the drug through the pores of the fibers is a distinct advantage of the fiber bundles as compared to other experimental models that have been developed to study the penetration of drugs into ISF. The rapid diffusion characteristic of the fiber bundles is of great importance in the study of drug disposition in tissues, especially in the study of drugs with a very short half-life such as penicillin G. This rapid diffusion characteristic of the fiber bundles is a result of the large surface area/volume ratio of 266 as compared to

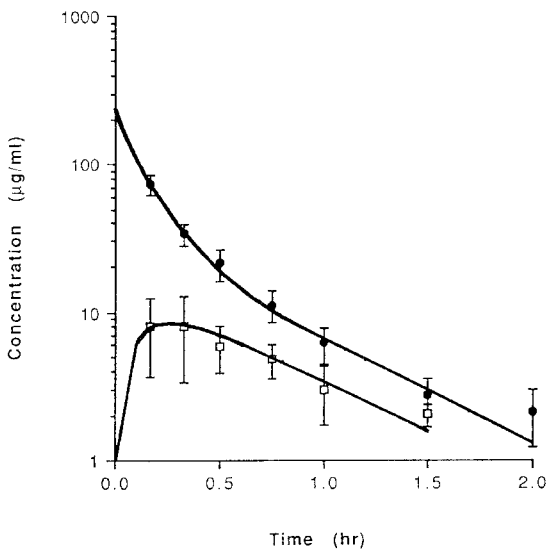
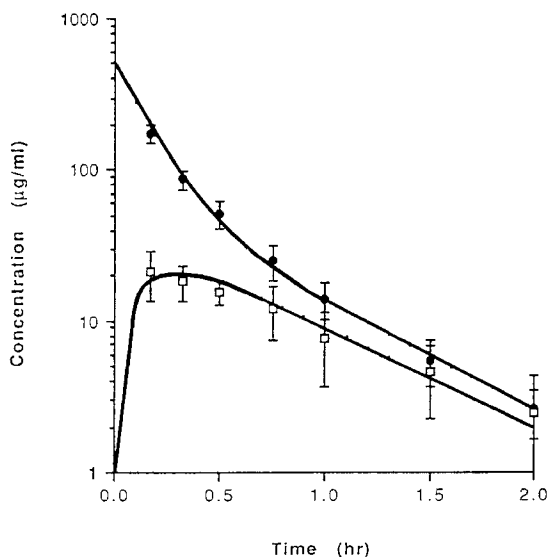


Fig 2. Mean (\pm SD) plasma (●) and interstitial fluid dialysate (□) concentrations of penicillin G in six sheep after its intravenous administration at 26.4 mg/kg. Simulated line (—) of best fit calculated from mean values of the pharmacokinetic parameters.

Fig 3. Mean (\pm SD) plasma (●) and interstitial fluid dialysate (□) concentrations of penicillin G in six sheep after its intravenous administration at 52.9 mg/kg. Simulated line (—) of best fit calculated from mean values of the pharmacokinetic parameters.



0.03 in some other chamber devices (Luthman and Jacobsson, 1986). The penicillin G concentrations in plasma and ISF dialysate were best described by a two-compartment open model. This again supports the concept of rapid diffusion across the fibers. Previous studies which utilized large chambers to study drug disposition in plasma and tissue fluid required a catenary three-compartment open model to adequately describe the data (Clarke et al, 1989). In this model drug concentrations in the plasma and chamber were represented in the first and

third compartments, respectively, and the predicted drug concentrations in the tissue fluid were represented by the second intermediate compartment.

The AUC for the free drug concentrations in plasma and ISF dialysate were not equal for either the low or high dose of penicillin G in this study as seen in tables II and III. Thus, it appears that the extent of diffusion of penicillin G into the fibers was not complete. Drug concentrations in ISF dialysate may have been higher if samples were obtained less frequently. The minimum collection interval of 5 min was based on the results of the fiber characterization study *in vitro*. However, it appears that longer collection intervals may have been more appropriate *in vivo*.

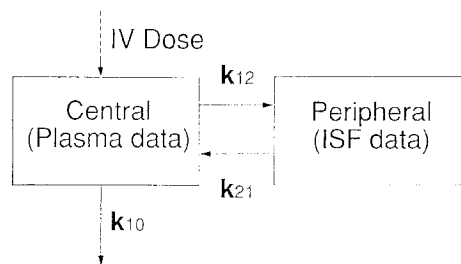


Fig 4. Diagram of the two-compartment open model illustrating the pharmacokinetics of penicillin G in the plasma and interstitial fluid of sheep.

No dose-dependent kinetics occurred within the range of doses used as there was no significant difference between values of the pharmacokinetic parameters obtained after administration of the low and high doses of penicillin G. Also, a proportional increase in penicillin G concentrations was observed when the high dose of

Table II. Values of pharmacokinetic parameters of penicillin G in six sheep after intravenous administration of 26.4 mg penicillin G/kg body weight.

Parameter	Sheep						Mean \pm SE
	422	421	426	425	431	423	
A ($\mu\text{g/mL}$)	298.37	97.25	125.89	140.69	228.97	132.65	170.64 \pm 31.34
B ($\mu\text{g/mL}$)	56.89	46.13	25.44	13.85	24.68	19.28	31.04 \pm 6.84
α (h^{-1})	13.71	6.22	4.66	5.48	6.49	6.42	7.16 \pm 1.34
β (h^{-1})	2.43	1.75	1.40	1.32	1.17	1.30	1.56 \pm 0.19
$t_{1/2\alpha}$ (h)	0.0505	0.1114	0.1486	0.1266	0.1068	0.1080	
$t_{1/2\beta}$ (h)	0.2856	0.3970	0.4949	0.5265	0.5948	0.5314	
AUC _{pl, total} ($\mu\text{g}\cdot\text{h/mL}$)	54.71	45.09	47.11	38.18	60.74	38.17	47.33 \pm 3.68
AUC _{pl, free} ($\mu\text{g}\cdot\text{h/mL}$)	38.30	31.56	32.98	26.73	42.52	26.72	33.13 \pm 2.58
AUC _{ISF, free} ($\mu\text{g}\cdot\text{h/mL}$)	9.82	6.02	9.27	7.19	6.98	10.47	8.29 \pm 1.80
CL ($\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$)	0.5840	0.6279	0.5846	0.7289	0.4675	0.7446	0.6229 \pm 0.0421
V_{Darea} (L/kg)	0.2407	0.3596	0.4175	0.5538	0.4013	0.5709	0.4240 \pm 0.0506
V_c (L/kg)	0.0743	0.1841	0.1745	0.1708	0.1041	0.1738	0.1469 \pm 0.0187
V_{ss} (L/kg)	0.1453	0.2635	0.2429	0.2554	0.1955	0.3057	0.2347 \pm 0.0230

A and B = zero-time plasma drug concentration intercepts of the biphasic disposition curve; α and β = first order distribution and elimination rate constants, respectively; $t_{1/2\alpha}$ and $t_{1/2\beta}$ = half-lives of distribution and elimination, respectively; V_{Darea} = apparent volume of drug distribution based on total area under the plasma drug concentration versus time curve; V_c = apparent volume of drug distribution in the central compartment; V_{ss} = apparent volume of drug distribution at steady-state drug concentrations; CL = total plasma clearance of the drug; AUC = area under the drug concentration versus time profile calculated by the trapezoidal rule extrapolated to infinity. The AUC was calculated for the total drug concentrations in plasma (AUC_{plasma, total}), free drug concentrations in plasma (AUC_{plasma, free}) and free drug concentrations in ISF dialysate (AUC_{ISF, free}).

Table III. Values of pharmacokinetic parameters of penicillin G in six sheep after intravenous administration of 52.9 mg penicillin G/kg body weight.

Parameter	Sheep						Mean \pm SE
	422	421	426	425	431	423	
A ($\mu\text{g/mL}$)	247.10	275.29	249.83	817.00	597.86	322.06	418.19 \pm 94.42
B ($\mu\text{g/mL}$)	28.46	94.75	30.06	71.78	69.16	64.90	59.85 \pm 10.34
α (h^{-1})	4.45	5.47	3.33	9.55	7.63	6.48	6.15 \pm 0.90
β (h^{-1})	1.54	1.72	1.06	1.87	1.66	1.53	1.56 \pm 0.11
$t_{1/2\alpha}$ (h)	0.1557	0.1266	0.2082	0.0726	0.0908	0.1069	
$t_{1/2\beta}$ (h)	0.4511	0.4039	0.6508	0.3713	0.4181	0.4521	
AUC _{pl, total} ($\mu\text{g}\cdot\text{h/mL}$)	78.02	109.99	109.36	142.47	131.31	97.50	111.44 \pm 9.45
AUC _{pl, free} ($\mu\text{g}\cdot\text{h/mL}$)	54.61	76.99	76.55	99.73	91.92	68.25	78.01 \pm 6.61
AUC _{ISF, free} ($\mu\text{g}\cdot\text{h/mL}$)	18.98	16.74	29.83	20.37	16.82	19.84	20.43 \pm 1.94
CL ($\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$)	0.7143	0.5014	0.5122	0.4266	0.4406	0.5748	0.5283 \pm 0.0422
V _{Darea} (L/kg)	0.4650	0.2922	0.4810	0.2285	0.2658	0.3750	0.3513 \pm 0.0424
V _c (L/kg)	0.1920	0.1430	0.1890	0.0595	0.0793	0.1367	0.1332 \pm 0.0219
V _{ss} (L/kg)	0.2367	0.1966	0.2433	0.1017	0.1301	0.2204	0.1881 \pm 0.0236

penicillin G was administered. Drug concentrations increased twofold in plasma and ISF dialysate when the dose was doubled.

Implanted dialysis fibers provided a simple, convenient and reliable technique to determine free antibiotic concentrations in ISF dialysate. The main advantages of this technique were the ease of use, rapid diffusion characteristic and the ability to obtain multiple samples. In addition, the dialysis fiber bundles can be easily adapted for a variety of potential applications. The fiber bundles may be implanted at different sites, organs or tissues. For example, they might be implanted in the peritoneal cavity for determination of drug concentrations during peritonitis. They might also be implanted in various muscles, organs or fat to detect tissue residue concentrations of drugs. This would obviate the animal sacrifices typical of conventional drug residue studies. Alternatively, the fibers could be used to create a localized site of infection in the body. Bacteria inoculated within implanted fibers would remain trapped due to the small size of the fiber pores and could serve as a model to study the antibacterial action of an antibiotic dosage regimen. The limitations in the use of the fiber bundle may be related more to assay requirements than any intrinsic limitation of the fiber bundle.

REFERENCES

- Baggot JD (1977) *Principles of Drug Disposition in Domestic Animals: the Basis of Veterinary Clinical Pharmacology*. WB Saunders Company, Philadelphia, PA, USA
- Beadle RE, Short CR, Corstvet RE et al (1989) Characterization of a soft-tissue infection model in the horse and its response to intravenous cephalosporin administration. *J Vet Pharmacol Therap* 12, 73-86
- Bengtsson B, Luthman J, Jacobsson SO (1984) Evaluation of a tissue cage model for use in cattle. *Acta Vet Scand* 25, 480-494
- Bergan T (1981) Pharmacokinetics of tissue penetration of antibiotics? *Rev Infect Dis* 3 (suppl 1), 45-66
- Bergan T, Engeset A (1987) Does serum protein binding inhibit tissue penetration of antibiotics. *Rev Infect Dis* 9 (suppl 4), 713-718
- Bergeron M, Nguyen BM, Trotter S, Gauvreau L (1977) Penetration of cefamandole, cephalothin, and desacetyl-cephalothin into fibrin clots. *Antimicrob Agents Chemother* 12, 682-687
- Berman M, Weiss MF (1977) Users' manual for SAAM. Laboratory of Theoretical Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA
- Bunnett NW, Walsh JH, Debas HT, Kauffman GL, Golanska EM (1983) Measurement of prostaglandin E2 in interstitial fluid from the dog stomach after feeding and indomethacin. *Gastroenterology* 85, 1391-1398
- Bunnett NW, Mogard M, Orloff MS, Corbet HJ, Reeve JR, Walsh JH (1984) Catabolism of neurotensin in interstitial fluid of the rat stomach. *Am J Physiol* 26, 675-682
- Burke JF (1964) An implanted reservoir for continuous sampling of interstitial fluid. *J Surg Res* 4, 195-199
- Calnan J, Ford PM, Holt PJJ, Pflugg JJ (1972) Implanted tissue cages—a study in rabbits. *Br J Plast Surg* 25, 164-174
- Carbon C, Contrepois A, Brian N, Lamotte-Barrillon S (1977) Penetration of cefazolin, cephaloridine and cefamandole into interstitial fluid in rabbits. *Antimicrob Agents Chemother* 11, 594-598
- Chisholm GD, Waterworth PM, Calnan JS, Garrod LP (1973) Concentration of antibacterial agents in interstitial fluid. *Am Med J* 1, 569-573
- Clarke CR (1989) Tissue-chamber modeling systems—applications in veterinary medicine. *J Vet Pharmacol Ther* 12, 349-368
- Clarke CR, Short CR, Bourne DWA, Usenik EA (1989) Subcutaneously implanted tissue chambers—a pharmacokinetic study. *J Vet Pharmacol Ther* 12, 312-389
- Cohen SH, Hoeprich PD, Demling R, Gunther R et al (1984) Entry of four cephalosporins into the ovine lung. *J Infect Dis* 149 (suppl 2), 264-270
- Courtice FC (1971) Lymph and plasma proteins: barriers to their movement throughout the extracellular fluid. *Lymphology* 4, 9-17
- Dan M, Halkin H, Rubinstein E (1981) Interstitial fluid concentrations of aminoglycosides. *J Antimicrob Chemother* 7, 551-558
- Eagle H, Fleischman R, Levy M (1953) 'Continuous' vs 'discontinuous' therapy with penicillin: the effect of the interval between injections on therapeutic efficacy. *N Engl J Med* 248, 481-488
- Engeset A, Hager B, Nesheim A, Kolbenstvedt A (1973) Studies on human peripheral lymph. *Lymphology* 6, 1-5

- Findlay CD, Wise R, Allcock JE, Durham SR (1981) The tissue penetration as measured by a blister technique and pharmacokinetics of cefulodin compared with carbenicillin and ticarcillin. *J Antimicrob Chemother* 7, 637-642
- Gardner WG, Prior RB, Perkins RL (1973) Fluid and pharmacological dynamics in a subcutaneous chamber implanted in rats. *Antimicrob Agents Chemother* 4, 196-197
- Gerding DN, Van Ette LL, Peterson LR (1982) Role of serum protein binding and multiple antibiotic doses in the extravascular distribution of ceftizoxime and cefotaxime. *Antimicrob Agents Chemother* 22, 844-847
- Gibaldi M, Perrier D (1982) Pharmacokinetics. In: *Drugs and the Pharmaceutical Sciences*. Vol 15 (J Swarbrick, ed), Marcel Dekker Inc, New York, NY, USA
- Gillette AP, Wise R (1978) Penetration of four cephalosporins into tissue fluid in man. *Lancet* I, 962-964
- Guyton C (1963) A concept of negative interstitial pressure based on pressures in implanted perforated capsules. *Circ Res* 12, 399-414
- Guyton C (1981) Capillary dynamics and exchange of fluid between the blood and interstitial fluid. In: *Textbook of Medical Physiology*, 6th ed (C Guyton, ed), WB Saunders Co, Philadelphia, PA, USA, 358-369
- Harding SM, Eilon LA (1977) Clinical pharmacology of cefuroxime. In: *The Early Evaluation of Cefuroxime* (ES Snell et al, ed), Glaxo Research Ltd, Granford, UK, 57-68
- Luthman J, Jacobsson SO (1986) Distribution of penicillin G in serum and tissue cage fluid in cattle. *Acta Vet Scand* 27, 313-325
- Pairet M, Meirieu O, Bardon T, Ruckebusch Y (1986) Cholinergic modulation of the release of serotonin in the gastric interstitial fluid. *Gastroenterology* 91, 1250-1257
- Peterson LR, Gerding DN (1978) Prediction of cefazolin into high and low protein containing extravascular fluid: new method for performing simultaneous studies. *Antimicrob Agents Chemother* 14, 533-538
- Raeburn JA (1971) A method for studying antibiotic concentrations in inflammatory exudates. *J Clin Pathol* 24, 633-635
- Ruckebusch M, Malbert CH (1987) Availability of 5-HTP in duodenal interstitial fluid of sheep and dogs. *J Vet Pharmacol Ther* 10, 254-256
- Ruckebusch Y, Meirieu O, Von Ritter C, Blum AL (1985) Measurement of monoamines and their metabolites in the interstitial fluid of the gut. *J Pharmacol Methods* 13, 167-177
- Ryan DM (1978) Implanted tissue cages: a critical evaluation of their relevance in measuring tissue concentrations of antibiotics. *Scan J Infect Dis [Suppl]* 14, 225-227
- Schreiner A, Hellum KB, Digranes A, Bergman I (1978) Transfer of penicillin G and ampicillin into human skin blisters induced by suction. *Scand J Infect Dis [Suppl]* 14, 233-237
- Sedman AJ, Wagner JG (1976) Autoan, a decision-making pharmacokinetic computer program. Publication Distribution Service, Ann Arbor, MI, USA
- Snedecor GW, Cochran WG (1980) *Statistical Methods*. Iowa State University Press, Ames, IA, USA, 233-234
- Smith JB, McIntosh GH, Morris B (1970) The traffic of cells through tissues: a study of peripheral lymph in sheep. *J Anat* 107 (suppl 1), 87-100
- Staub NC, Bland RD, Brigham KL, Demling R, Erdmann J, Woolverton WC (1975) Preparation of chronic lymph fistulas in sheep. *J Surg Res* 19, 315-320
- Tyckowska K, Aronson AL (1988) Ion-pair liquid chromatographic determination of some penicillins in canine and equine sera. *J Assoc Off Anal Chem* 71 (suppl 4), 773-775
- Weinstein L, Daikos GK, Perrin TS (1951) Studies on the relationship of tissue fluid and blood levels of penicillin. *J Lab Clin Med* 38, 712-718
- Wise R, Gillette AP, Cadge B, Durham SR, Baker S (1980) The influence of protein binding upon tissue levels of six β -lactam antibiotics. *J Infect Dis* 142, 77-82
- Ziv G, Sulman FG (1972) Binding of antibiotics to bovine and ovine serum. *Antimicrob Agents Chemother* 2, 206-213