THE PHARMACOKINETICS OF SULFACHLOR-PYRIDAZINE IN CHANNEL CATFISH (*ICTALURUS PUNCTATUS*)

BY

FEREIDON KHAN ALAVI

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Thesis Approved:

Lester L. Rolf Jr
Thesis Adviser

Subhash Samjiab

George E. Bowen

Norman N. Durham
Dean of the Graduate College

PREFACE

Fish culture, a growing industry, has increased the demand for therapeutic agents. Due to the lack of pharmacokinetic studies in fish, the use of effective antibacterial agents are either based on studies in other animals or prohibited by FDA. This study was undertaken to gain an understanding of the pharmacokinetics of the antimicrobial agent, sulfachlorpyridazine (SCP) in fish. Comparison of the pharmacokinetics of this drug with other well studied groups of animal were expected to provide evidence for or against the potential usefulness of SCP as a therapeutic agent for catfish.

This study was supported by professional developmental money from the College of Veterinary Medicine to Dr. Lester Rolf. Without his generous help and advice, this study would have never materialized. Thanks are extended to Dr. G. Burrows and Dr. S. Sangiah for their informative support and advice. The auther deeply appreciates the kind assistance of Drs. G. Gebhart and C. Kleinholz of the Oklahoma Cooperative Fish and Wild Life Research Unit in obtaining the catfish for this study. Many thanks to Dr. B. Lessley for his helpfull instruction in using the word processor. Graditude for kind moral support of dear friends Hamid Amouzadeh, Mary Bober and Michael Smith is expressed.

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A	Y-intercept of the alpha phase regression line (μg/ml)
AUC	area under the curve for concentration of drug in blood as a function of time measured by the trapezoid method (μg.min/ml)
α	apparent first order rate constant for distribution of drug in body calculated by equation (2), (min^{-1})
α phase	also known as distribution phase and applies to the portion of the semi-logarithmic graph during which the drug is being distributed in the body
B	Y-intercept of beta phase regression line (μg/ml)
β	apparent first order rate constant for elimination of drug from body calculated by equation (3), (min^{-1})
β phase	also known as elimination phase and applies to the portion of the semi-logarithmic graph during which the drug is being being eliminated from the body
$Cl_{B(area)}$	total body clearance, calculated by equation (10), (ml/min/kg)
$Cl_{B(\beta)}$	total body clearance, calculated by equation (11), (ml/min/kg)
C°_{P}	total (A+B) theoretical concentration of a drug in body at zero time (μg/ml)
Ic	abbreviation for intracardiac

k_{12}	first order rate constant for the movement of drug from central compartment (blood) into the tissue calculated by equation (6), (min^{-1})
k_{21}	first order rate constant for movement of drug from tissue to central compartment calculated by equation (4), (min^{-1})
k_{el}	first order rate constant for elimination of drug from the central compartment calculated by equation (5), (min^{-1})
Or	abbreviation for oral
P_{tn}	plasma concentration at the period immedialely before tn
SCP	abbreviation for sulfachlorpyridazine
$t_{½\alpha}$	the time required to reduce a given drug concentration to half it's value during the distribution phase calculated by equation (2), (min)
$t_{½\beta}$	the time required to reduce a given drug concentration to half it's value during the elimination phase calculated by equation (3), (min)
T_{tn}	tissue concentration at a specific time, tn
T_{tn-1}	tissue concentration at the period immediately before tn
Vc	volume of central compartment calculated by equation (7), (ml/kg)
$Vd_{(area)}$	apparent volume of distribution, calculated by equation (8), (ml/kg)
$Vd_{(\beta)}$	apparent volume of distribution, calculated by equation (9), (ml/kg)

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Antibacterials in Teleosts

Fish health is affected by a number of pathogenic microorganisms. Some of the most debilitating of these microorganisms are gram negative bacteria of the genera Aeromonas, Vibrio, Pseudomonas, Myxobacteria (Kingsford, 1975; Conroy, 1963; Van Duijan, 1973; Herman, 1970), Hemophilus and Edwardsiella (Aoki and Kitao, 1981). These bacteria are responsible for the common fish diseases, furunculosis, ulcer, cotton wool and tail or fin rot (Snieszko, et al., 1974; Van Duijan, 1973). Treatment of non-food fish for the above diseases with sulfonamides, tetracyclines, aminoglycosides and chloramphenicol has proved to be effective and is recommended accordingly (Kingsford, 1975; Herman, 1970).

Growing public concern about the possible unknown effects of drugs in fish intended for human consumption, has limited the use of various antibacterial agents. As more research and evaluation of antibiotics are carried out, an increasing number of these therapeutic agents may be approved by the United States Food and Drug Adminis-

tration (FDA) for use in aquaculture. At present, the FDA permits the use of salt, acetic acid, formalin, sulfamerazine and tetracycline in certain diseases of trout, salmon and catfish (Siderman, 1970; Snieszko, et al., 1974).

The most efficient and practical method of treatment of a large number of fish in aquaculture is by water treatment or medicated feed (Herman, 1970; Conroy, 1963; Herwing, 1979). This technique requires good water solubility, thermal stability and oral bioavailability of the theraputic agent. Water treatment tends to have a prophylactic effect on healthy fish, as well as the prevention of further progress of the disease in the unhealthy fish, allowing time for immunological defense mechanism activation. A theraputic agent, that meets at least some of these criteria, is sulfachlorpyridazine (SCP) In addition, very desirable pharmacokinetic parameters have been demonstrated in various animal species (Romvary and Horvay, 1976a, 1976b; Friis, et al., 1980). Sulfachlorpyridazine is one of the most effective sulfonamides for use in gram negative bacterial disease conditions (Neipp and Mayer, 1957; Domagk, 1957; Archibald, and McKay, 1964). It is highly water soluble and possesses a high therapeutic index. These characteristics rank SCP among the better antibacterial agents for treatment of gram negative bacterial disease in aquatic and non-aquatic animals. Rational treatment requires an appropriate pharmacokinetic study of that drug in that particular animal. Inter and

intraphylatic differences make it not only unreasonable but potentially dangerous to extrapolate pharmacokinetic parameters of an individual drug or class of agents. The objectives of this research were desiged to evaluate the following: (a) Bioavailability of orally administered SCP, (b) Variables of tissue and plasma disposition kinetics, (c) Protein and tissue binding of SCP and (d) Advantages of availability of kinetic parameters for application of SCP as a possible therapeutic agent in the treatment of channel catfish.

In 1932, the therapeutic value of prontosil, an azo dye was discoverd by German scientists (Struller, 1968). Subsequent research at the Pasture Institute showed that an in vivo metabolite of prontosil, para-aminobenzene-sulfonamide (Hawking, et al., 1950; Haviga, et al., 1946) was the molecule responsible for therapeutic efficacy. Shortly thereafter, recognition of the therapeutic value of sulfonamides was responsible for the initiation of research efforts that lead to the development of more active sulfonamides. The objectives of such research were development of an ideal sulfonamide, one that possesses: high efficacy, near complete oral bioavailability, high water solubility at neutral pH and low toxicity. These efforts lead to the synthesis of over 8000 derivitives of sulfanilamide (Northy, 1948; Archibald and McKay, 1964). At present, only eight or nine are marketed for human and veterinary use. The demonstration of a synergistic action

of sulfonamides with trimethoprim has brought about a resurgence in world wide use of sulfonamides during the last 15 years (Rosselet, et al., 1981; Miller and Greenbalt, 1979). Some of the sulfonamides used to effectively treat cotton wool disease, red mouth disease and ulcer diseases in fish are sulfamethzine, sulfisoxazole and sulfamerazine (Van Duijan, 1973; Snieszko, et al., 1974).

The variable pKa's of sulfonamides play an important role in their rates of elimination and nephrotoxicity (Notari, 1980). Sulfonamides, being weak acids, ionize in basic solutions. Ionization can decrease their reabsorption by the tubules in the kidney, which will increase the elimination rate and reduce the nephrotoxicity potential of the drug. Crystaluria, one of the primary potential problems associated with sulfonamide usage, occurs due to reduced solubility of the parent compound and it's N-4 acetylated (see figure 1) metabolite. To reduce precipitation, alkalinization of urine and intake of large quantities of water have been recommended (Goodman, et al., 1980; Gottschalk, et al., 1960).

Pharmacology of Sulfachlorpyridazine

Sulfachlorpyridazine, N(6-chloro-3-pyridazinyl)benzensulfanilamide, a drivitive of sulfanilamide, has a pKa of 5.9 (Wiseman, 1965; Struller, 1968; Nielsen and Rasmussen, 1977). The elimination half life of SCP varies from species to species. The reported half life in cattle, geese,

piglets and humans is 1.16, 1.89, 3.01 and 8 hours respectively (Nielsen and Rasmussen, 1977; Romvary and Horvay, 1976a, 1976b; Friis, et al., 1980). In one study a half life of 11.5 hours for normal adult men is indicated (Jones and Finland, 1975). Sulfachlorpyridazine is considered to be a short acting sulfonamide because of rapid renal clearance due to tubular secretion and glomerular filtration (Siderman, 1970; Friis, et al., 1980). Clearance of unbound drug by tubular secretion is noted to be six times faster than the clearance by way of glomerular filtration (Edelbeck Fredeuksen and Rasmussen, 1964).

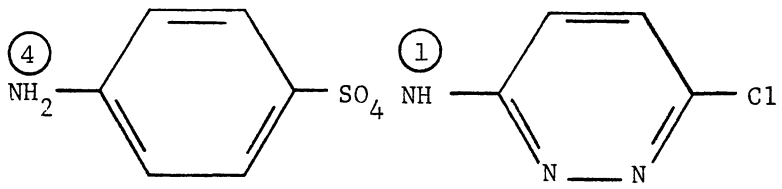

Figure 1. Chemical Structure of Sulfachlorpyridazine.

The antibacterial activity of SCP and other sulfonamides, resides in the sulfanilamide portion which resembles p-aminobenzoic acid (Figure 1). The 6-chloro-3-pyriazinyl side group of SCP determines the specific solubility, pKa and toxicity of this agent. Low pKa and high water solubility of SCP appear to decrease it's elimination half life.

The high water solubility may be partialy responsible for the reduced potential of nephrotoxicity when recommended doses are administered. The manufacturer's recommended intravenous dose of SCP (Vetisulid®*) for cattle and swine is 65-100 mg/kg and 45-75 mg/kg respectively, for treatment of collibacilosis. Variation in the recommended dose are based upon the animal species and microorganism relationships.

At present, most of the information on the pharmacokinetic parameters of SCP is derived from studies that have been conducted on mammals. Since blood, interstitial fluid and urine pH of some fish may be quite different from those of mammals, extrapolation from human and other mammals to fish is unwise. These differnces as well as differences in cardiovascular dynamics, tissue vascularity, metabolism, gill elimination, glomerular filtration rates, protein and tissue binding may have important effects on the rate of elimination, tissue distribution and therefore efficacy of sulfachlorpyridazine in fish (Nielsen and Rasmussen, 1977; Hoar, 1969a).

Metabolism

In mammals, sulfonamides undergo variable metabolic alteration especially in the liver. The primary metabolites

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*Vetisulid® is the tradmark for the sodium salt solution of sulfachlorpyridazine prepared by E.R.Squibb & Sons, Inc. Princeton, NJ 08540.

are N-4 acetylated derivitives (see Figure 1), many of which are less water soluble than the parent compound and pharmacologically inert. Acetylation is the result of conjugation with acetylcoenzyme A under the influence of the proper acetyltransferase. From the studies done on the metabolism of sulfonamides by rabbit liver, it has been concluded that the acetylation reaction takes place in the reticuloendothelial cells rather than the paranchymal cells of the liver and other organs (Govier, 1965). The short half life of SCP may lower the chance of acetylation. In man, SCP is reported to be acetylated to the extent of 20% and exhibits a half life of 8 hours, the longest of any species reported (Wolf, 1979). Sulfachlorpyridazine is considered to be less nephrotoxic than most other sulfonamides as these metabolites are very water soluble with little chance of crystaluria (Sieiro and Ameier, 1973).

A second major metabolite is the N-4 glucuoronide. The glucuoronic acid is transferred by glucuronyl transferase to the N-4 position of sulfonamides (Figure 1) in the liver. The metabolite is excreted into the bile and finally into the gastrointestinal tract, where it may be hydrolized by gastrointestinal microflora and excreted as such or may be reabsorbed (the unionized form) back into the systemic circulation as the active drug. The hydroxylation and N-1 acetylation products are pharmacologically inactive but of minor quantitive importance with respect to N-4 acetylation and glucuronidation.

Mechanism of Action and Bacterial Resistance

Investigations, as early as the 1940's, postulated that many chemotherapeutic agents produce their action through effects on essential bacterial metabolites. The resemblance between sulfanilamide and para-aminobenzoic acid (PABA), led to the theory that the two compounds must be competing for a critical enzymatic site and inhibition of bacterial growth resulted from reduced enzymatic activity (Hawking and Lawrance, 1950; Northy, 1948). This theory was strengthened by the discovery of folic acid which contains PABA as a portion of it's molecular structure. Bacteria synthesize reduced folic acid (tetrahydrofolic acid). The process starts by condensation of PABA with dihydropteridine and glutamic acid to form dihydrofolic acid, which then becomes reduced to tetrahydrofolic acid. Sulfonamides present in the environment, can competitively inhibit the synthesis of the dihydrofolic acid, therefore preventing formation of the coenzyme, tetrahydrofolic acid. Inhibition of the coenzyme by sulfonamides can be antagonized by minute quantities of PABA: thus inhibition can be abolished by as little as 10^{-4} moles of PABA per mole of sulfonamide (Struller, 1968). Reduced quantities or a complete absence of folic acid prevents one carbon transfer and subsequent nucleic acid synthesis by bacteria. This inhibitory process leads to a bacteriostatic rather than a bacteriocidal action on sensitive organisms. Since host

tissues acquire preformed folic acid from the environment, they are not affected by sulfonamides in this respect. With the production of refractive isoenzymes or mutations, the bacterial cells may acquire the capability of by-passing this "blockade" and developing partial or complete resistance.

Physiochemical Properties of Sulfachlorpyridazine

Passage of SCP and other chemicals across membranes, a lipid bilayer, is dependent on the lipid solubility and availability of a nonionized form of the chemical. The concentration of the nonionized portion depends on the pH of the environment and the pKa of the specific sulfonamide (Schanker, et al., 1957). Sulfachlorpyridazine as the sodium salt, is soluble to the extend of 20 % in water at a pH of 7.9. In a bufferd medium with a pH range of 6.5 to 7.0 and temperature of 37°C sulfachlorpyridazine is 25 times more soluble than sulfadiazine (Neip and Mayer, 1957) Lipid solubility can be measured by the partitioning of a chemical between an aqueous and oil phase. Frequently, 1-butanol is used as a representative of the microbial membrane bilayer. The nonionized portion of the drug will enter the oil phase. The relationship between the lipid solubility and the half life of several sulfonamides has been studied (Rieder, 1963; Schanker, et al., 1957). It is evident that long acting sulfonamides are in general more lipid soluable

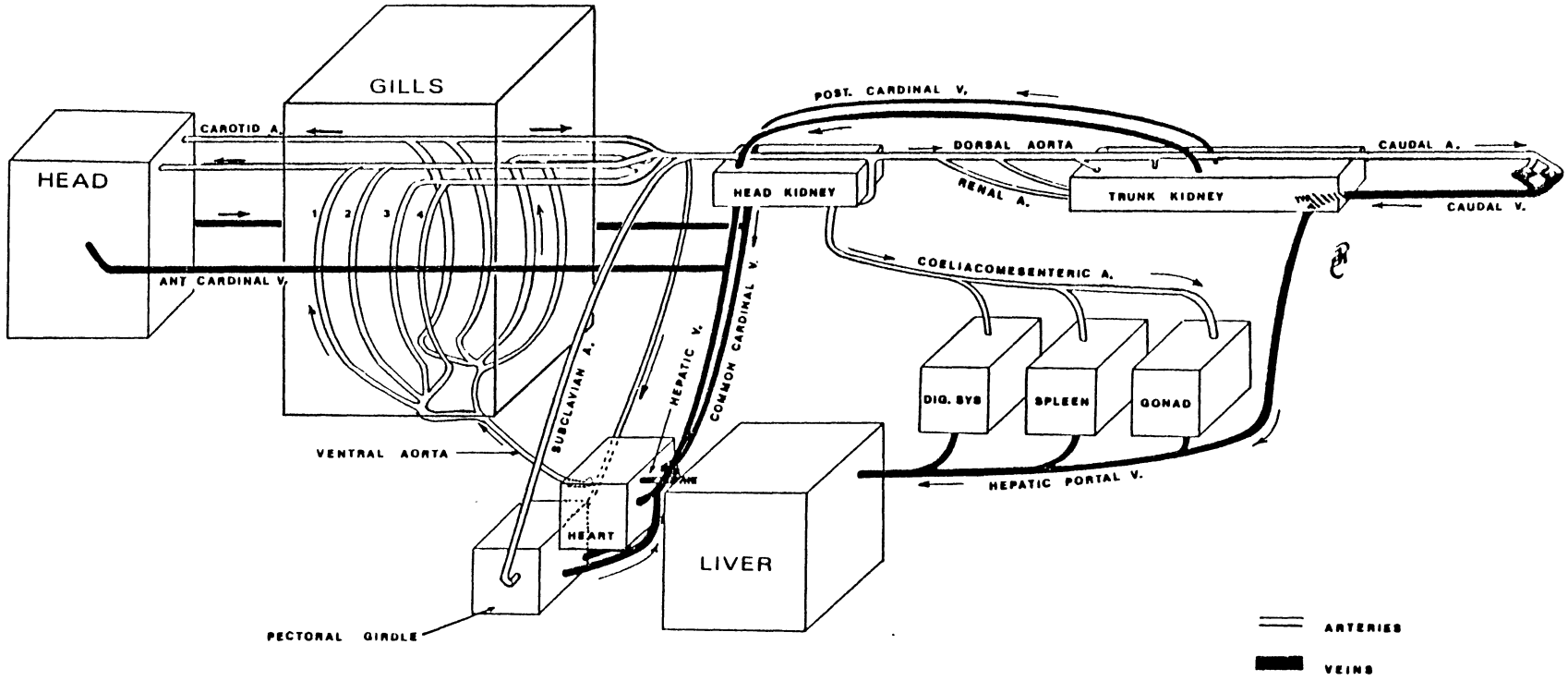
than short acting sulfonamides (Struller, 1968). This relationship is also affected by protein binding and the ambient pH. Protein binding of sulfonamides has always been considered very critical in the evaluation of their antibacterial action, since only the unbound sulfonamide finds it's way out of the blood vessels into the interstitial fluid. Bound sulfonamide is unable to exert it's antibacterial action. Available studies show that protein binding of sulfonamides becomes critical when binding is in the 80 % range (Nielson and Rasmussen, 1977; Anton, 1959). Sulfachlorpyridazine binding to plasma proteins of different species is enormously variable. In vitro studies have demonstrated plasma protein binding in the range of 54 to 59 % in pigs (Friis, et al., 1980) and a 80 to 85 % range in cows (Nielsen and Rasmussen, 1977). Binding of SCP to human plasma protein has been investigated by numerous researchers and the reported values show a range of 80 to 98 % (Marino, et al., 1979). The enhaced protein binding may advantage certain sulfonamides in their antibacterial activity. This suggestion is supported by a number of clinical studies which indicate that sulfonamides with a high degree of protein binding tend to be more rather than less effective antimicrobial agents (Witzgall, 1963)· The logic resides in the binding of sulfonamides to bacterial protein but findings on the affinity of sulfonamides to binding sites in bacteria may be relatively specific for certain compounds, and independent of their plasma protein binding

(Rieder and Bohni, 1963; Anton, 1960).

Renal and General Systemic Circulation in Channel Catfish

Channel catfish kidneys are divided into cranial (an endocrine organ) and caudal portions (excretory organ) and refered to as the head and trunk kidney respectively. The trunk kidney of catfish, in common with freshwater teleosts, are hyperosmotic regulators. The main function of these organs is the conservation of filtered electrolytes, filteration of waste in the blood and regulation of plasma pH to a small degree. In the catfish, renal ion regulating activity produces an alkaline urine with a pH of near 7.4 (Cameron, 1980; Hoar, 1969a).

The fish circulatory system is significantly different from that of mammals in several respects. First, there is only a two chamber heart. Secondly, the gill capillaries (see Figure 2) are in series with the rest of the systemic capilaries in contrast to the pulmonary and systemic circulatory capillaries in mammals which are parallel to one another. Pulse pressure in the dorsl aorta is usually less than 50 % of that in the ventral aorta. The capillary bed in the gills represents a considerable portion of the total vascular resistance. The blood volume of both marine and fresh water teleosts is about 3 % of the body weight with pH ranging from 7.5 to 7.9 (Hoar, 1969b). It has been shown that at 26° C the pH of the channel catfish's blood



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

Figure 2. A Diagram of Blood Flow in Major Arteries and Veins to the Vital Organs of Channel Catfish.

and intracellular spaces is 7.75 and 7.35 respectively. The alkalinity of these fluids will decrease at a rate of 0.018 pH units per 1° C of rise in the surrounding temperature (Cameron, 1980; Reeves, 1977).

CHAPTER II

MATERIALS AND METHODS

General Information

An injectable sodium salt preparation (Vetisulid®) of sulfachlorpyridazine, was obtained from the teaching hospital at the Veterinary School of Oklahoma State University. Each milliliter of Vetisulid® contained 215 mg/ml of sodium sulfachlorpyridazine equivalent to 200 mg/ml of SCP acid. Benzyl alcohol and sodium hydroxide are used as preservative and to adjust the pH of the solution to 9.10 respectively.

Channel catfish, Ictalurus punctatus, ranging in weight from 120-945 grams were obtained from Oklahoma State University Fisheries and Wildlife Unit and private aquaculturists in Oklahoma. The catfish were transported in a 10 gallon plastic can which was oxygenated via a small hose from a full oxygen cylinder. In the lab fish were gradually introduced into a 75 gallon tank to prevent sudden temperature shock. The water in the holding tank in the lab was continously filtered through a submerged charcoal and gravel cartridge filter. The chlorine level was minimized

by adding about 28 ml of 1 % solution of sodium thiosulfate and normally aerating the tank for a few days prior to introducing fish. Identification of individual fish in the tank was done by distinct clippings on the fins and recorded next to their weight in the notebook. All the fish were acclimated to the tank in the laboratory for at least 5 days and fasted a day prior to the experiment.

Colorimetric Chemical Analysis of Sulfachlorpyridazine

Sulfonamides and sulfachlorpyridazine can be quantitated by the colorimetric method of Bratton and Marshall (1939). In this method, the 4-amino group (Figure 1) of the SCP is diazotized with nitrous acid. The excess amount of nitrous acid is destroyed with ammonium sulfamate. The diazonium intermidiate is coupled with N-(naphtyl)ethylendiamine resulting in a pinkish purple color in the test tube. The reaction product is then measured spectrophotometerically at 540 nanometers. In these studies a Beckman DU-8 spectrophotometer was used. A standard curve was generated in the usual fashion, over a concentration range of 0.2 to 10 μg/ml of SCP. Exceptional accuracy and linearity of the method was evident at SCP concentrations to as low as 0.1 μg/ml. The color produced is stable for at least 24 hours (Rolf and Hudgins, 1979) but samples were usually evaluated spectrophotometrically within 2 hours of collection.

Micronized Analytical Procedure for Sulfachlorpyridazine

The procedure of the Bratton-Marshall method described previously was micronized to accomodate the small volumes (300 to 500 µl) of blood samples taken from the catfish. The blood withdrawn, was transfered to a couple of heparinized microhematocrit capillary tubes which were centrifuged to separate the plasma from the red cells. One hundred µl of plasma with 10 µl of 30 % trichloracetic acid were transfered into another capillary tube and centrifuged for period of 10 to 15 minutes to precipitate the protein. Precipitated protein was separated from the protein free extract by fracturing the tube and was then transfered into a clean watch glass. Ten to 25 µl of the extract, depending on the sample time and anticipated SCP concentration, were withdrawn by a micropipetter and trasfered into a test tube containing 0.5 ml of distilled water. The distilled water was used to add volume and to wash off any residue remaining in the tip of the micropipetter. Then, 100 µl of the nitrous acid were added to the test tube and mixed well so it could react with all the SCP present in the solution. After 4 minutes, 100 µl of the ammonium sulfamate were transfered into the test tube and mixed to remove excess nitrous acid. After 4 minutes 100 µl of the Bratton-Marshall reagent were transfered into the mixture followed by a quick mix for color development. The mixture was allowed to stand for 20 minutes before spectrophotometric measurement of the

optical density of the solution. Then the standard curve was used to determine the concentrations of SCP from the optical density of the solutions.

Fish Plasma Protein Analysis by the Hartree Method

The concentration of plasma and tissue protein was measured by the Hartree method (1972). Protein analysis by the Hartree method requires three different reagents (solutions A, B and C) which were made prior to the analysis:

Solution (A): 2.0 g of potassium sodium tartrate and 100.0 g of sodium carbonate dissolved in 500.0 ml. of 1.0 N sodium hydroxide and dilluted with deionized water to 1000 ml.

Solution (B): 1.0 g of cupric sulfate and 2.0 g of potassium sodium tatrate dissolved in 10 ml of 1.0 N sodium hydroxide and dilluted with deionized water to 100 ml.

Solution (C): This solution had to be freshly made by mixing 1 part Folin-Ciocalteu reagent to 15 parts distilled water. The normality of the solution was kept in the 0.15 to 0.18 range with 1.0 N sodium hydroxide. The normality of the solution was adjusted with 1.0 N sodium hydroxide .

To determine the protein concentration, 1.0 ml of the catfish plasma or crude tissue homogenate was dilluted with 1.0 ml of deionized water. To this volume 0.9 ml of solution

(A) was added and incubated in a preheated (50°C) water bath for 10 minutes. This sample was cooled at room temperature for 10 more minutes. To the cooled mixture, 0.1 ml of solution (B) was added and left at room temperature for 10 minutes. Finally, 3.0 ml of the solution (C) were transfered into the test tube and placed in the water bath for 10 minutes. The samples were cooled at room temperature for 30 minutes during which time a light blue color was developed. The optical density of the color developed was measured at 640 nanometers wavelength by a Beckman DU-8 spectrophotometer. To evaluate the measured optical density, a standard curve was made up from bovine serum albumin fraction a, in the concentration range of 1.66 to 8.33 µg of albumin per ml of distilled water.

Sulfachlorpyridazine Intracardiac Administration

Materials and Methods

A day prior to an experiment, 1 or 2 fish were marked, weighed and fasted. Individuals in these experiments were identified as having recieved an intracardiac (Ic) injection by including "Ic" after the fish number. The following day, a dose of 60 mg/kg of SCP was drawn into a 1.0 ml syringe for drug administration. Intracardiac puncture was done with a 3/4 inch, 27 gage disposable needle. This needle size prevented excessive damage to the heart and minimized hemolysis during repeated blood samplings.

To prevent excessive damage to the epithelial mucoid

coating which protects the catfish from bacterial infections, a very smooth cotton cloth was wetted and wrapped around the fish before handling. The wet cloth was quite effective in immobilization as well as protection. The intracardiac puncture was made at a 30 degree angle to the ventral surface, under the cleithrum and corocoid bone. To insure the placement of the tip of the needle in the heart, injection of the drug was preceeded by withdrawal of a small amount of blood into the visible part of the needle on the syringe. Using the first fish as a trial fish, an appropriate sampling schedule was established at 2, 5, 8, 14, 20, 60, 120, 240, 420, 600, 840, 1200 minutes post injection. At each time period withdrawn blood was transfered into heparinized capillary tubes and centrifuged for 3 to 5 minutes. After measuring the packed cell volume, plasma was transfered into another capillary tube and sealed from both ends. The sealed capillary tube, put into appropriately labled test tubes, were stored in a refrigerator till the end of the experiment. Concentrations of the SCP in the plasma samples were measured as described earlier. The intracardiac puncture study was performed on more than 14 catfish. Unfortunately in some instances the subject died in the process of the experiment or it appeared the drug may not have been injected into the heart.

The plasma SCP concentrations (μg/ml) at different sampling times of every individual catfish were used to generate a time concentration curve on four cycle semi-

logarithic graph paper. To illustrate the pharmacokinetic parameter findings, the time concentration profile for fish number 8 (F8Ic) is shown on page 22 (Figure 3). The time-concentration relationship in a two compartment open kinetic model, has two phases with an inflection point between the phases. The inflection point was determined by trial and error "best fit" of points that appeared to be on either side. Phase I represents a complex phase of distribution and elimination denoted as $\alpha + \beta$. Phase II is considered to represent the elimination component of the model, denoted as β. The procedure of "feathering" is used to subtract the minor elimination component that has occurred during distribution of the drug to produce the "pure" distribution phase, α (Jones, et al., 1977). After feathering, linear regression lines were ploted through the plasma values of the respective phases. Graphic display of the linear regression of the distribution phase was used to find the distribution half life, the time frame required for a reduction of the plasma concentration by one half, $t_{\frac{1}{2}}$ of alpha ($t_{\frac{1}{2}}\alpha$). From the $t_{\frac{1}{2}}$ alpha, alpha, a microconstant represnting the rate in reciprocal minutes at which the SCP was distributed within the body was calculated by Equation (2). Extrapolation of the alpha linear regression line back to the Y-intercept yields the theoretical zero time concentration, A.

Phase II, represents the total elimination of the drug from the central compartment. The regression of data points

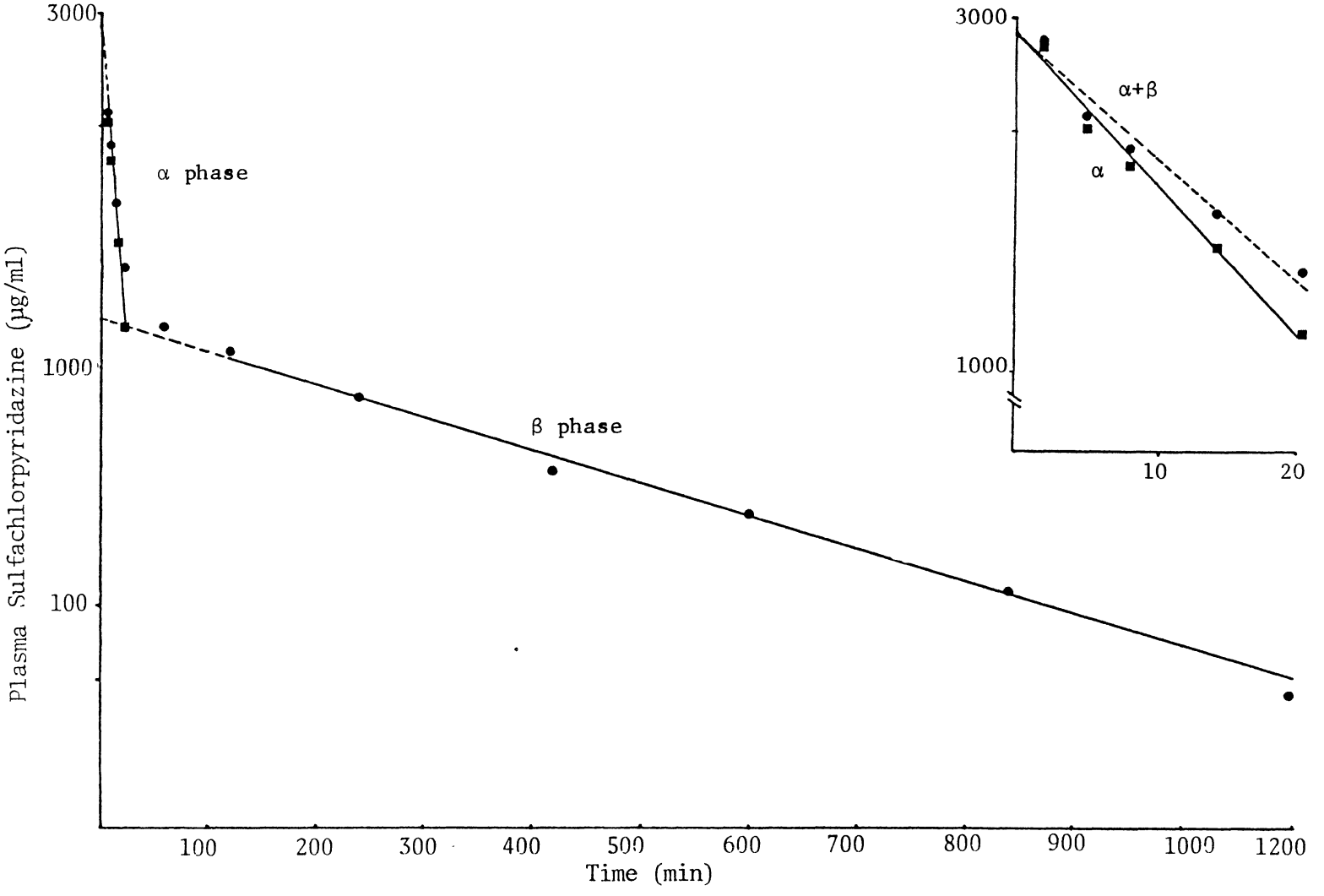


Figure 3. A Graph of Log Concentration-Time Curve for Sulfachlorpyridazine in F8Ic, Illustrating a Two Compartment Open Model.

in phase II is used to find the elimination half life ($t_{\frac{1}{2}\beta}$) which is used to calculate beta, β (beta, a microconstant similar to alpha) by Equation (3). The Y-intercept of this line is the hypothetical zero time SCP concentration of the elimination phase, designated as B. The sum of A and B is the total hypothetical concentration of SCP in the central compartment ($C\mathring{p}$) at time zero.

Kinetic microconstants of drug movement from the central compartment to the tissue (k_{12}) as well as the reverse (k_{21}) and egress from the central compartment via excretion (k_{el}) were calculated using Equations (4), (5) and (6) respectively (Appendix C). Area under the curve (AUC) for intracardiac administration was calculated mathematically by the trapezoid method for comparison with the AUC of the oral administration. The volume of distribution was calculated using the AUC generated by the trapezoid method multiplied by beta as in Equation (8), in Appendix C. The volume of the central compartment (Vc), representing the apparent blood volume in the catfish, was determined by the Equation (7). Sulfachlorpyridazine clearance was calculated by Equation (10) using the product of $AUC \cdot \beta$ and by Equation (11) using the product of $k_{el} \cdot Vc$.

Theoretical tissue SCP concentrations and fractional excretion rates were calculated by the Loo-Riegelman treatment, Equation (13), using the mean plasma SCP concentration of all fish given SCP by intracardiac route.

The mean and standard error (SE) of the raw data and

derived data were calculated and tabulated with the rest of the results in Appendix A and B.

Sulfachlorpyridazine Oral Administration

Materials and Methods

This experiment was designed to measure the oral bio-availability of SCP in catfish. To facilitate oral administration, a special dosing device was designed to deliver a SCP solution in a gelatin capsule. The device consisted of a 20 cm long outer tube, and a 30 cm long inner tube used as a plunger for the outer tube. The tip of the outer tube was cut at a 45 degree angle at one end to reduce resistance at the pharyngeal sphincter and minimize trauma. The day prior to experimentation 24 hour fasted fish were marked and weighed. Fish identification numbers contained (Or) to indicate the route of administration. The volume of SCP containing the calculated dose (60 mg/kg) was transfered into a gelatin capsule and placed inside of the angled end of the outer tube. The catfish was quickly restrained and the outer tube was inserted through the pharyngeal sphincter. The capsule was propelled from the outer tube with the inner plunger. The total time of administration was kept within a 2 minute time frame to prevent premature dissolution of the gelatin capsule. After administration, the fish were released into a small bucket with a known volume of water. Analysis of this water in the first 5-10 minutes indicated if drug was regurgitated.

Using the first fish as a trial fish, the blood sampling time after oral administration was scheduled for 30, 60, 90, 120, 150, 180, 240, 300, 420, 600, 840, 1200 minutes post administration. To calculate the individual oral bioavailability, every effort was made to repeat the oral adminstration experiment on a catfish that had already been used for an intracardiac experiment.

The blood samples were taken and analysed as in the intracardiac administration. The plasma concentration of the individual catfish were ploted on a 3 cycle semi-logarithmic graph paper. A representative plot for F80r is given in Figure 4. The log plasma concentration-time curve of the oral administration also has two phases, an absorption phase and elimination phase. Extrapolation of the linear regression line of the elimination phase to the Y-axes, was taken as the zero time concentration (B). The apparent volume of distribution and clearance were calculated by Equation (8) and (10) using the AUC values and the dose or beta. The AUC was calculated by the trapezoid method. Bioavailability of SCP in catfish was calculated by dividing AUC value of oral administration by the AUC value of intracardiac administration of the same fish. Fish 3, 5 and 7 in the oral studies did not have intracardiac injections, therefore the mean AUC value of all the fish given SCP by intracardiac injections were used in bioavailability calculations.

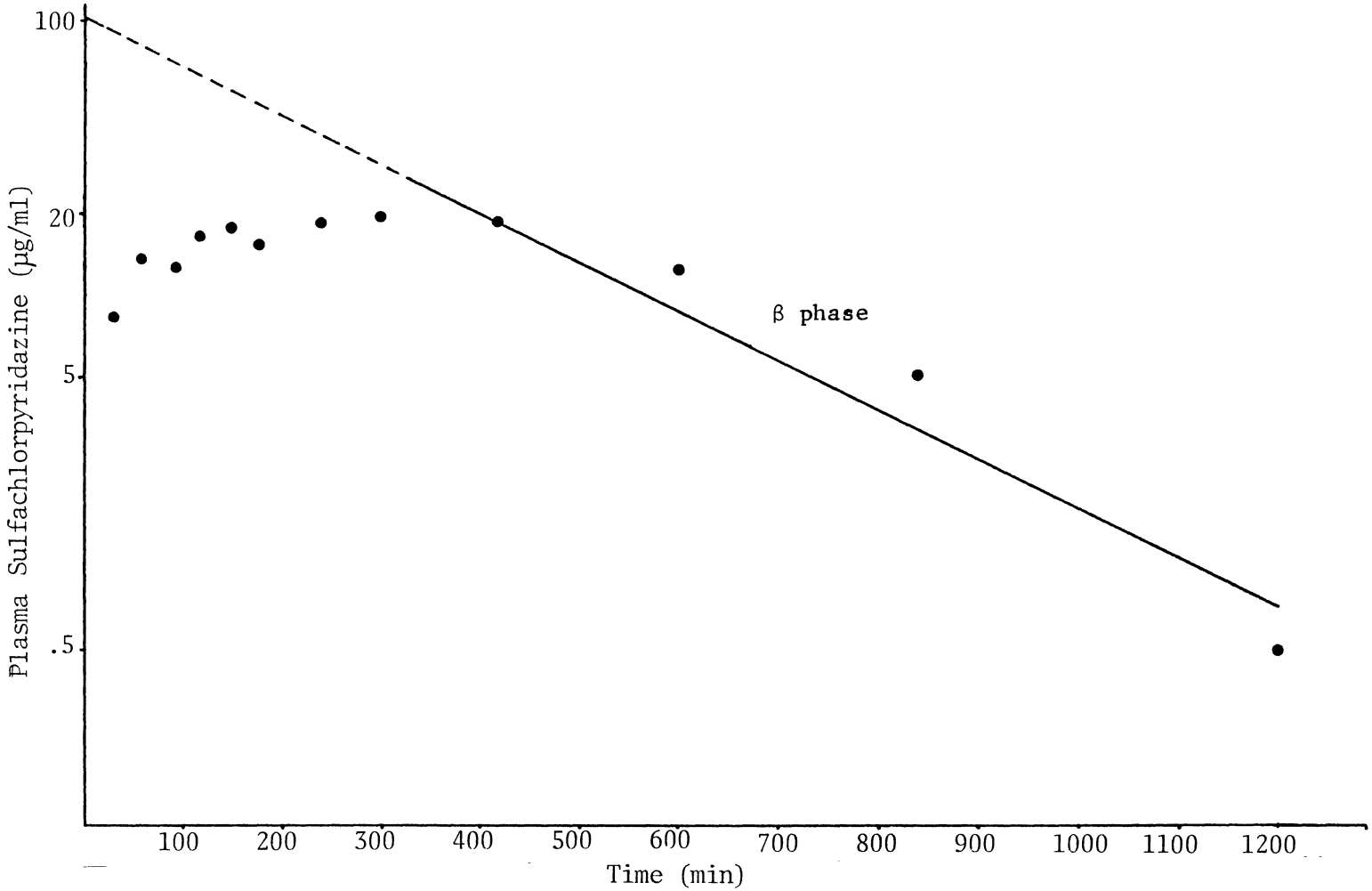


Figure 4. A Graph of Log Concentration-Time Curve for Sulfachlorpyridazine in F8Or, Illustrating the Two Phases of the Oral Administration Curve.

Lipid Solubility and pH Dependent Partitioning of Sulfachlorpyridazine

The oil:water partition experiments were designed to study the potential effects of the variable pH values of catfish body fluids on the ionization state of the SCP in relation to it's diffusion kinetics between the gastrointestinal tract, vascular compartment and tissues. The pH of separate volumes of monobasic phosphate buffer (0.154 M) was adjusted to 6.0, 7.4 and 7.8 by 0.154 M sodium hydroxide. Then, 13 small pyrex glass flasks along with rubber stoppers, were cleaned and divided into three groups of four. A flask filled with 20 ml of distilled water and 100 µl of SCP (200 mg/ml) were used as a control for the amount of SCP delivered by the micropipeter. Ten ml volumes of the specified phosphate buffer solution were placed into 50 ml Erhlenmeyer flask with 10 ml of 1-butanol or dichloroethylene. One hundred µl of SCP stock solution (200 mg/ml) were added. After periodic shaking for 5 days at room temperature, aliquots were withdrawn from the flasks. Sulfachlorpyridazine concentrations in the aqueous phase were determined by the Bratton-Marshall method. The concentration of SCP in the phosphate buffer as a percent of the amount added was calculated. The partitiion coefficient was calculated as the ratio of drug in oil (total drug - drug in the buffer) to drug in the phosphate buffer.

Plasma Protein Binding of Sulfachlorpyridazine

The conventional laboratory technique of equilibrium dialysis was used to determine the protein binding of SCP. This experiment was designed to evaluate the relationship between protein binding and varying both ambient pH and drug concentrations. Monobasic potassium phosphate buffers (0.154 M) with pH values of 7.8 and 6.0 were prepared. Cellophane dialysis tubing was cut into 8 cm lengths and moistened in distilled water over a period of time. In the first phase of the experiment 16 beakers with 10 ml capacity were divided into four groups : AI, BI and AII, BII. A and B represent the pH of the buffers 7.8 and 6.0 respectively and roman numbers indicate the SCP concentration. Into each beaker, 9.0 ml of the appropriate buffer were added. The dializing tubes were tied at one end to form a bag. Into each bag 1.0 ml of pooled catfish plasma along with four glass weights were added. The plasma had been previously dialysed against the phosphate buffer for 24 hours. The open end of the bags were tied up and immersed into the buffer containing the specified amount of SCP. Beakers were sealed to prevent water evaporation and set aside for two days at room temperature with periodic shaking. After 48 hours the buffer portion was analyzed by the Bratton-Marshall method. The pooled catfish plasma had a protein concentration of 4.8 gm/dl as determined by the Hartree method (1972). The concentration of SCP in the buffer

compartment was multiplied by 9.95 (9 ml buffer + 0.95 ml plasma water) to arrive at total unbound SCP. The amount of protein bound SCP was determined by subtracting total unbound SCP from total SCP added to the beaker. The percent binding was calculated by dividing the protein bound SCP by total SCP added to the beaker and multipling by 100.

Muscle Tissue Binding Of Sulfachlorpyridazine

Muscle tissue constitutes the largest mass of tissue in catfish and is the only part commonly consumed by people. The interest in this tissue from both a consumption and therapeutic point of view makes a binding study important. Monobasic potassium phosphate buffer (0.154 M) was adjusted to pH 6.0 and 7.8 with 0.154 M sodium hydroxide. Beakers with a 30 ml capacity were divided into 2 groups. After labeling, 8.0 ml of phosphate buffer were transferred into one beaker group with a buffer pH of 6.0 and second group with a pH of 7.8. A healthy catfish was killed by a blow into the head and about 15 to 20 g of muscle was removed from the sides close to the pelvic fin area. As in the plasma binding study, 12 dialysis bags were made containig 4 glass weights. After homogenization approximatly 1.0 g of the homogenate was transferred into each dialysis bag and the external surface was washed off with 1.0 ml appropriate buffer. The bags were tied up and immersed into the buffer. The beakers were sealed with parafilm, with

periodic shaking for the next 48 hours. After incubation,the buffer SCP concentration was analysed by the Bratton-Marshall method.

To calculate tissue water content, a known amount of muscle tissue was dried at 75°C for 48 hours to a constant weight. Percent water was calculated as the wet weight minus the dry weight divided by the wet weight and multiplied by 100. The percent binding of SCP was determined as in the plasma protein binding study. The protein concentration of the muscle homogenate was also measured by the Hartree method (1972).

CHAPTER III

RESULTS

General Information

The mean of the determined and calculated values are reported throughout this chapter. Sulfachlorpyridazine was administered at 60 mg/kg body weight. Using the SCP stock solution (200 mg/ml) a standard curve was produced with a correlation coefficient of 0.99989 and Y-intercept of 0.0092.

The hematocrit was measured for all the blood samples taken by intracardiac puncture and an initial mean hematocrit of 30 % was noted. In general, the hematocrit dropped to 2/3 of the initial volume by the end of the experiment after an initial small increase within the first hour.

The water content of muscle was calculated from the difference between the dry and wet weight. On the average, water constituted 81 % of the body weight. The protein concentration of the fish plasma and muscle as analyzed by the Hartree method was 4.8 g/dl and 8.9 g/100 g respectively.

Sulfachlorpyridazine Pharmacokinetic Parameters After Intracardiac Administration

The alpha (distribution) half life of SCP was 3.2 min with a zero time concentration, A of 2489.6 μg/ml. An alpha rate constant (α) of 0.25227 min^{-1} was calculated. The zero time concentration of the elimination phase, B, was 118.5 μg/ml. A beta half life of 252 min (4.12 hours) and beta rate constant (β) of 0.00303 min-1 was calculated. The theoretical total SCP concentration in the central compartment, $\overset{\circ}{Cp}$, at zero time was 2608.2 μg/ml. The kinetic microconstants k_{12}, k_{21} and k_{el} were found to be 0.18344, 0.01547 and 0.05640 min respectively. The apparent volume of distribution, Vd, was 441.8 ml/kg, calculated by dose divided by AUC β and 463.1 ml/kg calculated by $k_{el} \cdot$ Vc. The apparent volume of the central compartment, calculated from the plasma concentration of SCP was 25.7 ml/kg. Correcting the plasma value to a whole blood base, the volume of the central compartment would be 36.7 ml/kg. The area under the curve (AUC) was 514774.6 μg.min/ml. The rate of whole body clearance (Cl_B) of SCP from catfish calculated from $Vd_{(area)}$ times β and k_{el} times Vc, was 1.21 and 1.27 ml/min/kg respectively. The ratio of the two microconstants k_{12} over k_{21} was calculated to be 11.8. The mean plasma and tissue SCP concentration along with percent elimination over a 20 hour period is graphically presented in Figure 5.

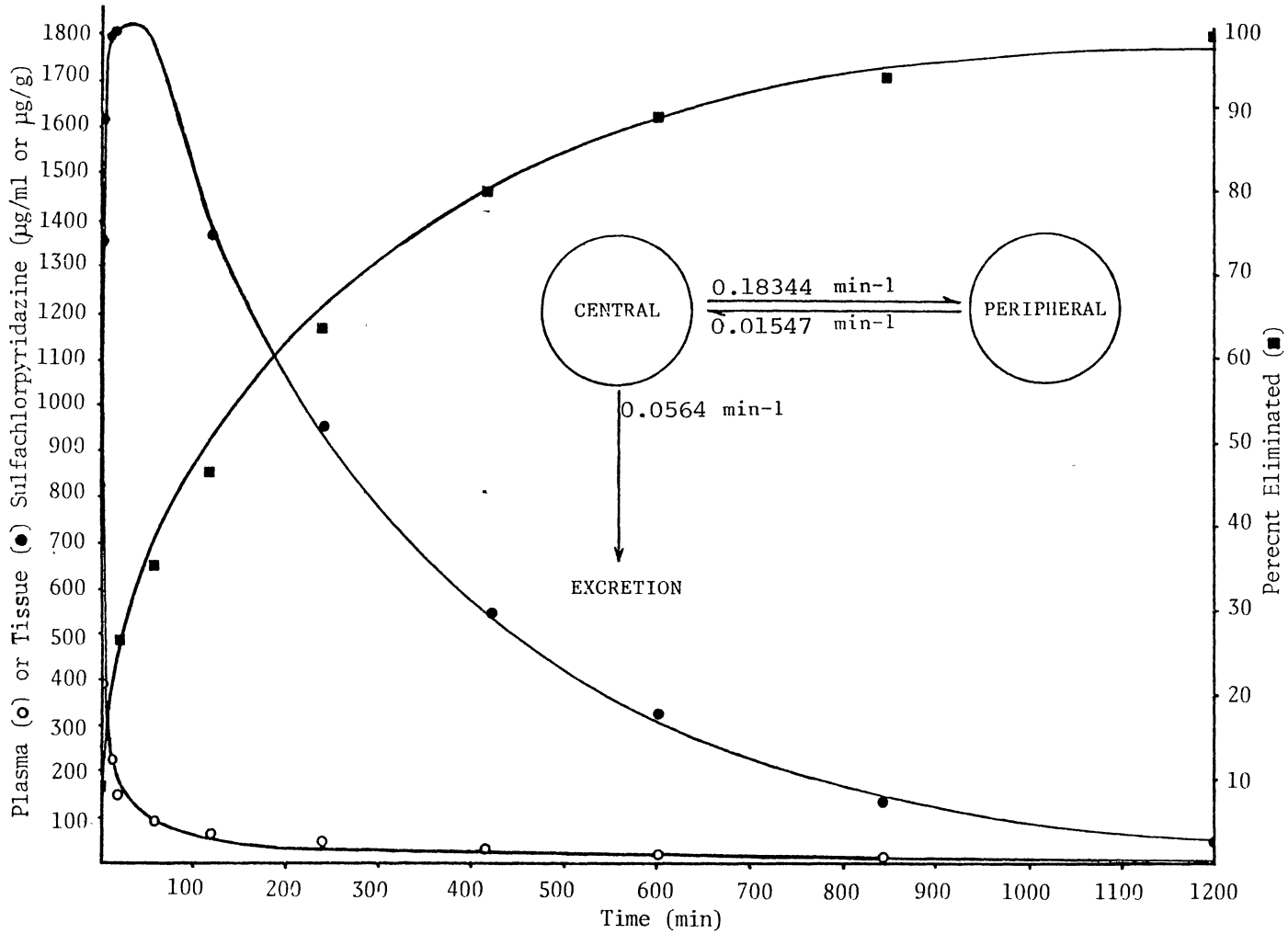


Figure 5. Disposition of Sulfachlorpyridazine (60 mg/kg) After a Single Intracardiac Administration to 9 Channel Catfish.

Sulfachlorpyridazine Pharmacokinetic Parameters After Oral Administration

The mean beta half life calculated from the slope of the beta phase was 265 min with zero time SCP concentration (B) of 35.3 µg/ml. The apparent volume of distribution (Vd) was 465.3 ml/kg. The whole body clearance, Cl_B value was calculated to be 1.20 ml/min/kg.

Theoretical tissue concentrations were calculated from the average values of required parameters from the oral and intracardiac studies. The results indicate that at a mean peak plasma concentration of 11.6 µg/ml, the tissue SCP concentration (Figure 6) can reach as high as 110 µg/ml.

The calculation of bioavailability for the 6 individual catfish was possible because the same fish were used for both treatments (intracardiac and oral). The mean bioavailability was 13.9 % with a maximum of 31.9 % in fish 9 and a minimum of 4.7 % in fish 1. A graphical depiction of mean values of SCP in plasma, tissue and a percent excretion is shown in Figure 6.

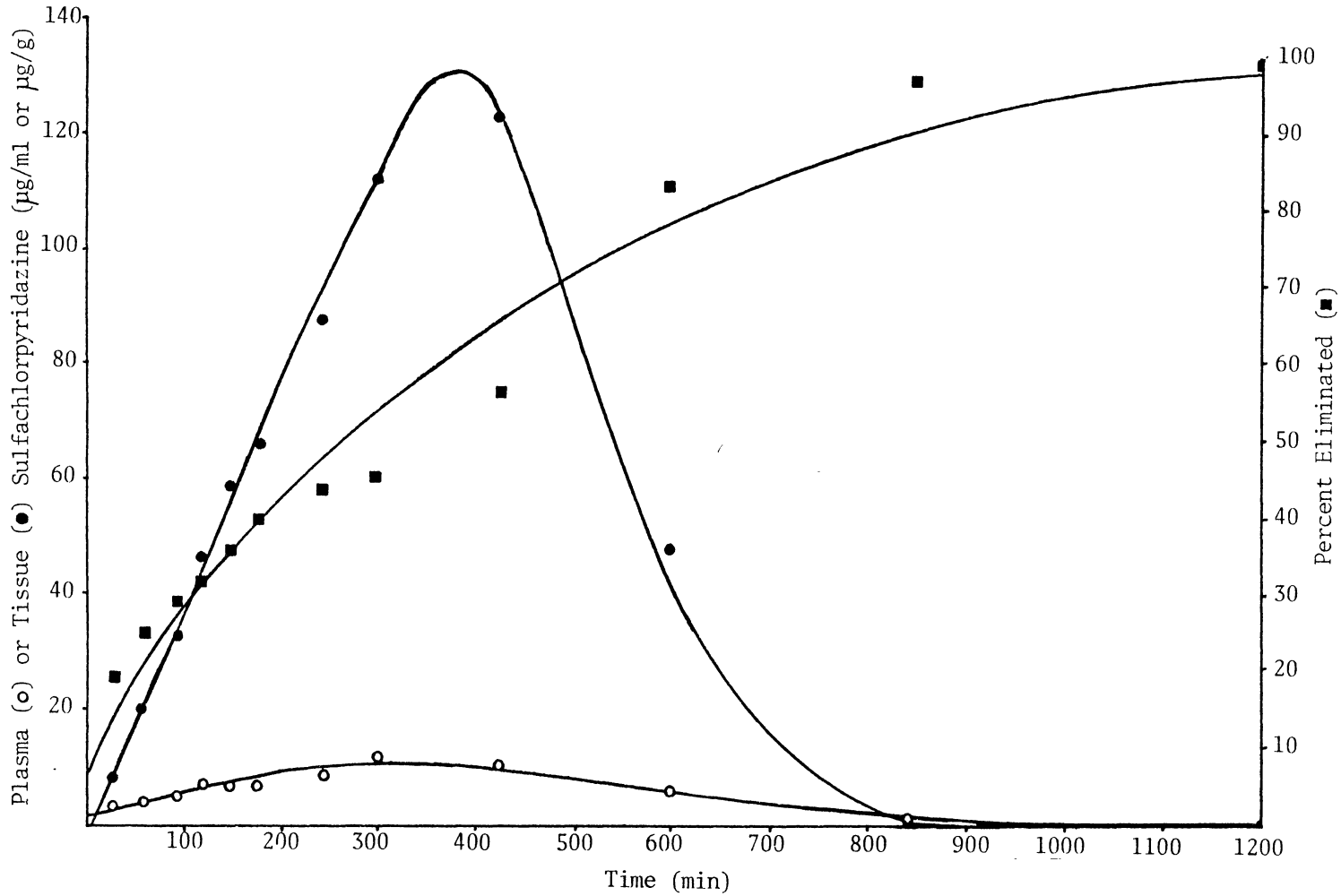
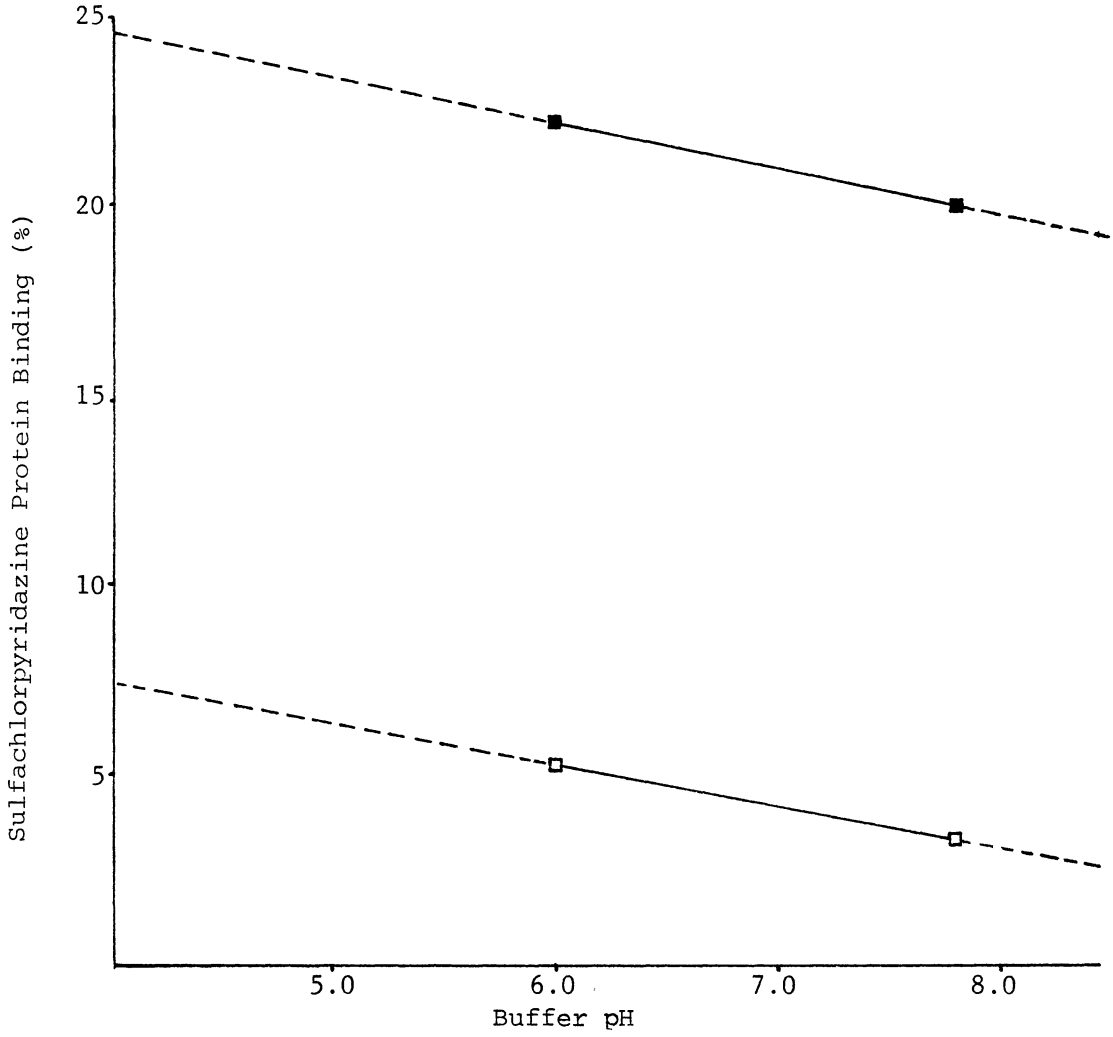


Figure 6. Disposition of Sulfachlorpyridazine (60 mg/kg) After a Single Oral Administration to 9 Channel Catfish.

Physiochemical Parameters of Sulfachlorpyridazine

Sulfachlorpyridazine equilibrated between phosphate buffer at pH 7.8 and 6.0 and 1-butanol, had a partition coefficient of 0.21 and 0.16 respectively. The concentration of SCP in buffer increased from 87.5 to 91.0 % when the pH of the phosphate buffer was reduced from 7.8 to 6.0. The partitioning of SCP between phosphate buffer and dichloroethylene had different absolute coefficients but followed the pattern established in 1-butanol. At buffer pH values of 7.8, 7.4 and 6.0, the partition coefficients were 0.43, 0.65 and 3.58 respectively. The percentage of SCP in the phosphate buffe increased from 21.6 % to 70.3 % as the pH was lowered from 7.8 to 6.0.

Tissue binding of SCP was performed on catfish white muscle. Binding studies revealed that at phosphate buffer pH of 7.8 and 6.0, the binding was about 19.9 and 22.2 % respectively (see Figure 7). Plasma protein binding was 4 to 5 times lower than tissue binding, at the same SCP concentrations. In phosphate buffer of pH 7.8 and 6.0, SCP binding to plasma protein was 3.7 and 5.5 % respectively. When the same experiment was repeated at lower SCP concentrations (about 238 µg) in phosphate buffer pH of 7.8, a binding of 1.9 % was observed. In similar experiments with 208.8 µg of SCP, a binding of 14.2 and 12.1 % was calculated at buffer pH values of 7.8 and 6.0.



A graphical presentation of tissue (■) and plasma (□) protein binding of sulfachlorpyridazine at different buffer pH indicates the difference in the binding to plasma and tissue protein.

Figure 7. Sulfachlorpyridazine Tissue and Plasma Protein Binding

CHAPTER IV

DISCUSSION

Sulfachlorpyridazine concentrations in plasma were analyzed with the Bratton-Marshall method and concentrations less than 0.2 μg/ml from small volumes of plasma (25 μl) were easily and accurately analyzed. Blood samples taken within the first 30 minutes after intracardiac injections of SCP provided distribution phase kinetics with an average half life of 3.2 min.

The zero time concentration (A) of the alpha (α) phase was variable from fish to fish. During injection, patency with the cardiac chamber required withdrawing small volumes of blood, including the dead space volume, into the needle hub which replaced some of the SCP solution. Upon injection then the calculated amount of drug, or more, may have been injected.

In most other SCP pharmacokinetic studies (man or animals), the kinetics of the distribution phase have not been done and/or reported. Kinetics of SCP have been studied most frequently on the basis of a one compartment, open model. With distinct distribution and elimination phases,

SCP in catfish behaves with disposition kinetics best fitted to a two compartment open model. The $t_{\frac{1}{2}\beta}$ of 4.20 hours after intracardiac administration was in close agreement with $t_{\frac{1}{2}\beta}$ of 4.41 hours after oral administration. The $t_{\frac{1}{2}\beta}$ of SCP in catfish was as variable within the species as reported from different animal species. In this study, a range of plasma half life values from 2.1 hours to 6.0 hours was observed. The reported $t_{\frac{1}{2}\beta}$ of SCP in 2 year old geese and piglets is 1.89 and 3.01 hrs respectively (Romvary and Horvay, 1976a; 1976b). It does seem surprising that these values are as low as reported. In the case of the piglets, the immature renal filteration/secretion capacity and probable acidic urine associated with a nursing omnivore should have prolonged plasma residence (Friis, et al., 1980). The alkaline urine of geese and the overall increased renal functional capacity in these mature animals is helpful in reducing the plasma concentration of SCP very rapidly in this species when compared to fish.

There was less than a 5% difference in the apparent volume of distribution for the intracardiac and oral administration of SCP. The values reported here are less than the intracellular water volumes reported for catfish of approximately 600 ml/kg (Cameron, 1980), but does indicate that SCP is present in much of cellular water. Comparatively, a value of about half that of catfish, 240 ml/kg has been reported in cattle (Nielson and Rasmussen, 1977)

Clearance of SCP has not been calculated in other

studies. Hoar (1969a) reported that the glomerular filtration rate (GFR) and renal blood flow in fish are approximately 0.13 and 3.1 ml/min/kg respectively. This GFR is almost 9 times lower than the Cl_B of 1.21 ml/min/kg reported for SCP in these fish, indicating a larger percentage of elimination by secretion than by filtration.

Critical to the use of any therapeutic agent is the maintenance of the plasma concentrations at levels sufficient to keep receptors occupied for a continued pharmacological effect. In the case of sulfonamides, the minimum inhibitory concentration (MIC) has generally been assumed to lie somewhere between 20 µg/ml and 50 µg/ml. The rapid elimination of SCP from fish plasma, obviously provides large amounts of drug in the urinary system. Studies of the theoretical tissue disposition indicate that this drug, given by either intracardiac or oral route, can exceed tissue levels of 25 µg/ml for 12 hours. Since tissue levels have not been correlated to plasma MIC values, it is not possible to speculate on the therapeutic relevance of these theoretical tissue concentrations. That evaluation awaits the actual simultaneous determination of plasma and tissue levels.

Three important factors that may be involved in the loculation of SCP in the tissue compartment as seen in these studies are ion trapping, tissue binding and lipid solubility. Plasma protein binding of SCP has been reported to be as high as 98 % in humans (Marino, et, al., 1979),

85 % in cattle (Nielsen and Rasmussen, 1977) to as low as 54 % in piglets (Friis, et al., 1980). In very sharp contrast, the results of this study indicate a very low protein binding of SCP to fish plasma protein that is relatively pH independent. While this allows a large amount of freely diffusible drug for elimination processes, it also provides a free moiety for a driving concentration gradient into tissue.

Tissue binding studies suggest that tissue has 4 to 5 times higher affinity for SCP than plasma proteins. The higher affinity of tissue may be regarded as reinforcement for SCP use in catfish, since tissue is the environment where SCP would be expected to exert it's antibacterial activity. A hypothesis supported by experimental findings of Witzgall (1963) suggests that the factors responsible for high protein binding of sulfonamides may also serve to increase their binding to bacterial membrane protein. Some of the differences in the affinity of SCP for tissue and plasma protein binding can be accounted for by previously reported selectivity of sulfanomides for different proteins (Rieder and Böhni, 1963).

Ambient pH may alter the ratio of unionized to ionized species and interrelate with the lipid solubility in that manner. The suggestion from the data is that even the ionized form of the SCP molecule, is lipid soluble enough to show a greater concentration in the oil phase than could be expected based on the pKa and pH. If the latter mechanism

is operating, then considerably more drug is available for membrane penetration and subsequent ion trapping in intracellular spaces. These mechanisms, ion trapping and lipid membrane penetration, are obviously important at the oral absorptive surface for achivement of bioavailability as well as increasing the chance for hepatocyte penetration and metabolism, intracellular movement with sequestering and reabsorption across renal tubular cells. Barriers such as the gastrointestnal mucosa and blood-brain barrier are more easily penetrated as lipophilicity of the nonionized molecule increases (Shanker, et al., 1957).

Hematocrit measurement indicated a decline in the packed cell volume over the course of blood sampling time. Whether the change in the PCV affected the concentrations of SCP in plasma is difficult to document. With an increased water content of the blood, the resistance to blood flow may have decreased, thus allowing better distribution. The volume of blood that was withdrawn at each sampling time was minute (about 40 µl) and less than 2 % of the total blood volume was removed with each experiment. Stress from the repeated blood sampling may have played a greater role than actual blood loss in the elimination and other kinetic parameters by changing the behavior of the animal.

CHAPTER V

CONCLUSION

This pharmacokinetic study of sulfachlorpyridazine (SCP) was oriented toward an understanding of the kinetic behavior of this drug in catfish and it's potential as a therapeutic agent. With a plasma half life of about 4.2 hours and an apparent volume of distribution of 450 ml/kg, SCP may prove to be a good therapeutic agent for catfish.

The very favorable tissue:plasma equilibrium provides for mean tissue concentrations in excess of 25 µg/ml for over 12 hours. It seems likely that some of this favorable equilibrium may enhance penetration of SCP into tissue compartments, the apparent volume of distribution having absolute significance. Tissue binding, though not pH depenent, has significance despite being a small absolute amount. The large percentage of the body mass occupied by the muscle coupled with ion trapping may explain the approximate 10 fold ratio of tissue ingress:egress of SCP.

Improvement of the 14 % bioavailability of SCP reported in these studies makes the therapeutic use of this sulfonamide even more appealing.

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APPENDIXES

APPENDIX A

RAW DATA

TABLE I

SULFACHLORPYRIDAZINE STANDARD CURVE

Sample Number	Concentration of SCP (μg/ml)	Optical Density at 540 nm
1	0.2	0.04440
2	1.0	0.20786
3	2.0	0.37420
4	4.0	0.74906
5	6.0	1.10890
6	8.0	1.50700
Slope		0.18566
Y-Intercept		0.00916
Correlation Coefficient		0.99989

TABLE II

BOVINE SERUM ALBUMIN STANDARD CURVE

Sample Number	Bovine Serum Albumin Concentration (μg/ml)	Optical Density at 640 nm
1	1.66	0.01952
2	3.33	0.04429
3	5.55	0.07605
4	6.66	0.09417
5	8.33	0.11731
Slope		0.00245
Y-Intercept		0.00492
Correlation Coefficient		0.99985

TABLE III

SULFACHLORPYRIDAZINE PARTITIONING BETWEEN POTASSIUM PHOSPHATE BUFFER AND 1-BUTANOL

Buffer pH	SCP Concentration (mg) Buffer	1-Butanol	Percent in Buffer	Partition Coefficient
6.0	4.51	15.49	22.5	3.44
6.0	4.59	15.41	23.0	3.35
6.0	4.20	15.80	21.0	3.76
6.0	4.18	15.92	20.9	3.78
Mean	----	-----	21.6	3.58
SE	----	-----	±0.5	± 0.11
7.4	11.75	8.25	58.6	0.70
7.4	12.64	8.36	63.2	0.58
7.4	11.97	8.03	59.9	0.67
7.4	12.05	7.95	60.3	0.66
Mean	-----	----	60.5	0.65
SE	-----	----	±1.0	± 0.03
7.8	14.14	5.85	70.8	0.41
7.8	13.43	6.57	67.2	0.49
7.8	13.76	6.24	68.8	0.45
7.8	14.83	5.17	74.2	0.39
Mean	-----	----	70.3	0.43
SE	-----	----	± 1.5	±0.02

TABLE IV

SULFACHLORPYRIDAZINE PARTITIONING BETWEEN POTASSIUM PHOSPHATE BUFFER AND DICHLOROETHYLENE (DCE)

Buffer pH	SCP Concentration (mg) Buffer	DCE	Percent in Buffer	Partition Coefficient
7.4	8.37	1.63	88.4	0.19
7.4	8.19	1.81	86.4	0.22
7.4	8.18	1.82	86.4	0.22
7.4	8.43	1.57	89.0	0.19
Mean	----	----	87.5	0.21
SE	----	----	±0.5	±0.01
7.8	8.81	1.19	93.0	0.14
7.8	8.45	1.55	89.0	0.18
7.8	8.37	1.63	88.4	0.20
7.8	8.94	1.06	94.4	0.12
Mean	----	----	91.3	0.16
SE	----	----	±1.5	±0.02

TABLE V

SULFACHLORPYRIDAZINE PLASMA PROTEIN BINDING
(PART I)

Buffer pH	Total SCP Added(μg)	Total SCP Measured(μg)	% Protein Binding
7.8	2300.1	2225.8	3.2
7.8	2300.1	2271.6	1.2
7.8	2300.1	2189.0	4.8
7.8	2300.1	2250.7	2.1
7.8	2300.1	2238.7	2.7
7.8	2300.1	2189.0	4.8
7.8	2300.1	2213.9	3.7
7.8	2106.5	1990.7	5.5
7.8	2106.5	1975.2	6.2
7.8	2106.5	1989.8	5.5
7.8	2106.5	2078.6	1.3
Mean	------	------	3.7
SE	------	------	±0.5
6.0	2106.5	2053.7	2.5
6.0	2106.5	1883.5	10.6
6.0	2106.5	1992.0	5.4
6.0	2106.5	2031.8	3.5
Mean	------	------	5.5
SE	------	------	±1.8

TABLE VI

SULFACHLORPYRIDAZINE PLASMA PROTEIN BINDING
(PART II)

Buffer pH	Total SCP Added(μg)	Total SCP Measured(μg)	% Protein Binding
7.8	238.0	235.4	1.1
7.8	238.0	234.7	1.4
7.8	238.0	228.9	3.8
7.8	238.0	236.1	0.8
7.8	238.0	233.2	2.0
7.8	238.0	233.2	2.0
Mean	-----	-----	1.9
SE	-----	-----	±0.4
7.8	208.8	178.3	14.6
7.8	208.8	178.3	14.6
7.8	208.8	171.0	13.1
7.8	208.8	178.5	14.5
Mean	-----	-----	14.2
SE	-----	-----	±0.4
6.0	208.8	182.6	12.6
6.0	208.8	192.4	7.8
6.0	208.8	187.6	10.2
6.0	208.8	172.0	17.6
Mean	-----	-----	12.1
SE	-----	-----	±2.1

TABLE VII

SULFACHLORPYRIDAZINE TISSUE BINDING

Buffer pH	Total SCP Added(µg)	Total SCP Measured(µg)	% Protein Binding
7.8	2230.0	1775.2	22.8
7.8	2230.0	1911.0	16.9
7.8	2299.9	1992.0	13.4
7.8	2301.3	1773.3	22.9
7.8	2299.9	1916.9	16.7
7.8	2299.9	1689.9	26.5
Mean	2300.2	1843.1	19.9
SE	±1.6	±46.6	±2.0
6.0	2300.1	2003.8	12.8
6.0	2299.9	1771.1	23.0
6.0	2300.0	1734.8	24.6
6.0	2300.0	1817.6	21.0
6.0	2298.7	1718.0	25.3
6.0	2300.0	1695.7	26.3
Mean	2299.8	1790.2	22.2
SE	±1.4	±46.2	±2.0

TABLE VIII

PLASMA SULFACHLORPYRIDAZINE CONCENTRATION IN INDIVIDUAL*
CATFISH AFTER INTRACARDIAC ADMINISTRATION

Time Post Injection (min)	Plasma Sulfachlorpyridazine Concentration (μg/ml)										
	F1Ic	F2Ic	F3Ic	F4Ic	F5Ic	F6Ic	F7Ic	F8Ic	F9Ic	Mean	SE
2	1487.6	1742.8	1737.3	1032.0	1304.5	1669.1	1796.3	2364.0	1848.0	1664.6	±124.3
5	495.0	735.0	917.0	548.9	587.1	989.3	989.3	1154.0	947.1	818.1	±77.3
8	258.0	212.0	568.0	241.0	-----	200.3	367.3	841.2	475.3	395.4	±79.1
14	-----	43.2	221.0	-----	261.5	85.0	310.1	452.6	279.8	236.2	±52.4
20	-----	----	162.1	124.1	134.4	76.8	140.6	260.7	91.5	141.4	±22.7
60	150.6	46.0	71.1	113.5	70.2	68.5	89.3	147.8	84.5	93.5	±12.1
120	-----	43.7	59.4	101.5	66.9	67.7	69.5	114.9	65.1	73.6	±8.2
180	87.5	----	----	-----	----	----	----	-----	----	----	---
240	----	41.1	50.9	112.9	44.9	56.7	56.7	73.0	39.1	59.4	±8.5
360	32.9	----	----	-----	----	----	----	----	----	----	---
420	----	26.3	28.6	40.4	32.2	44.2	46.0	37.2	17.2	34.0	±3.5
600	8.6	16.8	22.1	18.9	16.5	17.9	38.2	24.5	7.6	19.0	±3.0
840	---	7.5	10.5	7.9	6.8	8.3	19.2	11.6	3.6	9.4	±1.6
1200	---	7.0	----	1.9	6.5	2.0	6.1	4.3	2.6	4.7	±1.0

*Individual catfish in this experiment were used for oral administration as well except for F3Ic, F5Ic and F7Ic which died a week after the intracardiac experiment.

TABLE IX

PLASMA SULFACHLORPYRIDAZINE CONCENTRATION IN INDIVIDUAL*
CATFISH AFTER ORAL ADMINISTRATION

Time Post Injection (min)	Plasma Sulfachlorpyridazine Concentration (μg/ml)										
	F1Or	F2Or	F3Or	F4Or	F5Or	F6Or	F7Or	F8Or	F9Or	Mean	SE
30	3.6	2.0	1.5	3.4	1.5	3.0	0.7	8.4	1.8	2.9	±0.8
60	2.3	2.9	1.6	4.6	2.3	2.8	2.1	13.7	1.7	3.8	±1.3
90	3.8	---	1.8	5.2	1.7	3.3	7.1	12.7	2.8	4.8	±1.3
120	3.1	10.7	2.7	6.8	2.4	5.8	7.6	16.5	5.2	6.8	±1.5
150	5.3	---	3.6	5.2	1.6	4.7	9.6	17.8	4.7	6.6	±1.8
180	4.3	6.7	5.4	6.1	2.2	5.6	9.8	15.6	3.3	6.6	±1.3
240	3.8	4.7	9.6	10.3	1.8	7.1	10.4	18.4	11.3	8.6	±1.7
300	3.7	3.8	13.7	17.2	4.3	12.8	14.3	19.1	15.6	11.6	±2.0
420	4.0	2.4	8.8	11.1	4.8	6.6	11.9	18.6	27.1	10.6	±2.6
600	1.9	1.1	3.7	5.5	6.8	3.1	5.5	12.8	12.4	5.9	±1.4
840	0.2	1.4	2.3	2.3	5.5	1.6	1.7	5.2	4.6	2.8	±0.6
1200	---	0.5	1.7	1.9	1.9	1.3	1.2	0.5	3.2	1.4	±0.3

*Individuals in oral administrations were the same catfish used in intrcardiac administration except for F3 Or, F5 Or and F7 Oral.

APPENDIX B

DRIVED DATA

TABLE X

PHARMACOKINETIC PARAMETERS OF SULFACHLORPYRIDAZINE AFTER INTRACARDIAC ADMINISTRATION IN CHANNEL CATFISH

Catfish I.D.	A ($\mu g/ml$)	B ($\mu g/ml$)	C_p° ($\mu g/ml$)	α (min^{-1})	β (min^{-1})	k_{12} (min^{-1})	k_{21} (min^{-1})	k_{el} (min^{-1})
F1Ic	2769.3	218.9	2988.2	0.38077	0.00537	0.29106	0.03267	0.06221
F2Ic	4020.4	54.8	4075.2	0.38933	0.00193	0.27907	0.00713	0.10505
F3Ic	1979.4	83.3	2062.7	0.16579	0.00236	0.11556	0.00896	0.04363
F4Ic	1336.7	182.9	1519.6	0.25200	0.00377	0.19392	0.03364	0.02821
F5Ic	1413.2	78.8	1491.9	0.14903	0.00239	0.10613	0.01012	0.03517
F6Ic	3548.7	111.5	3660.2	0.37059	0.00315	0.27801	0.01434	0.08139
F7Ic	1993.1	97.1	2090.2	0.17678	0.00193	0.13479	0.01004	0.03387
F8Ic	2518.3	158.5	2676.8	0.16619	0.00309	0.11620	0.01275	0.04033
F9Ic	2827.9	81.0	2908.9	0.22000	0.00330	0.13619	0.00933	0.07778
Mean	2489.6	118.5	2608.2	0.25227	0.00303	0.18344	0.01547	0.05640
SE	± 304.0	± 18.5	± 300.5	±0.03366	±0.00036	±0.02622	± 0.00343	± 0.00886

TABLE X

(Continiued)

Catfish I.D.	$t_{\frac{1}{2}\alpha}$ (min)	$t_{\frac{1}{2}\beta}$ (min)	AUC (μg.min/ml)	V_c (ml/kg)	$V_{d(area)}$ (ml/kg)	$V_{d(\beta)}$ (ml/kg)	$Cl_{B(area)}$ (ml/min/kg)	$Cl_{B(\beta)}$ (ml/min/kg)
F1Ic	1.8	129	50906.3	20.1	219.5	232.5	1.18	1.24
F2Ic	1.8	360	40658.7	14.7	766.7	803.4	1.47	1.56
F3Ic	4.2	294	48807.0	29.1	521.6	538.2	1.23	1.27
F4Ic	2.8	184	56982.9	39.5	279.6	295.7	1.05	1.11
F5Ic	4.7	290	44786.0	40.2	560.8	591.9	1.34	1.41
F6Ic	1.9	220	47201.5	16.4	403.6	423.5	1.27	1.33
F7Ic	3.9	360	63948.4	28.7	487.4	504.9	0.94	0.97
F8Ic	4.7	224	70712.1	22.4	274.3	292.2	0.85	0.90
F9Ic	3.2	210	39269.2	20.6	463.0	486.0	1.52	1.60
Mean	3.2	252	51474.6	25.7	441.8	463.1	1.21	1.26
SE	±0.4	±26	±3520.6	±3.1	±57.0	±59.2	±0.08	±0.08

TABLE XI

PHARMACOKINETIC PARAMETERS OF SULFACHLORPYRIDAZINE AFTER ORAL ADMINISTRATION IN CHANNEL CATFISH

Catfish I.D.	B (μg/ml)	$t_{\frac{1}{2}\beta}$ (min)	β (min^{-1})	$AUC_{(area)}$ (μg.min/ml)	$V_{d(area)}$ (ml/kg)	$Cl_{B(area)}$ (ml/min/kg)	Percent Bioavail.
F1Or	4.6	282	0.00246	2401.9	479.8	1.18	4.7
F2Or	9.2	270	0.00257	3154.3	575.1	1.47	7.8
F3Or	21.2	291	0.00238	6194.9	488.5	1.16	12.0*
F4Or	29.9	271	0.00256	7791.6	411.7	1.05	13.7
F5Or	28.5	305	0.00227	5855.3	514.3	1.17	11.4*
F6Or	19.0	273	0.00254	5403.4	500.7	1.27	11.4
F7Or	34.4	266	0.00261	7291.6	448.4	1.17	14.2*
F8Or	102.4	170	0.00408	12848.6	208.2	0.85	18.2
F9Or	68.0	255	0.00272	12546.3	561.4	1.52	31.9
Mean	35.3	265	0.00269	7052.2	465.3	1.20	13.9
SE	± 10.4	± 12	± 0.00018	±1213.8	± 36.3	± 0.07	± 2.6

*The bioavailability for these 3 fish is calculated from the mean AUC value for all fish given intracardiac administrations.

TABLE XII

THEORETICAL TISSUE CONCENTRATION* AND PERCENT DOSE ELIMINATED AFTER INTRACARDIAC ADMINISTRATION OF SULFACHLORPYRIDAZINE

Time Post Injection (min)	Tissue Concentration (μg/ml)	Percent Dose Eliminated
2	748.0	8.9
5	1347.0	16.5
8	1618.5	20.4
14	1783.7	24.0
20	1794.4	25.9
60	1600.5	34.5
120	1334.5	45.4
240	927.7	62.0
420	537.7	79.0
600	311.7	87.2
840	150.6	93.8
1200	50.6	98.9

*Mean of the plasma SCP concentrations over the same time period were used in the calculations.

TABLE XIII

THEORETICAL TISSUE CONCENTRATION* AND PERCENT DOSE** ELIMINATED AFTER ORAL ADMINISTRATION OF SULFACHLORPYRIDAIZNE

Time Post Injection (min)	Tissue Concentration (µg/ml)	Percent Dose Eliminated
30	8.0	18.8
60	20.3	25.2
90	32.2	29.4
120	46.9	31.9
150	58.9	35.8
180	66.1	39.6
240	84.4	44.1
300	111.6	46.6
420	122.5	57.9
600	47.9	83.2
840	1.2	98.8

*Mean of the plasma SCP concentrations over the same time period were used in the calculations.

**Percent of the absorbed (13%) dose.

APPENDIX C

LIST OF PHARMACOKINETIC EQUATIONS GENERALLY APPLIED TO TWO COMPARTMENTAL OPEN MODEL

Dose to be administered= Equation (1)

$$\text{Body weight (kg)} \cdot \frac{\text{SCP Dose (60 mg)}}{\text{kg}} \cdot \frac{\text{ml}}{\text{SCP Conc. 200 mg}}$$

$$t_{\frac{1}{2}}\ (\alpha) = \frac{0.693}{\alpha} \qquad \text{Equation (2)}$$

$$t_{\frac{1}{2}}\ (\beta) = \frac{0.693}{\beta} \qquad \text{Equation (3)}$$

$$k_{21} = \frac{A \cdot \beta + B \cdot \alpha}{A+B} \qquad \text{Equation (4)}$$

$$k_{el} = \frac{\alpha \cdot \beta}{k_{21}} \qquad \text{Equation (5)}$$

$$k_{12} = \alpha + \beta - k_{el} - k_{21} \qquad \text{Equation (6)}$$

$$Vc = \frac{\text{Dose}}{\dot{Cp}} \qquad \text{Equation (7)}$$

$$Vd_{(area)} = \frac{\text{Dose}}{AUC \cdot \beta} \qquad \text{Equation (8)}$$

$$Vd_{(\beta)} = \frac{k_{el} \cdot Vc}{\beta} \qquad \text{Equation (9)}$$

$$Cl_{B(area)} = \beta \cdot Vd_{(area)} \qquad \text{Equation (10)}$$

$$Cl_{B(\beta)} = k_{el} \cdot Vc \qquad \text{Equation (11)}$$

$$\text{Bioavialibility} = \frac{AUC_{Or} \cdot \beta_{Or}}{AUC_{Ic} \cdot \beta_{Ic}} \qquad \text{Equation (12)}$$

Equation (13)

$$T = \left[\left(T_{tn-1}\right) e^{-k_{21}\Delta t}\right] + \left[\left(\frac{k_{12}}{k_{21}}\right) P_{tn-1} \left(1-e^{-k_{21}\ \Delta t}\right)\right] + \left[\frac{k_{12}\Delta p\ \Delta t}{}\right]$$

VITA

Fereidon Khan Alavi

Candidate for the Degree of

Master of Science

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

Major Field: Physiological Sciences (Pharmacology)

Biographical:

Personal Data: Born in Tabriz, Western Azarbijan, Iran, July 26, 1957, the son of Oscar and Farideh Alavi.

Education: Graduated from Pahlavi High School, Miandoab, Eastern Azarbijan, Iran, in May, 1975; recieved Bachelor of Science degree in Biology from Oklahoma State University in may, 1981; completed requirment for the Master of Science degree at Oklahoma State University in July, 1986.

Professional Experience: Graduate Research Assistant, Department of Physiological Sciences, Oklahoma State University, 1984-85 Lab Technician, Department of Animal Science, Oklahoma State University, 1985-86.