THE PHARMACOKINETICS OF GENTAMICIN IN CHANNEL CATFISH (*ICTALURUS PUNCTATUS*)

By

MARK DUANE SETSER

Bachelor of Science
Oklahoma State University
Stillwater, Oklahoma
1977

Doctor of Veterinary Medicine
Oklahoma State University
Stillwater, Oklahoma
1982

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 1983

THE PHARMACOKINETICS OF GENTAMICIN IN CHANNEL CATFISH (*ICTALURUS PUNCTATUS*)

Thesis Approved:

Lester L. Rolf Jr.
Thesis Adviser

James E. Breazile

George E. Burrows

Norman N. Durham
Dean of the Graduate College

PREFACE

This study uses the channel catfish as a poikilothermic model with which the pharmacokinetics of the aminoglycoside antibiotic, gentamicin, could be studied. The primary objectives of this study are to establish reliable pharmacokinetic parameters on which a rational course of antibiotic therapy can be based and to determine the effectiveness and reliability of selected enteral and parenteral routes of administration.

The author wishes first and foremost to express his deepest gratitude to Dr. Lester L. Rolf. Without his incredibly patient and generous support, this thesis could never have been completed. Thanks are also extended to Dr. George Burrows for his counsel and financial support, to Dr. James Blankemeyer for many late hours of computer programming work, to Dr. Bruce Lessley and Dr. James Breazile for their assistance in ironing out those nasty little academic quandries that tend to crop up with investigations into new areas of study, to Ms. LaVerne Jones for her indispensable assistance with the literature search, to Ms. Mary Bober and Dr. Jim Walker for the contribution of important journal articles and to Dr. Subbiah Sangiah for his advice and moral support.

Finally, special gratitude is expressed to my wife/typist/friend, Vicki, without whom it would have been most difficult to emerge from this experience employed, sane and mentally lucid.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

NOMENCLATURE

A	y intercept of alpha phase regression line; (μg/ml)
A_C	drug level in the central compartment of a two-compartment open model at the end of the interval τ; (μg/ml)
A_O	intracardiac bolus dose (mg/kg)
A_T	drug level in the peripheral compartment of a two-compartment open model at the end of the interval τ (μg/ml)
AUC	area under an elimination phase regression line; $\int_0^\infty C_p{}^t dt$
α	negative value of the slope of the alpha phase regression line (min^{-1})
$\alpha+\beta$ region	area under a plasma decay curve that includes both distribution and elimination phases
B	y intercept of beta phase regression line (μg/ml)
b	y intercept of a linear equation regression line
B_O	gamma emission count per minute for a 0 μg/ml standard (cpm)
$\%B/B_O$	percentage ^{125}I-labeled gentamicin bound to RIA antibody
$\%B/B_O$ of Y_{xo}	$\%B/B_O$ of a human serum standard regression line y-intercept
$\%B/B_O$ of Y_{xs}	$\bar{X}$ cpm of a catfish plasma sample divided by $\bar{x}$ cpm of a corresponding 0 μg/ml catfish plasma sample
β	negative value of the slope of the beta phase regression line (min^{-1})

β region	area under a plasma decay curve that includes only the elimination phase
C_p	calcuated plasma drug concentration at a given $\%B/B_o$ (μg/ml)
C_p^t	calculated plasma drug concentration at a given time (μg/ml)
C_p^{t*}	C_p^t calculated from one-compartment open model values (μg/ml)
C_p^o	theoretical C_p^t at time zero; y intercept of $\alpha+\beta$ regression line (μg/ml)
$C_{p(max)}^{\infty}$	maximum safe plasma drug concentration (μg/ml); maximum allowable peak plasma drug concentration
$C_{p(min)}^{\infty}$	minimum effective plasma drug concentration (μg/ml); maximum allowable trough plasma drug concentration
Cl_B	the rate at which a drug is eliminated from the central compartment (ml/min/kg)
D_i	loading dose calculated from two-compartment open model parameters (mg/kg)
D_i^*	loading dose calculated from one-compartment open model parameters (mg/kg)
D_m	maintenance dose calculated from two-compartment open model parameters (mg/kg)
D_m^*	maintenance dose calculated from one-compartment open model parameters (mg/kg)
e	the base of Naperian, or Hyperbolic, or Natural logarithms; 2.71828...
$E_*C_\#$	experimental subject identification; E_* = experiment no.*; $C_\#$ = catfish no. #
F	fraction of intramuscular dose reaching systemic circulation intact; an estimate of bioavailability
g	force due to gravity at sea level; $(6.673 \pm 0.003) \times 10^{-8}$ $(cm^3)(sec^2)/gm$
IM	intramuscular

K_{el}	elimination rate constant (min^{-1})
K_{12}	rate constant for the movement of drug from the central to the peripheral compartment in a two-compartment open model (min^{-1})
K_{21}	rate constant for the movement of drug from the peripheral to the central compartment in a two-compartment open model (min^{-1})
m	slope of a linear equation regression line
$\bar{m}$	mean value of a sample of population m's
m_c	slope of the unfeathered $\alpha+\beta$ regression line
n	number of items in a data set
pgc	plasma gentamicin concentration (μg/ml)
r	correlation coefficient; the square root of r^2
RIA	radioimmunoassay
Σ	symbol for sum the following
s	standard deviation of a sample
$s_{\bar{d}}$	standard deviation of the mean difference
s^2	variance of a sample
$s_{\bar{m}}^2$	the variance of the mean slope
$S_{Y_{xo}}^2$	the variance of the mean y intercept
t	time; Student's t value
t'	t value distributed as Student's t only when $n_1 = n_2$
$t_{\frac{1}{2}(\alpha)}$	the time required to reduce a given concentration to half its value by distribution (α) phase kinetics
$t_{\frac{1}{2}(\beta)}$	the time required to reduce a given concentration to half its value by elimination phase kinetics in a two-compartment open model

$t_{\frac{1}{2}(\beta)}^{*}$	the time required to reduce a given concentration to half its value by elimination phase kinetics in a one-compartment open model
τ	the dosage interval required to prevent trough plasma drug levels from exceeding $C_{p(min)}^{\infty}$ for a two-compartment open model
τ^{*}	τ as applied to the intramuscular dosage study using a one-compartment open model
V_{c}	apparent volume of the central compartment (ml/kg)
$V_{d(area)}$	apparent volume of distribution for a two-compartment open model (ml/kg)
$V_{d(B)}$	apparent volume of distribution for a one-compartment open model (ml/kg) as applied to the intracardiac bolus study
$V_{d(B)}^{IM}$	$V_{d(B)}$ as applied to the intramuscular dosage study
$\bar{x}$ cpm	average gamma emission counts per minute
$\bar{X}_{yo}$	mean x-intercept for a group of regression line x-intercepts
Y_{xo}	y-intercept of a regression line
$\bar{Y}_{xo}$	mean y-intercept for a group of Y_{xo}'s

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Antibiotics in Teleosts

The United States Food and Drug Administration has approved only salt, acetic acid and sulfamerazine for use in all food fishes and oxytetracycline for restricted use in trout, salmon and catfish.[1] No antibiotics are specifically marketed for fish species not intended for human consumption, but regulation of antibiotic use in these fishes is not widespread.[1,2] Sulfonamides, tetracyclines, chloramphenicol, erythromycin and various aminoglycosides have been recommended as antibiotics of value in the treatment of tank water for non-food fishes.[2,3] In the absence of controlled experimental data on the pharmacology of antibiotics in fish, case study and personal experience have frequently been the basis for drug therapy. It has recently been shown that some antibiotics once thought to be well absorbed following enteral administration are not (e.g. chloramphenicol in channel catfish), while others previously considered to be of little use in water treatment may actually be of therapeutic value (e.g. kanamycin in channel catfish).[4,5]

Aeromonas, Vibrio and Pseudomonas are the principal genera of pathogenic bacteria affecting fish.[3,4] Other gram negative bacteria considered important in aquaculture are Hemophilus, Corynebacterium[2], Myxobacteria[3], and Edwardsiella[6]. To combat infections caused by these pathogens, antibiotics are commonly administered by way of water treatment or medicated feed.[2,3] These methods of administration present two important problems. First, it is difficult to regulate dosage since an antibiotic added to water may not become evenly distributed throughout a tank or pond and medicated feed may be consumed by healthy fish to excess while diseased, anorectic individuals remain untreated. Second, the extensive use of an antibiotic armamentarium including only two bacteriostatic drugs would greatly increase the probability that resistant strains of bacterial pathogens would develop.[1,6,7]

The use of a broader range of antibiotics and the use of bactericidal agents has been suggested to aid in the prevention of resistant bacterial strain development.[3] Use of parenteral routes of drug administration further reduces the probability of resistant strain development by reducing the likelihood that potentially pathogenic bacteria in the environment would be exposed to sublethal amounts of antibiotic. Parenteral medication allows accurate and selective dosage of a therapeutic agent and is best suited for valuable breeding, aquarium or experimental fish.[2] The aminoglycoside antibiotic, gentamicin,

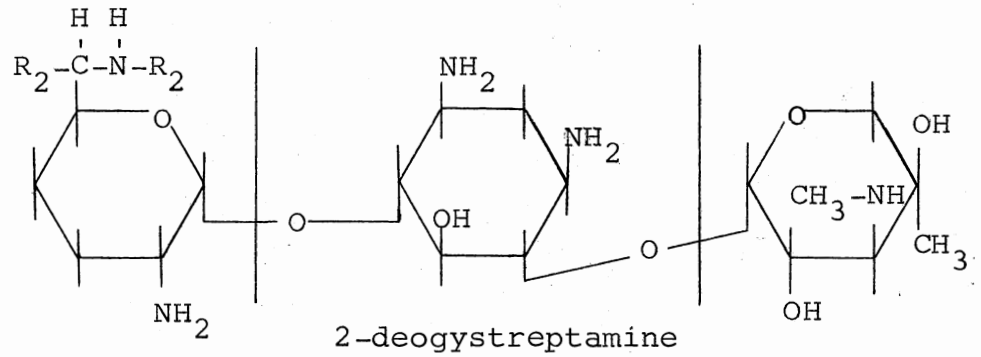
is known to be bacterial to the three principal genera of bacterial fish pathogens as well as other less commonly encountered gram negative pathogens.[5,8,9] Gentamicin's broad spectrum of activity against gram negative bacteria should render it an antibiotic of great potential value in aquaculture.

Gentamicin Pharmacology

The aminoglycoside antibiotic, gentamicin, was first described in 1963.[10] It is derived from the actinomycete Micromonospora purpurea and is actually a complex or family of closely related compounds.[9] The primary components of the gentamicin family are gentamicins C_1, C_{1a}, and C_2. These gentamicins are aminoglycosidic aminocyclitols consisting of two amino sugars joined in glycosidic linkage to a 2-deoxystreptamine nucleus (see Figure 1).[11] They are weak organic bases with pKa's of approximately 7.8[12] and are very similar in biological activity.[13]

Gentamicin is considered to be completely bioavailable in mammals when administered intravenously or intramuscularly,[12] but is not absorbed in significant amounts when given orally in mammals[9,13] or fish. Blood concentrations of the drug after parenteral administration are highly variable and are not dependably predictable from dosages based on body weight in man.[14,15,16] Calculation of dose by lean body weight has been proposed as a more dependable estimator of a dosage that will correspond to

plasma drug concentration in a patient with normal renal function, since aminoglycosides are minimally distributed in fatty tissue.[17] Packed cell volume has also been shown to affect peak serum levels of gentamicin by 2 g/ml for each five points percentage change in hematocrit.[16]



2-deogystreptamine

Gentamicin C_1: $R_1, R_2 = CH_3$. Gentamicin C_{1a}: $R_1, R_2 = H$
Gentamicin C_2: $R_1 = CH_3$, $R_2 = H$

Figure 1. Structures of the Three Primary Components of the Gentamicin Complex.

Studies in mammals indicate that the kinetics of gentamicin are consistent with a two-compartment pharmacokinetic model.[18,19] Because of its polar nature at normal body pH's, gentamicin is largely excluded from most cells,[13] but is distributed throughout the extracellular fluids of body tissues[16] other than the central nervous system and eye.[13,18] Gentamicin is not significanlty protein bound[20] and is excreted predominately by glomerular filtration[16,17,18] in an unmetabolized form. While some drug is eliminated through the biliary system, it is

considered a minor route of excretion.[9] The serum half-life of gentamicin ranges from 1.3-3.6 hours in man,[23] 1.8 hours in ewes,[17] 3 hours in calves,[24] 2.2-2.9 hours in horses[19] and 82 hours at 24°C in gopher snakes[25].

Gentamicin has been shown to cause nephrotoxicity, ototoxicity and neuromuscular blockade in mammals. Aminoglycoside concentration in the renal cortex is markedly higher than plasma concentrations.[21] Excessive drug concentration in the renal cortex can cause acute tubular necrosis with secondary interstitial damage.[13] These degenerative changes seem to be associated with peak blood concentrations of gentamicin (i.e. greater than 2 μg/ml) and the total time of renal exposure to this antibiotic.[14] Trough serum concentrations of gentamicin that consistently exceed 2 μg/ml are also believed to be related to the incidence of ototoxicity.[13] There is a linear relationship between dosage of gentamicin and drug concentration in the endolymph and perilymph.[26] With protracted gentamicin therapy, endolymph and perilymph drug concentrations are many times greater than gentamicin levels in the blood. The ototoxic process involves alteration of normal sodium and potassium transport in the spiral ligament and stria vascularis which in turn causes damage to the sensory cells of the spiral organ.[27] The mechanisms by which gentamicin elicits its ototoxic and nephrotoxic effects are thought to be closely related.

The effects gentamicin has on muscle tissue seem to be produced through mechanisms not related to those causing nephrotoxicity and ototoxicity. Aminoglycosides have been shown to inhibit presynaptic release of acetylcholine and reduce postsynaptic sensitivity to acetylcholine,[28] resulting in neuromuscular blockade that is reversible by the administration of a calcium salt.[13] Gentamicin has also been shown to have a direct myolitic effect on the smooth muscle of the biliary tract[29] and ureters of the urinary tract.[30]

Mechanism of Antibacterial Action and Bacterial Resistance to Gentamicin

Most of the work done on the mechanism of action of aminoglycoside antibiotics has been done on streptomycin. Because of this, this discussion of mechanisms of action will deal mainly with aminoglycosides as a group using streptomycin as a model, rather than with gentamicin specifically.

Aminoglycosides are rapidly bactericidal and act directly on bacterial ribosomes where they bind to the 30S and 50S ribosomal subunit,[30] cause formation of afunctional initiation complexes[31] and induce misreading of the bacterial mRNA templates[32]. In order to reach the bacterial ribosomes, the large, polar aminoglycoside molecules must first be transported across the cell membrane. A biphasic model of transport of streptomycin and gentami-

cin across bacterial cell membranes was proposed by Bryan and Van Den Elzen in 1977.[33] According to this model, the energy dependent phase I begins with low-affinity, divalent cation inhibited, binding of aminoglycodie molecules to portions of respiratory energization complexes that are transported across the cell membrane. Once across the cell membrane, the aminoglycosides bind with ribosomes associated with the membrane, triggering the energy dependent phase II transport. Phase II is associated with functional or architectural changes in the membrane that result in rapid influx of aminoglycoside and correspond with the onset of the inhibition of protein synthesis. The presence of other antibiotics that alter the structure of the cell wall (e.g. penicillin) act synergistically with the aminoglycosides by markedly accelerating their movement into the bacterial cells.[34] Since movement of aminoglycoside molecules across bacterial cell membranes is dependent on the presence of cell membrane complexes associated with aerobic metabolism, anaerobic bacteria are usually resistant to these antibiotics.[13,35] Other mechanisms of resistance include ribosomal mutation, a relatively rare phenomenon,[36] and the development of adenylylating, acetylating and/or phosphorylating enzymes in the bacterial cell membrane at or near aminoglycoside binding sites.[13]

Novokhatskii and Gerasimova[37] demonstrated that, at a concentration of 10 mg/ml, gentamicin inhibited the

cytoplasmic replication of Venezuelan Equine Encephalitis and Sindbis RNA viruses growing in chick embryon fibroblasts (CEF) without observable impairment of cell viability. The primary trypsinized CEF cultures' capacities for production of high titers of the model viruses were reduced after 24 hours of culture growth post gentamicin treatment and removal. Others have found that gentamicin at 100 μg/ml to 200 μg/ml levels effectively inhibit viral replication in tissue cell cultures.[38] Gentamicin's antiviral activity is presumably related to its ability to interfere with the intracytoplasmic ribosomal activity required for viral replication.

The antibacterial action of gentamicin is greatest at an alkaline pH.[39,40] Its clinical use is primarily directed towards aerobic gram negative bacteria,[8,13] although some species of gram positive bacteria are also susceptible.[8] The adverse effect of an acid pH on gentamicin[40] is a feature of potential importance for this antibiotic in channel catfish. It has been shown that the pH of the catfish's body fluids (at 26°C blood pH = 7.75 and intracellular fluid pH = 7.35) become less alkaline as its body temperature increases[41] at a rate of 0.018 pH units per 1°C change in temperature[42]. Cardiovascular changes associated with increased body temperature may also shift plasma gentamicin to other body-fluid compartments.[43] This compartmental shift would result in reduced

blood concentration of the drug and increased apparent volume of the central compartment.

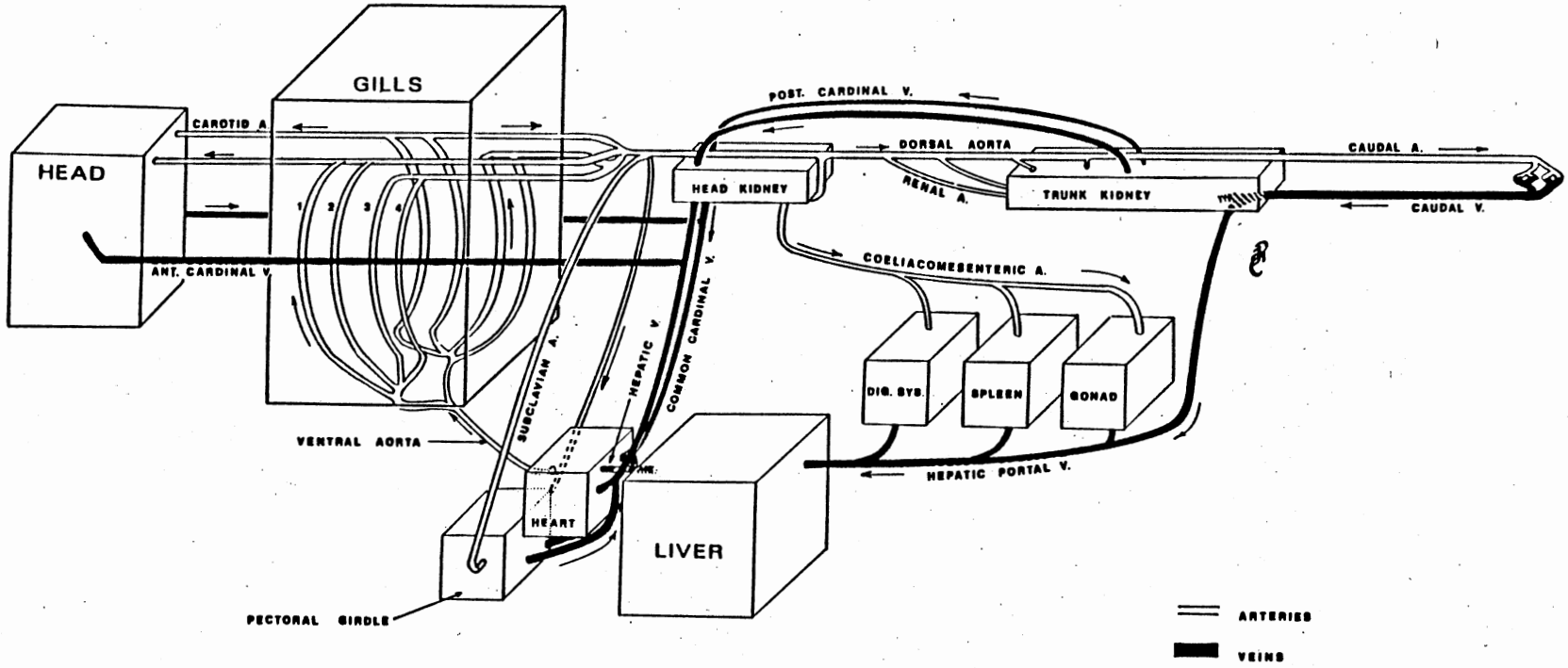
Renal Physiology and Circulatory Anatomy

The nephron of Ictalurus punctatus is representative of freshwater teleosts. It consists of a well developed glomerulus and Bowman's capsule, a neck segment of ciliated cuboidal epithelium, first and second proximal segments lined with cuboidal epithelium, an intermediate segment histologically similar to the neck segment, a distal tubule, and collecting tubule and duct.[44] The urinary bladder appears to function as a part of the nephron by selectively absorbing ions and water. The kidneys function primarily to excrete excess water while retaining most of the filtered solutes.[45] Secondarily, the kidneys supplement the gills' pH regulating activity and produce an alkaline urine (pH = 7.4 $\pm$ 0.18).[41]

The kidneys of the channel catfish are completely separated into cranial and caudal portions referred to as the head kidneys and trunk kidney, respectively. The renal corpuscles and convoluted tubules present in the head kidneys of juveniles less than four centimeters in total body length (TL), completely degenerate by the time the juvenile catfish reach four centimeters TL. The head kidneys of channel catfish greater than four centimeters TL are composed entirely of endocrine and hemopoietic tissue. The caudal kidney segments are fused caudally to

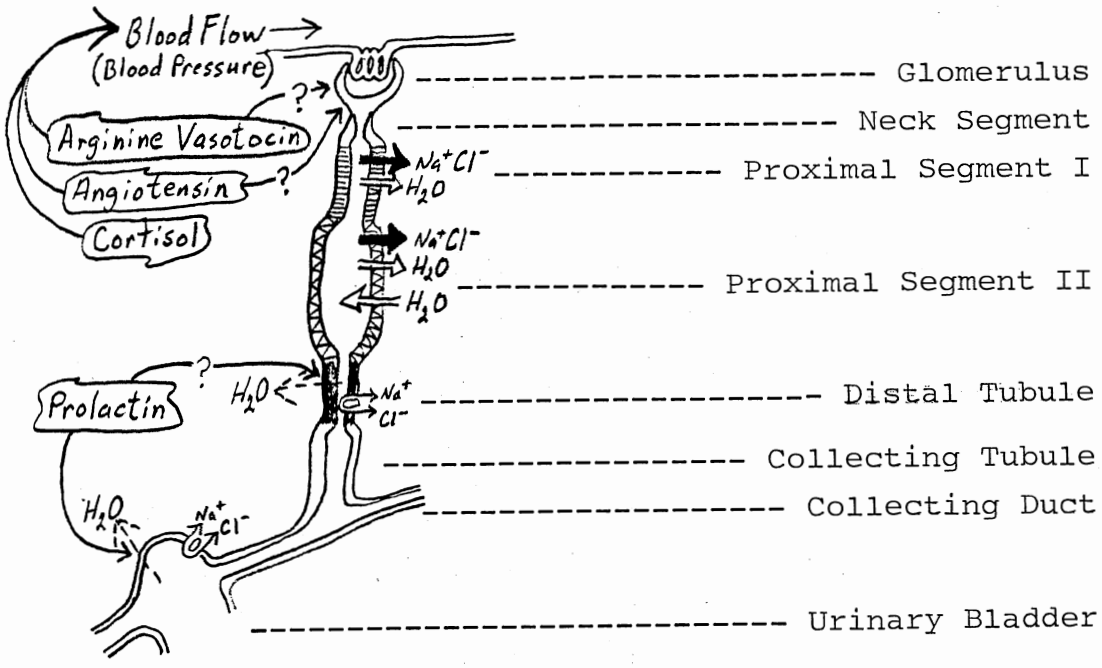
form a single Y-shaped mass known as the trunk kidney. Corpuscles of Stannius are located on the lateral margins of the trunk kidney and are thought to be associated with osmoregulation, steroid production or storage, renin production and the regulation of calcium metabolism.[44] Some hemopoietic tissue, similar to that found in the head kidneys is found in the trunk kidney. The head and trunk kidneys' arterial supply branches directly from the dorsal aorta. The trunk kidney also receives blood from the caudal vein. Efferent blood leaves the trunk kidney by way of the caudal cardinal veins, which deliver blood directly to the head kidneys. The head kidneys are drained by the common cardinal veins leading to the heart. A schematic diagram of the channel catfish circulatory system taken from Grizzle and Rogers[44] is illustrated in Figure 2.

The major determinant of urine flow is glomerular filtration rate (GFR). GFR is altered by arginine vasotocin, angiotensin and cortisol through their influences on systemic blood pressure and blood flow and/or their effect on the population of functioning nephrons[45] (see Figure 3). The GFR is largely unaffected by stressful events such as handling and surgery.[41]



Source: Grizzle, J. M. and Rogers, W. A.: Anatomy and Histology of the Channel Catfish. Department of Fisheries and Allied Aquacultures, Auburn University Agricultural Experiment Station. Auburn, Alabama: Auburn Printing, Inc., 1976.

Figure 2. A Diagram of the Major Arteries and Veins of the Channel Catfish.



Solid and open arrows indicate active and passive movements, respectively. Broken arrows indicate low permeability to water. U-shaped arrows indicate possible Na^+ and Cl^- cotransport. Question marks indicate sites of action that are likely, but unproven.

Source: Nishimura, H. and Imai, M.: Control of renal function in freshwater and marine teleosts. Federation Proc., 41(8):2355-2360, 1982.

Figure 3. A Schematic Illustration of Freshwater Teleost Nephron Function.

Assays for Gentamicin

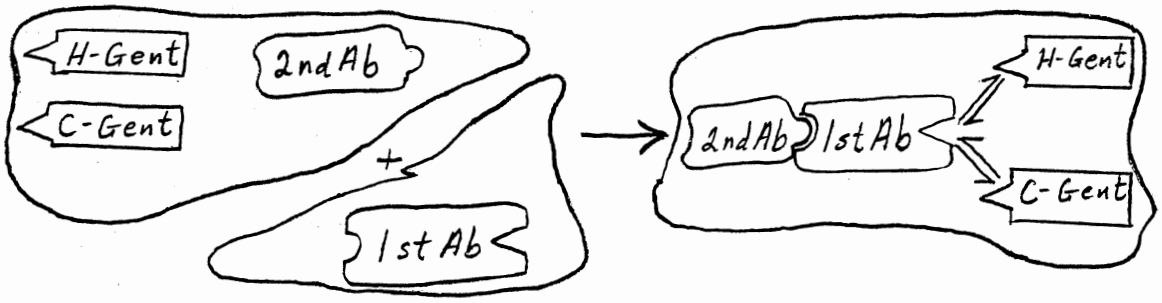
Until the early 1970's, the most commonly used assay procedure for gentamicin was the microbiological assay. In this assay, the concentration of gentamicin in a sample is correlated with the size of a zone of inhibited growth

on an agar plate containing a known type and number of bacteria. The advantage of this method is that it measures the total antibiotic activity in a given sample. The disadvantages of this method include low specificity, high variability, and a long assay time. The latter problem was resolved in the late 1960's when it was shown that the antibiotic activity of a sample inhibited long fermentation to cause a measurable pH change in the growth media in one to two hours.[46] Gentamicin concentration is correlated with the rate of pH change in the zone of inhibition over a 90 minute period. This rapid microbiological assay is still hindered by the problems of low specificity and high variability.

The problem of specificity was largely overcome in the early 1970's with the development of the enzymatic assays for gentamicin. The first enzymatic assay developed used ^{3}H-labeled ATP to adenylate the aminoglycoside.[47] The adenylation product is counted in a scintillation spectrometer and compared with counts from known standards. The specificity and reproducability of the enzymatic assay was further enhanced when a ^{14}C-labeled ATP adenylation agent replaced the ^{3}H-labeled ATP used earlier.[48]

In the mid-1970's, a radioimmunoassay (RIA) was developed using an ^{125}I-labeled acylating agent to iodinate gentamicin that was in turn mixed with rabbit antibody

and unlabeled gentamicin from a sample of unknown concentration.[49] ^{125}I-labeled and unlabeled gentamicin conjugated competitively with antibody. Free gentamicin was removed with dextan coated charcoal and the radioactivity of aliquots of supernatant was measured and compared to standards. This RIA was more specific and less variable than the previous enzymatic assays, but still required a few hours to set up and run. This final problem was finally resolved with the advent of the second antibody RIA. The ^{125}I-gentamicin, second antibody RIA system (see Figure 4) proved to be fast, sensitive, accurate, simple and able to produce very consistent assay values.[50]



H-Gent is ^{125}I-labeled gentamicin, C-Gent is sample gentamicin, 1stAb is the antibody that will bind with gentamicin and 2ndAb is the antibody that will bind with the 1stAb to form a precipitate. H-Gent and C-Gent will reach an equilibrium with the 1stAb, that is dependent on their respective concentrations. The percentage of 2ndAb-1stAb bound to H-Gent will be inversely proportional to the amount of C-Gent added from the sample of unknown concentration.

Figure 4. ^{125}I-Gentamicin Second Antibody RIA Reaction.

CHAPTER II

MATERIALS AND METHODS

General Information

The Schering veterinary product Gentocin®[a], gentamicin sulfate, was used as the gentamicin sulfate source for all the studies described in this chapter. Each milliliter of Gentocin® contained 50 mg gentamicin base. All fish used in these experiments were purchased from a single commercial source[b] in February and early March of 1982. "(^{125}I)-Gentamicin Second Antibody Buffer System", radioimmunoassay kits[c] (hereafter referred to as RIA kits) were used for all quantitative gentamicin assays mentioned in this chapter.

Assay Validity Study

Materials and Procedure

Three stock solutions (A, B and C) of gentamicin sulfate were made. Forty-nine ml of deionized water were

---

[a]Gentocin® (Control Nos. 9KMF29 P62883 and IKMF10 P66395), Schering Corp., Bloomfield, New Jersey.

[b]Crawford's Catfish Acres, Inc., Shawnee, Oklahoma.

[c]Antibodies Incorporated, Davis, California 95616.

added to 1 ml of Gentocin® to yield the 1 mg/ml solution, stock solution A. Four ml of deionized water was added to 1 ml of Gentocin® to yield the 10 mg/ml solution, stock solution B. One ml of deionized water was added to 1 ml of Gentocin® to yield the 25 mg/ml solution, stock solution C. These three stock solutions were used to spike 1 ml aliquots of heparinized catfish plasma to yield 0, 1, 2, 5, 10, 15 and 30 μg/ml standards (Table I). Additional catfish plasma standards were formulated (see Table I) for the supplemental assays that were performed to enhance the statistical significance of the standard curves generated by the initial human serum and catfish plasma standards. The catfish plasma was collected by exanguinating a number of 8 to 13 cm channel catfish. The 0, 1, 2, 4, 8, 16 and 32 μg/ml human serum standards from the RIA kits were used as packaged.

Assay Procedure

Each standard concentration sample was prepared for assay by adding 50 μl of sample to 10 ml of 0.9% saline in a 16 x 125 mm polystyrene test tube. Five sets human serum and five sets catfish plasma standard concentration samples were assayed.

TABLE I

ASSAY VALIDITY STUDY STANDARD FORMULATIONS

Catfish Plasma Standard Concentration (μg/ml)	Heparinized Catfish Plasma Volume (ml)	Volumes of Stock Solutions Added to Catfish Plasma
0	1.0	0 μl
1	1.0	1 μl A
2	1.0	2 μl A
6	0.5	3 μl A
5	1.0	5 μl A
10	1.0	1 μl B
15	1.0	5 μl A + 1 μl B
16	0.5	3 μl A + 0.5 μl B
30	1.0	5 μl A + 1 μl C
35	1.0	1 μl B + 1 μl C

A is a 1 mg/ml gentamicin sulfate solution; B is a 10 mg/ml gentamicin sulfate solution; C is a 25 mg/ml gentamicin sulfate solution.

The assay procedure was performed as described in the RIA kit's instruction booklet. The procedure is paraphrased as follows:

1. Label a duplicate set of 12 x 75 mm polystyrene test tubes for each human serum, catfish plasma and 0.9% saline standard.

2. Pipet 50 μl of each 1:201 standard dilution into

the appropriate tubes.

3. Pipet 500 μl of (^{125}I)-Gentamicin Second Antibody Buffer into each tube.

4. Pipet 500 μl of gentamicin antiserum into each tube. Thoroughly vortex each tube immediately after the addition of the antiserum.

5. Incubate all tubes at room temperature for at least 30 minutes.

6. Centrifuge all tubes for at least 10 minutes with a minimum force of 2000 x g.

7. Individually, thoroughly decant and discard the supernatant. Press each inverted tube against plastic backed absorbent paper to remove any residual supernatant.

8. Count the precipitate in a gamma counter for 1 minute or a time sufficient to accumulate a minimum of 10,000 counts, whichever is greater.

Assay Calculations

The average counts ($\bar{x}$ cpm) for the 0 μg/ml human and catfish standards were calculated and designated B_o for their respective standard sets. The (^{125}I) bound was calculated as a percentage of B_o (%B/B_o) (see equation 2-1).

$$\%B/B_o = \frac{\bar{x} \text{ cpm for a standard}}{B_o} \times 100\ \% \qquad [2\text{-}1]$$

The %B/B_o values and corresponding human serum and catfish plasma standard gentamicin concentrations were used to generate log-regression lines of best fit for each set of

standards using the equation: $y = m(\log x) + b$. In the regression equation, y was the $\%B/B_o$ of a given sample, x was the concentration of gentamicin corresponding to that $\%B/B_o$, m was the slope of the regression line (see equation 2-2) and b was the $\%B/B_o$ corresponding to the y

$$m = \frac{(\log x)y - \frac{\Sigma(\log x)\Sigma y}{N}}{\Sigma(\log x)^2 - \frac{[\Sigma(\log x)]^2}{N}} \qquad [2\text{-}2]$$

intercept of the regression line (see equation 2-3). The

$$b = \frac{\Sigma y - m[\Sigma(\log x)]}{N} \qquad [2\text{-}3]$$

accuracy of the calculated regression line's fit to the data and, subsequently, the reliability of the regression line as a predictor of values was expressed as a fraction of one, called the correlation coefficient (r) (see equation 2-4). An r value of one represented 100% reliability and fit.

$$r = \frac{m(\text{s of sample gentamicin concentrations})}{\text{s of } \%B/B_o\text{'s}} \qquad [2\text{-}4]$$

Mean y intercept ($\bar{Y}_{xo}$) and slope ($\bar{m}$) was calculated for both of the standard groups. The number of values (n), variance (s^2) and standard deviation ($s = \sqrt{s^2}$) of both groups were also calculated for the mean values reported (see equations 2-5 and 2-6). The averaged slopes

$$S_{\bar{Y}_{xo}}^2 = \frac{\Sigma(Y_{xo})^2 - \frac{(\Sigma Y_{xo})^2}{n}}{n - 1} \qquad [2\text{-}5]$$

$$S_{\bar{m}}^2 = \frac{m^2 - \frac{(\Sigma m)^2}{n}}{n - 1} \qquad [2\text{-}6]$$

and y intercepts of the two mean regression lines were compared as unpaired observations with unequal variances. Confidence statements about the homogeneity of the slopes and y intercepts were developed using t' tests (see equation 2-7). The t' criterion was distributed as

$$t' = \frac{\text{Mean Value}_1 - \text{Mean Value}_2}{s_{\bar{d}}} \qquad [2\text{-}7]$$

Student's t at n-1 degrees of freedom when $n_1 = n_2$. The standard deviation of the mean difference ($s_{\bar{d}}$) was calculated as the square root of the sum of the variance of the means of the catfish plasma and human serum standard sets (see equation 2-8).

$$s_{\bar{d}} = \sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}} \qquad [2\text{-}8]$$

For confidence intervals stating there is more than a 10% chance that the difference in reported values is not due to random chance alone, calculated values (i.e. plasma concentrations) were compared to support or refute the assertion that there was no significant difference between values.

Gentamicin Intracardiac Bolus Pharmacokinetic Study

Materials and Procedure

A microliter pipette[d] was used to measure 1.5 ml of Gentocin into a 10 ml volumetric flask[e]. The 1.5 ml Gentocin was diluted to 10 ml with deionized[f] and filtered[g] water. This 7.5 mg/ml stock solution was used as the gentamicin source for all intracardiac and intramuscular dosages. The 10 ml, ground glass stoppered volumetric flask, containing the 7.5 mg/ml gentamicin stock solution, was sealed with Parafilm[h] and held at room temperature (20°C to 24°C) when not in use.

Channel catfish weighing between 0.3 kg and 1.7 kg were held in a 1.5' x 3' x 4', epoxy sealed, aerated holding tank for at least 3 days before use as experimental subjects. The holding tank water was filtered through a submerged, activated charcoal and gravel cartridge filter. A covered, 20 gallon all-glass aquarium

---

[d] Oxford Sampler® micropipettes, Oxford Laboratories, Foster City, California 94404.

[e] Kimax, No. 28017-A.

[f] Barnstead "ROpure" (mod. D2610), Sybron Corp., Boston, Massachusetts.

[g] Barnstead "NANOpure" (Mod. D2782), Sybron Corp., Boston, Massachusetts.

[h] Parafilm "M" Laboratory Film, American Can Co., Dixie/Marathon, Greenwich, Connecticut 06830.

was used as the experimental tank. Experimental tank water was filtered through a bonded filter pad[i] and activated charcoal gravel circulating filter system[j] and was aerated. No fish were fed during holding or experimentation and no fish were held longer than six days before use and disposal. Holding and experimental tank temperatures were maintained at 22°C ± 2°C. Tank water pH's were monitored[k] and maintained at 6.9 ± 0.1.

The first catfish, E_1C_1, was run as a trial fish and was used to establish an appropriate sampling schedule for the other nine fish. A 100 µl heparinized intracardiac blood sample was taken through a 25 gauge hypodermic needle at time zero. A calculated dose (see equation 2-9) of the 7.5 mg/ml gentamicin stock solution, rounded

$$\text{Calculated Dose} = \text{fish weight (in grams)} \times \frac{0.13\ \mu\text{l stock solution}}{\text{gram of fish}} \quad [2\text{-}9]$$

to the nearest 75 micrograms, was injected through a 25 gauge needle as an intracardiac bolus immediately thereafter. Disposable tuberculin syringes[l] and 25 gauge needles[m] were used to obtain 100 µl heparinized blood

---

[i] Marineland Aquarium Products Division of Aquaria, Inc., Van Nuys, California 91406. Cat. No. 100.

[j] The Aquology Corp., USA, model 10-120.

[k] Corning Digital 112 Research pH Meter.

[l] 1.0 cc tuberculin syringe, Becton-Dickinson, Rutherford, New Jersey 07070.

[m] Monoject 200, 25 GA 5/8 A hypodermic needles, Sherwood Medical Industries, Inc., Deland, Florida 32720.

samples by cardiac puncture at 15 minute intervals for the first three hours of the experiment. Subsequent samples were taken every 30 minutes for the next two hours and a last time twenty-four hours after time zero. Blood samples were stored and centrifuged in glass microhematocrit tubes[n] blocked on one end with clay[o]. The catfish plasma was stored at 5°C until assayed. E_1C_1 was hand held for bolus administration and sampling, as were all the catfish used in this experiment.

From the kinetic curve generated from catfish E_1C_1, it was determined that the sampling schedule for the other nine fish used in this study would be every 15 minutes for the first two hours, every 30 minutes for the next three hours, at six and eight hours post injection, and one or two times over the next sixteen hours. It was also decided that, because of the sampling schedule, three catfish could be sampled during each experimental trial. The catfish used in each trial were identified by colored tags[p] attached to their adipose fins with 3-0 silk suture[q]. All fish were weighed in a dry plastic tub on a

---

[n]Heparinized Capillary Tubes, Stock No. 30-2501, General Scientific, Richmond, Virginia 23228.

[o]Seal-Ease, Ct. No. A-2980, Clay-Adams, Inc., New York, New York.

[p]Scientific Products labeling tape.

[q]Black braided Type B, Ethicon, Inc., Somerville, New Jersey.

top-loading balance[r] before being placed in the experimental tank.

Assay Procedure

The RIA kit's assay protocol was modified and applied to each group of standards and samples assayed at a given time with reagents from a single RIA kit. The modified assay protocol was as follows:

1. Label a duplicate set of 16 x 125 mm polystyrene test tubes for each human serum standard from the RIA kit and for each catfish plasma sample.

2. Break hematocrit (Ht) tubes on the plasma side of the packed cell fractions and pool the plasma fractions of each sample. Discard any hemaglobin stained plasma fraction.

3. Pipet 50 μl of each human serum or catfish plasma standard sample into the appropriately labeled 16 x 125 mm polystyrene test tube.

4. Add 10 ml of 0.9% saline to all 16 x 125 mm polystyrene test tubes to yield 1:201 dilutions of standards and samples.

5. Label a duplicate set of 12 x 75 mm polystyrene test tubes for each 1:201 dilution of human serum standard or catfish plasma.

---

[r]Sartorius-Werke Kilomat, Type 2113 gram scale.

6. Pipet 50 μl of each of the 1:201 human serum standard and catfish plasma sample dilutions into the appropriate 12 x 75 mm tube and its contents.

7. Pipet 500 μl of (^{125}I)-Gentamicin Second Antibody Buffer into each 12 x 75 mm tube.

8. Pipet 500 μl of gentamicin antiserum into each tube. Thoroughly vortex each tube immediately after the addition of the antiserum.

9. Incubate all tubes at room temperature for at least 30 minutes.

10. Centrifuge all tubes for at least 10 minutes with a minimum force of 2000 x g.

11. Thoroughly decant and discard the supernatant. Press each inverted tube against plastic backed absorbent paper to remove any residual supernatant.

12. Count the gamma emissions from the precipitate in a gamma counter for 1 minute or a time sufficient to accumulate a minimum of 10,000 counts, whichever yields the larger gamma count. Recount each tube at least one time within six hours of the initial gamma count.

Assay Calculations

The $\%B/B_o$ vs. log human serum gentamicin concentration, linear regression line for each assay group was used to estimate the gentamicin concentrations in the catfish plasma samples of each assay group (see equation 2-10).

$$C_p = \frac{\log^{-1}(\%B/B_o \text{ of } Y_{xs} - \%B/B_o \text{ of } Y_{xo})}{m} \quad [2\text{-}10]$$

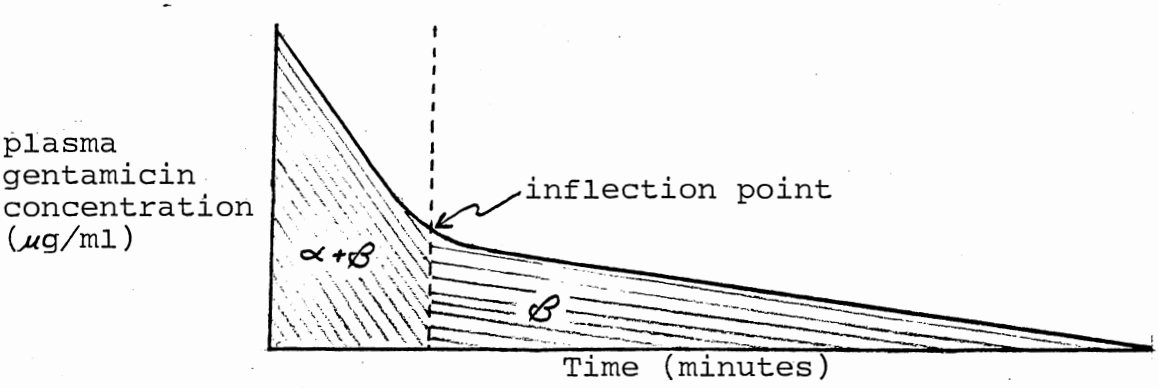
The catfish plasma gentamicin concentration (C_p) was calculated as the antilog of the sum of the negative value of the $\%B/B_o$ of the human serum standard regression line y intercept ($-\%B/B_o$ of Y_{xo}) and the fraction ($\%B/B_o$ of Y_{xs}) in which the $\bar{x}$ cpm of the catfish plasma sample is the numerator and the $\bar{x}$ cpm of the 0 μg/ml (i.e. pre-injection) catfish plasma sample is the denominator. Equations 2-2 and 2-3 from the Assay Validity Study were applied to determine the slope and y intercept of the linear regression line for each assay group. A unique regression line was calculated for each assay group from RIA kit human serum standards assayed with that particular set of catfish plasma samples.

The plasma gentamicin concentration curve (hereafter referred to as the concentration curve) for each catfish was plotted on three cycle semi-logarithmic graph paper[s] with plasma gentamicin concentration (hereafter referred to by the acronym pgc), in μg/ml, plotted on the dependent (y) axis and time, in minutes, plotted on the independent (x) axis. The inflection point at which the concentration curve appeared to change slope in an abrupt fashion was determined by visual inspection of the curve of best fit

---

[s] K-E Semi-logarithmic, 3 cycles x 70 divisions. Keuffel & Esser Co., USA.

plotted through the data points. The slope of the concentration curve after this inflection point was considered to be the slope of the elimination phase of the curve. The slope of the concentration curve prior to this inflection point was considered to be the sum of the slopes of the distribution and elimination phases and was referred to as the $\alpha+\beta$ portion of the curve (see Figure 5). The distribution phase was labeled as the alpha phase and the elimination phase was labeled as the beta phase.



β is the region of the curve where the slope is due to elimination phase kinetics only. The elimination phase is called the beta phase. $\alpha+\beta$ is the unfeathered region of the curve where the slope is the sum of the slopes of distribution and elimination phase kinetics. The distribution phase is called the alpha phase. These two regions are separated by a vertical line passing through the inflection point of the curve.

Figure 5. Hypothetical Plasma Gentamicin Concentration Kinetic Curve.

A least squares regression line was calculated for the region of the concentration curve identified as the beta phase. From this line of best fit, the y intercept, B (μg/ml), and the negative value of the slope of the beta phase, β(min^{-1}), could be calculated. β was the microconstant representing the rate, in reciprocal minutes, at which gentamicin was eliminated from the blood plasma. A second least squares regression line was calculated for the assayed gentamicin plasma concentration points in the $\alpha+\beta$ portion of the concentration curve. The y intercept of this regression line was the theoretical zero time pgc in micrograms per milliliter, C_p^o. The slope and y intercept of the hypothetical regression line describing the distribution phase was derived by a process called feathering wherein the β phase regression line was subtracted from the $\alpha+\beta$ regression line. The y intercept of the alpha phase regression line, A (μg/ml), was determined by subtracting B from C_p^o (see equation 2-11). The

$$A = C_p^o - B \qquad [2\text{-}11]$$

negative value of the slope of the alpha phase regression line, α(min^{-1}), was derived by subtracting β from the negative value of the slope of the least squares regression line through the assayed pgc points in the $\alpha+\beta$ portion of the concentration curve, m_c(min^{-1}) (see equation 2-12). α was the constant representing the rate, in

$$\alpha = m_c - \beta \qquad [2\text{-}12]$$

reciprocal minutes, at which gentamicin was distributed throughout the central and peripheral compartments.

The experimental constants, A, B, α and β, were used to calculate the pharmacokinetic microconstants K_{21}, K_{el} and K_{12} (see equations 2-13, 2-14 and 2-15) in reciprocal

$$K_{21} = \frac{A(\beta) + B(\alpha)}{C_p^o} \qquad [2\text{-}13]$$

$$K_{el} = \frac{(\alpha)(\beta)}{K_{21}} \qquad [2\text{-}14]$$

$$K_{12} = \alpha + \beta - K_{21} - K_{el} \qquad [2\text{-}15]$$

minutes.

The alpha half-life, $t_{\frac{1}{2}(\alpha)}$, and beta half-life, $t_{\frac{1}{2}(\beta)}$, were calculated by dividing α and β into the natural logarithm of 2 (see equations 2-16 and 2-17) and

$$t_{\frac{1}{2}(\alpha)} = \frac{(\ln 2)}{\alpha} \qquad [2\text{-}16]$$

$$t_{\frac{1}{2}(\beta)} = \frac{(\ln 2)}{\beta} \qquad [2\text{-}17]$$

reported in minutes.

The apparent volume of the central compartment, V_c (in ml/kg body weight), was calculated by dividing the intracardial gentamicin dose, in mg/kg, by the peak plasma gentamicin concentrations, in μg/ml (see equation 2-18).

$$V_c = \frac{\text{Dose}}{C_p^o} \times 1000 \text{ ml/l} \qquad [2\text{-}18]$$

The volume of fluid, in ml/kg, required to contain the amount of gentamicin in a given catfish, if it had been uniformly distributed throughout that fluid at a

concentration equal to that in the plasma (i.e. C_p^o) was called the apparent volume of distribution. The area method of calculating the apparent volume of distribution was utilized in these calculations (see equation 2-19).

$$V_{d(area)} = \frac{Dose}{(A/\alpha + B/\beta)} \times 1000 \text{ ml/l} \qquad [2\text{-}19]$$

The extrapolation method was also used to calculate an apparent volume of distribution (see equation 2-27) that could later be compared to the $V_{d(B)}$'s of the intramuscular injection study.

The product of the apparent volume of the central compartment, V_c, and the overall elimination rate constant, K_{el}, was the volume of the blood, in ml/kg, cleared of gentamicin per minute, Cl_B (see equation 2-20).

$$Cl_B = K_{el} \cdot V_c \qquad [2\text{-}20]$$

Plasma gentamicin concentrations (μg/ml), C_p^t, were calculated at t = 240, 360, 480, 720 and 1440 minutes post injection (see equation 2-21). The letter e was used as a

$$C_p^t = Ae^{-\alpha t} + Be^{-\beta t} \qquad [2\text{-}21]$$

symbol for the base for hyperbolic logarithms, 2.71828....

In an attempt to propose a rational course of multiple dose gentamicin therapy, minimum (2 μg/ml) and maximum (12 μg/ml) therapeutic gentamicin plasma concentratins were used to arithmetically predict and evaluate dosage strength and frequency. In equation 2-22, the initial dose, D_i (mg/kg), was the product of the maximum allowable

$$D_i = \frac{C^{\infty}_{p(max)} \cdot V_{d(area)}}{1000\ \mu g/mg} \qquad [2\text{-}22]$$

plasma gentamicin concentration, $C^{\infty}_{p(max)}$, and the apparent volume of distribution. The subsequent dosage frequency in minutes, (see equation 2-23), and maximum

$$\tau = t_{\frac{1}{2}(\beta)} \times \frac{\log(C^{\infty}_{p(max)} \div C^{\infty}_{p(min)})}{\log 2} \qquad [2\text{-}23]$$

effective plasma gentamicin concentration $C^{\infty}_{p(max)}$, were used to calculate the maintenance dosage, D_m (mg/kg), amount (see equation 2-24) for continuing intravenous

$$D_m = \frac{C^{\infty}_{p(max)} \cdot V_{d(area)} \cdot (1 - e^{-\beta\tau})}{1000\ \mu g/mg} \qquad [2\text{-}24]$$

(e.g. intracardiac) therapy.

The central and peripheral compartment gentamicin concentrations (μg/ml) at the end of the calculated multiple dose time interval, τ, were arithmetically estimated for a given initial intracardiac dose, A_o. The central compartment concentration was used as an indicator of plasma concentration (see equation 2-25) and the peri-

$$A_C = \frac{A_o(\alpha - K_{21})}{\alpha - \beta} \cdot e^{-\alpha\tau} + \frac{A_o(K_{21} - \beta)}{\alpha - \beta} \cdot e^{-\beta\tau} \qquad [2\text{-}25]$$

pheral compartment concentration was used as an indicator of the average tissue concentration (see equation 2-26).

$$A_T = \frac{K_{12} \cdot A_o}{\beta - \alpha} \cdot e^{-\alpha\tau} + \frac{K_{12} \cdot A_o}{\alpha - \beta} \cdot e^{-\beta\tau} \qquad [2\text{-}26]$$

All pharmacokinetic calculations were performed and values reported for each experimental subject. Individual $B, \beta, C_p^o, A, \alpha, K_{21}, K_{el}, K_{12}, t_{\frac{1}{2}(\alpha)}, t_{\frac{1}{2}(\beta)}, V_c, V_{d(area)}, V_{d(B)}, Cl_B, C_p^t, D_i, \tau, D_m, A_c$ and A_T values were averaged and reported with standard deviations as experimental estimates of population mean values. Values for $t_{\frac{1}{2}(\alpha)}, t_{\frac{1}{2}(\beta)}, V_c, V_{d(area)}, V_{d(B)}, Cl_B, C_p^t, D_i, \tau, D_m, A_c$ and A_T were also calculated from the mean values for administered dose, $B, \beta, C_p^o, A, \alpha, K_{21}, K_{el}$ and K_{12}.

Gentamicin Intramuscular Dose Pharmacokinetic Study

Materials and Procedure

The experimental materials and procedures outlined for the Gentamicin Intracardiac Bolus Pharmacokinetic Study were utilized in this study with the following modifications.

A catfish weighing between 1.4 kg and 1.5 kg was used as the trial fish labeled E_2C_1. The other eight catfish used in this study weighed between 0.3 kg and 0.5 kg.

The first catfish, E_2C_1, was run as a trial fish and was used to establish an appropriate sampling schedule for the other eight fish. A 100 μl heparinized intracardiac blood sample was drawn immediately before the calculated dose (see equation 2-9) of gentamicin was injected into the epaxial musculature in the region bounded cranially

by the pectoral fins and caudally by the dorsal fin. The 100 μl heparinized intracardiac blood samples were drawn at fifteen minute intervals for the first four hours after the intramuscular (IM) injection, at thirty minute intervals for the next three hours, then at 14.5, 27.75, 28 and 29 hours post IM injection. From the kinetic curve generated with catfish E_2C_1, it was determined that the sampling schedule for the other eight catfish used in this study would be every sixteen minutes for the first two and one-half hours, every half hour for the next five and one-half hours, and at nine hours post IM injection. From ten to 24 hours post IM injection, one or two samples were to be taken. Because of the sampling schedule, it was possible to sample four catfish during each experimental trial.

Assay Procedure

The assay procedure outlined for the Gentamicin Intracardiac Bolus Pharmacokinetic Study was also used for this study.

Assay Calculations

Catfish plasma gentamicin concentrations were determined as outlined in the Gentamicin Intracardiac Bolus Pharmacokinetic Study.

The plasma gentamicin concentration curve (hereafter referred to as the concentration curve) for each catfish was plotted on three cycle semi-logarithmic graph paper with plasma gentamicin concentration, in μg/ml, plotted on the dependent (y) axis and time, in minutes, plotted on the independent (x) axis. The portion of the curve exhibiting a constant negative slope was determined by visual inspection of the curve of best fit plotted through the data points. The slope of the concentration curve in the constant slope region was considered to be the slope of the elimination phase after distribution equilibrium had been reached.

A least squares regression line was calculated for the constant slope region of the concentration curve. From this line of best fit, the y intercept, B (μg/ml), and the negative value of the slope, β(min^{-1}), could be calculated. β was the overall elimination constant representing the rate, in reciprocal minutes, at which gentamicin was eliminated from the blood plasma. The beta half-life, $t^{*}_{\frac{1}{2}(\beta)}$, was the quotient of the Naperian logarithm of two divided by β(see equation 2-17).

The extrapolation method of calculating apparent volume of distribution, $V_{d(B)}$ (see equation 2-27), was applied in this study.

$$V_{d(B)} = \frac{Dose}{B} \qquad [2\text{-}27]$$

The product of the apparent volume of distribution, $V_{d(B)}^{IM}$, and the microconstant $\mathcal{B}$, was the volume of blood, in ml/kg, cleared of gentamicin per minute, Cl_B^* (see equation 2-28).

$$Cl_B^* = \mathcal{B} \cdot V_{d(B)}^{IM} \qquad [2\text{-}28]$$

Plasma gentamicin concentrations, C_p^{t*}, were calculated at t = 240, 360, 480, 720 and 1440 minutes post injection (see equation 2-29). The time, t, after intramuscular

$$C_p^{t*} = Be^{-\mathcal{B}t} \qquad [2\text{-}29]$$

injection for each of these C_p^{t*}'s were considered to be sufficiently long for distribution equilibrium to have been reached.

Dosage frequency, τ^*, was calculated (see equation 2-30) for maintenance of plasma gentamicin levels between 12

$$\tau^* = t^*_{\frac{1}{2}(\mathcal{B})} \times \frac{\log(C^{\infty}_{p(max)} \div C^{\infty}_{p(min)})}{\log 2} \qquad [2\text{-}30]$$

μg/ml ($C^{\infty}_{p(max)}$) and 2 μg/ml ($C^{\infty}_{p(min)}$) during multiple dose intramuscular therapy. The initial or loading dosage, D_i^*, and subsequent maintenance dosages, D_m^*, were determined on the basis of therapeutic range of gentamicin plasma concentrations and apparent volume of distribution. The initial dose was calculated to produce the maximum desirable steady state level of 12 μg of gentamicin per ml of plasma (see equation 2-31). Calculation of the mainten-

$$D_i^* = \frac{C^{\infty}_{p(max)} \cdot V_{d(B)}^{IM}}{1000\ \mu g/mg} \qquad [2\text{-}31]$$

ance dosage was based on the maximum plasma gentamicin level, 12 μg/ml, to be achieved during each dosage interval, τ^* (see equation 2-32).

$$D_m^* = \frac{C_{p(max)}^{\infty} \cdot V_{d(B)}^{IM} \cdot (1 - e^{-\beta\tau^*})}{1000\ \mu g/mg} \qquad [2\text{-}32]$$

All pharmacokinetic calculations were performed and values reported for each experimental subject. Individual B, β, $t_{\frac{1}{2}(\beta)}^*$, $V_{d(B)}$, Cl_B^*, C_p^{t*}, D_i^*, τ^* and D_m^* values were averaged and reported with standard deviations as experimental estimates of population mean values. Values for $t_{\frac{1}{2}(\beta)}^*$, $V_{d(B)}$, Cl_B^*, C_p^{t*}, D_i^*, τ^* and D_m^* were also calculated from the mean values for administered dose, B and . An estimate of gentamicin intramuscular bioavailability (F) was calculated by dividing the area under the mean elimination phase regression line for the intramuscular dosage study, $(AUC)_{IM}$, by the area under the mean elimination phase regression line for the intracardiac bolus study, $(AUC)_{IC}$ (see equation 2-34). The apparent volume of distribution value $V_{d(B)}$ was used as an estimate of the area under the mean elimination phase regression line, AUC, that was defined as the integrated product of $\int_o^{\infty} C_p^t dt$ where $C_p^t dt$ was the product of B and the mean value for C_p^t at $t = \infty$ (see equation 2-33).

$$AUC = (B)(\beta) + \frac{C_p^t}{\beta} \approx V_{d(B)} \qquad [2\text{-}33]$$

$$F = \frac{(AUC)_{IM}}{(AUC)_{IC}} \times 100\% \qquad [2\text{-}34]$$

Gentamicin Tank Dosage
Pharmacokinetic Study

Materials and Procedure

Gentocin (equivalent to 50 mg/ml gentamicin) was used as the gentamicin source for tank dosage. Tank water was dosed at 6.6 mg/l (0.5 ml/gal). An aerated, 20 gallon, all-glass aquarium was used as the experimental tank. The holding tank was set up and used as previously described in the Gentamicin Intracardiac Bolus Pharmacokinetic Study. Holding and experimental tank temperatures were maintained at $22^{o}C \pm 2^{o}C$ and tank water pH's were monitored.

A catfish weighing between 0.7 kg and 0.8 kg was used as the trial fish labeled E_3C_1. The other nine catfish used in this study weighed between 0.8 kg and 1.1 kg. All catfish were weighed and identified, as outlined in the intracardiac study, before use as experimental subjects.

The first catfish, E_3C_1, was run as a trial fish and was used to establish an appropriate sampling schedule for the other nine fish. A 100 μl heparinized intracardiac blood sample was drawn immediately before E_3C_1 was introduced into the experimental tank containing a calculated dose of 7 μg of gentamicin per milliliter of tank water. A 25 GA, 7/8 inch hypodermic needle was used to draw each 100 μl heparinized intracardiac blood sample. Blood

samples were drawn at fifteen minute intervals for the first four hours, at half-hour intervals for the next 4.5 hours, and at 10.5, 12.5 and 27 hours after introduction into the experimental tank. Water samples were taken hourly for the first seven hours and at 8.5, 10.5, 12.5 and 27 hours after E_3C_1 was introduced into the experimental tank.

From the data generated from catfish E_3C_1, it was determined that blood was to be drawn from the remaining nine catfish at hourly intervals for the first thirteen hours, at seventeen hours and once again 21 to 24 hours after introduction into the experimental tank. Water samples were to be taken each time blood samples were taken.

Assay Procedure

The assay procedure outlined for the Gentamicin Intracardiac Bolus Pharmacokinetic Study was also used for this study.

Assay Calculations

Catfish plasma gentamicin concentrations were determined as outlined in the Gentamicin Intracardiac Bolus Pharmacokinetic Study.

The plasma gentamicin concentration curve (hereafter referred to as the concentration curve) for each catfish was plotted on three cycle semi-logarithmic graph paper

with plasma gentamicin concentration, in μg/ml, plotted on the dependent (y) axis and time, in minutes, plotted on the independent (x) axis. The water gentamicin concentration curve corresponding to each catfish was plotted on the same graph as that individual catfish's plasma concentration curve.

The time of peak plasma gentamicin concentration and level of the average steady state plasma gentamicin concentration were to be determined. The relationship between water gentamicin levels and average steady state plasma gentamicin levels were to be elucidated.

CHAPTER III

RESULTS

General Information

Raw data is listed in Appendix A. Values derived from the raw data are tabulated in Appendix B.

Assay Validity Study

Regression lines were generated for five different human serum and five different catfish plasma standard sets. The mean y intercept ($\%B/B_o$) for the human serum standards was 92.34% (s = 3.57%) and the mean slope was -40.52 (s = $\pm$ 0.99). For the five different catfish plasma standard regression lines, the mean y intercept was 82.05% (s = $\pm$ 6.34%) and the mean slope was -37.98 (s = $\pm$ 3.51). All standard set regression line correlation coefficients were in excess of 0.96.

There was less than a 4% chance that the difference in $\bar{Y}_{xo}$'s for the human serum standards and catfish plasma standards was attributable to anything other than random chance alone. There was more than an 80% chance that

random sampling error alone, rather than a real difference in population means, was responsible for the difference in $\bar{m}$'s between the human serum standards and catfish plasma standards.

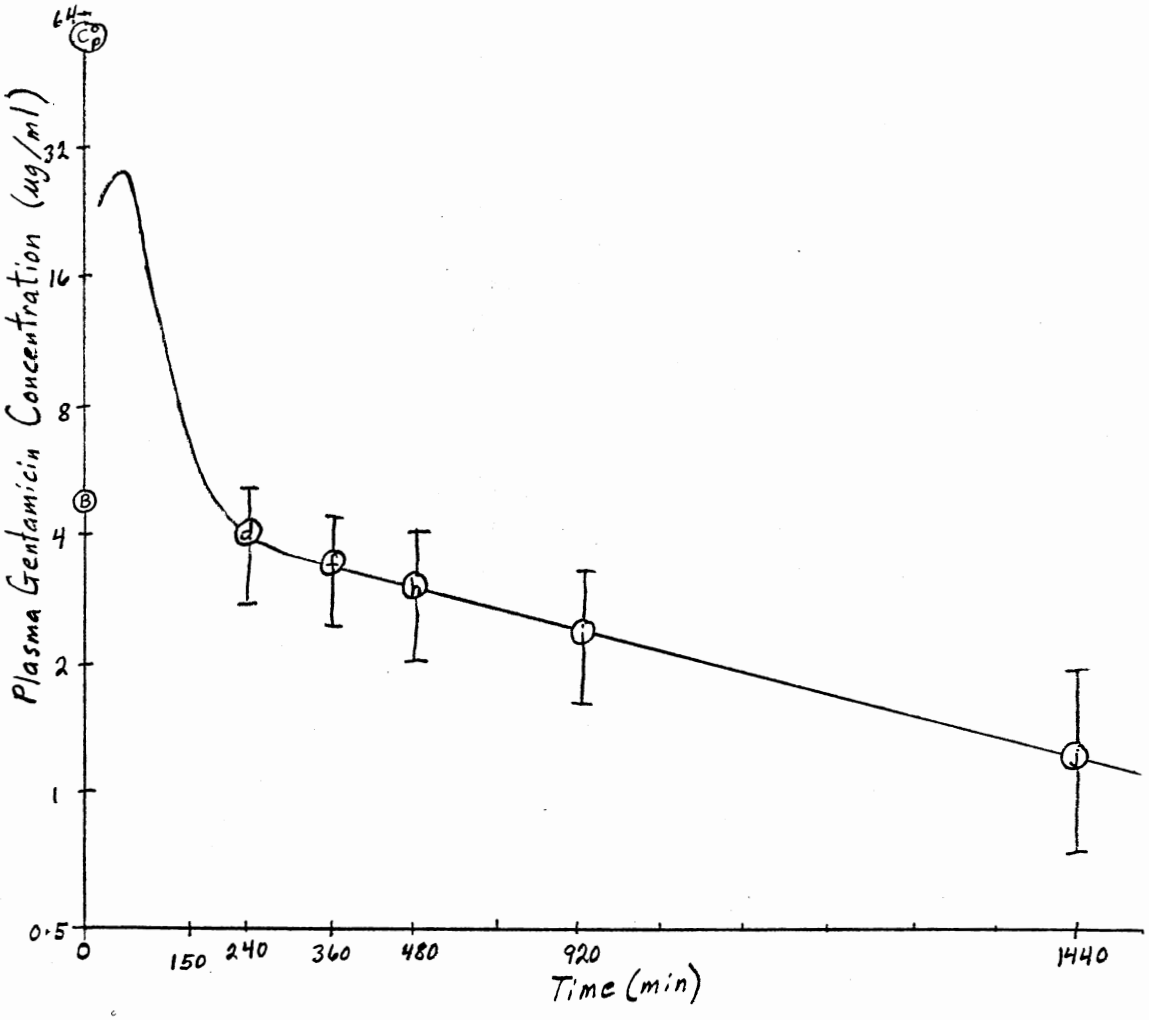
The $\bar{m}$'s of the human serum standards and catfish plasma standards have overlapping value ranges at $\pm$ 0.6 standard deviation units. At a hypothetical common $\%B/B_o$ of 90%, the difference in calculated gentamicin sample concentrations are less than 1 μg/ml at binding values greater than 55% (i.e. less than 8 μg/ml).

Gentamicin Intracardiac Bolus Pharmacokinetic Study

The mean beta half-life of gentamicin given as an intracardiac bolus was 13.8 (s = $\pm$ 4.7) hours and the mean alpha half-life of gentamicin was 54.12 (s = $\pm$ 61.96) minutes. The apparent volume of the central compartment was 61.46 (s = $\pm$ 49.8) ml/kg. The volume of fluid required to contain the dose of gentamicin given, if it were uniformly distributed at a concentration equal to that in the plasma, ($V_{d(area)}$) was calculated to be 212.04 (s = $\pm$ 96.92) ml/kg. The microconstants K_{12}, K_{21} and K_{el} were calculated to be 0.195 (s = $\pm$ 0.196) min^{-1}, 0.0048 (s = $\pm$ 0.0025) min^{-1} and 0.0074 (s = $\pm$ 0.0089) min^{-1}, respectively. Gentamicin was cleared from catfish plasma at a rate of 0.1983 (s = $\pm$ 0.1608) ml/min/kg after intracardiac bolus administration. The value of α was 0.0307

(s = ± 0.028) min^{-1}, of β was 0.0009 (s = ± 0.0003) min^{-1}, of A was 54.62 (s = ± 101.18) μg/ml and of B was 4.85 (s = 1.96) μg/ml. A graphic representation of the plasma decay curve described by mean C_p^t values generated from intracardiac bolus injection study data is given in Figure 6.

The initial intracardiac dose of gentamicin required to yield a peak plasma level of 12 μg/ml was calculated to be 2.43 (s = ± 1.12) mg/kg. The intracardiac dosage interval, τ, required to prevent trough plasma levels in excess of 2 μg/ml was calculated to be 35.71 (s = ± 12.16) hours. The maintenance intracardiac dose was calculated to be 2.06 (s = ± 0.93) mg/kg when administered at the dosage interval, τ. At the end of each dosage interval the gentamicin concentration in the central compartment (e.g. blood plasma) was predicted to be 0.133 (s = ± 0.308) μg/ml. The average tissue concentration at the end of the dosage interval was expected to be 0.295 (s = ± 0.616) μg/ml, given the assumption that the calculated value for peripheral compartment drug level, A_T, accurately predicted tissue levels.



This curve is derived from the time ending the $\alpha+\beta$ phase (156 ± 36 minutes) and the mean values, in μg/ml, for C_p^o (59.37 ± 102.2), B (4.85 ± 1.96) and C_p^t at t = 240, 360, 480, 720 and 1440 minutes. Peak values at 240 minutes were estimated from raw data values. Standard deviation ranges are given with C_p^t values: d = 4.02 ± 1.48; f = 3.49 ± 1.34; h = 3.22 ± 1.17; i = 2.49 ± 0.95; j = 1.33 ± 0.54.

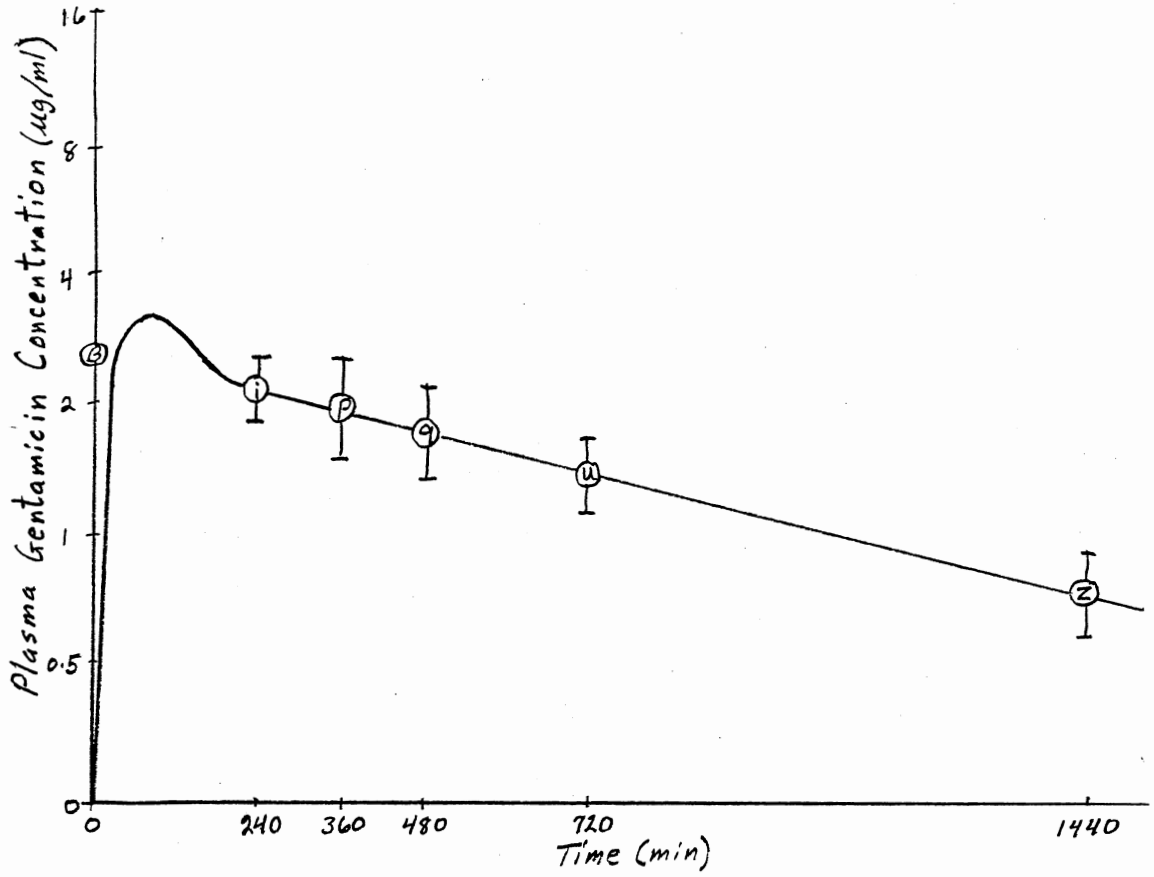
Figure 6. Composite Intracardiac Bolus Gentamicin Plasma Decay Curve.

Gentamicin Intramuscular Dose Pharmacokinetic Study

The mean beta half-life of gentamicin given intramuscularly was 9 (s = ± 1.94) hours. The apparent volume of distribution was calculated to be 365.78 (s = ± 74.83) ml/kg. Gentamicin was cleared from catfish plasma at a rate of 0.3484 (s = ± 0.0777) ml/min/kg after intramuscular administration. The mean value of β was 0.0009 (s = ± 0.0002) min^{-1} and the mean value of B was 2.86 (s = ± 0.51) μg/ml. A graphic representation of the plasma decay curve described by mean C_p^t values generated from intramuscular injection study data is given in Figure 7.

The initial intramuscular dose of gentamicin required to yield a peak plasma level of 12 μg/ml was calculated to be 4.39 (s = ± 0.90) mg/kg. The intramuscular dosage interval, τ^*, required to prevent trough plasma levels in excess of 2 μg/ml was calculated to be 31.83 (s = ± 5.01) hours. The maintenance intramuscular dose was calculated to be 3.64 (s = ± 0.74) mg/kg when administered at the dosage interval, τ^*.

The estimate of the bioavailability of intramuscularly administered gentamicin in the channel catfish was 59%.



This curve is derived from the mean values, in μg/ml, for B (2.86 $\pm$ 0.56) and C_p^t at t = 240, 360, 480, 720 and 1440 minutes. Peak values at t 240 minutes were estimated from raw data values. Standard deviation ranges are given with C_p^t values: 1 = 2.26 $\pm$ 0.38; p = 2.02 $\pm$ 0.34; q = 1.79 $\pm$ 0.30; u = 1.43 $\pm$ 0.25; z = 0.72 $\pm$ 0.17.

Figure 7. Composite Intramuscular Dose Gentamicin Plasma Decay Curve.

Gentamicin Tank Dosage

Pharmacokinetic Study

The catfish identified as E_3C_1, E_3C_2, E_3C_5, and E_3C_8 did not show significant elevation in plasma gentamicin levels. The catfish labeled E_3C_4, E_3C_7 and E_3C_{10} did show some elevation in plasma gentamicin levels. At greater than 240 minutes, E_3C_4 had an average plasma gentamicin level of 1.0 (s = $\pm$ 1.4) μg/ml. Before 450 minutes, E_3C_9 averaged 1.1 (s = $\pm$ 1.3) μg gentamicin per ml blood plasma. When averaged over the entire span of time blood levels were monitored though, the mean values for E_3C_4 and E_3C_9 fell below the 1 μg/ml level considered to be the lower end of the regression analysis' range of reliable correlation of $\%B/B_o$ to sample concentration. The mean plasma level for E_3C_7 was 3.3 (s = $\pm$ 0.28) μg/ml and was 1.5 (s = $\pm$ 0.8) μg/ml for E_3C_{10}. These plasma levels for E_3C_7 and E_3C_{10} were averaged over the entire sampling time for which each catfish was monitored and were above the 1 μg/ml regression line minimum reliably predictive value.

Regression lines developed for tank water gentamicin concentrations indicated that catfish E_3C_1 was exposed to a tank dosage range of 8 μg/ml to 6 μg/ml; E_3C_2, E_3C_3 and E_3C_4 were exposed to a dosage range of 8.4 μg/ml to 6 μg/ml; E_3C_5, E_3C_6 and E_3C_7 were exposed to gentamicin concentrations ranging from 7.3 μg/ml to 6.9 μg/ml, and E_3C_8, E_3C_9 and E_3C_{10} were exposed to dosages of gentamicin ranging from 6.5 μg/ml to 6.0 μg/ml.

CHAPTER IV

DISCUSSION

General Information

In order to minimize the variability in experimental subjects, all catfish used in this study were acquired from a single source, during a single season, and were of a limited size range. They were maintained without feeding for the brief period they were kept before use to minimize the chances of fouling the water. It was believed that no significant adverse effects resulted from the brief period of fasting. The holding tank was coated with epoxy paint and sealed with silicone sealant to minimize exposure to exogenous toxic substances. An activated charcoal filter and extensive aeration were used to control biological waste products produced by the fish. Since the pH of a catfish's intracellular fluids drops 0.018 units for every 1^{o}C temperature increase,[42] and gentamicin is sensitive to pH levels,[13] the holding tank temperature was controlled as best possible.

Assay Validity Study

The high correlation coefficients corresponding to the regression lines developed for the human serum and catfish plasma standards indicated that these regression lines were very reliable for predicting gentamicin concentrations from given $\%B/B_o$'s.

The t test used to calculate t' values for unpaired observations of unequal variances (equation 2-7) was taken from Steel and Torrie.[51] The tabular values of t' were distributed as Student's t at n-1 degrees of freedom since the same number of standard sets were used in the calculation of catfish plasma and human serum $\bar{Y}_{xo}$'s and $\bar{m}$'s.[52] The human serum and catfish plasma $\bar{Y}_{xo}$'s were considered as estimates of a common population mean since there was a 96% chance that the difference in the $\bar{Y}_{xo}$'s of the human serum standard regression line and the catfish plasma standard regression line was due to sampling error alone. While there was a 20% chance that the difference between $\bar{m}$'s of the catfish human standard regression lines was due to a real difference in population mean slopes, the difference in slope values was within 0.6 standard deviation units of the respective slopes. The differences in calculated sample gentamicin concentrations due to the differences in $\bar{m}$ values were less than 1 μg/ml at these plasma concentrations.

It was decided to assume that the human serum standards could be used to reliably predict catfish plasma gentamicin levels at concentrations ranging from 1 μg/ml to 8 μg/ml and could be used to predict values outside this range with the understanding that the reliability of the numbers generated decreased as their values diverged from the 1 to 8 μg/ml range. It was believed that formulation error due to small plasma volumes in catfish plasma standards could offset the small theoretical advantage of using catfish plasma standards instead of the human serum standards provided in the RIA kits. The human serum standards were used because of convenience, adequate reliability and high standard concentration reliability.

Gentamicin Intracardiac Bolus Pharmacokinetic Study

The calculated dose of gentamicin administered to the catfish in this study was determined by multiplying each fish's body weight, in grams, by the product of 0.2 ml extracellular fluid per gram of catfish body weight[41] and the arbitrary gentamicin blood level of 5 μg per ml extracellular fluid, then dividing by the concentration of the gentamicin stock solution, 7.5 mg/ml. Doses were calculated volumetrically and rounded to the nearest 10 μl. This 10 μl was equivalent to about 0.08 mg of gentamicin and was responsible for the variation of dosage about the 1.0 mg/kg mean value. Aspiration of blood into the 25

gauge needle hub was considered verification that the gentamicin dose was being delivered directly into the circulatory system. Work done by Cameron[41] suggested that it was not likely that renal blood flow or renal output were significantly altered by repeated manual restraint and cardiac puncture. Post mortem examination of the channel catfish used in this study did not reveal any gross ventricular lesion or pericardial blood accumulation from repetitive cardiac puncture.

The $\%B/B_o$ vs. log human serum gentamicin standards regression line slope and y intercept values were used to calculate catfish plasma sample gentamicin concentrations. A unique regression line was developed for each assay set. Although sample gentamicin concentrations were comparable between assay sets, percent binding values were only meaningful when they were compared to other binding values within the same set.

Gentamicin kinetics were thought to be adequately described by a two-compartment open model[12,26], even though it had been shown that this antibiotic was concentrated in the renal cortex[13,14] and that as much as 10% of blood gentamicin may be found associated with red blood cells[15,16]. Because of these compartments of selective drug concentration, it might have been more theoretically accurate to utilize a more complicated multicompartment open model, but the rapidity with which the red blood cell compartment was thought to equilibrate with blood

plasma and the very slow rate of accumulation of drug in renal tissue prevented either compartmental equilibration rate from significantly affecting the kinetics described by a simple two compartment open model over the calculated dosage interval of 33 hours.

The mean value for K_{12} (0.0195 min^{-1}) was nearly five times the mean value for K_{21} (0.0048 min^{-1}). This indicated that gentamicin moved from the central compartment to the peripheral compartment a great deal more rapidly than from the peripheral to the central compartment. The mean values for A_C (0.133 $\mu g/ml$) and A_T (0.295 $\mu g/ml$) supported this assertion.

The $V_{d(area)}$ value included both distribution and elimination phase kinetics in the calculation of the apparent volume of distribution. $V_{d(B)}$ was also calculated, but was of value primarily as an apparent volume of distribution value that could later be compared to that calculated for the intramuscular dosage study. The larger the ratio of $\alpha:\beta$, the smaller the area of the $\alpha+\beta$ region of a kinetic curve when compared to the β region of that same curve. The total area under a kinetic curve was inversely proportional to the corresponding apparent volume of distribution (see equations 2-19 and 2-27). The $V_{d(B)}$ value was expected to overestimate the true apparent volume of distribution of a drug whose kinetics could best be described by a two compartment open model since the one

compartment model extrapolation method did not take distributive phase kinetics into consideration.

The difference in the calculated mean estimates of $V_{d(area)}$ (156.56 ml/kg) and $V_{d(B)}$ (208.25 ml/kg) was comparable to that seen in the dog.[12] These calculated estimates of mean apparent volumes of distribution compared to the fluid compartment values of 680 ml/kg for total body water, 190 ml/kg for extracellular fluid and 490 ml/kg for intracellular fluid in channel catfish.[41] Since the calculated estimate of $V_{d(area)}$ value did not exceed the expected volume of extracellular fluid in the channel catfish, it was assumed that gentamicin was not significantly partitioned into a subdivision of the central compartment such as red blood cells endothelium or perivascular fluid.

The $t_{\frac{1}{2}}$ values calculated from mean α and β values showed a beta half-life (770.2 min) more than 34 times the alpha half-life (22.6 min). This was a greater difference than normally seen in mammals.[12,13,23,24] Following intracardiac bolus administration, the long alpha half-time and the delayed (35 $\pm$ 15 min) peak plasma concentration could have been due to the disequilibrium partitioning of gentamicin into red blood cells accompanied by a protracted bolus dispersal period. As plasma gentamicin from the bolus dispersed, aminoglycoside associated with red blood cells would move back into the plasma. This would cause an apparent rise in the blood gentamicin concentration and prolong the time required for complete

distribution of drug between the central and peripheral compartments. The high concentration of antibiotic in an injected bolus could have also caused movement of drug to other areas of the central compartment such as endothelial cells, perivascular fluid or blood proteins.

The mean value for K_{el} was about 25% of the amount reported for dogs.[12] The low Cl_B accompanying this K_{el} value indicated that a smaller fraction of total body gentamicin per unit time was eliminated by catfish than by homothermic animals. This resulted in the extension of the beta half-life beyond that seen in mammals. The Cl_B was calculated as the product of the mathematically independent parameters K_{el} and V_c. This method of calculation was considered superior to the alternative product of the arithmetically related parameters β and $V_{d(area)}$. The preferential partitioning of gentamicin into the peripheral compartment, as reflected by the small value of K_{21} relative to K_{12}, also increased the beta half-time of gentamicin.

The dosage interval for channel catfish at 22 $\pm$ 2°C calculated from a $t_{\frac{1}{2}(\beta)}$ based on the mean β value and the maximal safe peak and trough plasma values was 33 hours. This compared with a value of 72 hours reported for gopher snakes at 24°C[25] and a range of four to six hours for man[16]. The calculated dosage of gentamicin used in this study was expected to produce blood levels around the 5

μg/ml level. The D_i, τ and D_m values were calculated to allow the maximum peak and trough gentamicin concentrations considered allowable without significant risk of aminoglycoside toxicity. The peak plasma concentration of 12 μg/ml was above the minimum inhibitory concentration reported to be effective against many gram negative and some gram positive bacteria.[8,9] The initial dosage required to achieve a peak plasma concentration of 12 μg/ml, was 2.4 mg/kg. Maintenance dosages required to attain peak plasma concentrations of 12 μg/ml were slightly smaller (2.1 mg/kg). This reduction of the dosage required for maintenance of desired plasma drug concentration was attributed to saturation of the peripheral compartment by the initial dose.

The risk and technical difficulty involved in cardiac puncture in channel catfish made intracardiac bolus injection an impractical route of parenteral administration for use outside the laboratory setting. The practical worth of this study was the establishment of basic pharmacokinetic parameters from which information of clinical value, such as dosage schedule, bioavailability, clearance rate and drug distribution, could be determined.

Gentamicin Intramuscular Dose Pharmacokinetic Study

The same considerations concerning dosage calculation and determination of plasma sample gentamicin concentration previously mentioned in the intracardiac bolus study discussion apply to the intramuscular dose study.

The mean value for β (0.0009 ± 0.0002 min^{-1}) was the same as reported for the β of the intracardiac bolus study (0.0009 ± 0.0003 min^{-1}). This indicated that the true β for gentamicin in channel catfish at $22 \pm 2^{o}C$ was probably very near the 0.0009 min^{-1} value. Using the value 0.0009 min^{-1} in the equation for beta half-life (see equation 2-17), a mean value of $t_{\frac{1}{2}(\beta)}$ = 770.16 minutes was derived. Recalculating τ (see equation 2-30), a new value of 33.18 hours was indicated. $V_{d(B)}^{IM}$ was recalculated to be 353.15 ml/kg by dividing the mean administered dose (1.01 mg/kg) by the mean experimental value of B (2.86 μg/ml).

$V_{d(B)}$ was an extrapolated estimator of $V_{d(area)}$ that did not consider the distributive phase (i.e. $\alpha = \infty$) of drug disposition.[54] Because of this, $V_{d(B)}$ consistently overestimated the apparent volume of distribution for drugs like gentamicin that were best described by a two-compartment open model. The assumption was made that the ratio of $V_{d(area)}$:$V_{d(B)}$ from the intracardiac bolus study could be used to estimate a $V_{d(area)}$ for the intramuscular dosage study. By this method of approximation, the value

for $V_{d(B)}^{IM}$ of 353.15 ml/kg would correspond with a calculated $V_{d(area)}$ of 265.64 ml/kg. The Cl_B corresponding to this calculated $V_{d(area)}$, was 0.2391 ml/min/kg. This value was within 25% of one standard deviation of the experimental mean Cl_B value for the intracardiac study. Similar clearance rates would be expected for a drug with equal intracardiac and intramuscular beta half times.

The mean intramuscular dosage study experimental values for B and $\mathcal{B}$ were used to calculate D_i* (4.24 mg/kg) and D_m* (3.54 mg/kg). These values deviated less than a single standard deviation unit from the mean experimental values of 4.39 mg/kg and 3.46 mg/kg, respectively.

Bioavailability of a drug was a function of both the rate of drug absorption and the fraction of the administered dose that reached the systemic circulation intact. It was determined by administering equal doses of a drug intramuscularly and intravenously at different times in the same animal.[12] This was not done in these experiments. As an estimate of the extent of absorption (F), the areas under the composite mean value curves (AUC) from the intracardiac and intramuscular studies were compared.

The apparent volume of distribution value, $V_{d(B)}$, was used as an estimate of the mean AUC (see equation 2-33). The fraction of drug that reached the systemic circulation after intramuscular administration was calculated to be 0.59 (59%). This value for bioavailability in

channel catfish was only 60% of that reported for mammals ($>90\%$).[12] Because of the large variances associated with the $V_{d(B)}$'s used to calculate F, it was perhaps most appropriate to report that the bioavailability of gentamicin in channel catfish was near 60%.

Gentamicin was easily administered as an intramuscular drug. This study revealed adequate absorption and a long plasma half-life. The dosage frequency of 33 hours and the small amount of antibiotic required for dosage rendered gentamicin a drug of practical use in a clinical setting for experimental fish, aquarium fish or aquatic breeding stock.

Gentamicin Tank Dosage Pharmacokinetic Study

Two of the ten catfish involved in this study, E_3C_7 and E_3C_{10}, developed significant blood plasma levels with exposure to water gentamicin levels between 7.3 and 6.0 μg/ml. It was not discovered what unique feature common to these two fish could have resulted in significant absorption of gentamicin from the tank water. It had been long assumed that fresh water teleosts did not drink a great deal, in an attempt to prevent the relatively hypotonic environmental water from diluting the relatively hypertonic internal body fluids. An interesting explanation for the variability in absorption from tank water

could have been that only two of the ten fish used in this study actually drank the tank water in significant quantities during the course of an experimental trial.

Because of the undependable and low-grade absorption from tank water and the high potential cost of water dosage, gentamicin was not found to be an acceptable antibiotic for tank water treatment of systemic bacterial infection. These conclusions are in keeping with those of Gilmartin and coworkers.[6]

CHAPTER V

SUMMARY AND CONCLUSIONS

The purpose of this study was to establish values for the common pharmacokinetic parameters applicable to a two-compartment open model as they apply to intracardiac, intramuscular and oral routes of gentamicin administration. It was hoped that these values could be used to propose a rational course of therapy. The beta half-life of gentamicin in channel catfish was estimated to be 770 minutes. The loading dosage for intracardiac administration was 2.5 mg/kg and was estimated to be 4.2 mg/kg for intramuscular administration. At dosage intervals of 33 hours, a maintenance intramuscular dosage of 3.5 mg/kg could be used to achieve peak serum levels of 12 μg/ml and prevent trough serum levels in excess of 2 μg/ml.

Further investigation into the distribution of gentamicin in the various body tissues would be a valuable addition to this body of information and prove helpful in better directing use of this aminoglycoside antibiotic in the treatment of susceptible gram negative infections.

BIBLIOGRAPHY

1. Sinderman, C. J. (ed): Disease Diagnosis and Control in North American Marine Aquaculture. New York: Elsevier Scientific Publishing Co., 1977.

2. Herman, R. L.: Chemotherapy of fish diseases: a review. J. Wildl. Dis., 6:31-34, 1970.

3. Kingsford, E.: Treatment of Exotic Marine Fish Diseases. New York: Arco Publishing Co., Inc., 1975.

4. Conroy, D. A.: Studies on the application of kanamycin to the control and treatment of some bacterial diseases of fish. J. Appl. Bact., 26(2):182-192, 1963.

5. Gilmartin, W. G.; Camp, B. J. and Lewis, D. H.: Bath treatment of channel catfish with three broad-spectrum antibiotics. J. Wildl. Dis., 12:555-559, 1976.

6. Aoki, T. and Kitao, T.: Drug resistance and transferable R plasmids in Edwardsiella tarda from fish culture ponds. Fish Pathol., 15(3/4):227-281, 1981.

7. Jones, L. M.; Booth, N. H. and McDonald, L. E. (ed): Veterinary Pharmacology and Therapeutics, 4th ed. Ames, Iowa: The Iowa State University Press, 1977.

8. Weinstein, M. J.; Wagman, G. H.; Oden, E. M. and Marquex, J. A.: Biological activity of the antibiotic components of the gentamicin complex. J. Bact., 94:789-790, 1967.

9. Black, J.; Calesnick, B.; Williams, D. and Weinstein, M. J.: Pharmacology of gentamicin, a new broad-spectrum antibiotic. Antimicrobial Agents and Chemotherapy, 138-147, 1963

10. Weinstein, M. J.; Luedemann, G. M.; Oden, E. M.; Wagman, G. H.; Rosselot, J. P.; Marquex, J. A.; Conglio, C. T.; Charney, W.; Herzog, H. L. and Black, J.: Gentamicin, a new antibiotic complex from Micromonospora. J. Med. Chem., 6:463-464, 1963.

11. Cooper, D.; Daniels, P.; Yudis, M.; and Marigliano, J., and in part Guthrie, R. D. and Baukharie, S.: The gentamicin antibiotics, part III. The gross structures of the gentamicin C components. J. Chem. Soc. (C), 3126, 1971.

12. Baggot, J. D.: Principles of Drug Disposition in Domestic Animals: The Basis of Veterinary Clinical Pharmacology. Philadelphia: W. B. Saunders Co., 1977.

13. Gilman, A. G.; Goodman, L. S. and Gilman, A. (ed.): The Pharmacological Basis of Therapeutics, 6th ed. New York: Macmillan Publishing Co., Inc., 1980.

14. Barza, M. and Lauerman, M.: Why monitor serum levels of gentamicin? Clinical Pharmacokinetics, 3:202-215, 1978.

15. Barza, M.; Brown, R. B.; Shen, D.; Gibaldi, M. and Weinstein, L.: Predictability of blood levels of gentamicin in man. J. Infect. Dis., 132:165-174, 1975.

16. Riff, L. J. and Jackson, G. G.: Pharmacology of gentamicin in man. J. Infect. Dis., 142:S98-S105, 1971.

17. Ziv, G. and Sulman, F. G.: Distribution of aminoglycoside antibiotics in blood and milk. Res. Vet. Sci., 17:68-74, 1974.

18. Federspil, P.: Experimentelle Untersuchungen zur Pharmakokinetik des Gentamycins in Liquor und Augenflüssigkeiten. Arzneim.-Forsch., 24(5): 808-814, 1974.

19. Pedersoli, W. M.; Belmonte, A. A.; Purohit, R. C. and Ravis, W. R.: Pharmacokinetics of gentamicin in the horse. Am. J. Vet. Res., 41(3):351-354, 1980.

20. Gordon, B. C.; Regamey, C. and Kirby, W. M. M.: Serum protein binding of aminoglycoside antibiotics. Antimicrob. Agents Chemother., 2:214-216, 1972.

21. Barza, M. and Scheife, R. T.: Antimicrobial spectrum, pharmacology and therapeutic use of antibiotics - part 4: aminoglycosides. Am. J. Hospital Pharm., 34:723-737, 1977.

22. Gyselynck, A. M.; Forrey, A. and Cutler, R.: Pharmacokinetics of gentamicin: distribution and plasma and renal clearance. J. Infec. Disease, 124:S70-S76, 1971.

23. Kaye, D.; Levison, M. E. and Labovitz, E. D.: The unpredictability of serum concentrations of gentamicin: pharmacokinetics of gentamicin in patients with normal and abnormal renal function. J. Infec. Disease, 130(2):150-154, 1974.

24. Ziv, G.; Storper, M.; Wanner, M. and Nicolet, J.: Vergleichende pharmakologische Untersuchungen von Gentamicin and Tobramycin bei neugelborenen Kälbern. Schweiz. Arch. Tierheilk., 122:417-426, 1980.

25. Bush, M.; Smeller, J. M.; Charache, P. and Arthur, R.: Biological half-life of gentamicin in gopher snakes. Am. J. Vet. Res., 139(1):171-173, 1978.

26. Federspel, P.; Schätzyl, W. and Tiesler, E.: Pharmakokinelische, histologische und histochemische Untersuchungen zur Ototoxizität des Gentamicins, Tobramycins und Amikacins. Arch. Oto-Rhino-Laryng., 217:147-166, 1977.

27. Hawkins, E.: Ototoxic mechanisms, a working hypothesis. Audiology, 12:383-393, 1973.

28. Pittinger, C. and Adamson, R.: Antibiotic blockade of neuromuscular function. Annu. Rev. Pharmacol. 12:169-184, 1972.

29. Benzi, G. M.; Arrigoni, E.; Firgo, G. M.; Ferrara, A.; Panceri, P.; Berte', F. and Crema, A.: Antibiotics excreted into the bile - action on the tone and motility of he bile duct. Digestion, 7: 54-73, 1972.

30. Benzi, G. M.; Arrigoni, E. Panceri, P.; Panzarasa, R.; Berte', F. and Crema, A.: Effect of antibiotics on ureter motor activity. Japan J. Pharmacol., 23:599-607, 1973.

31. Luzzatto, L.; Apirion, D. and Schlessinger, D.: Polyribosome depletion and blockage of the ribosome cycle by streptomycin in Escherichia coli. J. Mol. Biol., 42:315-335, 1969.

32. Tai, P. C.; Wallace, B. J. and Davis, B. D.: Streptomycin causes misreading of natural messenger by interacting with ribosomes after initiation. Proc. Natl. Acad. Sci. U.S.A., 75:275-279, 1978.

33. Bryan, L. E. and Van Den Elzen, H. M.: Effects of membrane-energy mutations and cations on streptomycin and gentamicin accumulation by bacteria: a model for E entry of streptomycin and gentamicin in susceptible and resistant bacteria. Antimicrob. Agents Chemother., 12:163-177, 1977.

34. Moellering, R. C. and Weinberg, A. N.: Studies on antibiotic synergism against Enterococci. II. Effect of various antibiotics on the uptake of ^{14}C-labeled streptomycin by Enterococci. J. Clin. Invest., 50:2580-2584, 1971.

35. Bryan, L. E. and Van Den Elzen, H. M.: Streptomycin accumulation in susceptible and resistant strains of E. coli and P. aeruginosa. Antimicrob. Agents Chemother., 9:928-938, 1976.

36. Zimmerman, R. A.; Moellering, R. C., Jr. and Weinberg, A. N.: Mechanism of resistance to antibiotic synergism in Enterocci. J. Bacteriol., 105:873-879, 1971.

37. Novokhatskii, A. S. and Gerasimova, S. S.: Study of the anti-viral effect of gentamicin. Antibiotiki. Ministerstvo Zdravookharaneniia. USSR, 20 (5):428-433, 1975.

38. Castro, A. E.: Personal Interview. Oklahoma Animal Disease Diagnostic Laboratory, Department of Microbiology. Stillwater, Oklahoma, March 15, 1983.

39. Rubenis, M.; Kozig, V. and Jackson, G. G.: Laboratory and pharmacological studies on gentamicin. Antimicrob. Agents Chemother., 153-156, 1963.

40. Strausbaugh, L. J. and Sande, M. A.: Factors influencing the therapy of experimental Proteus mirabilis meningitis. J. Infec. Dis., 137:251-260, 1978.

41. Cameron, J. N.: Body fluid pools, kidney function, and acid-base regulation in the freshwater catfish Ictalurus punctatus. J. Exp. Biol., 86:171-185, 1980.

42. Reeves, R. B.: The interaction of body temperature and acid-base balance in ectothermic vertebrates. A. Rev. Physiol., 39:559-586, 1977.

43. Pennington, J. E.; Dale, D. C.; Reynolds, H. Y. and MacLowry, J. D.: Gentamicin sulfate pharmacokinetics: lower levels of gentamicin in blood during fever. J. Infec. Dis., 132(3):270-275, 1975.

44. Grizzle, J. M. and Rogers, W. A.: Anatomy and Histology of the Channel Catfish. Department of Fisheries and Allied Aquacultures, Auburn University Agricultural Experiment Station. Auburn, Alabama: Auburn Printing, Inc., 1976.

45. Nishimura, H. and Imai, M.: Control of renal function in freshwater and marine teleosts. Federation Proc., 41(8):2355-2360, 1982.

46. Paine, S. and Knight, D. C.: Rapid microbiological assay of antibiotics in blood and other body fluids. Lancet, 2:375-378, 1968.

47. Daigneault, R.; Gagne', M. and Brazeau, M.: A comparison of two methods of gentamicin assay: an enzymatic procedure and an agar diffusion technique. J. Infec. Dis., 130(6):642-645, 1974.

48. Gramarellou, H.; Zimelis, V. M.; Matulionis, D. O. and Jackson, G. G.: Assay of aminoglycoside antibiotics in clinical specimens. J. Infec. Dis., 132(4):399-404, 1975.

49. Broughton, A. and Strong, J. E.: Radioimmunoassay of iodinated gentamicin. Clinica. Chimica Acta, 66:125-129, 1976.

50. (^{125}I)-Gentamicin Second Antibody Buffer System. Antibodies, Inc. RIA Kit, Davis, CA., 1978.

51. Steel, R. G. D. and Torrie, J. H.: Principles and Procedures of Statistics. New York: McGraw-Hill Book Co., Inc., 1960.

52. Cochran, W. G. and Cox, G. M.: Experimental Designs, 2nd ed. New York: John Wiley and Sons, Inc., 1957.

53. Jusko, W. J. and Gibaldi, M.: Effects of change in elimination on various parameters of the two-compartment open model. J. Pharm. Sci., 61:1270-1273, 1972.

54. Nelson, E.: Kinetics of drug absorption, distribution, metabolism, and excretion. J. Pharm. Sci., 50:181-192, 1961.

APPENDIXES

APPENDIX A

RAW DATA

Assay Validity Study

TABLE II

HUMAN SERUM STANDARD %B/B_o'S

%B/B_o's for Standard Lot	Sample Gentamicin Concentration (μg/ml)						
	0	1	2	4	8	16	32
1	100	95.8	86.2	83.9	54.8	45.5	38.0
2	100	88.4	82.5	64.3	53.1	44.4	30.8
3	100	89.9	79.5	65.6	49.8	41.3	29.0
4	100	88.6	78.6	68.3	52.1	40.4	27.4
5	100	91.1	79.9	67.6	54.3	42.8	32.4

TABLE III

CATFISH PLASMA STANDARD %B/B_o'S

%B/B_o's for Standard Lot	Sample Gentamicin Concentration (μg/ml)									
	0	1	2	5	6	10	15	16	30	35
1	100	82.4	71.4	56.2	-	44.1	39.1	-	28.1	-
2	100	81.9	79.3	46.1	-	40.7	37.4	-	28.5	-
3	100	89.2	85.7	-	58.0	45.2	-	39.5	-	28.6
4	100	74.0	63.4	-	47.1	41.4	-	31.0	-	23.7
5	100	84.1	61.2	-	51.3	42.7	-	33.1	-	22.5

- = no value tabulated

Gentamicin Intracardiac Bolus

Pharmacokinetic Study

TABLE IV

INTRACARDIAC BOLUS STUDY RAW DATA

Time Post Injection (min)	Plasma Gentamicin Concentrations, in μg/ml, for catfish									
	E_1C_1	E_1C_2	E_1C_3	E_1C_4	E_1C_5	E_1C_6	E_1C_7	E_1C_8	E_1C_9	E_1C_{10}
15	-	10.6	10.7	61.5	11.2	5.0	4.1	6.7	5.1	24.1
31	23.6	-	-	-	-	-	-	-	-	-
30	-	7.5	8.5	35.2	90.6	5.9	7.0	45.4	8.5	12.8
45	-	16.6	8.0	27.4	50.1	5.7	14.2	15.1	7.5	9.8
46	10.7	-	-	-	-	-	-	-	-	-
60	-	10.5	12.3	19.4	13.8	3.5	4.9	8.7	5.8	8.6
63	8.1	-	-	-	-	-	-	-	-	-
75	-	10.3	13.1	12.5	-	2.9	4.0	8.8	9.2	5.2

TABLE IV

(Continued)

Time Post Injection (min)	Plasma Gentamicin Concentrations, in μg/ml, for catfish									
	E_1C_1	E_1C_2	E_1C_3	E_1C_4	E_1C_5	E_1C_6	E_1C_7	E_1C_8	E_1C_9	E_1C_{10}
78	7.3	-	-	-	-	-	-	-	-	-
90	-	8.2	9.8	12.4	9.6	1.7	3.6	3.4	6.7	8.3
105	-	6.2	8.2	5.7	2.6	1.7	3.0	6.2	4.6	6.5
108	7.7	-	-	-	-	-	-	-	-	-
120	-	5.4	12.9	5.9	4.3	1.8	3.9	3.5	5.2	6.8
124	8.7	-	-	-	-	-	-	-	-	-
140	6.2	-	-	-	-	-	-	-	-	-
150	-	4.5	8.6	6.5	-	1.5	3.2	4.1	5.6	6.8
170	6.1	-	-	-	-	-	-	-	-	-
180	-	5.5	5.5	4.7	4.5	1.4	3.4	4.5	3.9	3.9
185	7.9	-	-	-	-	-	-	-	-	-
210	-	6.0	6.6	2.8	2.0	1.4	2.6	2.5	3.0	5.2

TABLE IV

(Continued)

Time Post Injection (min)	Plasma Gentamicin Concentrations, in μg/ml, for catfish									
	E_1C_1	E_1C_2	E_1C_3	E_1C_4	E_1C_5	E_1C_6	E_1C_7	E_1C_8	E_1C_9	E_1C_{10}
240	-	4.9	6.1	4.0	1.2	1.6	2.6	4.1	3.4	3.9
247	5.2	-	-	-	-	-	-	-	-	-
270	-	3.8	6.0	2.9	5.5	1.3	2.8	2.7	2.4	6.4
277	5.2	-	-	-	-	-	-	-	-	-
300	-	2.4	4.9	3.2	1.8	1.3	2.5	2.2	3.1	4.9
330	-	3.3	4.5	2.5	4.6	0.8	2.3	2.7	3.4	5.1
360	-	3.1	5.2	1.5	-	1.2	2.5	1.8	3.2	6.0
480	-	3.8	-	2.6	2.0	1.0	3.1	-	-	-
540	-	-	-	-	-	-	-	1.9	3.2	4.2
720	-	2.9	2.8	3.0	-	-	-	-	-	-
1005	-	-	-	-	1.6	-	2.1	-	-	-
1320	-	-	-	-	-	-	-	-	-	-

TABLE IV

(Continued)

Time Post Injection (min)	Plasma Gentamicin Concentrations, in μg/ml, for catfish									
	E_1C_1	E_1C_2	E_1C_3	E_1C_4	E_1C_5	E_1C_6	E_1C_7	E_1C_8	E_1C_9	E_1C_{10}
1380	-	1.3	1.7	1.5	-	-	-	-	-	-
1440	1.9	-	-	-	-	-	-	-	-	-
mg/kg Dose*	1.3	1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0	0.9

- = no value tabulated

* Dose of Gentamicin

Gentamicin Intramuscular Bolus

Pharmacokinetic Study

TABLE V

INTRAMUSCULAR DOSAGE STUDY RAW DATA

Time Post Injection (min)	Plasma Gentamicin Concentrations, in μg/ml, for catfish								
	E_2C_1	E_2C_2	E_2C_3	E_2C_4	E_2C_5	E_2C_6	E_2C_7	E_2C_8	E_2C_9
15	4.6	-	-	-	-	-	-	-	-
16	-	0.6	3.2	1.2	4.2	1.7	2.2	4.0	1.0
30	2.6	-	-	-	-	-	-	-	-
32	-	3.8	3.0	4.6	3.8	3.8	0.9	3.5	2.7
48	-	2.2	1.9	4.8	4.4	3.6	1.5	3.1	3.6
50	5.0	-	-	-	-	-	-	-	-
60	1.3	-	-	-	-	-	-	-	-
64	-	3.3	3.8	3.8	3.3	2.8	1.8	3.5	3.1
75	2.9	-	-	-	-	-	-	-	-
80	-	3.7	2.8	2.8	3.7	3.4	1.9	3.2	-
90	1.7	-	-	-	-	-	-	-	-
96	-	2.8	2.6	-	-	4.1	1.7	3.0	2.8
105	3.0	-	-	-	-	-	-	-	-
112	-	3.4	3.1	3.4	2.8	-	-	-	-
120	1.8	-	-	-	-	5.9	2.1	2.6	2.9
135	2.0	-	-	-	-	-	-	-	-
150	1.6	-	-	-	-	2.8	1.7	2.7	-

TABLE V

(Continued)

Time Post Injection (min)	Plasma Gentamicin Concentrations, in μg/ml, for catfish								
	E_2C_1	E_2C_2	E_2C_3	E_2C_4	E_2C_5	E_2C_6	E_2C_7	E_2C_8	E_2C_9
165	1.4	-	-	-	-	-	-	-	-
180	2.1	-	2.8	2.5	3.6	2.7	1.7	2.3	-
195	2.5	-	-	-	-	-	-	-	-
208	-	2.4	2.2	2.3	3.6	-	-	-	-
210	1.8	-	-	-	-	3.0	2.4	-	3.0
225	2.1	-	-	-	-	-	-	-	-
240	2.2	1.6	2.0	2.2	2.5	2.8	1.4	1.7	2.0
268	-	-	2.4	-	3.6	-	-	-	-
270	2.8	-	-	-	-	2.6	2.6	-	1.6
300	-	1.8	2.4	2.7	2.3	2.3	1.8	-	1.5
328	-	1.5	1.6	2.3	2.2	-	-	-	-
330	-	-	-	-	-	1.8	2.1	1.8	2.1
335	3.0	-	-	-	-	-	-	-	-
360	2.4	1.6	2.0	2.6	2.1	1.8	1.7	1.6	1.6
390	1.5	-	-	-	-	2.0	1.0	3.5	1.4
392	-	1.9	1.3	2.6	2.0	-	-	-	-
415	1.4	-	-	-	-	-	-	-	-
420	-	-	-	-	-	1.5	1.4	1.5	1.5
424	-	1.7	1.4	1.8	1.6	-	-	-	-
450	-	-	1.6	2.2	1.9	-	-	-	-

TABLE V

(Continued)

Time Post Injection (min)	Plasma Gentamicin Concentrations, in μg/ml, for catfish								
	E_2C_1	E_2C_2	E_2C_3	E_2C_4	E_2C_5	E_2C_6	E_2C_7	E_2C_8	E_2C_9
480	-	1.6	1.9	1.8	-	1.9	1.2	2.2	1.5
540	-	1.4	1.8	1.9	-	2.5	1.2	1.5	1.5
660	-	1.2	2.0	1.8	2.6	-	-	-	-
855	1.0	-	-	-	-	-	-	-	-
1080	-	-	-	-	-	1.2	0.6	0.9	0.9
1380	-	0.7	1.0	1.0	1.2	-	-	-	-
1665	0.9	-	-	-	-	-	-	-	-
Gentamicin Dose (mg/kg)	1.8	1.0	1.0	1.0	0.9	1.1	1.0	1.0	1.1

- = no value tabulated

Gentamicin Tank Dosage

Pharmacokinetic Study

TABLE VI

TANK DOSAGE STUDY PLASMA GENTAMICIN CONCENTRATION RAW DATA

Time Post Injection (min)	Plasma Gentamicin Concentrations, in μg/ml, for catfish									
	E_3C_1	E_3C_2	E_3C_3	E_3C_4	E_3C_5	E_3C_6	E_3C_7	E_3C_8	E_3C_9	E_3C_{10}
15	*	-	-	-	-	-	-	-	-	-
30	*	-	-	-	-	-	-	-	-	-
45	*	-	-	-	-	-	-	-	-	-
60	*	-	-	-	-	-	-	-	-	-
75	*	-	-	-	-	-	-	-	-	-
90	*	-	-	-	-	-	-	-	-	-
105	*	*	*	*	*	*	1.1	-	-	-
120	*	-	-	-	-	-	-	1.2	1.0	*

TABLE VI

(Continued)

Time Post Injection (min)	Plasma Gentamicin Concentrations, in μg/ml, for catfish									
	E_3C_1	E_3C_2	E_3C_3	E_3C_4	E_3C_5	E_3C_6	E_3C_7	E_3C_8	E_3C_9	E_3C_{10}
135	*	-	-	-	-	-	-	-	-	-
150	*	-	-	-	-	-	-	-	-	-
165	*	-	-	-	-	-	-	-	-	-
180	*	*	*	*	*	*	1.4	*	*	1.4
195	*	-	-	-	-	-	-	-	-	-
210	*	-	-	-	-	-	-	-	-	-
225	*	-	-	-	-	-	-	-	-	-
240	*	*	*	*	*	*	2.2	*	1.6	1.2
270	*	-	-	-	-	-	-	-	-	-
300	*	*	*	*	*	-	1.3	-	1.3	1.2
330	*	-	-	-	-	-	-	-	-	-
360	-	*	*	*	*	*	1.1	-	*	3.1

TABLE VI

(Continued)

Time Post Injection (min)	Plasma Gentamicin Concentrations, in μg/ml, for catfish									
	E_3C_1	E_3C_2	E_3C_3	E_3C_4	E_3C_5	E_3C_6	E_3C_7	E_3C_8	E_3C_9	E_3C_{10}
390	*	-	-	-	-	-	-	-	-	-
420	*	*	1.2	*	*	*	1.1	-	4.0	1.6
450	4.5	-	-	-	-	-	-	-	-	-
480	4.2	*	*	1.4	*	*	1.4	-	1.1	1.6
510	3.5	-	-	-	-	-	-	-	-	-
546	-	*	4.1	*	*	*	1.3	-	*	1.6
600	-	*	*	1.0	2.8	*	1.5	-	*	1.5
630	2.4	-	-	-	-	-	-	-	-	-
660	-	*	*	1.3	*	*	1.3	-	*	-
720	-	*	-	*	*	*	1.2	-	*	-
750	*	-	-	-	-	-	-	-	-	-
780	-	-	-	-	*	*	1.4	-	*	-

TABLE VI

(Continued)

Time Post Injection (min)	Plasma Gentamicin Concentrations, in μg/ml, for catfish									
	E_3C_1	E_3C_2	E_3C_3	E_3C_4	E_3C_5	E_3C_6	E_3C_7	E_3C_8	E_3C_9	E_3C_{10}
810	-	*	-	1.0	-	-	-	-	-	-
1020	-	*	-	*	*	-	1.3	-	-	-
1380	-	*	-	4.3	-	-	-	-	-	-
1470	-	-	-	-	1.3	-	1.1	-	-	-
1820	*	-	-	-	-	-	-	-	-	-

* = value < 1.0 μg/ml

- = no value tabulated

TABLE VII

TANK DOSAGE STUDY GENTAMICIN WATER CONCENTRATION RAW DATA

Time Post Injection (min)	Catfish Group Exposed; Values in μg/ml			
	E_3C_1	E_3C_2, C_3, C_4	E_3C_5, C_6, C_7	E_3C_8, C_9, C_{10}
0	9.3	-	7.8	6.6
40	-	11.4	-	-
60	6.2	-	7.8	-
85	-	6.5	-	-
105	-	-	7.1	-
120	8.8	-	-	6.2
160	-	7.0	-	-
180	9.7	-	8.9	6.1
220	-	6.0	-	-
240	7.9	-	6.1	7.0
280	-	6.5	-	-
300	7.2	-	-	6.2
340	-	15.5	-	-
360	7.2	-	6.8	6.8
400	-	7.7	-	-
420	8.7	-	-	6.6
460	-	7.2	-	-
480	-	-	7.0	6.8
510	5.7	-	-	-
540	-	-	-	6.8
550	-	-	7.2	-
580	-	7.3	-	-

TABLE VII

(Continued)

Time Post Injection (min)	Catfish Group Exposed; Values in μg/ml			
	E_3C_1	E_3C_2, C_3, C_4	E_3C_5, C_6, C_7	E_3C_8, C_9, C_{10}
600	-	-	6.4	6.1
630	8.2	-	-	-
640	-	6.8	-	-
660	-	-	6.7	5.4
700	-	7.1	-	-
720	-	-	6.8	6.3
750	5.6	-	-	-
780	-	-	6.1	6.8
790	-	9.4	-	-
1000	-	5.4	-	-
1020	-	-	10.7	-
1360	-	6.4	-	-
1470	-	-	6.4	-
1620	6.8	-	-	-

- = no value tabulated

APPENDIX B

DERIVED DATA

Assay Validity Study

TABLE VIII

SAMPLE REGRESSION COEFFICIENTS

	Y_{xo}	-m	r
Human Serum Standard Lot #1	98.67	40.93	0.972
Human Serum Standard Lot #2	90.12	39.25	0.994
Human Serum Standard Lot #3	90.25	41.28	0.997
Human Serum Standard Lot #4	90.43	41.46	0.998
Human Serum Standard Lot #5	91.21	39.68	0.999
Catfish Plasma Stan. Lot #1	82.24	37.00	1.000
Catfish Plasma Stan. Lot #2	82.90	39.44	0.965
Catfish Plasma Stan. Lot #3	92.32	42.82	0.989
Catfish Plasma Stan. Lot #4	73.50	33.23	0.997
Catfish Plasma Stan. Lot #5	79.27	37.43	0.984

TABLE IX

MEAN REGRESSION COEFFICIENTS

	$\bar{Y}_{xo}$	S_y	S_y^2	n	df	$-\bar{m}$	S_m	S_m^2	n	df
Human Serum Standards	92.34	3.574	12.78	5	4	40.52	0.993	0.99	5	4
Catfish Plasma	82.05	6.838	46.77	5	4	37.98	3.514	12.35	5	4

TABLE X

DATA PAIRS DERIVED FROM MEAN REGRESSION LINES

	x (μg/ml)	y (%B/B_o)		x (μg/ml)	y (%B/B_o)
Human Serum Standards	0	-	Catfish Plasma Standards	0	-
	1	92.34		1	82.05
	2	80.14		2	70.62
	4	67.94		4	59.18
	8	55.75		8	47.75
	16	43.55		16	36.32
	32	31.35		32	24.88
$\%B/B_o = -40.52(\log x) + 92.34$			$\%B/B_o = -37.98(\log x) + 82.05$		

TABLE XI

CALCULATED GENTAMICIN CONCENTRATIONS AND DIFFERENCES BETWEEN LINE HS* AND LINE CP* VALUES

$\%B/B_o$	Line HS Calculated Gentamicin Concentration (μg/ml)	Line CP Calculated Gentamicin Concentration (μg/ml)	Difference (μg/ml)
90	0	0	0
80	1.06	1.83	0.77
70	3.12	3.36	0.24
60	5.50	6.16	0.66
55	7.31	8.35	1.04
50	9.71	11.30	1.59
40	17.14	20.72	3.58

*HS = Human Serum Standard Regression Line:

$$\%B/B_o = -40.52(\log x) + 90$$

*CP = Catfish Plasma Standard Regression Line:

$$\%B/B_o = -37.98(\log x) + 90$$

TABLE XII

PHARMACOKINETIC PARAMETERS FROM THE INTRACARDIAC BOLUS STUDY

Catfish I.D.	C_p^o (μg/ml)	A (μg/ml)	α (min^{-1})	B (μg/ml)	β (min^{-1}	K_{12} (min^{-1})	K_{21} (min^{-1})	K_{el} (min^{-1})	Time Ending $\alpha+\beta$ Phase (min)
E_1C_1	339.41	331.47	0.0995	7.93	0.0010	0.0668	0.0033	0.0304	100
E_1C_2	15.44	10.01	0.0170	5.42	0.0010	0.0088	0.0067	0.0026	180
E_1C_3	9.56	2.07	0.0036	7.49	0.0011	0.0015	0.0026	0.0016	210
E_1C_4	98.94	94.89	0.0319	4.05	0.0008	0.0187	0.0020	0.0120	150
E_1C_5	43.51	40.21	0.0160	3.30	0.0007	0.0087	0.0019	0.0062	180
E_1C_6	14.69	12.77	0.0440	1.92	0.0015	0.0290	0.0070	0.0094	180
E_1C_7	7.59	4.35	0.0175	3.24	0.0005	0.0092	0.0078	0.0011	120
E_1C_8	22.54	18.82	0.0304	3.72	0.0011	0.0200	0.0059	0.0055	180
E_1C_9	7.10	2.93	0.0048	4.17	0.0007	0.0013	0.0031	0.0011	150
E_1C_{10}	34.93	28.66	0.0424	6.27	0.0007	0.0312	0.0082	0.0038	110
Mean Value	59.37	54.62	0.0307	4.85	0.0009	0.0195	0.0048	0.0074	156
Standard Deviation	102.20	101.18	0.0280	1.96	0.0003	0.0196	0.0025	0.0089	36

TABLE XIII

CALCULATED VALUES FROM THE INTRACARDIAC BOLUS STUDY

Catfish I.D.	$t_{\frac{1}{2}}(\alpha)$ (min)	$t_{\frac{1}{2}}(\beta)$ (min)	V_c (ml/kg)	Cl_B (ml/min/kg)	$V_{d(area)}$ (ml/kg)	$V_{d(B)}$ (ml/kg)
E_1C_1	6.97	684.03	3.83	0.1164	114.83	163.84
E_1C_2	40.66	669.51	64.78	0.1716	165.75	184.33
E_1C_3	191.89	611.07	104.62	0.1657	146.15	133.48
E_1C_4	21.70	905.59	10.11	0.1210	158.19	247.05
E_1C_5	43.29	958.02	20.68	0.1273	176.01	273.04
E_1C_6	15.77	458.63	68.08	0.6412	424.36	521.52
E_1C_7	39.58	1566.15	131.68	0.1405	297.35	308.11
E_1C_8	22.83	650.68	44.35	0.2430	228.15	268.63
E_1C_9	143.08	946.43	140.76	0.1586	216.59	239.58
E_1C_{10}	16.32	938.80	25.76	0.0980	132.92	143.48
Mean Value	54.21	838.89	61.46	0.1983	212.04	248.31
Standard Deviation	61.96	282.30	49.80	0.1608	96.92	112.90
Value Calculated from Table XIII Mean Values	22.58	770.16	17.01	0.1259	156.56	208.25

TABLE XIV

CALCULATED DOSAGE AND COMPARTMENT CONCENTRATION VALUES FROM THE INTRACARDIAC BOLUS STUDY

Catfish I.D.	D_i (mg/kg)	D_m (mg/kg)	τ (hr)	A_C (μg/ml)	A_T (μg/ml)
E_1C_1	1.38	1.15	29.47	0.005	0.147
E_1C_2	1.99	1.66	28.84	0.058	0.091
E_1C_3	1.75	1.46	26.33	1.006	2.046
E_1C_4	1.90	1.58	39.02	0.007	0.100
E_1C_5	2.11	1.76	41.27	0.011	0.085
E_1C_6	5.09	4.24	19.76	0.022	0.114
E_1C_7	3.57	2.97	63.16	0.071	0.090
E_1C_8	2.74	2.28	28.03	0.028	0.114
E_1C_9	2.60	2.16	40.77	0.098	0.053
E_1C_{10}	1.60	1.33	40.45	0.027	0.112
Mean Value	2.43	2.06	35.71	0.133	0.295
Standard Deviation	1.12	0.93	12.16	0.308	0.616
Value Calculated from Tables XII & XIV Mean Values	1.88 (2.50†)	1.56 (2.08†)	33.18	0.041 (0.054†)	0.205 (0.273†)

† Calculated using $V_{d(B)}$ rather than $V_{d(area)}$

TABLE XV

CALCULATED PLASMA GENTAMICIN CONCENTRATIONS AT GIVEN TIME INTERVALS AFTER INTRACARDIAC BOLUS ADMINISTRATION

Catfish I.D.	Dose (mg/kg)	C_p^{240min} (μg/ml)	C_p^{360min} (μg/ml)	C_p^{480min} (μg/ml)	C_p^{720min} (μg/ml)	$C_p^{1440min}$ (μg/ml)
E_1C_1	1.3	6.22	5.51	4.88	3.82	1.84
E_1C_2	1.0	4.40	3.76	3.30	2.57	1.22
E_1C_3	1.0	5.71	4.98	4.35	3.31	1.46
E_1C_4	1.0	3.41	3.07	2.80	2.33	1.34
E_1C_5	0.9	3.63	2.67	3.45	1.96	1.16
E_1C_6	1.0	1.33	1.11	0.93	0.65	0.22
E_1C_7	1.0	2.96	2.74	2.59	2.31	1.64
E_1C_8	1.0	2.90	2.54	2.23	1.73	0.80
E_1C_9	1.0	4.42	3.72	3.22	2.55	1.46
E_1C_{10}	0.9	5.26	4.81	4.40	3.69	2.17
Mean Value	1.01	4.02	3.49	3.22	2.49	1.33
Standard Deviation	0.11	1.48	1.34	1.17	0.95	0.54
Value Calculated from Table XII Mean Values		3.94	3.51	3.15	2.53	1.33

TABLE XVI

PHARMACOKINETIC PARAMETERS AND CALCULATED VALUES FROM THE INTRAMUSCULAR DOSAGE STUDY

Catfish I.D.	B (μg/ml)	β (min^{-1})	$t_{\frac{1}{2}(\beta)}^{*}$ (min)	Cl_B^{*} (ml/min/kg)	$V_{d(B)}^{IM}$ (ml/kg)	D_i^{*} (mg/kg)	D_m^{*} (ml/kg)	τ^{*} (hr)
E_2C_1	2.51	0.0007	946.27	0.2915	397.98	4.78	3.92	40.77
E_2C_2	2.49	0.0010	718.16	0.3880	402.09	4.82	4.07	30.94
E_2C_3	2.77	0.0008	817.28	0.3058	360.64	4.33	3.53	35.21
E_2C_4	3.14	0.0008	809.45	0.2729	318.77	3.82	3.18	34.87
E_2C_5	3.54	0.0009	745.65	0.2365	254.48	3.06	2.52	32.12
E_2C_6	3.37	0.0011	653.18	0.3467	326.78	3.92	3.31	28.14
E_2C_7	2.00	0.0009	756.68	0.4589	501.05	6.01	4.98	32.60
E_2C_8	3.31	0.0013	538.01	0.3889	301.89	3.62	3.03	23.18
E_2C_9	2.57	0.0010	665.09	0.4463	428.37	5.14	4.22	28.65
Mean Value	2.86	0.0009	538.86	0.3484	365.78	4.39	3.64	31.83
Standard Deviation	0.51	0.0002	116.22	0.0777	74.83	0.90	0.74	5.01

TABLE XVI

(Continued)

Catfish I.D.	B (μg/ml)	β (min^{-1})	$t_{1/2(\beta)}^{*}$ (min)	Cl_B^{*} (ml/min/kg)	$V_{d(B)}^{IM}$ (ml/kg)	D_i^{*} (mg/kg)	D_m^{*} (ml/kg)	τ^{*} (hr)
Value Calculated from Table XVI B and β Values			770.16	0.3182	353.54	4.24 (3.19†)	3.54 (2.66†)	33.18

† Calculated from $V_{d(area)}^{*}$ rather than $V_{d(B)}^{IM}$;

$$V_{d(area)}^{*} = \frac{V_{d(area)} \cdot V_{d(B)}^{IM}}{V_{d(B)}} = 2.65.79 \text{ ml/kg}$$

TABLE XVII

CALCULATED PLASMA GENTAMICIN CONCENTRATIONS AT GIVEN TIME INTERVALS AFTER INTRAMUSCULAR DOSE ADMINISTRATION

Catfish I.D.	Dose (mg/kg)	C_p^{240min} (μg/ml)	C_p^{360min} (μg/ml)	C_p^{480min} (μg/ml)	C_p^{720min} (μg/ml)	$C_p^{1440min}$ (μg/ml)
E_2C_1	1.0	2.11	1.93	1.77	1.48	0.88
E_2C_2	1.0	1.97	1.76	1.56	1.24	0.62
E_2C_3	1.0	2.26	2.04	1.84	1.50	0.82
E_2C_4	1.0	2.55	2.30	2.08	1.69	0.91
E_2C_5	0.9	2.83	2.53	2.26	1.81	0.93
E_2C_6	1.1	2.61	2.30	2.02	1.57	0.73
E_2C_7	1.0	1.60	1.44	1.28	1.03	0.53
E_2C_8	1.0	2.43	2.08	1.78	1.31	0.52
E_2C_9	1.1	2.00	1.76	1.56	1.21	0.57
Mean Value	1.01	2.26	2.02	1.79	1.43	0.72
Standard Deviation	0.06	0.38	0.34	0.30	0.25	0.17
Value Calculated from Table XVI Mean Values		2.30	2.07	1.86	1.50	0.78

TABLE XVIII

GENTAMICIN WATER CONCENTRATIONS EXTRAPOLATED FROM TANK DOSAGE STUDY RAW DATA

Group of Catfish Exposed to Tank Dosage	Regression Line Developed from Water Assay Raw Data	Correlation Coefficient	Gentamicin Concentration (μg/ml) at t=				
			240 min	360 min	480 min	720 min	1440 min
E_3C_1	$d=\log^{-1}(-0.00007(t)+8\mu g/ml)$	0.31	7.7	7.6	7.4	7.1	6.3
E_3C_2, E_3C_3, E_3C_4	$d=\log^{-1}(-0.0001(t)+8.4\mu g/ml)$	0.29	8.0	7.8	7.6	7.2	6.2
E_3C_5, E_3C_6, E_3C_7	$d=\log^{-1}(-0.00002(t)+7.3\mu g/ml)$	0.09	7.25	7.22	7.2	7.1	7.0
E_3C_8, E_3C_9, E_3C_{10}	$d=\log^{-1}(-0.00002(t)+6.5\mu g/ml)$	0.15	6.5	6.4	6.4	6.3	6.1

d = gentamicin tank dose in μg/ml

t = time in minutes

VITA

Mark Duane Setser

Candidate for the Degree of

Master of Science

Thesis: THE PHARMACOKINETICS OF GENTAMICIN IN CHANNEL CATFISH (ICTALURUS PUNCTATUS)

Major Field: Physiological Sciences

Biographical:

Personal Data: Born in Tulsa, Oklahoma, August 8, 1954, the son of Ralph and Joan Setser.

Education: Graduated from Nathan Hale High School, Tulsa, Oklahoma, in May, 1973; received Bachelor of Science degrees in Physiology and Zoology from Oklahoma State University in 1977; received Doctor of Veterinary Medicine degree from Oklahoma State University in 1982; completed requirements for the Master of Science degree at Oklahoma State University in May, 1983.

Professional Organizations: American Veterinary Medical Association; Tulsa County Veterinary Medical Association; American Association of Zoo Veterinarians; Association of Avian Veterinarians; Oklahoma Herpetological Society; American Society of Veterinary Physiologists and Pharmacologists.

Professional Experience: Graduate Teaching Assistant, Department of Physiology, Oklahoma State University, 1977-78; Graduate Teaching Assistant, School of Biological Sciences, Oklahoma State University, 1979; Curator for Herpetarium, School of Biological Sciences, Oklahoma State University, 1979-81; Research Fellow, Department of Physiology, Oklahoma State University, 1980; Veterinarian, Alta View Animal Hospital, 1982-

present; Guest Lecturer, College of Veterinary Medicine, Oklahoma State University, 1983.