THE EFFECT OF EXPERIMENTALLY INDUCED BRONCHOPNEUMONIA ON THE PHARMACOKINETICS AND TISSUE DEPLETION OF GENTAMICIN IN HEALTHY AND PNEUMONIC CALVES

A Thesis

by

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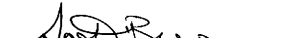
Approved as to style and content by:



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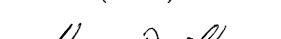

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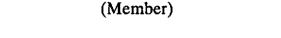

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ABSTRACT

The Effects of Experimentally Induced Bronchopneumonia on the Pharmacokinetics and Tissue Depletion of Gentamicin in Healthy and Pneumonic Calves. (August 1989)

Robert Paul Hunter, B.S., Angelo State University

Chair of Advisory Committee: Dr. Scott A. Brown

The effects of a bovine bronchopneumonia model on the pharmacokinetics and tissue residue depletion profiles of gentamicin in calves weighing 90-140 kg was explored. Two groups of heifer calves were used. The first was a normal control group, while the other group had bronchopneumonia induced. A scoring system was developed to evaluate the extent of disease in the two groups. A bimodal distribution of the serum pharmacokinetic parameters in the pneumonic group was caused by the effects of dehydration on the parameters. A significantly higher serum clearance was seen of gentamicin in the pneumonic group than the normal group ($P < 0.05$). The pharmacokinetic models used to fit the tissue concentrations varied from tissue to tissue and between groups. The power function was the best fit for normal group's renal cortex concentrations and lung concentrations and the grossly appearing diseased lung in the pneumonic group. A one-compartment model was the best fit for the pneumonic group's renal cortex concentrations, renal medulla concentrations in both groups, liver concentrations in both groups, and the grossly normal appearing lung in the pneumonic groups. Because the best model of a particular tissue's concentrations varied between groups, withdrawal periods normally determined in healthy animals may be inappropriate in diseased animals.

Addition of several parameters (serum creatinine, serum urea nitrogen, albumin, fibrinogen, and total protein concentrations, along with white blood cell counts, and central fluid volume, volume of distribution at steady state, the area under the serum concentration vs. time curve, serum clearance of gentamicin, and the elimination rate constant) to these tissue depletion models using multiple regression improved the prediction of a given tissue's concentration. Certain parameters (serum creatinine and severity of disease score on the kill day) consistently improved the prediction of the concentrations in different tissues.

DEDICATION

I would like to dedicate the following manuscript to the two people who made it worth the effort. First to my wife Trisha (SB), the woman who was always there for me and understood when I wasn't. The other person is my son Mark. Someday he will read this and realize that his father probably isn't as dumb as he thought.

ACKNOWLEDGEMENTS

There are many people that gave their help to this project and deserve credit. First is Dr. Scott Brown. When I first came under his guidance in June, 1987, he treated me not as another worker, but as a person. His vast amount of patience, understanding, and TIME really made my work at TAMU (and this project) a learning experience. Scott, I cannot say enough to thank you for being my friend, mentor, and counselor.

The friendship of Lynn Friedlander should also be recognized here. Lynn, thanks for listening to my gripes and complaints, and for showing me the ins and outs of A&M.

Gary Smith was also a very big help with this project. His patience in teaching me how to use the TDx is greatly appreciated. Gary, thanks for teaching me more about computers and biochemistry.

Eric Ramirez was a high school student who worked on the animal part of this study. Eric learned a lot about working with animals, and I learned a lot about working with people.

To the people at Innotron of Oregon, thanks for all the technical advice with the tissue standard curves. Without that help, the tissue residue analysis would have taken much longer.

Jennifer Rollins, for all the words that were said, thank you very much for the help!

To Drs. Craig and Thomson, the help given to me with the many revisions to this thesis will always be remembered.

Finally, to Dave Nelligan, since the bear didn't get you, you came back to let the calves do you in. Thank you so much for being my friend!

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INTRODUCTION

Tissue drug residues are a major concern to consumers, livestock producers, and veterinarians. The occurrence of residues has steadily increased over the past several years. Even though drug companies must place a proven withdrawal time on the label of all drug formulations approved for use in food animals, altered physiological functions due to disease states may change these withdrawal periods by either shortening them, or worse, increasing their length.

Bronchopneumonia (BP), or "shipping fever", is one of the most prevalent diseases that affect feedlot cattle. These animals may be treated with many different pharmacologic agents. Both acute and chronic dosage regimens may be employed in a feedlot setting. The absorption, distribution, metabolism, and excretion and hence tissue concentrations and depletion profiles could be changed by BP. The possibility exists that tissues may be contain a drug above the tolerance limit, though unintentionally, due to the effects of BP.

The objectives of this project were: 1) to determine the changes in tissue drug residue profiles caused by experimentally induced BP for a water-soluble, non-metabolized xenobiotic (gentamicin) and 2) to correlate the serum pharmacokinetics with the gentamicin depletion profiles in various tissues in both healthy and pneumonic calves.

The journal model is the *Journal of Veterinary Pharmacology and Therapeutics*.

REVIEW OF LITERATURE

Tissue Residues

The public perception that pharmacologic agents are present in edible tissues of food-producing animals has caused both an increase in concern among consumers and an increase in residue screening by the Food Safety and Inspection Service of the United States Department of Agriculture (Engel, 1988; Marteniuk *et al.*, 1988; Van Houweling, 1981). This concern must be based on an accurate estimate of the residue levels present in the final food product available for consumption to the consumer (Braun & Waechter, 1983).

When a new animal drug application (NADA) is made, drug companies must determine the time required for tissue residues to decrease below the tolerance level for the dosage regimen stated on the drug label. This period of time is referred to as the withdrawal period (Booth, 1988) and is typically determined in normal, healthy animals by the pharmaceutical company. Since veterinarians and livestock producers usually treat diseased animals rather than normal animals, the labeled withdrawal time may not be valid in all situations.

The method used to establish a withdrawal time for a drug is almost universal in the drug industry. This protocol involves the administration of a radiolabeled dose of the compound, via the intended route. The only difference between this formulation and the one used by producers and veterinarians is that it contains a ^{14}C- or ^{3}H-label. This allows the compound and its metabolites to be easily detected in the target animals' edible tissues and also allows for estimation of the major routes of metabolism. The target organ, which is usually the organ in which the

highest concentration of drug and/or metabolite is found, is also determined by this experimental technique. The stated method is extremely accurate for determining the withdrawal time for the dosage regimen tested. However, it has been shown that the data obtained from these experiments cannot be extrapolated to other dosage regimens (higher dose or different route of administration) (Brown *et al.*, 1986a; TerHune & Upson, 1989).

Bronchopneumonia

Cattle are one of the major food-producing animals in the United States. The prevalence of respiratory disease, especially BP, in cattle is fairly common. Approximately 64% of feedlot cattle deaths are due to respiratory disease. Bronchopneumonia accounts for 75% of these cases (Frank & Smith, 1983; Jensen *et al.*, 1976). Costs incurred by feedlot operators due to BP can be large because of the expense of treating animals, decreased weight gain, carcass condemnation, and/or loss of revenue due to animal deaths. Veterinarians must attempt to treat BP in cattle while not endangering public health by causing violative drug residues in these food-producing animals.

Many different models for inducing BP have been described. Some have used both viruses and bacteria (Jericho & Langford, 1978); others have used bacteria and temperature-induced stress (Slocombe *et al.*, 1984); and finally, some models use bacteria as the sole pathogenic agent (Ames *et al.*, 1983; Ames & Patterson, 1985; Burrows, 1985; Burrows *et al.*, 1986a; Burrows *et al.*, 1986b; Farrington *et al.*, 1987; Gibbs *et al.*, 1984; Panciera & Corstvet, 1984). Methods of induction have varied because the first method (administration of viruses and bacteria) did not induce a consistent disease state. Research also showed that

stress played an important role in disease among feedlot cattle (Cole *et al.*, 1988). For this reason, a few early methods used some form of stress to help induce BP (Slocombe *et al.*, 1984). Since *Pasteurella haemolytica* is known to be the primary pathogen in BP, newer methods of induction have attempted to suppress the immune system and use *P. haemolytica* as the sole pathogenic agent. These methods produce a consistent BP.

Pharmacokinetics and Disease

Pharmacokinetic models have the potential to predict the serum drug concentrations in animals (both healthy and diseased). Furthermore, pharmacokinetic dogma says that, for many drugs, tissue concentrations parallel serum concentrations; therefore, pharmacokinetic modeling of tissue depletion profiles may also predict withdrawal periods for altered dosage regimens and disease (Dittert, 1977). It is impossible to isolate the effect of disease on the pharmacokinetic parameters of a given drug without relating them to those parameters in normal animals (Benet *et al.*, 1984). When the pharmacokinetic parameters and tissue depletion profiles are compared between healthy and diseased animals, prediction of the effects of disease on withdrawal periods may be more effectively extrapolated from the pharmacokinetic parameters obtained from the tissue depletion profile (Mercer *et al.*, 1977). The goal of pharmacokinetic modeling of tissue depletion is to model the tissue drug profile seen in a group of animals; then, using this information, the model could be used to predict when the concentration will be below the tolerance limit set by the United States Food and Drug Administration in a larger population in that particular species and tissue (Braun & Waechter, 1983; Weber, 1983).

The effects of disease upon the pharmacokinetics of certain classes of drugs have been well-reviewed (Baggot, 1980; Benet *et al.*, 1984). Since *P. haemolytica* is a gram-negative bacteria, endotoxin may be released and influence pharmacokinetic parameters and tissue concentrations. Many studies have shown that endotoxin alters the pharmacokinetics of gentamicin. In rabbits, significant differences in the elimination rate constant β, half-life ($t_{1/2\beta}$), volume of the central compartment (V_c), and area-derived volume of distribution ($V_{d(area)}$) were seen between control and endotoxin groups (Halkin *et al.*, 1981). In cats, differences were seen between control and endotoxin groups with respect to mean residence time (MRT) and $t_{1/2}$ (Jernigan *et al.*, 1988b). Sheep also showed differences in their pharmacokinetic parameters when given endotoxin. Significant differences in distribution rate constant α, concentration at time zero (C_0), V_c, and volume of the peripheral compartment (V_p) were reported between control and endotoxin group (Wilson *et al.*, 1984). The pharmacokinetic parameters in horses given gentamicin and endotoxin were also altered. Those parameters in which there were observed differences were C_0, $V_{d(area)}$, and V_c (Wilson *et al.*, 1983). Alterations in gentamicin tissue concentrations have also been reported in the literature. Both renal cortex and medulla concentration in rats given endotoxin were significantly higher than the control group (Bergeron *et al.*, 1982). Renal medulla concentrations were also higher in cats given endotoxin when compared to a control group (Jernigan *et al.*, 1988a).

The effects of bovine BP on the pharmacokinetics of various xenobiotics is documented to a lesser extent (Ames *et al.*, 1983; Burrows, 1985; Burrows *et al.*, 1986a; Burrows *et al.*, 1986b). In all of these reports, but not all drugs tested, BP

had an influence on pharmacokinetic parameters. The parameters that were altered included the distribution rate constant α (gentamicin and erythromycin), elimination rate constant β (gentamicin, oxytetracycline, and erythromycin), $t_{1/2\alpha}$ (gentamicin), $t_{1/2\beta}$ (tylosin and oxytetracycline), $V_{d(area)}$ (oxytetracycline), and steady-state volume of distribution ($V_{d(ss)}$) (oxytetracycline). Tissue concentrations were reported in all studies, but in no instance were tissue depletion profiles studied and used to estimate a withdrawal period. Also, serum pharmacokinetics were not correlated with tissue depletion profiles in healthy and bronchopneumonic calves. It may be hypothesized that changes in tissue residue profiles may have been present due to the alteration in the pharmacokinetic parameters described. With this information in mind, there seems to be a strong likelihood that gentamicin tissue depletion profiles and pharmacokinetic parameters may be altered by *P. haemolytica*-induced pneumonia.

Gentamicin

Gentamicin is an aminoglycoside antibiotic. It is water-soluble, excreted by glomerular filtration, and actively accumulates in renal tissue by binding to, specifically, renal brush border membranes (Sastrasinh *et al.*, 1982). Renal gentamicin residues can persist for several months (Brown & Baird, 1988; Burrows, 1979). In food-producing animals, gentamicin has been approved by the FDA and is currently marketed for use in swine, chickens, and turkeys. The injectable formulation for swine has a label-prescribed withdrawal period of 40 days (Sundlof *et al.*, 1988).

The pharmacokinetics of gentamicin have been described in a large variety of food-producing species, such as swine, cattle, sheep, rabbits, and catfish (Brown

et al., 1985; Burrows *et al.*, 1987; Clarke *et al.*, 1985; Curl & Curl, 1988; Riond & Riviere, 1988; Setser, 1985; Wilson *et al.*, 1981). Serum levels of gentamicin can best be described using a three- or four-compartment open model. The tissue residues have also been investigated in food animals in which the drug is not approved. These species include cattle and sheep (Brown & Baird, 1988; Brown *et al.*, 1986a; Haddad *et al.*, 1986; Haddad *et al.*, 1987; Takahashi *et al.*, 1985; Ziv *et al.*, 1982). In sheep, after a dose of 3 mg/kg IV every 12 hours for 10 days, renal concentrations ranged from 183 μg/g of tissue on day 1 post-injection to 2.86 μg/g of tissue on day 77 (Brown & Baird, 1988). Renal cortex concentrations in sheep were approximately 120 μg/g on post-injection day 1 and decreased to approximately 5.1 μg/g by day 12 after a single intramuscular (IM) injection of 6 mg/kg (Brown *et al.*, 1986a). Renal concentrations varied from 0.74 μg/g on day 3 after injection to 0.02 μg/g on day 30 of a study done in calves that had received a single oral dose of 1 mg/kg (Takahashi *et al.*, 1985). Renal concentrations in another study were reported to range from 320 μg/ml 4 hours after single gentamicin administration of 3 mg/kg IV to 45 μg/ml at 48 hours (Ziv *et al.*, 1982). Renal cortex concentrations ranged from 557 μg/g at 24 hours after injection IM of 5 mg/kg every 8 hours for 10 days to 139 μg/g after 144 hours and an IM dose of 3.5 mg/kg every 8 hours for 10 days in cattle (Haddad *et al.*, 1987). Renal medulla concentrations were reported to be approximately 5.5 μg/g in sheep 1 day after gentamicin administration of 6 mg/kg IM in sheep (Brown *et al.*, 1986a). The renal medulla concentrations in cattle were reported as approximately 400 μg/g 24 hours after gentamicin administration of 5 mg/kg IM every 8 hours for 10 days (Haddad *et al.*, 1987). The renal tissue depletion profiles have been modeled using

both exponential equations and the power function (Brown & Baird, 1988; Brown *et al.*, 1986b; Brown *et al.*, 1985). Skeletal muscle concentrations of gentamicin have been reported for sheep as approximately 0.23 μg/g at 1 day post-administration of 6 mg/kg IM (Brown *et al.*, 1986a). The concentration of gentamicin in skeletal muscle of cattle was approximately 0.82 μg/g 25 hours after gentamicin administration of a single dose of 2,500 mg intrauterinally (Haddad *et al.*, 1986). Liver concentrations of gentamicin were approximately 1.72 μg/g 24 hours post-injection of 6 mg/kg IM in sheep (Brown *et al.*, 1986a), while concentrations in cattle were 13.5 μg/g at 24 hours post-gentamicin administration of 5 mg/kg every 8 hours for 10 days (Haddad *et al.*, 1987). Since gentamicin is frequently used in veterinary medicine against known or suspected gram-negative infections (Brown, 1988), gentamicin tissue residues need to be investigated in food animals to help estimate withdrawal periods and prevent contamination of meat products sold to consumers.

MATERIALS AND METHODS

Animal Use and Care

Cross-bred, beef heifer calves (90-140 kg body weight) were acquired from commercial sources and determined to be free of disease by physical examination, complete blood count (CBC) (which included fibrinogen concentration), and serum biochemical panel (including the serum activities of aspartate aminotransferase, γ-glutamyltransferase, alkaline phosphatase, and creatine phosphokinase; and the concentrations of glucose, total protein, albumin, calcium, inorganic phosphorus, total bilirubin, urea nitrogen, and creatinine). The heifers were housed outdoors in concrete pens with shelter provided. Calves were fed alfalfa hay and had access to fresh water *ad libitum*. Calves were observed for alterations in disposition and clinical signs daily, and pens were cleaned daily.

Acute Bronchopneumonia

The method used to induce BP in this study was one modified from Farrington *et al.* (1987). Each calf in the pneumonic group received an early morning dose of 10 mg dexamethasone (Azium^R, Schering Corp., Kenilworth, NJ 07033) intramuscularly, followed in the afternoon by administration of 4 ml of 5% acetic acid into the right cranial lung lobe via bronchoscopy under xylazine (Rompun^R, Mobay Corp., Shawnee, KS 66201) sedation and analgesia. The next afternoon a suspension of *Pasteurella haemolytica* (in trypticase soy broth), Biotype 1A, containing 2-8 x 10^8 colony forming units per ml, 16.7% of which was in the logarithmic phase of growth, was inoculated into the right cranial lobe of the lung via bronchoscopy at a rate of 0.22 ml suspension/kg body weight, again using

xylazine sedation and analgesia to control the animals. The course and progress of this experimentally induced BP was predictable in these calves. Historically, within 24-36 hours, animals typically displayed signs of dyspnea, depressed appetite and attitude, increased rectal temperature ($\geq$ 40°C), and auscultable changes in bronchovesicular sounds (Friedlander, personal communication, 1988). Clinical signs were evaluated using a scoring system similar to that described for this *P. haemolytica* model (Farrington *et al.*, 1987) and for intensive care patients in human hospitals (Knaus *et al.*, 1985). The scoring system is described later.

Experimental Design

Twenty-four calves were assigned to two treatment groups. The animals were blocked over time so that 8 calves, 4 from each treatment group, were studied in any one block. Group 1 (G1) served as normal controls; within G1, each calf received 5 mg gentamicin (Gentocin^R, Schering Corp., Kenilworth, NJ 07033)/kg body weight intravenously (IV) via jugular venipuncture. Each calf in group 2 (G2) had acute BP induced by the method described previously. At the onset of clinical signs (rectal temperature $\geq$ 40°C and change in lung auscultation from before BP induction), each calf was given 5 mg gentamicin/kg body weight IV.

Blood samples for CBC and serum biochemical panel were again taken immediately prior to administration of gentamicin in all calves. Severity of disease was determined by a systematic scoring of the clinical indices of disease (pulse, respiration rate, quality of respiration, nasal discharge, cough, temperature, auscultation, attitude, appearance, appetite, posture, open mouth breathing, change in body weight, and diarrhea). Each indicator or test was scored from 0 to 3 with 0 being normal and 3 being the most severe (Table 1). A sum of the scores (0 - 28

Table 1. Scoring system for bovine pneumonia, scores for individual indices summed together to give final disease score

CLINICAL INDICES	Score 0 (least severe)	1	2	3 (most severe)
temperature:	normal (37.3-39.9°C)	sl.elevated (40-41°C)	mk.elevated (>41°C)	
respiration: (rate)	low/normal (≤36)	sl.elevated (37-60)	mk.elevated (>60)	
(quality)	normal	labored		
nasal discharge: (amount)	none	light	heavy (mucopurulent)	
cough: (frequency)	none	sporadic productive	persistent non-productive	
auscultation·	normal	dry/wheeze	moist/rales	marked consolidation
posture: (head & neck)	normal	abnormal (neck extended & head down)		
open mouth breathing:	no	yes		
pulse:	low/normal (<100)	sl.elevated (100-120)	mk.elevated (>120)	
attitude:	alert	sl.depressed	md.depressed	sv.depressed
appearance: (hair coat & mucous membranes)	normal	fair	poor	cyanotic
appetite:	normal to increased	observably decreased	anorectic	
wt. gain:	normal	low	none	loss
diarrhea:	no	yes		

maximum) was used to differentiate more severely affected animals from those that were mildly ill. Those indices which were more important to diagnosis of pneumonia (temperature, auscultation) were allowed a higher maximum score than other indices. Calves were scored on each day that a blood sample was taken. The scores taken were used to correlate the severity of disease to the changes in

pharmacokinetic parameters and tissue residue profile for each calf.

Blood samples were taken from all calves at 0, 0.17, 0.33, 0.5, 0.67, 1, 1.5, 2, 4, 6, 8, and 12 hour(s) and 1, 2, 3, 5, 7, 9, 11, 14, 17, 21, 26, 31, 36, and 42 day(s) after gentamicin administration. For gentamicin analysis, blood samples were allowed to clot at room temperature (22 - 25°C) for approximately 1 hour, centrifuged at 1000 x g for 10 minutes. The serum was then aspirated and stored in plastic tubes at -20°C until analyzed for gentamicin. All calves were killed using captive-bolt stunning followed immediately by exsanguination. Six animals (3 from each group) were killed on days 1, 11, 21, and 42 after gentamicin administration. Tissues that were collected for drug analysis at the time of necropsy included renal cortex, renal medulla, liver, right cranial lung lobe (normal and diseased where appropriate), left ventricle of the heart, skeletal muscle (semimembranosus and semitendinosus area), and perirenal fat. These tissue samples were blotted dry and frozen at -20°C until time of analysis. Also, complete gross and microscopic post-mortem evaluation of each calf were performed at the time of euthanasia to assess the severity of the experimentally induced BP and any effects that drug administration may have had on each animal.

Gentamicin Analysis

Serum gentamicin samples were analyzed in singlet. All tissue samples were analyzed in duplicate. Samples of renal cortex, renal medulla, skeletal muscle, heart, liver, lung, and fat were analyzed using a modification of a method developed in this laboratory (Brown *et al.*, 1988). For skeletal muscle, liver, lung, and fat, 600 mg of tissue (wet weight) was weighed into a plastic test tube and 1.8 ml of 1 N NaOH was added to the tube. For renal medulla, renal cortex, and heart, 600 mg

of tissue was weighed into a plastic test tube and 2.4 ml of physiological saline solution (PSS) was added to each sample. An equal volume of 2 N NaOH was then added. All samples were incubated at 70°C for 20 minutes, then cooled to room temperature. The pH was adjusted to 7.5 $\pm$ 0.2 with either glacial acetic acid or 1% acetic acid. If the pH decreased below 7.0, the solution became cloudy and the pH was increased back to 7.5 $\pm$ 0.2 with 1 N NaOH. At this point, the lung samples were homogenized with an ultra-sonic tissue homogenizer (Vibra-Cell, Sonics & Materials Inc., Danbury, CT, 06810) for 1 minute with the instrument set at 80% of capacity and power switch set at 40. An aliquot of each extract and all serum samples were assayed for gentamicin using an automated fluorescence polarization immunoassay (TDx, Abbott Diagnostics, Irving, TX 75015; Innoflour™, Innotron of Oregon, Portland, OR 97292). This assay system has been shown to have a coefficient of variation of less than 10% both in intra- and inter-run assay analysis (Cheng *et al.*, 1987; Jolley *et al.*, 1981). Since the tissue matrix has different effects upon assay concentrations when analyzed using a standard curve in serum (Kaplan *et al.*, 1973), a standard curve in each tissue matrix was generated. Gentamicin-naive tissues were analyzed and tissues containing known amounts of gentamicin were also analyzed to validate the assay and to determine the limit of sensitivity of the assay for each tissue. Limit of sensitivity was defined as the concentration which could be determined as different from zero with 95% confidence. This was done by determining the 95% confidence interval for a zero sample run on four consecutive days and calculating the upper limit of that 95% confidence interval as the limit of sensitivity. The coefficient of variation (CV) for serum was <3% for within day samples and <5% for between day samples. Kidney

tissue had a CV for within day samples of <10% and between day CV of <8%. Skeletal muscle samples had a CV for between day samples of <15% and within day was <11%. The CV for heart tissue was <10% for between day samples. Liver tissue had a CV for within day samples of <8% while between day CV was <14%. Lung tissue had a within day CV of <9% and a between day CV of <13% (except for 100 ng/g standard which was 29%). Gentamicin in fat tissue was detectable, but not quantifiable. Data for standard curves of each matrix is shown in Appendix 1.

Pharmacokinetic Analysis

Serum concentrations were fitted with a sum of exponential terms by a curve-feathering computer program (RSTRIP, MicroMath, Salt Lake City, UT 84121). Once those initial estimates had been determined, the final fits were determined using a weighted least-squares nonlinear regression computer routine (PCNONLIN, Statistical Consultants, Inc., Lexington, KY 41017). The optimum model was determined by means of residual trend analysis and minimization of Akaike's information criterion (AIC), a statistical parameter derived from maximum likelihood estimations that determines the minimum model that maximizes the predictive potential of the model (Yamaoka *et al.*, 1978). The equation for AIC is:

$$AIC = (n)(\ln(ss_R)) + 2p$$

where n equals the number of observations, ss_R is the sum of the squared residuals, and p is the number of parameters in the model. The model with the lowest AIC is the model which best estimates the data. Once the final fits were determined, noncompartmental pharmacokinetic parameters were calculated (Gibaldi & Perrier, 1982) including area under the serum concentrations vs. time curve (AUC), area

under the first moment curve (AUMC), C_0, V_c, $V_{d(area)}$, $V_{d(ss)}$, serum clearance (Cl_s), MRT, elimination rate constant (k_{el}), the various coefficients and exponents of the model and their respective half-lives, and the percentages of the AUC contributed by each phase. The equations used to calculate the serum pharmacokinetic parameters are shown in Appendix 2.

Tissue concentrations, in particular renal, liver, and lung, were mathematically modeled over time using several models that have previously been reported (Brown & Baird, 1988; Brown *et al.*, 1986a; Brown *et al.*, 1986b). Included in these models was a one-compartment model and a power function. The equation for the one-compartment model is:

$$C_t = C_0 e^{-kt}$$

The equation for the two-compartment model is:

$$C_t = Ae^{-\alpha t} + Be^{-\beta t}$$

The equation for the power function is:

$$C_t = At^{-\alpha}$$

The power function is linear when plotted as ln(tissue concentration) vs. ln(time). The one-compartment model is linear when plotted as ln(tissue concentration) vs. time. In each instance, tissue and serum concentrations were correlated to determine if changes in serum pharmacokinetic parameters are direct indicators of the changes in the depletion kinetics in any or all tissues.

Statistical Analysis

The study was blocked into studies with 4 calves (one for each time of necropsy) from each group (G1 or G2) included in each block. Therefore, comparisons of all pharmacokinetic values between groups were analyzed using a randomized complete block design. For pharmacokinetic values that were normally distributed, analysis of variance was used; for pharmacokinetic values that were not normally distributed, Kruskal-Wallis nonparametric analog of analysis of variance was used. In all instances, differences were considered significant at the 0.05 level. When either parametric or nonparametric analysis of variance showed a significant difference, multiple comparisons between treatment groups were made using Student-Newman-Keuls multiple comparisons test or an appropriate nonparametric multiple comparisons test. Harmonic means were calculated and used for all $t_{1/2}$'s because, by definition, they are not normally distributed. Complete blood count data, serum biochemical panel data, and severity of disease score were analyzed using analysis of variance with repeated measures. A non-paired t-test was used to compare tissue concentrations between the two groups. A paired t-test was used to compare normal and diseased lung concentrations of gentamicin in animals from the pneumonic group.

Multiple regression was used to relate a single dependent variable (ln(tissue concentration)) to more than one independent variable. Multiple regression was used to relate the natural logarithm of tissue concentration of renal cortex, renal medulla, liver, and all lung concentrations to time or ln(time), severity of disease scores on day 0 and the day the heifer was killed, AUC, C_0, $V_{c\beta}$ $V_{d(ss)}$, Cl_B, k_{el}, albumin concentration, total protein concentration, white blood cell count, packed

cell volume, and fibrinogen concentration. Parameters were included in the equation if $P < 0.15$ for significant contribution to the model and AIC was calculated for all equations. The decision on which equation was the best fit was the same as that used for the pharmacokinetic modeling of the tissue concentrations, except that the AIC value for the multiple regression equations used the sum of squares for the residuals and not the weighted sum of squares. All values are reported as mean $\pm$ standard deviation ($X \pm SD$) unless otherwise noted.

RESULTS

One heifer, #209, in the pneumonic group, scheduled to be killed on day 42, died on day 3 of the study and was not exsanguinated until approximately 4 hours after death. The data obtained from her was used in the study and another animal was added to replace her. Another heifer (#39) died approximately four hours before she was scheduled to be killed on day 1 and therefore was not exsanguinated until 4 hours after death. Also, the two heifers in the third block which were scheduled to be killed on day 11 both had BP induced since the first animal (#220) did not show clinical signs of BP. When the other animal was tried (#221), again, no effect was seen. But after approximately a week, both calves showed very mild clinical signs (scores ranged from 2 to 6 for calf #220 and from 2 to 10 for calf #221). Thus, there were 11 animals in the normal group and 14 animals in the pneumonic group for a total of 25 heifers in the study.

Post-mortem Evaluations

In the healthy group, no significant lesions were reported. In the pneumonic group, 8 of the 14 calves had marked consolidation of approximately 60% of their lungs. Six of the 14 had prominent abscesses primarily in the right anterior lung lobe upon gross examination. Histopathology revealed that 6 of the 14 calves had severe pulmonary necrosis. Fibrosis of the lungs (principally the right lung) was also present in 9 of the 14 calves in the pneumonic group.

Severity of Disease Scores

A graph representing this data is shown in Figure 1. On post-injection day 1 of the study, the disease severity score of the pneumonic group was higher than

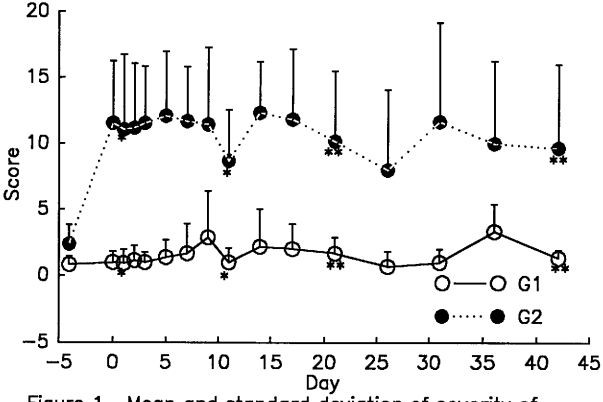

Figure 1. Mean and standard deviation of severity of disease scores for normal (G1) and pneumonic (G2) groups (* = P < 0.0001; ** = P < 0.05)

the normal group ($P < 0.0001$). On day 11 of the study, the diseased scores for the two groups were still significantly different ($P < 0.0001$) from each other. By day 21 of the study, the groups' scores were starting to converge but were still different ($P = 0.0005$). On the final day of the study, the scores of the pneumonic group were still higher than those from the normal group ($P = 0.0155$).

Serum Biochemical Panel and CBC Data

Normal ranges for serum biochemical and CBC parameters in cattle are shown in Appendix 3. The means and standard deviations of all serum panel values in both groups are shown in Appendix 4. Those parameters in which significant differences were seen are shown in Table 2. Albumin concentrations were significantly lower ($P = 0.0011$) after BP in the pneumonic group when compared to the normal group. Albumin concentrations also showed a significant decrease ($P = 0.0009$) over time in the pneumonic group. Alkaline phosphatase activity differed significantly ($P = 0.0018$) between the two groups after BP. Alkaline phosphatase activity significantly decreased ($P = 0.0478$) over time in the pneumonic group. A significant difference ($P = 0.0077$) was also shown between groups. Calcium concentrations were significantly lower in the pneumonic group both before ($P = 0.0013$) and after ($P < 0.0001$) BP. The pneumonic group had a significant decrease ($P = 0.0039$) in calcium concentrations over time. The decrease in γ-glutamyltransferase in both groups with respect to time approached significance ($P = 0.0760$). The mean glucose concentration in the pneumonic group was significantly lower ($P = 0.0257$) after BP. Glucose concentrations were significantly decreased ($P = 0.0272$) over time in the pneumonic group. Phosphorous concentrations displayed a significant decrease ($P = 0.0469$) over time

Table 2. Differences in serum biochemical panel data before and after bronchopneumonia and prior to gentamicin treatment

Indices	Group	Before Mean ± SD	After Mean ± SD
Albumin (g/dl)	G1	3.15 ± 0.25	3.15 ± 0.24[a]
	G2	3.08 ± 0.25[1]	2.71 ± 3.32[2, b]
Alkaline phosphatase (U/l)	G1	133 ± 52.7	138 ± 57.4[a]
	G2	99.8 ± 34.5[1]	77.8 ± 25.3[2, b]
Calcium (mg/dl)	G1	10.7 ± 0.62[a]	10.6 ± 0.27[a]
	G2	9.91 ± 0.44[1, b]	8.96 ± 0.82[2, b]
γ-glutamyltransferase (U/l)	G1	35.8 ± 3.76[3]	34.1 ± 3.53[4]
	G2	36.1 ± 6.62[3]	33.8 ± 3.14[4]
Glucose (mg/dl)	G1	80.3 ± 8.44	74.5 ± 22.6[a]
	G2	79.6 ± 13.8[1]	54.8 ± 18.7[2, b]
Phosphorous (mg/dl)	G1	6.62 ± 0.79[1]	5.70 ± 0.74[2]
	G2	6.66 ± 1.42[1]	5.46 ± 3.07[2]
Total bilirubin (mg/dl)	G1	0.25 ± 0.08[a]	0.23 ± 0.12[a]
	G2	0.45 ± 0.25[b]	0.59 ± 0.32[b]
Total protein (g/dl)	G1	6.24 ± 0 23	6.10 ± 0.23[a]
	G2	6.39 ± 0.40[1]	5.75 ± 0.48[b]

[1, 2] = If numbers are different, significant difference ($P < 0.05$) within groups from before to after

[3, 4] = Decreased in value over time, approached significance ($P = 0.0760$)

[a, b] = If letters are different, significant difference ($P < 0.05$) between groups at the same time

G1 = normal calves (n = 11)

G2 = pneumonic calves (n = 14)

in both groups. Total bilirubin showed significantly lower concentrations in the normal group both before ($P = 0.0163$) and after ($P = 0.0015$) BP. Total bilirubin was also significantly different ($P = 0.0011$) between the two groups. Total protein differed between the normal group and the pneumonic group after BP ($P = 0.0374$). Total protein also showed a significant decrease ($P = 0.0122$) over time in the pneumonic group. The only significant correlation seen was serum albumin

to severity of disease score on day 0 and is shown in Figure 2. ($r = -0.5251$ and $P = 0.0083$).

The CBC data for both groups are shown in Appendix 5. Parameters which had significant differences are shown in Table 3. Fibrinogen concentrations were significantly higher ($P = 0.0301$) after BP in the pneumonic group. Hemoglobin values were significantly lower ($P = 0.0281$) in the pneumonic group after BP. Hemoglobin values also significantly decreased ($P = 0.0049$) over time in both groups. Lymphocyte cell counts showed significantly lower values in the pneumonic group both before ($P = 0.0232$) and after ($P = 0.0081$) BP. Both groups had a significant decrease ($P = 0.0050$) in number of lymphocytes over time. Monocyte numbers were significantly lower ($P = 0.0429$) after BP in the pneumonic group. Monocyte cell count significantly decreased ($P = 0.0492$) over time in the pneumonic group. The lower packed cell volume in the pneumonic group both before ($P = 0.0505$) and after ($P = 0.0558$) BP approached significance. Packed cell volume, in both groups, significantly decreased ($P = 0.0016$) over time. Packed cell volume was also significantly different ($P = 0.0315$) between the groups. Plasma protein values were significantly higher ($P = 0.0278$) before BP in the pneumonic group. Plasma protein concentrations significantly decreased ($P = 0.0036$) in the pneumonic group over time. Red blood cell count decreased over time in both groups, but this change was not significant ($P = 0.0604$). White blood cell count was lower in the pneumonic group before BP, but was not significant ($P = 0.0700$). White blood cell count was significantly lower ($P = 0.0340$) in the pneumonic group after BP. White blood cell numbers were significantly different ($P = 0.0263$) between the normal and pneumonic groups. The decreased white

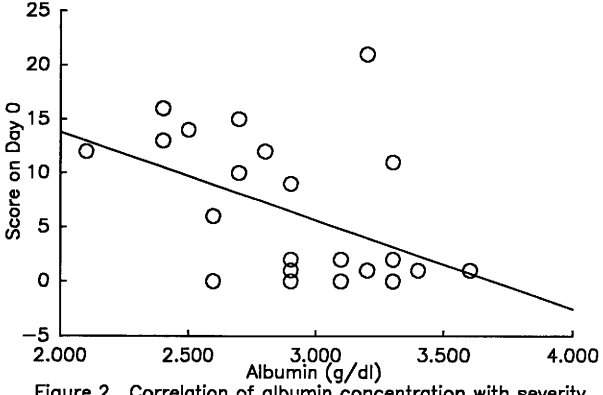

Figure 2. Correlation of albumin concentration with severity of disease score on day 0 ($r = -0.5251$; $P < 0.05$)

Table 3. CBC data in which differences were seen before and after bronchopneumonia and prior to gentamicin treatment

Indices	Group	Before Mean ± SD	After Mean ± SD
Fibrinogen (mg/dl)	G1	445 ± 202	490 ± 129[a]
	G2	607 ± 289	692 ± 250[b]
Hemoglobin (g/dl)	G1	14.7 ± 1.73[1]	14.0 ± 1.80[2, a]
	G2	13.6 ± 2.01[1]	12.3 ± 1.59[2, b]
Lymphocytes (#/μl)	G1	$7.34 \times 10^3 \pm 2.44 \times 10^3$ [1, a]	$6.32 \times 10^3 \pm 2.10 \times 10^3$ [2, a]
	G2	$5.59 \times 10^3 \pm 1.60 \times 10^3$ [1, b]	$4.16 \times 10^3 \pm 1.44 \times 10^3$ [2, b]
Monocytes (#/μl)	G1	402 ± 219	602 ± 572[a]
	G2	393 ± 247[1]	231 ± 218[2, b]
Packed cell volume (%)	G1	42.3 ± 2.72[1, c]	39.4 ± 3.92[2, g]
	G2	39.4 ± 5.58[1, d]	35.5 ± 4.94[2, h]
Plasma protein (g/dl)	G1	7.17 ± 0.40[1, a]	7.11 ± 0.25[2, b]
	G2	7.65 ± 0.56[1, b]	6.92 ± 0.61[2, b]
Red blood cells (#/μl)	G1	$9.56 \times 10^6 \pm 1.09 \times 10^6$ [4]	$9.31 \times 10^6 \pm 1.91 \times 10^5$ [5]
	G2	$8.95 \times 10^6 \pm 6.15 \times 10^5$ [4]	$7.73 \times 10^6 \pm 3.32 \times 10^5$ [5]
White blood cells (#/μl)	G1	$9.31 \times 10^3 \pm 2.67 \times 10^3$ [6, e]	$8.89 \times 10^3 \pm 2.63 \times 10^3$ [7, a]
	G2	$7.68 \times 10^3 \pm 2.22 \times 10^3$ [6, f]	$6.45 \times 10^3 \pm 2.50 \times 10^3$ [7, b]

[1, 2, 3] = If numbers are different, significant difference ($P < 0.05$) from before to after within the same group

[4, 5] = Difference between groups over time, but not significant ($P = 0.0604$)

[6, 7] = Difference over time, but not significant ($P = 0.0808$)

[a, b] = If letters are different, significant difference between groups within a given time

[c, d] = Difference between groups, but not significant ($P = 0.0505$)

[e, f] = Difference between groups, but not significant ($P = 0.0700$)

[g, h] = Difference between groups, but not significant ($P = 0.0558$)

G1 = normal group ($n = 11$)

G2 = pneumonic group ($n = 15$)

blood cell count over time in both groups approached significance ($P = 0.0808$). The only significant correlation was severity of disease score on day 0 with fibrinogen concentrations and is shown in Figure 3 ($r = 0.6786$; $P = 0.0007$).

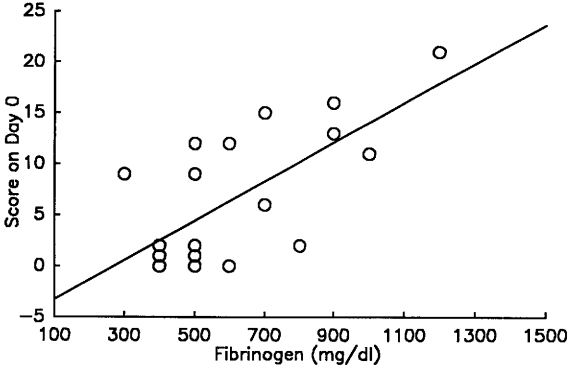

Figure 3. Correlation of fibrinogen concentrations with severity of disease score (Day 0) ($r = 0.6787$; $P < 0.001$)

Gentamicin Analysis

Limit of sensitivity for serum was 17.9 ng/ml. Renal cortex and medulla had a limit of sensitivity of 17.0 ng/g of kidney tissue. Skeletal muscle had a limit of sensitivity of 10.8 ng/g of muscle tissue. The limit of sensitivity for liver tissue was 4.25 ng/g of tissue. Heart muscle had a limit of sensitivity of 27.3 ng/g of tissue. Normal and diseased lung had a limit of sensitivity of 20.70 ng/g of lung tissue. Typical standard curves are shown in Appendix 1.

Serum Pharmacokinetic Values

Serum concentrations for each group are shown in Figure 4. There were no significant differences in serum pharmacokinetic values between the normal group and the pneumonic group (Table 4). Closer observation of the individual data indicated that the pneumonic group had a bimodal distribution. Because of that, nonparametric analysis was done on all serum pharmacokinetic parameters; no significant differences were seen. The pneumonic group was tested for outliers. First, #39 was determined to be an outlier, then #42 was found to be an outlier. For these reasons, the data for animals #39 and #42 were removed and analysis of variance was done again on the remaining heifers. This yielded a significant difference ($P = 0.0222$) in Cl_s between the normal group and the pneumonic group, with the pneumonic group having the higher Cl_s. The smaller serum AUC in the pneumonic group compared to the normal group approached significance ($P = 0.0679$). Correlations of the serum pharmacokinetic parameters Cl_s, $V_{d(\infty)}$, k_{el}, and AUC with serum urea nitrogen, total protein, packed cell volume, and creatinine were done both with and without #39 and #42. In both cases, significant correlations were seen. The following correlations contain animals #39 and #42.

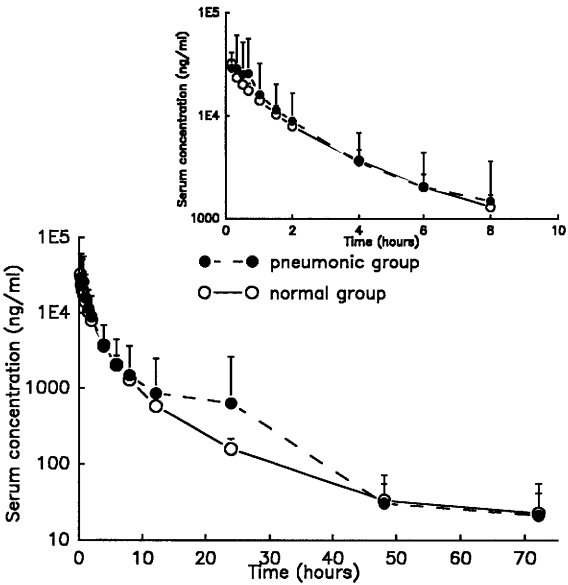

Figure 4. Gentamicin serum concentrations vs. time for normal and pneumonic groups

Table 4. Serum pharmacokinetic values in normal and bronchopneumonic groups

Values **	G1 Mean ± SD	G2 Mean ± SD	G2a Mean ± SD	#39	#42
A (μg/ml)	24.5 ± 6.19	77.7 ± 163	22.5 ± 3.96	619	200
B (μg/ml)	8.70 ± 6.20 [1]	9.87 ± 7.60	9.34 ± 7.83	18.2	7.87
C (μg/ml)	0.85 ± 0.98 [2]	0.72 ± 0.53 [1]	0.78 ± 0.52 [3]	----	0.13
α (hr^{-1})	1.42 ± 1.02	2.12 ± 1.44	1.97 ± 1.26	4.98	1.09
β (hr^{-1})	0.28 ± 0.21 [1]	0.33 ± 0.26	0.37 ± 0.26	0.080	0.20
γ (hr^{-1})	0.058 ± 0.060 [2]	0.080 ± 0.052 [1]	0 087 ± 0.050 [3]	----	0.020
C_0 (μg/ml)	33.1 ± 11.4	88.1 ± 165	32.4 ± 10.6	638	208
AUC (μg x hr/ml)	61.8 ± 14.1	82.9 ± 92.6	48.4 ± 18.7 [b]	352	228
AUMC (μg x hr^2/ml)	489 ± 302	594 ± 800	397 ± 492	2870	686
MRT (hr)	7.87 ± 5.19	7.82 ± 10.23	8.20 ± 11.02	8.16	3.00
V_c (ml/kg)	169 ± 60.5	150 ± 81.4	173 ± 63.3	7.84	24.1
$V_{d(area)}$ (ml/kg)	4080 ± 4680	3920 ± 7470	4460 ± 7980	178	1100
$V_{d(ss)}$ (ml/kg)	660 ± 507	859 ± 1600	988 ± 1700	116	65 8
Cl_s (ml/hr/kg)	85.5 ± 22.6	99 9 ± 44.8	113.5 ± 30.9 [c]	14.2	21.9
k_{el} (hr^{-1})	0.19 ± 0.13	0.25 ± 0.14	0.25 ± 0.14	0.12	0.33
$t_{1/2\alpha}$ (hr)	0.38 - 1.73 [a]	0.19 - 1.02 [a]	0.21 - 0.98 [a]	0.14	0.64
$t_{1/2\beta}$ (hr)	1.40 - 10.4 [1, a]	1.18 - 8.86 [a]	1.11 - 6.68 [a]	8.67	3.41
$t_{1/2\gamma}$ (hr)	0.00 - 5.85 [2, a]	5.23 - 24.5 [1, a]	5.04 - 18.9 [3, a]	----	34 9
% AUC α	44.3 ± 29.6	42.9 ± 28.4	40.4 ± 28.5	35.4	80.2
% AUC β	46.2 ± 16.0 [1]	44.7 ± 21.2	45.3 ± 20.6	64.6	16.9
% AUC γ	18.9 ± 10.0 [2]	17.5 ± 7.80 [1]	19.1 ± 6.22 [3]	----	2.85

** = all values defined in Appendix 2
[a] = range of half-lives
[b] = difference between G1 and G2a (P = 0.0679)
[c] = significant difference between G1 & G2a (P < 0.05)
[1] = (n = 10)
[2] = (n = 8)
[3] = (n = 9)
G1 = normal group (n = 11 unless otherwise noted)
G2 = pneumonic group (n = 14 unless otherwise noted)
G2a = pneumonic group without #39 & #42 (n = 12 unless otherwise noted)

Correlation of Cl_s with serum creatinine (Figure 5) was significant ($r = -0.6769$; $P = 0.0002$). The correlation of Cl_s with serum urea nitrogen (Figure 6) was also significant ($r = -0.6025$; $P = 0.0014$). Correlation of serum AUC with serum urea nitrogen (Figure 7) was significant ($r = 0.7234$; $P < 0.0001$). The correlation of serum creatinine with AUC (Figure 8) was significant ($r = 0.7079$; $P < 0.0001$). There were no significant correlations of serum pharmacokinetic parameters with severity of disease scores on days 0, 1, or on the days calves were killed.

Tissue Concentrations and Pharmacokinetic Parameters

Due to the fact that calves #39 and #209 were not completely exsanguinated at the time of death, their tissues were not used in modeling of tissue concentrations or in the multiple regression equations. The concentrations for each tissue and day are shown in Tables 5 and 6. Tissue concentrations seen were ranked highest to lowest as follows: renal cortex, renal medulla, liver, lung, skeletal muscle, and perirenal fat. No differences were seen in renal cortex, liver, normal-appearing lung, and "diseased" lung (G2) concentrations between groups on any day after dosing. Renal medulla concentrations were significantly different ($P = 0.0475$) between groups on day 21 of the study. No significant differences were seen between the "diseased" lung concentrations and "normal" lung concentrations in the same animals.

A one- and two-compartment model and the power function were used to fit the data from each tissue. The two-compartment model was inappropriate due to a large variance of the parameter estimates obtained. The parameters for one-compartment model and the power function are shown in Table 7. For renal cortex, the data from the normal group was best fitted (lowest AIC) by the power

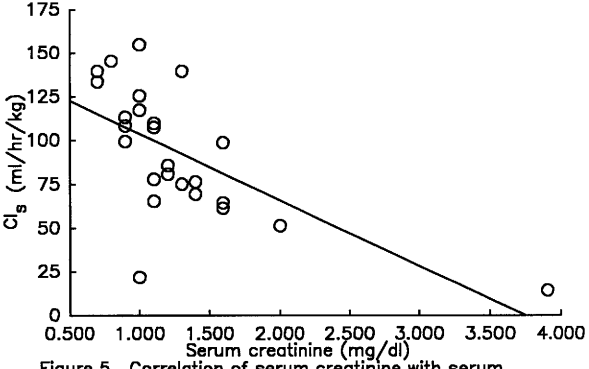

Figure 5. Correlation of serum creatinine with serum clearance (Cl_s) ($r = -0.6769$; $P < 0.005$)

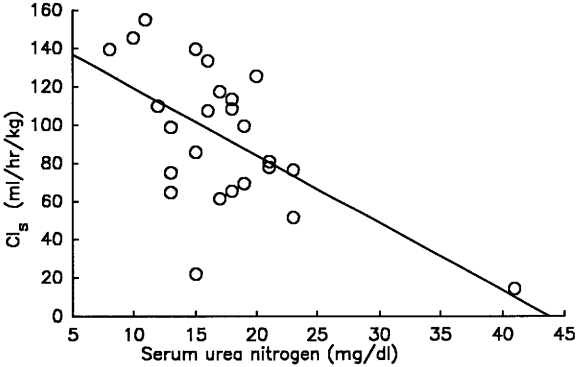

Figure 6. Correlation of serum urea nitrogen with serum clearance (Cl_S) ($r = -0.6025$; $P < 0.005$)

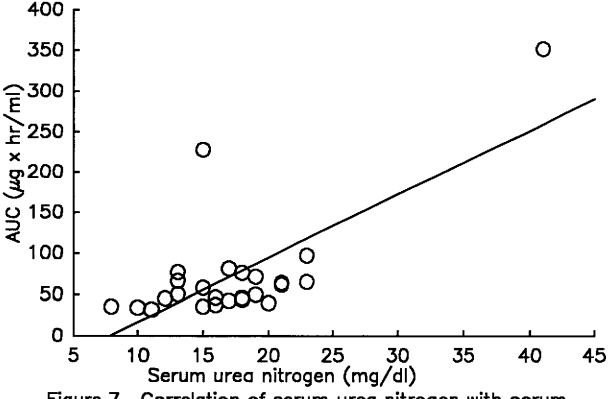

Figure 7. Correlation of serum urea nitrogen with serum area under the curve (AUC) ($r = 0.7234$; $P < 0.0001$)

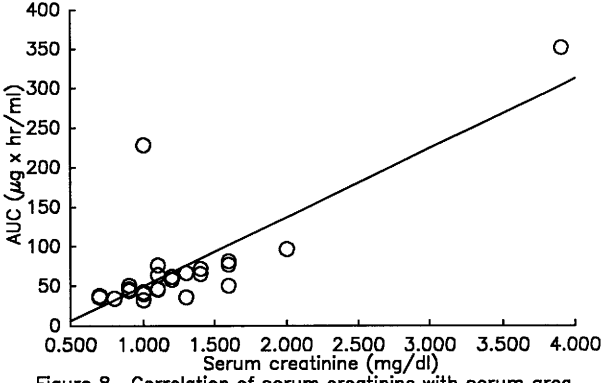

Figure 8. Correlation of serum creatinine with serum area under the curve (AUC) ($r = 0.7079$; $P < 0.0001$)

Table 5. Gentamicin tissue concentrations for renal cortex, renal medulla, liver, normal lung, and diseased lung

Tissue	Day	Group	n	Concentration (ng/g of tissue)
Renal cortex	1	G1	3	$1.05 \times 10^5 \pm 2.33 \times 10^4$
		G2	2	$7.11 \times 10^4 \pm 997$
	11	G1	2	$7.49 \times 10^3 \pm 8.85 \times 10^3$
		G2	4	$4.82 \times 10^4 \pm 3.92 \times 10^3$
	21	G1	3	939 ± 343
		G2	3	$2.54 \times 10^3 \pm 1.39 \times 10^3$
	42	G1	3	489 ± 345
		G2	3	637 ± 360
Renal medulla	1	G1	3	$7.32 \times 10^3 \pm 2.67 \times 10^3$
		G2	2	$1.76 \times 10^4 \pm 1.44 \times 10^4$
	11	G1	2	$6.32 \times 10^3 \pm 6.77 \times 10^3$
		G2	4	$1.55 \times 10^3 \pm 1.17 \times 10^3$
	21	G1	3	523 ± 248
		G2	3	$1.36 \times 10^3 \pm 448$
	42	G1	3	404 ± 87.3
		G2	3	809 ± 673
Liver	1	G1	3	$1.10 \times 10^3 \pm 455$
		G2	2	630 ± 86.2
	11	G1	2	$1.09 \times 10^3 \pm 735$
		G2	4	753 ± 119
	21	G1	3	570 ± 159
		G2	3	706 ± 350
	42	G1	3	436 ± 51.8
		G2	3	353 ± 74.9
Normal lung	1	G1	3	720 ± 369
		G2	2	385 ± 76.8
	11	G1	2	410 ± 392
		G2	4	155 ± 87.4
	21	G1	3	140 ± 48.3
		G2	3	129 ± 59.5
	42	G1	3	98.6 ± 26.4
		G2	3	25.3, ND, ND
Diseased lung	1	G2	2	703 ± 380
	11	G2	4	115 ± 31.7
	21	G2	3	127 ± 31.2
	42	G2	3	105 ± 30.9

G1 = normal group (n = 11)

G2 = pneumonic group (n = 12)

ND = not detectable

Table 6. Gentamicin tissue concentrations for skeletal muscle, fat, and heart

Tissue	Day	Group	n	Concentration (ng/g of tissue)
Skeletal muscle	1	G1	3	88.1, ND, ND
		G2	2	47.4 ± 15.4
	11	G1	2	all ND
		G2	4	all ND
	21	G1	3	all ND
		G2	3	all ND
	42	G1	3	all ND
		G2	3	all ND
Fat	1	G1	3	35.9 ± 18.9
		G2	2	46.7, ND
	11	G1	2	all ND
		G2	4	all ND
	21	G1	3	all ND
		G2	3	all ND
	42	G1	3	all ND
		G2	3	all ND
Heart	1	G1	3	all ND
		G2	2	all ND
	11	G1	2	all ND
		G2	4	all ND
	21	G1	3	all ND
		G2	3	all ND
	42	G1	3	all ND
		G2	3	all ND

G1 = normal group (n = 11)

G2 = pneumonic group (n = 12)

ND = not detectable

Table 7. Tissue depletion model parameters and associated Akaike's Information Criterion (AIC) values

Tissue	Group	Model	Parameters	AIC Value
Renal cortex	G1	Power	$A = 0.0203$ $\alpha = 1.57$	188.6
		one-compartment	$C_0 = 1.48 \times 10^5$ $k_{el} = 0.374$	190.1
	G2	Power	$A = 0.0143$ $\alpha = 8.36$	231.0
		one-compartment	$C_0 = 9.53 \times 10^4$ $k_{el} = 0.297$	221.5
Renal medulla	G1	Power	$A = 1.33 \times 10^{-3}$ $\alpha = 0.755$	179.7
		one-compartment	$C_0 = 7.42 \times 10^3$ $k_{el} = 0.117$	178.9
	G2	Power	$A = 2.35 \times 10^{-3}$ $\alpha = 9.76$	238.1
		one-compartment	$C_0 = 1.47 \times 10^4$ $k_{el} = 0.233$	237.9
Liver	G1	Power	$A = 2.17 \times 10^{-4}$ $\alpha = 0.212$	210.5
		one-compartment	$C_0 = 1.00 \times 10^3$ $k_{el} = 0.0256$	208.9
	G2	Power	$A = 1.23 \times 10^{-4}$ $\alpha = 11.5$	187.6
		one-compartment	$C_0 = 780$ $k_{el} = 0.0175$	160.9
Lung	G1	Power	$A = 1.10 \times 10^{-4}$ $\alpha = 0.456$	135.4
		one-compartment	$C_0 = 525$ $k_{el} = 0.0589$	136.5
	G2 "normal"	Power	$A = 7.4 \times 10^{-5}$ $\alpha = 11.8$	149.4
		one-compartment	$C_0 = 355$ $k_{el} = 0.0724$	134.0
	G2 "diseased"	Power	$A = 1.15 \times 10^{-4}$ $\alpha = 0.582$	150.2
		one-compartment	$C_0 = 622$ $k_{el} = 0.188$	153.6

G1 = normal group (n = 11) G2 = pneumonic group (n = 12)

A = model coefficient (ng/g) α = distribution rate constant (day^{-1})

C_0 = concentration at time zero (ng/g) k_{el} = elimination rate constant (day^{-1})

function, while the pneumonic group was better represented by a one-compartment model. A graph of the one-compartment model is shown in Figure 9 and the power function for renal cortex is shown in Figure 10. Renal medulla concentrations were

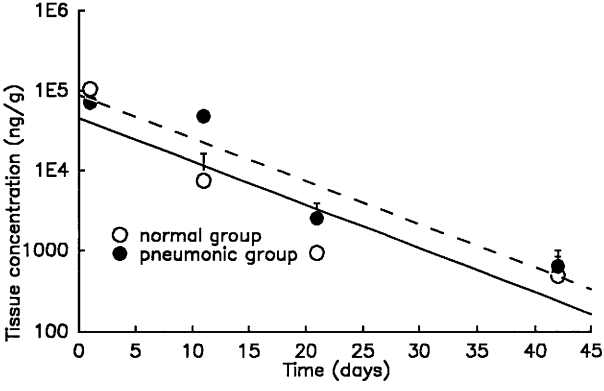

Figure 9. One-compartment model of renal cortex concentrations

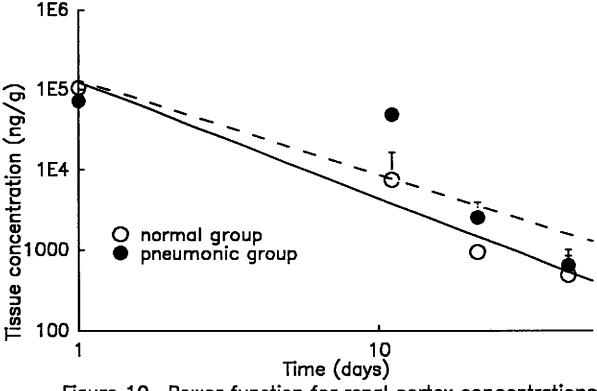

Figure 10. Power function for renal cortex concentrations

best modeled using a one-compartment model in both groups. One-compartment model for renal medulla concentrations is shown in Figure 11 and the power function is shown in Figure 12. Gentamicin depletion in liver was also best represented by a one-compartment model in both groups. One-compartment model is shown in Figure 13 and the power function is shown in Figure 14. Lung concentrations in the normal group was best represented by the power function, the normal appearing lung tissue of the pneumonic group was best represented by a one-compartment model. Gentamicin concentrations of diseased lung of the pneumonic group were best described using the power function. One-compartment model is shown in Figure 15 and the power function for lung tissue is shown in Figure 16.

Multiple Regression

The equations for each tissue's concentrations are shown in Tables 8 and 9. Creatinine concentrations were a frequent parameter of the multiple regression equations. This parameter appeared in 11 of the 14 equations. Serum urea nitrogen concentrations, V_c, and albumin concentrations each contributed to 4 of the 14 equations. Packed cell volume, AUC, severity of disease score on day 0, severity of disease score on kill day, Cl_p, total protein concentrations, and $V_{d(\infty)}$ each appeared in 2 of the 14 equations. White blood cell count and k_{el} were each in one of the 14 multiple regression equations. The ln(time) was used in 2 of the equations which had the lowest AIC values in a particular tissue (renal cortex and renal medulla). Time was used in the equations with the lowest AIC for liver, normal lung (G1), normal appearing lung from G2, diseased lung, and skeletal muscle.

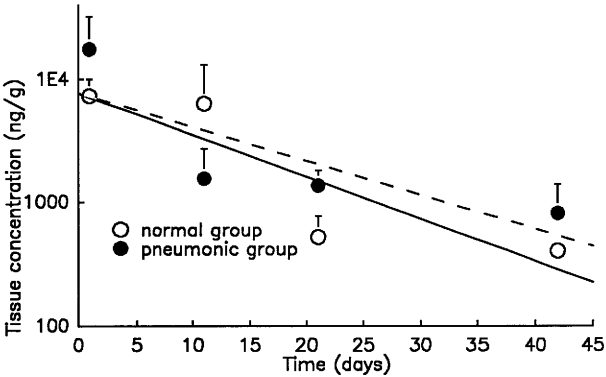

Figure 11. One-compartment model for renal medulla concentrations

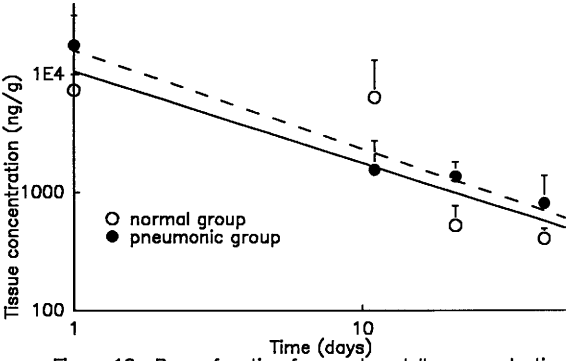

Figure 12. Power function for renal medulla concentrations

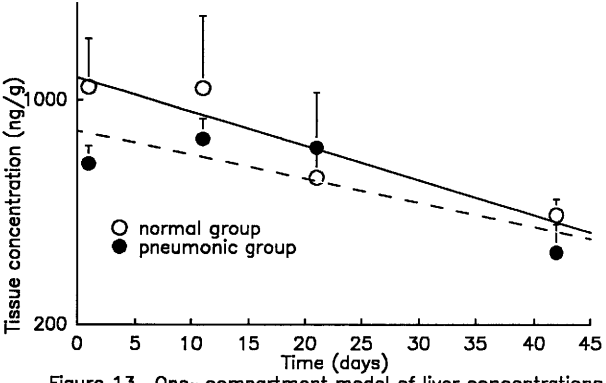

Figure 13. One-compartment model of liver concentrations

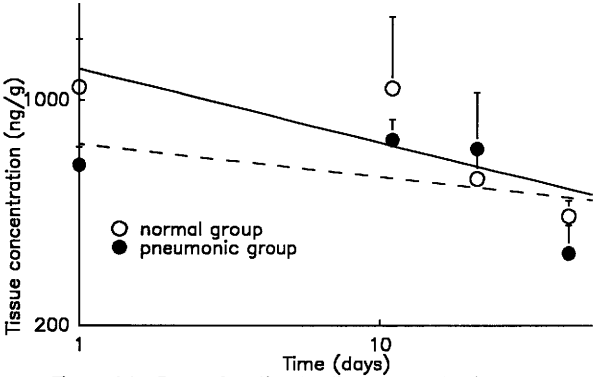

Figure 14. Power function for liver concentrations

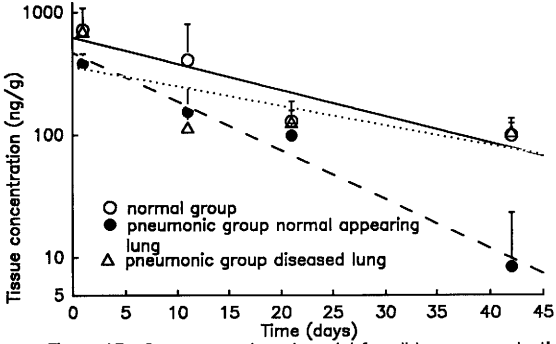

Figure 15. One-compartment model for all lung concentrations

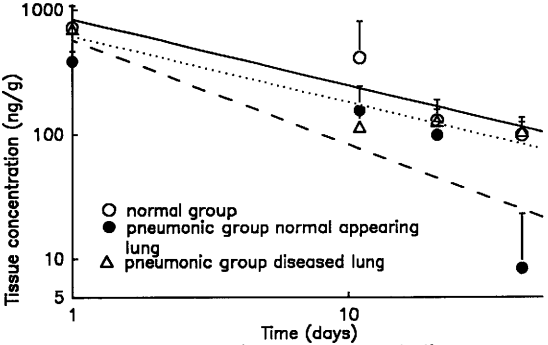

Figure 16. Power function for lung concentrations

Table 8. Multiple regression of ln(concentration) with time, severity of disease score, pharmacokinetic parameters, and clinical laboratory parameters for renal cortex, renal medulla, liver, and normal lung (from G1)

Equation: $\ln(\text{tissue concentration}) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_i x_i$

Tissue	Parameters *	P value	R^2	AIC value
Renal cortex	$\beta_1 = 8.96$	(P < 0.0001)	0.934	80.4
	$\beta_1 = -1.37$	(P < 0.0001)		
	$\beta_3 = 1.90$	(P = 0.0012)		
	$\beta_6 = 0.0380$	(P = 0.0814)		
	$\beta_0 = 13.2$	(P < 0.0001)	0.814	51.1
	$\beta_1 = -0.0819$	(P < 0.0001)		
	$\beta_4 = -0.131$	(P = 0.0491)		
	$\beta_5 = 0.00380$	(P = 0.1281)		
	$\beta_9 = -0.0112$	(P = 0.0100)		
Renal medulla	$\beta_0 = 4.69$	(P = 0.0035)	0.778	37.3
	$\beta_1 = -0.727$	(P < 0.0001)		
	$\beta_3 = 2.46$	(P = 0.0029)		
	$\beta_7 = 0.0146$	(P = 0.0282)		
	$\beta_0 = 11.2$	(P < 0.0001)	0.694	43.0
	$\beta_2 = -0.0377$	(P = 0.0053)		
	$\beta_4 = -0.147$	(P = 0.0093)		
	$\beta_9 = -0.00571$	(P = 0.0596)		
Liver	$\beta_0 = 6.12$	(P < 0.0001)	0.628	14.5
	$\beta_1 = -0.277$	(P = 0.0008)		
	$\beta_3 = 0.897$	(P = 0.0089)		
	$\beta_0 = 5.10$	(P < 0.0001)	0.814	4.04
	$\beta_2 = -0.0225$	(P < 0.0001)		
	$\beta_3 = 0.764$	(P = 0.0050)		
	$\beta_{12} = 0.315$	(P = 0.0851)		
Normal lung (G1)	$\beta_0 = 4.24$	(P = 0.0129)	0.837	6.273
	$\beta_1 = -0.438$	(P = 0.0266)		
	$\beta_3 = 1.75$	(P = 0.0577)		
	$\beta_0 = 8.88$	(P = 0.0020)	0.9999996	-87 6
	$\beta_2 = -0.360$	(P = 0.0020)		
	$\beta_3 = 0.832$	(P = 0.0052)		
	$\beta_5 = 0.00925$	(P = 0.0072)		
	$\beta_8 = -0.650$	(P = 0.0030)		
	$\beta_9 = -0.00464$	(P = 0 0020)		
	$\beta_{10} = -0.0000114$	(P = 0.0183)		
	$\beta_{11} = 2.35$	(P = 0.0020)		

* = abbreviations
β_0 = intercept (µg/g)
β_1 = ln(time) (days)
β_2 = time (days)
β_3 = creatinine concentrations (mg/dl)
β_4 = serum urea nitrogen concentrations (mg/dl)
β_5 = AUC (µg x hr/ml)
β_6 = severity of disease score on day animal was killed
$\beta_7 = Cl_t$ (ml/hr/kg)
β_8 = total protein concentration (g/dl)
$\beta_9 = V_c$ (ml/kg)
β_{10} = white blood cell count (#/µl)
$\beta_{11} = k_{el}$ (hr^{-1})
β_{12} = albumin concentration (g/dl)

Table 9. Multiple regression of ln(concentration) with time, severity of disease scores, pharmacokinetic parameters, and clinical laboratory parmeters for normal lung (from G2), diseased lung, and skeletal muscle

Equation: $\ln(\text{tissue concentration}) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_i x_i$

Tissue	Parameters *	P value	R^2	AIC Value
Normal lung (G2)	$\beta_0 = 5.88$	(P = 0.0236)	0.819	26.3
	$\beta_1 = -2.08$	(P = 0.0022)		
	$\beta_2 = -0.192$	(P = 0.0567)		
	$\beta_9 = 5.22$	(P = 0.0191)		
	$\beta_0 = 3.36$	(P = 0.0469)	0.857	22.2
	$\beta_2 = -0.130$	(P = 0.0004)		
	$\beta_9 = 3.00$	(P = 0.0460)		
Diseased lung	$\beta_0 = 3.79$	(P = 0.0068)	0.754	0.570
	$\beta_1 = -0.240$	(P = 0.0459)		
	$\beta_8 = 0.616$	(P = 0.0799)		
	$\beta_0 = 5.37$	(P = 0.0180)	0 999992	-80.1
	$\beta_2 = 0.0160$	(P = 0.0190)		
	$\beta_3 = -0.0305$	(P = 0.0103)		
	$\beta_4 = -0.122$	(P = 0.0142)		
	$\beta_5 = -0.00512$	(P = 0.0430)		
	$\beta_6 = 1.42$	(P = 0.0069)		
	$\beta_8 = -1.15$	(P = 0.0126)		
	$\beta_{11} = 0.000675$	(P = 0.1267)		
	$\beta_{12} = -0.0000294$	(P = 0.0445)		
Skeletal muscle	$\beta_0 = -1.45$	(P = 0.2959)	0.805	30.1
	$\beta_1 = -0.334$	(P = 0.0078)		
	$\beta_2 = 0.0878$	(P = 0.0006)		
	$\beta_8 = 1.29$	(P = 0.0050)		
	$\beta_9 = -1.51$	(P = 0.0064)		
	$\beta_0 = -0.850$	(P = 0.6231)	0 948	12.2
	$\beta_2 = -0.0119$	(P = 0.0559)		
	$\beta_3 = -0.0387$	(P = 0.0953)		
	$\beta_4 = -0.188$	(P < 0.0001)		
	$\beta_6 = 0.0937$	(P = 0.0003)		
	$\beta_8 = -1.18$	(P = 0.0303)		
	$\beta_{10} = 1.19$	(P = 0.0007)		
	$\beta_{12} = -0.000454$	(P = 0.0007)		

* = abbreviations
β_0 = intercept (µg/g)
β_1 = ln(time) (days)
β_2 = time (days)
β_3 = packed cell volume (%)
β_4 = serum urea nitrogen concentrations (mg/dl)
β_5 = severity of disease score on day 0
β_6 = severity of disease score on day animal was killed
$\beta_7 = Cl_s$ (ml/hr/kg)
β_8 = albumin concentration (mg/dl)
β_9 = creatinine concentration (mg/dl)
β_{10} = total protein concentration (mg/dl)
$\beta_{11} = V_c$ (ml/kg)
$\beta_{12} = V_{d(ss)}$ (ml/kg)

DISCUSSION AND CONCLUSIONS

Correlation of Severity of Disease Scores

The significant differences between severity of disease scores in the two groups on days 1, 11, 21, and 42 show that the scoring system could represent the severity of the induced pneumonia with a numerical value. This is supported by the significant correlation of fibrinogen and albumin concentrations, parameters which typically change with bronchopneumonia, with the severity of disease scores on day 0 in the two groups. Still, the system has minor problems that need to be rectified. The most important parameter that was not included in the scoring system was the hydration status of the animal. Two animals were documented to have been at least 10% dehydrated at the time of gentamicin administration, but their scores at that time were not any higher than the mean scores of the animals in the pneumonic group. The AUC and Cl, values for these animals were also much different from the mean of the normal group. With this information in mind, these two animals, #39 and #42, were viewed as a subgroup of the pneumonic calves. Because we could quantitate disease severity, correlation of severity of disease scores with the serum pharmacokinetic parameters and multiple regression were used to use the information maximally for prediction.

Serum Pharmacokinetics

The lack of significance of the serum pharmacokinetic parameters between the two groups, before #39 and #42 were removed, was due to the large variation in the pneumonic group. The standard deviations, in most cases, were 1 to 2 times the means of a given parameter. But, when #39 and #42 were excluded from the

pneumonic group, the variation greatly decreased in all but MRT, $V_{d(area)}$, and $V_{d(ss)}$. Serum clearance showed a significant increase from the normal group to the pneumonic group. An increase in Cl_t in the pneumonic group was also seen by Burrows *et al.* (1986a), but the increase was not significant. The increase of Cl_t in the current study may be due to the fact that the diseased animals were in a mild to moderate state of pneumonia without marked dehydration. Since gentamicin is eliminated primarily by glomerular filtration, an increase in renal blood flow, caused by an increase in heart rate, could explain an increase in Cl_t of gentamicin. The pneumonic group showed an increase in heart rate as a response to the pneumonia. Since animals #39 and #42 had Cl_t values that were approximately 21% of the normal group, increased renal blood flow probably did not take place. Rather, since these animals were severely dehydrated, their blood pressure may have been reduced, possibly causing a sympathetic vasoconstriction of the renal arteries leading to decreased renal blood flow. If small amounts of blood reach the kidney, then small amounts of gentamicin would be removed from the body (Chiu & Long, 1978).

The means of V_c (central fluid volume) in both groups (minus the two dehydrated heifers) was approximately 170 ml/kg, but it was 7.84 ml/kg and 24.1 ml/kg in the two dehydrated calves. Because V_c is inversely related to initial serum concentrations, the central fluid volume is directly affected by hydration state. If central fluid volume (which is approximately the serum volume) is greatly decreased, then drug may be forced because of the increased concentration gradient into areas outside of this volume, possibly the edible tissues.

The difference in AUC between the two groups was attributed to the increased clearance in the pneumonic group. If gentamicin is removed from a diseased animal at a higher rate than in a normal animal and total distribution volume does not change, then the AUC will be smaller. The large AUC of the two outlying animals was probably caused by their dehydration, which resulted in smaller distribution and smaller Cl_s.

The significant correlation of creatinine and serum urea nitrogen concentrations with Cl, supports a role of dehydration on the magnitude of that pharmacokinetic parameter. As creatinine concentrations increased, gentamicin Cl_s decreased. Serum urea nitrogen concentrations also would follow the same principle as creatinine in respect to its ability to predict gentamicin clearance. But, serum urea nitrogen is typically not as reliable an indicator as creatinine in ruminants due to the fact that urea nitrogen freely diffuses across the rumen (Lloyd, 1986).

Since no correlations were seen between severity of disease scores on day 0, 1, or the day the animal was killed with any of the serum pharmacokinetic values, some of the serum biochemical parameters or the hydration status of the animal may need to be included into the scoring system to give a more sensitive score of disease. Alternatively, the disease severity may truly be unrelated to the serum pharmacokinetic values.

Tissue Concentrations and Multiple Regression

Tissue gentamicin concentrations, ranked from highest to lowest were as follows: renal cortex, renal medulla, liver, lung, skeletal muscle, and perirenal fat. The rank of the various tissues from highest to lowest is similar to that reported

in sheep by Brown *et al.* (1986a) and in cattle (Haddad *et al.*, 1987). There was a large variance in all but one of the tissue concentrations. Overall, it appeared that the normal group had higher tissue concentrations. The variability of the pneumonia between animals in the pneumonic group may have played a role in the high variability of tissue concentrations within each tissue and day. However, disease severity scores should account for those changes.

Based on AIC values of the pharmacokinetic models, the best fit of the renal cortex concentrations in normal animals was the power function, where as in the pneumonic animals, a one-compartment model was the most appropriate. Renal medulla concentrations were best expressed using a one-compartment model in both groups, but the AIC values of the different models were nearly identical. Liver concentrations also favored the one-compartment model, but again, the AIC values were similar. Lung concentrations were modeled using the power function for the normal group and diseased lung in the pneumonic group, but a one-compartment model best fit the normal appearing lung tissue in the pneumonic group. The AIC values for each model, with respect to group, were very close to one another in all of the tissues.

The fact that some tissues were best fit using one model in normal animals and another model in pneumonic animals has great implications. Withdrawal times for a drug are determined in healthy animals and the tissue concentrations are predicted by a given model. If the drug is given to diseased animals whose tissue depletion follows a different model, then there is a likelihood that the withdrawal time predicted in diseased animals would be incorrect. Simple alteration of the

magnitude of the parameters of a model would not allow for appropriate prediction of tissue residues in food-producing animals.

A main goal of this study was to find a way to predict when the concentration of a xenobiotic in a given tissue will decrease below the tolerance limit in pneumonic calves. Multiple regression incorporated several different parameters (creatinine, serum urea nitrogen, severity of disease score on day 0, V_d etc.) that affected tissue concentration (in addition to time), into an equation that more accurately predicted tissue concentration (Tables 8 & 9). Renal cortical concentrations were best predicted when ln(time) was used (analogous to the power function) instead of time. Renal medulla concentrations were best predicted by a one-compartment model in both groups, but when other parameters such as creatinine concentration and Cl, were added to the equation, ln(time) (power function) was a better choice to predict these concentrations. Concentrations in liver, normal lung, and diseased lung were better predicted using a multiple regression equation which utilized time instead of the ln(time), implying first-order elimination from those tissues.

Conclusions

The fact that tissue concentrations can be predicted using a combination of factors is an important achievement. A large number of different parameters were initially included in the multiple regression equation, but only those which significantly contributed to the predictability of the final equation were included. This model could be used by a veterinarian in the field to predict if the drug concentrations in the edible tissues are below the tolerance limit of a drug administered to a diseased animal before the animal is slaughtered. In the case of

renal cortex concentrations, the veterinarian would determine ln(number of days since the drug was given), obtain a blood sample to measure the serum creatinine concentrations on the day the drug was given, and evaluate a severity of disease score the day the animal was killed. An estimate of the renal cortex concentration would be obtained from the multiple regression equation. The advantage is that all of the values needed, except one, can be obtained when the animal receives the drug. No extra time or manpower is needed to take multiple blood samples over time or to get the animal out of the feedlot every day. Finally, this approach may lead to a better understanding of the pathophysiologic changes that alter the depletion of drugs from animal tissues.

Possible Changes in this Study

Initial problems were seen with the model of pneumonia. Too many bacteria in the logarithmic phase of growth caused very quick deaths in the first two calves in which BP induction was attempted. These two calves did not live long enough for gentamicin to be administered. The brain-heart infusion media used in the first two calves may have also contributed the initial deaths. When the media was changed to trypticase soy broth, these acute deaths ceased. Also, the ability to induce a consistent, moderate degree of pneumonia may have helped to decrease the degree of variability seen in the tissue concentrations of both groups and in the serum pharmacokinetic parameters of the pneumonic group.

The scoring system should have taken into account the variability of the extent of BP and yielded a score that represented the extent of disease and not just the fact that the animal was sick. Possible changes to the scoring system include adding hydration state (which includes evaluating skin tint, mucous membranes, and

appearance of ocular area). Auscultation of each lung should be scored separately. Temperature should also be weighted more heavily in the scoring system. A selection for a markedly lowered temperature should be added which as a higher point value than an elevated temperature.

The last two animals that had BP induced probably had some form of resistance to *P. haemolytica*. The concentrations of bacteria given to them were within the range given to the other calves, but pneumonia was not easily induced in them. An antibody titer for *P. haemolytica* or a nasal swab culture should be done on each calf to determine the degree of resistance of each calf. Bacterial cultures should also be done to show that the pneumonia induced was strictly due to *P. haemolytica* and not another pathogen.

To achieve a power of 75%, it would take approximately 14,000 animals in each group based on the variation observed and the desire to detect a 10% difference between the means of the pharmacokinetic values and tissue concentrations seen in the two groups. However, the current expense of cattle and possibility of undue suffering on a large population of animals should be considered.

REFERENCES

Ames, T.R., Larson, V.L., & Stowe, C.M. (1983) Oxytetracycline concentrations in healthy and diseased calves. *American Journal of Veterinary Research*, 44, 1354-1357.

Ames, T.R. & Patterson, E.B. (1985) Oxytetracycline concentrations in plasma and lung of healthy and pneumonic calves, using two oxytetracycline preparations. *American Journal of Veterinary Research*, 46, 2471-2473.

Baggot, J.D. (1980) Distribution of antimicrobial agents in normal and diseased animals. *Journal of the American Veterinary Medical Association*, 176, 1085-1090.

Benet, L.Z., Massoud, N., & Gambertoglio, J.G. (1984) *Pharmacokinetic Basis for Drug Treatment*, Raven Press, New York.

Bergeron, M.G., Trottier, S., Lessard, C., Beauchamp, D., & Gagnon, P.M. (1982) Disturbed intrarenal distribution of gentamicin in experimental pyelonephritis due to *Escherichia coli*. *Journal of Infectious Diseases*, 146, 436-439.

Booth, N.H. (1988) Drug and chemical residues in the edible tissues of animals. In *Veterinary Pharmacology and Therapeutics*, Eds. Booth, N.H. and McDonald, L.E., 6th edn., pp. 1149-1205, Iowa State University Press, Ames.

Braun, W.H. & Waechter, J.M. (1983) Sources of uncertainty in pharmacokinetic prediction. *Journal of Animal Science*, 56, 235-243.

Brown, S.A. (1988) Treatment of gram-negative infections. *Veterinary Clinics of North America: Small Animal Practice*, 18, 1141-1165.

Brown, S.A. & Baird, A.N. (1988) Evaluation of renal gentamicin depletion kinetic properties in sheep, using serial percutaneous biopsies. *American Journal of Veterinary Research*, 49, 2056-2059.

Brown, S.A., Coppoc, G.L., & Riviere, J.E. (1986a) Effects of dose and duration of therapy on gentamicin tissue residues in sheep. *American Journal of Veterinary Research*, 47, 2373-2379.

Brown, S.A., Riviere, J.E., Coppoc, G.L., & Dix, L.P. (1986b) Superiority of the power function over exponential functions for prediction of renal gentamicin residues in sheep. *Journal of Veterinary Pharmacology and Therapeutics*, 9, 341-346.

Brown, S.A., Riviere, J.E., Coppoc, G.L., Hinsman, E.J., Carlton, W.W., & Steckel, R.R. (1985) Single intravenous and multiple intramuscular dose

pharmacokinetics and tissue residue profile of gentamicin. *American Journal of Veterinary Research*, 46, 69-74.

Brown, S.A., Sugimoto, K., Smith, G.G., & Garry, F.B. (1988) Improved sodium hydroxide digestion method without homogenization for extraction of gentamicin from renal tissue. *Antimicrobial Agents and Chemotherapy*, 32, 595-597.

Burrows, G.E. (1979) Gentamicin. *Journal of the American Veterinary Medical Association*, 175, 301-302.

Burrows, G.E. (1985) Effects of experimentally induced *Pasteurella haemolytica* pneumonia on the pharmacokinetics of erythromycin in the calf. *American Journal of Veterinary Research*, 46, 798-803.

Burrows, G.E., Barto, P.B., & Martin, B. (1986a) Antibiotic disposition in experimental pneumonic pasteurellosis: gentamicin and tylosin. *Canadian Journal of Veterinary Research*, 50, 193-199.

Burrows, G.E., Barto, P.B., & Martin, B. (1987) Comparative pharmacokinetics of gentamicin, neomycin and oxytetracycline in newborn calves. *Journal of Veterinary Pharmacology and Therapeutics*, 10, 54-63.

Burrows, G.E., Barto, P.B., & Weeks, B.R. (1986b) Chloramphenicol, lincomycin and oxytetracycline disposition in calves with experimental pneumonic pasteurellosis. *Journal of Veterinary Pharmacology and Therapeutics*, 9, 213-222.

Cheng, A.F., Lam, A.W., & French, G.L. (1987) Comparative evaluation of the Abbott TDx, the Abbott ABA200, and Syva LAB5000 for assay of serum gentamicin. *Journal of Antimicrobial Chemotherapy*, 19, 127-133.

Chiu, P.J.S. & Long, J.F. (1978) Effects of hydration on gentamicin excretion and renal accumulation in furosemide-treated rats. *Antimicrobial Agents and Chemotherapy*, 14, 214-217.

Clarke, C.R., Short, C.R., Hsu, R.-C., & Baggot, J.D. (1985) Pharmacokinetics of gentamicin in the calf: developmental changes. *American Journal of Veterinary Research*, 46, 2461-2466.

Cole, N.A., Camp, T.H., Rowe, L.D., Stevens, D.G., & Hutcheson, D.P. (1988) Effect of transport on feeder calves. *American Journal of Veterinary Research*, 49, 178-183.

Curl, J.L. & Curl, J.S. (1988) Pharmacokinetics of gentamicin in laboratory rabbits. *American Journal of Veterinary Research*, 49, 2065-2067.

Dittert, L.W. (1977) Pharmacokinetic prediction of tissue residues. *Journal of Toxicology and Environmental Health*, 2, 735-756.

Engel, R.E. (1988) Quality assurance through residue screening. *Journal of the American Veterinary Medical Association*, 192, 268-270.

Farrington, D.O., Jackson, J.A., Bentley, O.E., & Barnes, H.J. (1987) Efficacy of sulbactam-ampicillin in an induced *Pasteurella haemolytica* pneumonia model in calves. *American Journal of Veterinary Research*, 48, 1684-1688.

Frank, G.H. & Smith, P.C. (1983) Prevalence of *Pasteurella haemolytica* in transported calves. *American Journal of Veterinary Research*, 44, 981-985.

Gibaldi, M. & Perrier, P. (1982) *Pharmacokinetics*, 2nd edn., Marcel Dekker, Inc., New York.

Gibbs, H.A., Allan, E.M., Wiseman, A., & Selman, I.E. (1984) Experimental production of bovine pneumonic pasteurellosis. *Research in Veterinary Science*, 37, 154-166.

Haddad, N.S., Pedersoli, W.M., Carson, R.L., & Ravis, W.R. (1986) Concentrations of gentamicin in serum, milk, urine, endometrium, and skeletal muscle of cows after repeated intrauterine injections. *American Journal of Veterinary Research*, 47, 1597-1601.

Haddad, N.S., Ravis, W.R., Pedersoli, W.M., & Carson, R.L. (1987) Pharmacokinetics and tissue residues of gentamicin in lactating cows after multiple intramuscular doses are administered. *American Journal of Veterinary Research*, 48, 21-27.

Halkin, H., Lidji, M., & Rubinstein, E. (1981) The influence of endotoxin-induced pyrexia on the pharmacokinetics of gentamicin in the rabbit. *Journal of Pharmacology and Experimental Therapeutics*, 216, 415-418.

Jensen, R., Pierson, R.E., Braddy, P.M., Saari, D.A., Lauerman, L.H., England, J.J., Keyvanfar, H., Collier, J.R., Horton, D.P., McChesney, A.E., Benitez, A., & Christie, R.M. (1976) Shipping fever pneumonia in yearling feedlot cattle. *Journal of American Veterinary Medical Association*, 169, 500-506.

Jericho, K.W.F. & Langford, E.V. (1978) Pneumonia in calves with aerosols of bovine herpesvirus 1 and *Pasteurella haemolytica*. *Canadian Journal of Comparative Medicine*, 42, 269-277.

Jernigan, A.D., Hatch, R.C., Wilson, R.C., Brown, J., & Crowell, W.A. (1988a) Pathologic changes and tissue gentamicin concentrations after intravenous gentamicin administration to clinically normal and endotoxemic cats. *American Journal of Veterinary Research*, 49, 613-617.

Jernigan, A.D., Hatch, R.C., Wilson, R.C., Brown, J., & Tuler, S.M. (1988b) Pharmacokinetics of gentamicin in cats given *Escherichia coli* endotoxin. *American Journal of Veterinary Research*, 49, 603-607.

Jolley, M.E., Stroupe, S.D., Wang, C.-H.J., Panas, H.N., Keegan, C.L., Schmidt, R.L., & Schwenzer, K.S. (1981) Fluorescence polarization immunoassay I. monitoring aminoglycoside antibiotics in serum and plasma. *Clinical Chemistry*, 27, 1190-1197.

Kaplan, J.M., McCracken, G.H., & Snyder, E. (1973) Influence of methodology upon apparent concentrations of antibiotics in tissue. *Antimicrobial Agents and Chemotherapy*, 3, 143-146.

Lloyd, W.E. (1986) Urea and other nonprotein nitrogen sources. In *Current Veterinary Therapy: Food Animal Practice 2*, Ed. Howard, J.L., pp. 354-356, W.B. Saunders Company, Philadelphia.

Knaus, W.A., Draper, E.A., Wagner, D.P., & Zimmerman, J.E. (1985) APACHE II: a severity of disease classification system. *Critical Care Medicine*, 13, 818-829.

Marteniuk, J.V., Ahl, S.A., & Bartlett, P.C. (1988) Compliance with recommended drug withdrawal requirements for dairy cows sent to market in Michigan. *Journal of the American Veterinary Medical Association*, 193, 404-407.

Mercer, H.D., Baggot, J.D., & Sams, R.A. (1977) Application of pharmacokinetic methods to the drug residue profile. *Journal of Toxicology and Environmental Health*, 2, 787-801.

Panciera, R.J. & Corstvet, R.E. (1984) Bovine pneumonic pasteurellosis: model for *Pasteurella haemolytica*- and *Pasteurella multocida*-induced pneumonia in cattle. *American Journal of Veterinary Research*, 45, 2532-2537.

Riond, J.-L. & Riviere, J.E. (1988) Multiple intravenous dose pharmacokinetics and residue depletion profile of gentamicin in pigs. *Journal of Veterinary Pharmacology and Therapeutics*, 11, 210-214.

Sastrasinh, M., Knauss, T.C., Weinberg, J.M., & Humes, H.D. (1982) Identification of the aminoglycoside binding site in rat renal brush border membranes. *Journal of Pharmacology and Experimental Therapeutics*, 222, 350-358.

Setser, M.D. (1985) Pharmacokinetics of gentamicin in channel catfish (*Ictalurus punctatus*). *American Journal of Veterinary Research*, 46, 2558-2561.

Slocombe, R.F., Derksen, F.J., Robinson, N.E., Trapp, A., Gupta, A., & Newman, J.P. (1984) Interactions of cold stress and *Pasteurella haemolytica* in the pathogenesis of pneumonic pasteurellosis in calves: method of induction and

hematologic and pathologic changes. *American Journal of Veterinary Research*, 45, 1757-1763.

Sundlof, S.F., Riviere, J.E., & Craigmill, A.L. (1988) *Food Animal Residue Avoidance Databank Trade Name File*, pp. 147-150, University of Florida, Gainesville.

Takahashi, Y., Kido, Y., Naoi, M., & Kokue, E. (1985) The serial biopsy technique for estimation of drug residue in calf kidney using gentamicin as a model drug. *Japanese Journal of Veterinary Science*, 47, 179-183.

TerHune, T.N. & Upson, D.W. (1989) Oxytetracycline pharmacokinetics, tissue depletion, and toxicity after administration of a long-acting preparation at double the label dosage. *Journal of the American Veterinary Medical Association*, 194, 911-917.

Van Houweling, C.D. (1981) The magnitude of the sulfa residue problem. *Journal of the American Veterinary Medical Association*, 178, 464-466.

Weber, N.E. (1983) Metabolism and kinetics in the regulation of animal drugs. *Journal of Animal Science*, 56, 244-251.

Wilson, R.C., Goetsch, D.D., & Huber, T.L. (1984) Influence of endotoxin-induced fever on the pharmacokinetics of gentamicin in ewes. *American Journal of Veterinary Research*, 45, 2495-2497.

Wilson, R.C., Moore, J.N., & Eakle, N. (1983) Gentamicin pharmacokinetics in horses given small doses of *Escherichia coli* endotoxin. *American Journal of Veterinary Research*, 44, 1746-1749.

Wilson, R.C., Whelan, S.C., Coulter, D.B., Mahaffey, E.A., Mahaffey, M.B., & Huber, T.L. (1981) Kinetics of gentamicin after intravenous, intramuscular, and intravenous administration in sheep. *American Journal of Veterinary Research*, 42, 1901-1904.

Yamaoka, K., Nakagawa, T., & Uno, T. (1978) Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. *Journal of Pharmacokinetics and Biopharmaceutics*, 6, 165-176.

Ziv, G., Nouws, J.F.M., & Van Ginneken, C.A.M. (1982) The pharmacokinetics and tissue levels of polymyxin B, colistin and gentamicin in calves. *Journal of Veterinary Pharmacology and Therapeutics*, 5, 45-58.

APPENDIX 1

Serum Standard Curve

Concentration (ng/ml)	Average Polarization	Predicted Polarization
0.00	187.13	187.13
500.00	177.36	178.59
1500.00	152.72	151.30
3000.00	117.88	118.77
6000.00	87.79	87.52
10000.00	72.45	72.51

Kidney Tissue Standard Curve

Concentration (ng/g)	Average Polarization	Predicted Polarization
0.00	184.86	184.86
100.00	171.03	171.96
250.00	150.22	149.32
750.00	102.55	102.92
1500.00	77.36	77.37
3000.00	62.23	62.14

Skeletal Muscle Standard Curve

Concentration (ng/g)	Average Polarization	Predicted Polarization
0.00	185.06	185.06
100.00	171.08	170.25
250.00	144.51	144.92
750.00	96.83	97.33
1500.00	74.86	73.73
3000.00	59.87	60.56

Heart Tissue Standard Curve

Concentration (ng/g)	Average Polarization	Predicted Polarization
0.00	187.67	187.67
100.00	174.67	172.34
250.00	150.17	149.31
750.00	99.29	105.81
1500.00	88.27	81.85
3000.00	61.94	66.82

Liver Standard Curve

Concentration (ng/g)	Average Polarization	Predicted Polarization
0.00	185.04	185.04
100.00	166.28	164.71
250.00	140.76	142.59
750.00	103.88	102.46
1500.00	76.12	76.78
3000.00	56.80	56.78

Lung Tissue Standard Curve

Concentration (ng/g)	Average Polarization	Predicted Polarization
0.00	194.78	194.78
100.00	183.08	180.72
250.00	156.85	158.39
750.00	111.21	110.84
1500.00	81.45	80.90
3000.00	59.85	60.41

Fat Tissue Standard Curve

Concentration (ng/g)	Average Polarization	Predicted Polarization
0.00	178.53	178.53
100.00	158.72	156.76
250.00	126.06	128.20
750.00	87.63	85.77
1500.00	66.31	67.33
3000.00	57.17	57.19

APPENDIX 2

Serum Pharmacokinetic Equations *

C_0 =	A	(one compartment model)
	A + B	(two compartment model)
	A + B + C	(three compartment model)
AUC =	A/α	(one compartment model)
	$A/\alpha + B/\beta$	(two compartment model)
	$A/\alpha + B/\beta + C/\gamma$	(three compartment model)
AUMC =	A/α^2	(one compartment model)
	$A/\alpha^2 + B/\beta^2$	(two compartment model)
	$A/\alpha^2 + B/\beta^2 + C/\gamma^2$	(three compartment model)
MRT =	AUMC/AUC	
V_c =	$dose/C_0$	
$V_{d(area)}$ =	$dose/(AUC * \alpha)$	(one compartment model)
	$dose/(AUC * \beta)$	(two compartment model)
	$dose/(AUC * \gamma)$	(three compartment model)
$V_{d(ss)}$ =	$(dose * AUMC)/AUC^2$	
Cl_s =	dose/AUC	
k_{el} =	AUC/AUMC	
$t_{1/2\alpha}$ =	$0.693/\alpha$	
$t_{1/2\beta}$ =	$0.693/\beta$	
$t_{1/2\gamma}$ =	$0.693/\gamma$	
% AUC α =	$((A/\alpha)/AUC) * 100$	
% AUC β =	$((B/\beta)/AUC) * 100$	
% AUC γ =	$((C/\gamma)/AUC) * 100$	

* see next page for abbreviations

C_0 = concentration at time zero
AUC = area under the concentration vs. time curve
AUMC = area under the first moment curve
MRT = mean residence time
V_c = central fluid volume
$V_{d(area)}$ = area-derived volume of distribution
$V_{d(ss)}$ = volume of distribution at steady state
Cl_s = serum clearance
k_{el} = elimination rate constant
$t_{1/2}$ = half-life
% AUC = percentage of each compartment's contribution to AUC

APPENDIX 3

Normal Values in Adult Cattle for Serum Biochemical Panel and CBC

Parameter	Units	Range
Albumin	g/dl	3.3 - 4.1
Alkaline phosphatase	U/l	0 - 127
Aspartate aminotransferase	U/l	30 - 94
Calcium	mg/dl	8.0 - 10.0
Creatine phosphokinase	U/l	0 - 165
Creatinine	mg/dl	0.2 - 2.2
γ-glutamyltransferase	U/l	0 - 100
Glucose	mg/dl	30 - 106
Phosphorous	mg/dl	4.6 - 8.6
Total bilirubin	mg/dl	0.3 - 0.7
Total protein	g/dl	5.8 - 8.2
Urea nitrogen	mg/dl	10 - 26
Band neutrophils	#/μl	0 - 120
Basophils	#/μl	0 - 200
Eosinophils	#/μl	0 - 2400
Fibrinogen	mg/dl	300 - 700
Hemoglobin	g/dl	8 - 15
Lymphocytes	#/μl	2500 - 7500
Monocytes	#/μl	25 - 840
Packed cell volume	%	24 - 46
Plasma protein	g/dl	6.5 - 8.5
Red blood cells	#/μl	5.0×10^6 - 10×10^6
Segmented neutrophils	#/μl	600 - 4000
White blood cells	#/μl	4×10^3 - 12×10^3

APPENDIX 4

Serum Biochemical Data

Indices	Group	Before BP Mean ± SD	After BP Mean ± SD
Albumin (g/dl)	G1	3.15 ± 0.25	3.15 ± 0.24
	G2	3.08 ± 0.25	2.71 ± 3.32
Alkaline phosphatase (U/l)	G1	133 ± 52.7	138 ± 57.3
	G2	99.8 ± 34.5	77.8 ± 25.3
Aspartate aminotransferase (U/l)	G1	72.9 ± 13.2	90.9 ± 23.0
	G2	94.0 ± 53.4	147 ± 224
Calcium (mg/dl)	G1	10.7 ± 0.62	10.6 ± 0.27
	G2	9.91 ± 0.44	8.96 ± 0.82
Creatine phosphokinase (U/l)	G1	157 ± 51.8	361 ± 363
	G2	169 ± 132	$1.40 \times 10^3 \pm 4.66 \times 10^3$
Creatinine (mg/dl)	G1	1.34 ± 0.18	1.20 ± 0.27
	G2	1.34 ± 0.31	1.33 ± 0.82
γ-glutamyltransferase (U/l)	G1	35.8 ± 3.76	34.1 ± 3.53
	G2	36.1 ± 6.62	33.8 ± 3.14
Glucose (mg/dl)	G1	80.3 ± 8.44	74.5 ± 22.6
	G2	79.6 ± 13.8	54.8 ± 18.7
Phosphorous (mg/dl)	G1	6.62 ± 0.79	5.70 ± 0.74
	G2	6.66 ± 1.42	5.46 ± 3.07
Total bilirubin (mg/dl)	G1	0.25 ± 0.08	0.22 ± 0.12
	G2	0.45 ± 0.25	0.59 ± 0.32
Total plasma protein (g/dl)	G1	6.24 ± 0.23	6.10 ± 0.23
	G2	6.39 ± 0.40	5.75 ± 0.48
Urea nitrogen (mg/dl)	G1	15.6 ± 2.54	17.0 ± 3.63
	G2	14.6 ± 3.41	17.5 ± 7.93

APPENDIX 5

CBC Data

Indices	Group	Before BP Mean ± SD	After BP Mean ± SD
Band neutrophils (#/μl)	G1	0.00 ± 0.00	0.00 ± 0.00
	G2	0.00 ± 0.00	29.5 ± 66.4
Basophils (#/μl)	G1	48.1 ± 108	10.8 ± 34.2
	G2	0.00 ± 0.00	9.85 ± 24.2
Eosinophils (#/μl)	G1	94.7 ± 173	37.1 ± 66.9
	G2	30.4 ± 70.3	23.6 ± 43.0
Fibrinogen (mg/dl)	G1	445 ± 202	490 ± 129
	G2	607 ± 289	692 ± 250
Hemoglobin (g/dl)	G1	14.7 ± 1.73	14.0 ± 1.80
	G2	13.7 ± 2.01	12.3 ± 1.59
Lymphocytes (#/μl)	G1	$7.34 \times 10^3 \pm 2.44 \times 10^3$	$6.31 \times 10^3 \pm 2.10 \times 10^3$
	G2	$5.59 \times 10^3 \pm 1.60 \times 10^3$	$4.16 \times 10^3 \pm 1.44 \times 10^3$
Monocytes (#/μl)	G1	402 ± 219	602 ± 572
	G2	393 ± 247	231 ± 218
Packed cell volume (%)	G1	42.3 ± 2.72	39.4 ± 3.92
	G2	39.4 ± 5.58	35.5 ± 4.94
Plasma protein (g/dl)	G1	7.17 ± 0.40	7.11 ± 0.25
	G2	7.65 ± 0.56	6.92 ± 0.61
Red blood cells (#/μl)	G1	$9.56 \times 10^6 \pm 1.09 \times 10^6$	$9.31 \times 10^6 \pm 1.91 \times 10^5$
	G2	$8.95 \times 10^6 \pm 6.15 \times 10^5$	$7.73 \times 10^6 \pm 3.32 \times 10^6$
Segmented neutrophils (#/μl)	G1	$1.42 \times 10^3 \pm 876$	$1.90 \times 10^3 \pm 1.56 \times 10^3$
	G2	$1.64 \times 10^3 \pm 1.05 \times 10^3$	$1.98 \times 10^3 \pm 1.50 \times 10^3$
White blood cells (#/μl)	G1	$9.31 \times 10^3 \pm 2.67 \times 10^3$	$8.89 \times 10^3 \pm 2.63 \times 10^3$
	G2	$7.68 \times 10^3 \pm 2.22 \times 10^3$	$6.45 \times 10^3 \pm 2.50 \times 10^3$

VITA

Robert Paul Hunter, the son of Ray and Hazel Hunter, was born October 30, 1964 in Phoenix, Arizona. He graduated from high school in 1983 from L.D. Bell High School in Hurst, Texas. His undergraduate education included one year at Texas Wesleyan College in Fort Worth, Texas, two summers at Tarrant County Junior College, and the remaining three years at Angelo State University, where he received a Bachelor of Science degree in Animal Science on May 15, 1987.

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