DRUG RESIDUES IN FOOD PRODUCING ANIMALS

DISSERTATION

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By

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* * * * * *

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iii


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TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>VITA</td>
<td>iii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xiii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xv</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II. LITERATURE REVIEW AND BACKGROUND INFORMATION</td>
<td>9</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>16</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>24</td>
</tr>
<tr>
<td>Potassium Penicillin G</td>
<td>28</td>
</tr>
<tr>
<td>III. MATERIALS AND METHODS</td>
<td>34</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>35</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>40</td>
</tr>
<tr>
<td>Potassium Penicillin G</td>
<td>43</td>
</tr>
<tr>
<td>IV. RESULTS</td>
<td>48</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>48</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>54</td>
</tr>
<tr>
<td>Potassium Penicillin G</td>
<td>59</td>
</tr>
<tr>
<td>V. DISCUSSION AND CONCLUSIONS</td>
<td>83</td>
</tr>
<tr>
<td>APPENDICES</td>
<td>104</td>
</tr>
</tbody>
</table>

Figure 9. The two-compartment open model consists of a central (1) and a peripheral (or tissue, 2) compartment. The constants $k_{12}$ and $k_{21}$ are the first-order rate constants for the passage of the drug between the two compartments; $k_{1}$ is the first-order rate constant for elimination of the drug from the central compartment. A single compartment open model consists of the central (1) compartment only. 105
Table 13. Linear regression analysis of plasma concentration of SMZ (mg/100 ml) and amount of SMZ to be excreted (percent of dose) versus the concentration of the drug (ppm) in various times following IV administration of sulfa-methazine (107.25 mg/kg body weight) .............. 106

Table 14. Linear regression analysis of average plasma concentration of sulfathiazole and rate of excretion of unchanged sulfathiazole versus the concentration of sulfathiazole in various tissues following intravenous administration of sodium sulfathiazole (72 mg/kg) to swine .... 107

Table 15. Linear regression analysis of average plasma concentration of sulfathiazole and rate of excretion of unchanged sulfathiazole versus the concentration of sulfathiazole in various tissues following intravenous administration of sulfathiazole (72 mg/kg) ...................... 108

Table 16. Definition of Terms ............ 109

Table 17. Chloramphenicol plasma concentrations in swine following intravenous injection of a single dose (22 mg/kg). Units in ug/ml ........ 110

Table 18. Plasma concentrations of oxytetracycline in young swine following an intravenous dose of 11 mg/kg ......................... 111

Table 19. Plasma concentrations of penicillin G in swine dosed intravenously with 5,000 units/lb or equivalent to 7.64 mg/kg body weight ........ 112

Table 20. Total tissue and body fluid levels of chloramphenicol in pigs at selected intervals following a single intravenous dose (22 mg/kg). Total in milligrams (mg), and percent of dose ......................... 113

Table 21. Total tissue and body fluid levels of oxytetracycline in pigs at selected intervals following a single intravenous dose (11 mg/kg). Total in milligrams (mg), and percent of dose .... 114
Table 22. Total tissue body fluid levels of penicillin G in pigs at selected intervals following a single intravenous or oral dose (7.64 mg/kg). Total in units, and percent total dose.

| BIBLIOGRAPHY | 116 |
10. Concentrations of penicillin G in selected extracellular fluid compartments and associated organs from pigs treated intravenously and orally with a single dose (7.64 mg/kg) of the drug.............. 80

11. Concentration of penicillin G in selected organs of pigs treated intravenously and orally with a single dose (7.64 mg/kg) of the drug................ 81

12. Comparisons between biological half-lives and elimination constants (Beta) of mean plasma concentrations of penicillin G and concentrations in major organs and body fluids of swine treated with a single intravenous bolus of the drug (dose = 7.64 mg/kg) ......................... 82
<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Semilogarithmic plot of chloramphenicol concentration in serum versus time after intravenous administration of a single dose of the drug (22 mg/kg). The circle dots (o) represent actual concentrations of drug in serum; the crosses (x) are the concentrations obtained by the feathering technique. Least square regression lines describe the α- and β- phases of the biexponential drug concentration-time profiles. A and B are the intercept values for pig No. 5</td>
<td>63</td>
</tr>
<tr>
<td>2. Semilogarithmic plot of chloramphenicol concentration in serum versus time after intravenous administration of a single dose of the drug (22 mg/kg). This plot illustrates the fitting of the single compartment model to the data from pig No. 10. The circle dots (o) represent actual concentrations of drug in serum</td>
<td>64</td>
</tr>
<tr>
<td>3. Semilogarithmic plot of chloramphenicol concentration in serum versus time obtained after intravenous administration of a single dose of chloramphenicol (22 mg/kg). Plot represents a single animal (pig No. 3) which illustrates the enterohepatic recycling effect on plasma levels between 30 to 60 minutes post-dose as observed in 16 of 17 animals</td>
<td>65</td>
</tr>
<tr>
<td>4. The concentration of chloramphenicol in pig tissues, plasma and urine at various times following the intravenous administration of chloramphenicol</td>
<td>66</td>
</tr>
<tr>
<td>5. Semilogarithmic plot of oxytetracycline concentration in serum versus time obtained after administration of a single dose of the drug (11 mg/kg) as an intravenous bolus to pig F. The closed circles (o) represent measured concentration of antibiotic in serum; the open squares (□) are the concentrations obtained by the feathering technique. Least square regression lines describe the α- and β- phases of the biexponential concentration-time profile. A and B describe the concentrations at zero time ( (C^0_p) )</td>
<td>67</td>
</tr>
</tbody>
</table>
6. The concentration of oxytetracycline in pig tissues, plasma and urine at various times following the intravenous administration of oxytetracycline 68

7. Semilogarithmic plot of penicillin G concentration in serum versus time obtained after administration of a single dose of the drug (7.64 mg/kg). The circle dots (○) represent the measured activity of antibiotic in serum; the open squares (□) are the concentrations obtained by the feathering technique. Least square regression lines describe the α- and β-phases of the biexponential drug concentration-time profile. A and B are the concentration at zero time (C° intercepts). 69

8. The concentration of penicillin in pig tissues, plasma and urine at various times following the intravenous administration of the drug 70
CHAPTER I

INTRODUCTION

During 1975, United States farmers raised 69 million hogs, 128 million head of beef and dairy cattle, 275 million laying hens, and 3 billion broilers. These industries are worth 40 billion dollars a year to the U.S. economy and support a 1 billion dollar a year animal health industry. Raising animals and poultry to provide food for the ever-increasing U.S. and world populations is an extremely complex undertaking. Fewer than one in twenty Americans are now farming. Grains for feeding animals must be used more efficiently. More poultry and animals are raised to market weight within smaller land areas than ever before. Modern agriculture is no longer a family farm with a few cows, a few chickens, a few hogs and a field or two of corn, wheat or hay.

The fact that large volumes of drugs are used in food-producing animals is well documented. It has been estimated that 80% of the animal protein consumed in the United States originates from animals fed medicated feeds for part or for their entire life period. Within the next one or two decades, it is anticipated that nearly 100% of the animals will have been exposed to some form of medication in the control, prevention and treatment of diseases to enable ample quantities of protein in the human diet. Consequently, the
justification for the use of drugs in animal production is also a well documented fact.\(^3\)

When it is considered that animal drugs may be administered in feed, in drinking water, parenterally, topically and by intramammary or intrauterine infusion, the potential for the deposition of drug residues in edible tissues of animals becomes enormous.\(^1\)

Antimicrobial agents are widely used to control and treat animal diseases, and as growth promoting agents. Many drugs such as the steroids, are used to enhance growth and to control estrus cycles. Anthelmintics are well established as necessary tools in animal production. There is little question that more effective and efficient usage of drugs in food producing animals is desirable, but regardless of new developments and scientific accomplishments, it is envisioned that the production of animal origin protein will require the continued use of drugs.

Problems associated with the use of drugs in food producing animals not only revolve about the detection of drug residues in tissues, but also involve the detection of metabolites which may be of greater significance from a public health standpoint than the parent or unmetabolized drug. For this reason, pharmacokinetic studies need to be conducted in a number of animal species to fully comprehend the differences that exist in the metabolic transformation of drugs. It is well known that marked differences exist among the various animal species in the metabolism of drugs and chemicals. For example, glucuronide synthesis is virtually absent in one of the animal species (i.e., the cat). In the rabbit, rat, dog, cat and pig, mercapturic
acid synthesis occurs rapidly compared to the rate in the guinea pig and man. Synthesis of ethereal sulfate occurs in the majority of animal species, but only at an extremely low level in swine. The dog and guinea pig have defective acetylating mechanisms compared to the rat, rabbit, pig, monkey and man. Similar differences exist for phenomena associated with oxidation, hydrolysis and reduction. In general, our knowledge is limited relative to the fate of drugs in the various animal species and particularly in species such as poultry, swine, sheep and cattle.

When domestic food-producing animals are treated with veterinary drugs, significant concentrations of the drugs may be retained in tissues for varying periods of time. The potential hazards of consuming meat containing such residues include the development of hypersensitivities to drugs that may be needed therapeutically and in the case of antimicrobial drugs, the preferential selection of bacterial mutants that may be resistant to drugs used in the treatment of human disease. Thus, drug withdrawal periods must be established and may also become a criterion for the use of drugs in food animals.

The search for new drugs and their subsequent development follows a long and intense scientific pathway. Development of manufacturing methods, analytical methods, formulations, stability, posology, toxicology, packaging and marketing are only a few of the major scientific endeavors required for new drugs. In food animals, the Food and Drug Administration (FDA) adds several additional requirements including drug residue studies in target animals, metabolism studies and data relating to human toxicity potentials. The development of the drug
residue data is one of the single most costly steps in drug development for food animals. The establishment of the drug withdrawal requirements and setting of tolerances which insure that the intended drug usage will not pose a threat to public health is the single most important aspect of FDA's responsibilities in approving new drugs for use in food animals.

The conventional methods used to determine the residual nature of a drug in food animals involves the following general sequence of events:

1. Develop an analytical method for quantitative determination of the parent drug in biologic fluids and tissues.
2. Dose a series of animals via intended route with proposed formulation.
3. At various intervals after dosing (i.e., at 0, 1, 3, 5, 7, 9, 12 days), slaughter at least three animals, collect a variety of tissues, including specifically liver, muscle, fat, and kidney, then conduct numerous assays to determine the necessary withdrawal interval.
4. Provide supporting toxicology data for establishing tolerances.
5. Refine the analytical procedure to the desired sensitivity level (parent drug and/or metabolites must be considered).
6. Establish withdrawal requirements, which then become an important part of the product labeling.
Inherent in a residue study conducted in this manner are a number of potential disadvantages and problem areas as defined below.

1. Numerous assays and extensive manpower expenditures have been made. (Drug extractions from tissues still remain a tedious, and in many cases, a non-exacting scientific endeavor).

2. Considerable animal to animal variation is often observed in drug excretion patterns and residual tissue levels, but the design of these conventional residue experiments does not allow a critical evaluation of this parameter.

3. Data which may be used to project therapeutic effectiveness of the compound are scanty to nonexistent.

4. The data developed are only applicable to a single dose in a single formulation, and for a specific dosing interval.

5. Lower doses of drug can comply with withdrawal periods established at the higher dose, but extrapolations cannot be made either upward or horizontal to other routes of administration and other formulations.

6. There is no specific data base on the behavior of the drug in the system, i.e., absorption rates, extent of distribution and elimination rates.

7. The drug withdrawal period may be established on the basis of the performance of the poorest animal in the experiment in terms of its metabolic capabilities in the handling of the drug. Application of the withdrawal time to the broader population of animals in a typical feedlot
situation is questionable.

The potential use of pharmacokinetics to better define the residual nature of a drug in tissues of food animals has been discussed in detail in a recent literature report.\(^{34}\)

The withdrawal time is only effective if it is observed in actual use. Studies of the number of carcasses containing illegal concentrations of antimicrobial agents suggest that the incidence of failure to observe required withdrawal times is quite high.\(^{24}\) As a result, the United States Department of Agriculture (USDA) has a program of inspection of carcasses for illegal drug residues and may condemn carcasses in which residues exceed established tolerance limits. Because current methods for determining the concentrations of drugs in animal tissues are expensive and time-consuming, surveillance is inefficient and costly. In addition, detection of a contaminated carcass requires condemnation which is a direct monetary loss to the producer and packer and adds to the cost of meat. If a method could be developed to detect those animals whose meat contained more than the tolerance limit of a drug before slaughter, it would be possible to delay slaughtering until the drug is at tolerated levels thereby saving the carcass from needless destruction. Furthermore, if the detection method utilized blood or urine instead of tissue specimens, it would be possible to reduce the cost and time involved in assay and thereby increase the efficiency of surveillance.

Pharmacokinetics deals with the time course of drug and metabolite concentrations in plasma, urine, and other body tissues and fluids. The detailed use of this mechanism in determining the drug
residue profile has been reported. If a relationship could be estab-
lished between tissue concentrations and plasma and/or urine concen-
trations of the drug, and if the pharmacokinetics of the drug were
known, it would be possible to predict when the tissue concentrations
in an animal had reached pre-set tolerance limits\(^3\). 

In summary,
on several problem areas associated with the use of drugs in food
animals are as follows:

1. Better and more precise methods need to be developed
to determine the metabolic and residual nature of a drug
in food animals. The use of pharmacokinetics appears to
be one method which will improve the quality of the data
base.

2. There needs to be a better understanding of both plasma
and tissue kinetics of commonly used veterinary drugs.
The literature is seriously deficient on the tissue kinetics
of drugs when used in food animals.

3. Once residue profiles have been established, there need
to be better and more efficient methods for screening for
drug residue violations in order to more efficiently pro-
tect the consumer.

4. Plasma and tissue kinetics of drugs are important to a
residue profile, but may also provide important insight
into the potential effectiveness of a compound. Thus,
residue studies properly designed, can contribute to a
better understanding of the therapeutic use of drugs in food animals.

The specific objectives of this study are:

1. Determine the elimination kinetics from plasma of chloramphenicol, oxytetracycline, and penicillin G in young crossbred swine.

2. Estimate the elimination rate of above three drugs from major tissues and body fluids.

3. To propose readily available body fluids to predict tissues residues.
CHAPTER II

LITERATURE REVIEW AND BACKGROUND INFORMATION

It is clear to the experienced observer that the conventional methods for determining drug residue patterns in food animals "evolved" rather than developed as a result of systematic research planning. Consequently, these methods have several serious deficiencies and in many instances, lack a broad data base for use for interpretive purposes. This has led, and continues to lead to enumerable problems in the regulation of drugs for use in food animals. As an emphasis to this point, during the Spring of 1977, the incidence of violative sulfonamide residues in swine in the Midwest was occurring at the rate of 10 - 15% in slaughter carcasses, and may be occurring at a rate of 35 - 40% in isolated instances. In anticipation of such an event, and because the sulfonamide drugs are a much needed and widely used class of drugs, and also because of recognized deficiencies in our knowledge about their residual profile, a series of studies was initiated five years ago by the Food and Drug Administration. These investigations were designed to (1) develop or improve analytical methods for sulfonamides and their metabolites in the plasma, urine, and tissues of domestic animals; (2) develop pharmacokinetic models describing the distribution, metabolism, and elimination of sulfonamides in domestic animals following intravenous and oral administration, and; (3) establish
a correlation between plasma, urine and tissue concentrations of several commonly used sulfonamides.

Since the published literature is deficient in this area, and because of the importance and relationship of this work to the work to be subsequently reported, a brief review of these recently obtained results for the sulfonamide drugs will be provided.

Sulfamethazine was administered intravenously (IV) at a dose rate of 107 mg/kg body weight to three calves. The intravenous plasma data were described in terms of a one-compartment pharmacokinetic model with an elimination half-life of about 9 hours and a volume of distribution of 0.35 l/kg body weight. Elimination of sulfamethazine in cattle was found to include excretion of the unchanged drug into urine and the formation of three metabolites in the central compartment. Urinary excretion data were used to calculate the formation and excretion rate constants for the metabolites.

Sulfamethazine was also administered orally to the same three calves as a 12.5% solution of sodium sulfamethazine, a rapid release bolus of sodium sulfamethazine, and a sustained release bolus of sulfamethazine. The orally administered solution was absorbed by a simple first-order process, whereas the two solid dosage forms demonstrated a kinetically distinct dissolution phase preceding the absorption process. The half-life for absorption of sulfamethazine from the oral solution was about 6 hours.

Both the solution and the rapid release bolus were well absorbed (99% and 82%, respectively) and gave peak plasma concentrations of 12 - 13 mg%. The sustained release bolus was not completely
absorbed (46%) and gave peak plasma levels of about 9.5 mg%. However, this dosage form did produce effective blood levels (about 5 mg%) for about twice as long as two oral dosage forms.

The metabolic pattern of unchanged drug and metabolites in urine observed following intravenous administration of sulfamethazine was different from the pattern observed following oral administration. The proportion of sulfamethazine excreted unchanged after oral administration was less than the proportion excreted unchanged after intravenous administration. This result suggested that, in cattle, sulfamethazine was partially metabolized during the absorption process following oral administration. This "first pass" metabolism possibly occurs in the rumen, the gut wall, or the liver.

The overall elimination rate constant ($k_{e1}$) was calculated to be 0.0744 hr.$^{-1}$.

Thirteen young ewe lambs were dosed with sulfamethazine IV at a dose rate of 107 mg/kg body weight$^5, 52$. The time course of the unchanged sulfamethazine in plasma and urine can be described in terms of a one-compartment model. The biologic half-life of unchanged sulfamethazine in sheep was approximately 7 hours.

The formation of the acetyl, hydroxy, and polar metabolites of sulfamethazine in the body and their excretion in urine can be described in terms of a linear pharmacokinetic model. There was no evidence of saturable metabolic or urinary elimination steps.

Both sulfamethazine concentrations in plasma and urine outputs of sulfamethazine were correlated with sulfamethazine residues in eight tissues at slaughter, demonstrating that sulfamethazine obeys a
one-compartment pharmacokinetic model in sheep and that the washout of sulfamethazine from various food tissues was accurately reflected by the plasma and urine concentrations of the drug (Appendix Table 2).

Sulfamethazine was administered intravenously to five swine at the 107 mg/kg body weight dose level, and plasma and urine specimens were collected at various times following administration \(^4\), \(^19\).

The plasma concentration-time data and the urinary rate of excretion-time data appeared biexponential; therefore, the disposition of sulfamethazine in swine was described with a two-compartment pharmacokinetic model. The \(\alpha\) and \(\beta\) rate constants were 2.33 and 0.0406 hr\(^{-1}\), respectively. Approximately 21% of the intravenous dose was excreted unchanged in urine, 42% of the dose was excreted as acetyl sulfamethazine in urine, and 5.7% of the dose was excreted as a polar metabolite in urine.

Sulfamethazine was administered orally to four swine at the 107 mg/kg body weight dose level, and plasma and urine specimens were collected at various times following administration \(^19\).

The two-compartment pharmacokinetic model previously described with an added first-order absorption process was fit to the plasma level data. The orally administered dose appeared to be completely absorbed. The average value of the absorption rate constant was found to be 2.21±1.26 hr\(^{-1}\) (half-life 0.31±0.18 hr). Approximately 27% of the absorbed dose was excreted into urine as the parent drug, 45% as the acetyl metabolite, and 10% as a polar metabolite.

The tissue, urine and plasma concentrations of sulfathiazole were determined at various times following intravenous administration
to fifteen swine. The average plasma and urine data were found to fit a two-compartment pharmacokinetic model with a biological half-life of 2.6 hour and a total apparent volume of distribution of 0.625 liter/kg body weight. The second compartment was found to be "shallow", that is, the apparent volume of the second compartment was only 23% of the central compartment.

Sulfathiazole was eliminated by excretion of unchanged drug into urine (48%) and formation of an acetyl metabolite (19%). Twelve of the swine appeared to be divided into six "rapid" and six "slow" acetylators. The data obtained from seven tissue sites paralleled the plasma concentrations and confirmed that tissue residues of sulfathiazole can be calculated from plasma and/or urine concentrations of the drug.

The correlation between plasma and tissue levels of sulfathiazole is presented in Appendix Table 3.

The plasma, urine and tissue concentrations of sulfathiazole were determined at various times following intravenous administration of 72 mg/kg body weight to twelve sheep. The plasma and urine data were consistent with a one-compartment pharmacokinetic model with a half-life of elimination of 1.1 hours and a volume of distribution of 0.37 l/kg body weight. The overall elimination ($k_e$) was found to be 0.63 hr$^{-1}$. Sulfathiazole was eliminated by excretion of unchanged drug into urine (67%) and by formation of two metabolites. The data obtained from eight tissue sites were consistent with the one-compartment pharmacokinetic model presented and confirmed that tissue
residues of sulfathiazole can be calculated from serum and urine concentrations of the drug.

The correlation coefficients determined between plasma and tissue concentrations of sulfamethazine can be found in Appendix Table 4.

Similar pharmacokinetic data have been developed for sulfamerazine in cattle and sheep, sulfadimethoxine in cattle and swine, sulfaethoxypyridazine in sheep and cattle, and sulfapyridine in swine, sheep, and cattle. Results are not yet complete, but will be available in the near future.

The correlation coefficients between plasma and the various body tissues obtained with sulfamethazine and sulfathiazole in several species of animals were greater than expected, and in many cases were 1.000. Results of this nature were highly stimulatory to the desire to examine other classes of drugs of equal importance in veterinary medicine. Preliminary evidence was clear that plasma may have predictive value regarding tissue levels for these specific sulfonamide drugs. This information could have a tremendous impact upon both the methods used in determining drug residue profiles as well as upon the expensive, time consuming, and inefficient methods used for monitoring drug residues in the nation's meat supply. This desire to examine other major classes of drugs commonly used in veterinary medicine provided the stimulus for the work reported herein.

The kinetic nature of the drugs to be selected for these studies was of utmost importance. They should be representative of their
respective classes, but they also needed to possess certain kinetic properties which should technically separate them with regard to their behavior in the live animal system. The following three drugs and a brief description of their kinetic properties were selected for these studies:

1. Chloramphenicol, while not approved for use in food animals, is nevertheless commonly used to treat neonatal calf and swine enteric diseases. It characteristically demonstrates a rapid biological half-life;\(^{10}\) has a high volume of distribution;\(^{10,23}\) is rapidly and rather completely absorbed from the intestinal tract;\(^{23}\) has a high degree of tissue penetration;\(^{23}\) is eliminated primarily by tubular excretion of metabolites\(^{23}\).

2. Oxytetracycline is one of the most commonly used antibiotics in veterinary medicine. It characteristically demonstrates a long and variable biological half-life;\(^{23}\) has a high volume of distribution;\(^{23}\) is incompletely absorbed from the gastrointestinal tract;\(^{23}\) has a high degree of tissue penetration;\(^{23}\) is eliminated by mainly the kidney by glomerular filtration and in the feces following biliary excretion\(^{23}\).

3. Penicillin G is also a commonly used veterinary drug. It has a rapid biological half-life;\(^{23}\) generally has a low volume of distribution;\(^{23}\) does not penetrate tissues to a great
extent; is almost exclusively eliminated by the kidneys through filtration and excretion.

Chloramphenicol Literature

Chloramphenicol is unique among natural compounds in that it contains a nitrobenzene moiety and is a derivative of dichloroacetic acid. The biologically active form is levorotatory. It is only slightly soluble in water (1:400). The antibiotic is extremely stable. Chloramphenicol is inactivated by enzymes present in filtrates of certain bacteria which reduce the nitro group and hydrolyze the amide linkage and it is acetylated also.

Chloramphenicol is rapidly absorbed from the gastrointestinal tract. In man, significant plasma concentrations were reached within 30 minutes with peak values obtained within 2 hours. Peak values range from 20-40 μg/ml after a 4g dose. The half-life of the drug is about 1.5 to 3.5 hours, thus it becomes nondetectable after 12 to 18 hours. In man, it is estimated that about 60% is bound to plasma albumin.

Plasma concentrations of chloramphenicol and the kinetics of its disappearance from plasma after intravenous administration were determined in dogs, cats, swine, goats and ponies; plasma half-life values varied from 0.9 hours in ponies to 5.1 hours in cats. The half-life in swine was found to be 1.3 hours with a peak plasma concentration of 21.0 μg/ml immediately following IV injection. Apparent specific volumes of distribution varied from 1.02 l/kg in ponies to 2.36 l/kg
in cats. Swine were determined to have a volume of distribution of 1.05 L/kg. The drug was bound to plasma protein at the rate of 30 to 46%, and the extent of binding was independent of drug concentration\textsuperscript{10}.

Chloramphenicol and its metabolites are rapidly excreted in the urine. Over a 24-hour period, 80 to 90% of an orally administered dose is excreted; about 5 to 10% is in the biologically active form, whereas the remainder is inactive and consists of a hydrolysis product and a glucuronic acid conjugate. The unaltered antibiotic is eliminated mainly by glomerular filtration. The inactive degradation products are eliminated primarily by tubular secretion\textsuperscript{23}.

Chloramphenicol appears in most tissues within 0.5 to 1 hour after oral ingestion. It reaches the highest concentration in the liver, bile and kidneys. Chloramphenicol apparently is present in the intracellular as well as the extracellular fluid. It is effective for use in eye infections since it readily penetrates ocular tissues. Chloramphenicol diffuses into the cerebrospinal and pleural fluids and diffuses across the placenta. It reaches a concentration in fetal blood of approximately 75% of that in maternal blood 2 hours after administration\textsuperscript{25}.

Chloramphenicol is eliminated from blood within 4 to 6 hours after IV injection in dogs, and 12 to 16 hours after oral administration. Only 6.3% of the drug excreted is active chloramphenicol. The inactive form appears to be excreted largely by the renal tubules and the active form by the renal glomeruli. In the dog and rat, a large portion of the inactive metabolized drug is excreted in the bile.
as aromatic amines derived from chloramphenicol by reduction of the nitro group. In a recent study, chloramphenicol was found to attain a distribution equilibrium between blood and tissues in less than 30 minutes. If the observed values for drug concentration at zero time are used and a blood volume equivalent to 7% of the body weight is assumed, it can be calculated that after distribution equilibrium was attained, there were 4.0%, 4.0%, 6.7%, 5.2%, and 6.8% of the dose in the blood of dogs, cats, swine, goats and ponies, respectively. These findings were in agreement with earlier investigations. They observed that the drug attained concentrations in kidney, liver, lung, spleen, heart and muscle that exceeded the concentration in plasma. Further corroboration that chloramphenicol is sequestered in extravascular tissues was provided in a subsequent study by the relatively large values for apparent specific volumes of distribution (1.46, 1.61, 1.91, 2.96, and 3.69 L/kg in ponies, swine, goats, dogs and cats, respectively). These volumes were in excess of the total body weight in all species studies. Tissue chloramphenicol concentrations studied in three horses, destroyed 5 and 6 hours after being treated intramuscularly at a rate of 20 mg/kg, were found to be much higher than serum concentrations. Serum concentrations were 0.0 at 3 hours after injection, whereas levels in muscle, lung, liver, kidney, spleen, brain and aqueous humor were 7.01, 0.00, 8.41, 6.03, 0.00, 10.50 and 0.80 μg/gm, respectively at 5 and 6 hours after injection. The most outstanding feature of
these results was that tissue chloramphenicol levels were compara-
tively high after serum levels had fallen to zero. The authors sug-
gested that chloramphenicol in the horse must quickly leave the blood
and find its way to extracellular or intracellular locations in tissues.
It should be pointed out, however, that these investigators found levels
of chloramphenicol in injection sites (mean 417 µg/gm tissue) up to 3
days after injection. With such an injection site depot, and given
the credibility of analytical methods for chloramphenicol in 1959, it
possibly cannot be concluded that zero levels in blood necessarily mean
a total exodus to regional tissue sites. These findings may simply have
been a function of an extremely slow absorption constant\textsuperscript{14}.

Work was carried out on tissue levels in guinea pigs, rats,
and dogs. Two dogs were treated at the rate of 35 mg/kg subcutaneously;
one was destroyed at 1 1/2 and the other at 3 hours after treatment at
which time blood levels of chloramphenicol were still quite high. At
1 1/2 hours, most tissue levels were twice as high as those at 3 hours
and, furthermore, at the times of destruction, blood levels were
higher than tissue levels in muscle and brain; about the same as in
lung and spleen; but less than half that in liver and kidney. In one
dog, it appeared as though fall in tissue and blood levels were
parallel\textsuperscript{22}.

The speedy disappearance of chloramphenicol from lung tissue
would suggest the necessity for frequent dosage when using this drug
to treat pulmonary infections in the horse\textsuperscript{14}.

It has been observed in the cow that transient elevations of
chloramphenicol blood levels occur at 1 1/2 and 3 hours after IV
administration of the drug. These periodic rises of the blood level suggest reentry of the drug into the bloodstream through a recirculation cycle approximately every 90 minutes. Since high concentrations of chloramphenicol and its metabolites are excreted in bile, but very little in feces, it suggests that almost all of the drug (or metabolites) excreted via bile are reabsorbed by the intestines into blood circulation. It was concluded that the entero-hepatic cycle was affecting chloramphenicol blood levels. Chloramphenicol has been reported to concentrate 30x in bile versus plasma. This cycle is of significance because it should prolong the sojourn of the drug in the body and would also be expected to provide high intraluminal levels of the antibiotic in small and upper large intestines. In this respect, chloramphenicol is similar to the tetracyclines.

Recommended therapeutic levels in plasma (>5 μg/ml) of chloramphenicol were maintained in the cow for 4 to 5 hours following an IV dose of 11 mg/kg. However, the same dose intramuscular (IM) produced blood levels only in the range of 1.4 to 2.0 μg/ml which is less than the recommended therapeutic range. An IV dose of chloramphenicol (22 mg/kg) is required to provide therapeutic levels in milk for between 2 to 8 hours after medication.

Recent studies in cows and horses have determined the half-life of chloramphenicol in cows to be 3.5 hours, and in horses 1 hour. The volume of distribution in the cow was 0.814 l/kg and 1.82 l/kg in horses. These two species metabolized this drug differently. The higher fraction of metabolites in horse plasma compared to cattle plasma shows that the metabolic transformation was more rapid in
horses, both in absolute terms and relative to the renal handling of the drug and its metabolites between the two species. Seventy-five percent of the water soluble compounds in urine were accounted for by glucuronides as shown by the treatment with β-glucuronidase⁴¹.

A study was conducted in swine comparing plasma and tissue levels of chloramphenicol after oral, intramuscular and intraperitoneal administration of 25 and 50 mg/kg. Comparisons of results obtained after dosage at the 50 mg/kg rate showed that (1) the mean serum concentration 1 hour after intraperitoneal treatment was significantly higher (10.28 versus 2.28 μg/ml) than oral treatment; whereas oral treatment gave levels (2.28 versus 0.76 μg/ml) significantly higher than intramuscular; (2) both oral and intraperitoneal levels at 3 hours post-dose were clearly superior (3.54 to 5.61 versus 0.85 μg/ml) to the IM route; (3) results were similar with all 3 routes at 6 hours, but at 12 hours, the IM treated pigs still had measurable levels (0.39 μg/ml). The same general relationships were found with the 25 mg/kg dose, but the magnitude of the plasma levels were about 50% lower¹⁵.

Tissues were sampled in two pigs from each route of administration at 6 hours post-dosing. In the pig, unlike the horse, blood samples were still positive at time of slaughter. The following range of tissue levels were found from the three routes of administration and 50 mg/kg dose: muscle 0 - 3.5 μg/gm; heart 0 - 6.2 μg/gm; kidney 0.8 - 10 μg/gm; brain 1.5 - 2.7 μg/gm; skin 0 - 1.2 μg/gm; spleen 0 - 3.6 μg/gm; stomach wall 0 - 8.6 μg/gm; testes 2.0 - 4.2 μg/gm; lung 0 - 3.6 μg/gm; and liver 0 - 1.4 μg/gm. The average serum concentration at 6 hours was 0.66 μg/ml. Although there was some variation
lung 31.2, 8.9, 8.7 and 11.8; lymph node 56.3, 15.9, 17.7 and 5.4; muscle 24.6, 8.2, 5.1 and 2.7; pancreas 43.6, 18.7, 22.7 and 5.1; spleen 48.1, 9.6, 2.3, and 12.2.  

The distribution of chloramphenicol in the body was not uniform. In all sampled tissues except brain, the initial corrected values were higher than concentrations in blood. The tissue sampled in diminishing order of chloramphenicol concentrations were: lymph node, spleen, pancreas, liver, kidney, lung, muscle and brain. These results support the contention that chloramphenicol may penetrate tissue cells. If this were not so, the concentrations of chloramphenicol in tissue interstitial fluids would be 3 times those found in tissue homogenates and, therefore, about 4 to 8 times those in plasma. This is not the case because it has been shown that chloramphenicol concentrations in extravascular fluids are, in fact, lower than those in plasma as determined by simultaneous assay.

These investigations suggest that because of the different rates of loss of chloramphenicol from various tissues and extravascular fluids, it may be advantageous during therapy to select dosage frequencies according to site of infection. However, before one attempts to do this, information on specific activity potential and better data on the tissue kinetics of chloramphenicol would be required.

A recent study on the pharmacokinetics of chloramphenicol in tissues and plasma of baby pigs was reported. The distribution and elimination of chloramphenicol and its metabolites were determined following a single IV dose of 22 mg/kg. Disappearance of chloramphenicol from plasma was biplastic; an initial component was rapid (half-life
about 9 min) and a second component had a half-life of 55 minutes. The total chloramphenicol levels in tissues and body fluids had half-life periods of 1-1.5 hours and dropped below limits of detectability within 9 hours after injection. About 80% of the injected dose was recovered from urine over the first 6 hours, and none thereafter. No levels were detected in feces. At 6 hours, muscle tissue contained 200 ppb chloramphenicol which was determined to be mostly non-metabolized drug.

Oxytetracycline Literature

All the tetracyclines are adequately but incompletely absorbed from the gastrointestinal tract. Most absorption takes place from the stomach and upper small intestine. Absorption is greater in the fasting state and it is much less complete from the lower portions of the intestinal tract. The greater the dose, the more the drug is unab­sorbed, amounting to more than 50% with large doses. Calcium and other divalent cations form insoluble complexes with the tetracyclines, accounting for the decreased absorption which occurs when feed or milk is given with the drug.\(^{23, 26, 35}\)

Peak serum levels after single oral doses are reached in 2 to 4 hours and decrease slowly over the next 12 to 24 hours. The primary excretion is urinary, however, the tetracyclines are removed by the liver, where they are concentrated and then excreted by way of bile into the intestine where they are partially reabsorbed. Biliary concentrations may average 5 to 10 times higher than simultaneous values in plasma.\(^1\) This provides an effective enterohepatic cycle which
maintains small amounts of tetracycline in the body and delays its ultimate removal. The half-lives of the several common tetracyclines vary from 6 to 19 hours. The volume of distribution of the tetracyclines is relatively larger than that of body water, indicating good tissue penetration and possibly sequestration in some tissues. Highest concentrations generally occur in kidney, liver, spleen and lung.

Ten to 35% of a dose of oxytetracycline is excreted in active form in the urine, in which it is detectable within one-half hour and reaches peak concentrations in about 5 hours after administration.

Considerable effort has been made to examine distribution of various tetracyclines into body tissues. Although the value of volume of distribution (Vd) is a valuable tool in calculating infusion rates and dosage regimens, predicting multiple dose plasma levels, as an indicator of protein binding, and so on, Vd does not indicate where the drug has been distributed once it has left plasma. By analyzing surgically removed tissues of treated animals, the ratio of total tetracycline in muscle tissue to the concentration in canine serum has been correlated with chloroform/water partition coefficients. The tetracycline with the highest partition coefficient had the highest muscle concentration. The observed order of the tetracyclines studied was 6-demethyl-6-deoxytetracycline > doxycycline > tetracycline > demethylchlortetracycline = methacycline > oxytetracycline. In another study, concentrations of tetracycline, demethylchlortetracycline and chlorotetracycline were determined in about 47 organs, tissues or fluids by use of radiolabeled compounds. Results indicated that all nonfat tissues...
are penetrated by the antibiotics within 4.5 hours after intravenous injection and that tissue distribution of tetracycline is generally less than that of the other two.

Doxycycline content in 12 organs sampled operatively in 81 patients was compared with the serum levels at the time of tissue removal and the tissue to serum ratios calculated for each organ. These ratios varied from approximately 2 for kidney, lung and bladder to less than 0.7 for the appendix and adipose tissue. When the serum concentrations were corrected for protein binding, the ratios became 13.4 for the kidney, 12.2 for the lungs, 6.3 for muscle, and 3.9 for lymph node. The concentration in all organs usually fell in the range of 2 to 4 µg/g of tissue and always was above 1 µg/g which was considered to be amply bacteriostatic.

The emphasis on improved tissue distribution of antimicrobial agents has increased in the past several years. It has been stated that "bacteria germinate more frequently in tissues than in blood" and that "bacteria are more common in other tissues than in blood". It has also been stated that antibiotic effectiveness depends upon penetration into tissues and particularly inflamed tissues. The fact that the tetracyclines as a class have this property has made them especially valuable as therapeutic agents.

Serum and milk concentrations of 9 tetracycline analogues, e.g., tetracycline, oxytetracycline, chlortetracycline, demethyl-chlortetracycline, methacycline, doxycycline, minocycline, pyrrolidino-methyl tetracycline and tetracycline-L-methyl-lysine, were determined
in lactating cows and ewes given a single intravenous injection (20 mg/kg)\textsuperscript{65}.

Oxytetracycline and tetracycline were estimated on the basis of the two-compartment open model to occupy the largest distribution volume ($V'_d$) which was 535% of body weight; the smallest $V'_d$ was obtained for pyrrolidinomethyltetracycline and tetracycline-$L$-methyllysine at approximately 128% of body weight. The most lipophilic analogues, e.g., doxycycline, metacycline and minocycline were recovered in the greatest amounts in the milk after IV injection and attained the highest ratios of milk-to-serum ultrafiltrate concentrations during equilibrium\textsuperscript{65}.

The elimination half-life of oxytetracycline in cows and ewes was found to be 4.10 hours, whereas in horses it was found to be 10.5 and 15.7 hours following IM and IV injections, respectively. The data indicated that oxytetracycline was rapidly distributed in the body, but was rather slowly eliminated\textsuperscript{56, 65}.

The ratio of individual rate constants $k_{12}$ and $k_{21}$ ($k_{12}:k_{21}$) which reflects the rate of distribution of the drug into and out of the peripheral compartment was highest (1.46) for chlortetracycline in cows and ewes and was close to unity for tetracycline and oxytetracycline, indicating that these 3 analogues returned rapidly from their distribution sites for elimination from the body. This does not appear to be the case with oxytetracycline in the horse\textsuperscript{56, 65}.

The pharmacokinetics of oxytetracycline were determined in horses and cows following an intravenous dose of 2.5 mg/kg body weight. The elimination half-life in cows was found to be $9.12\pm0.67$ hours and
in horses 10.5±1.3 hours. Oxytetracycline was found to be bound to plasma proteins at the rate of 79.3 g/L in cows and 64.2 g/L in horses. It was concluded on the basis of these high protein bound values and relatively low plasma concentrations at zero time \( C^0_p \) that the currently recommended doses of oxytetracycline in cattle and horses (2.5 mg/kg) could well underestimate the amount of drug necessary for successful treatment of infections due to bacteria of average or low sensitivity$^{42}$.

Penicillin Literature

Penicillins are a most important class of antibiotics in veterinary medicine; however, there are few published reports of pharmacokinetic studies in domestic animals to support therapeutic regimens or residue profiles.

Penicillin G is widely distributed throughout the body, while concentrations in various fluids and tissues differ widely. Its apparent volume of distribution is in about 50% of total body water. More than 90% of the penicillin G in blood is in plasma and less than 10% is in erythrocytes. In man, approximately 65% is reversibly bound to plasma albumin. Significant amounts of penicillin G appear in liver, bile, kidney, semen, lymph and intestine$^{27}$. Comparatively, in horses, about 59% is bound, in calves 63%, sheep 70% and rabbits 65%$^{49}$. Penicillin does not readily enter the cerebrospinal fluid when the meninges are normal; however, when the meninges are inflamed and fever is present, penicillin may penetrate the cerebrospinal fluid
more readily. Recent data clearly support the concept of an active transport system for pumping penicillin out of cerebrospinal fluid, despite differences in concentration gradients.

Under normal conditions, penicillin G is rapidly eliminated from the body, mainly by the kidney, but in small part in the bile and by other channels. Approximately 10 to 20% is eliminated by glomerular filtration and 80 to 90% by tubular secretion. A major site of extra renal disposition of penicillin is the liver. Biliary excretion of the drug is directly proportional to the adequacy of hepatic function. Because the duodenum is the main site for enteric absorption, it is possible that some of the drug excreted in bile is absorbed by the intestinal mucosa.

Experiments with S-labeled penicillin demonstrate that the concentration of penicillin is consistently higher in the kidneys than in other body tissues, although in the liver it is occasionally as high. The diffusion of penicillin into tissues and fluids occurs as long as the unbound plasma concentration exceeds that of the tissues and fluids.

The administration of penicillin along with chymotrypsin to rats for 7 days allowed for increased penetration of penicillin into organs and granulomas when compared to treatment with penicillin alone.

Penicillin G has been compared to a variety of other penicillins. In man, the renal clearance of penicillin G following steady state administration IV was found to be the highest of all penicillins studies, e.g., 386 ml/min compared to the next highest, ampicillin at
the movement of drug from serum to tissues back to serum, was found to be approximately one.

The volume of distribution (liters) was found to be as follows: penicillin G, 33; ampicillin, 27; oxacillin, 30; dicloxacillin, 12; and methicillin 21. Based upon the ratios determined between volumes of distribution in central compartment and extravascular tissue compartments, it was concluded that during the β phase (elimination), more than half the drug in the body might be in the extravascular tissue compartment. There were no tissue concentrations available to confirm this suspicion. The clearance rate for penicillin G was found to be (as recalculated for a 70 kg man), $7.85 \text{ ml/kg/min}^{12}$. Clearance rates of most penicillins have been shown to be greatly reduced by the concurrent use of probenecid, which is an active competitor for tubular secretory functions.$^{59}$

Twenty-four cross-bred pigs were given recommended therapeutic doses of procaine penicillin G and dihydrostreptomycin intramuscularly. The absorption half-life for penicillin was estimated to be 18 to 28 minutes. Penicillin concentrations peaked in serum in 1 to 2 hours (7.7 to 8.5 units/ml) and at 24 hours had decreased to 0.04 units/ml.$^{38}$ Other investigators have found that at a dose rate of 10,000 units of procaine penicillin G/kg body weight given to pigs subcutaneously resulted in therapeutic concentrations of 0.04 - 0.84 units/ml serum, maintained for 18 to 24 hours.$^{48}$ It has been suggested that there is little to be gained in terms of blood concentrations by increasing doses of either crystalline penicillin or procaine benzylpenicillins beyond 6000 units/kg.$^{16}$
A preparation containing a combination of benzathine penicillin, procaine penicillin, and dihydrostreptomycin was administered to calves by intramuscular injection and also via subcutaneous injection. Residues were determined and blood concentrations from the two routes were compared. Residues of penicillin and dihydrostreptomycin were found at injection sites in muscle at 30 and 45 days after dosing. There were no residues in any other tissues. A most important observation was the fact that urine remained positive for penicillin for the duration of the study, but was negative for dihydrostreptomycin, despite positive concentrations in the injection sites. Since there were no significant differences in blood concentration profiles from either route, it was recommended that this type of long-acting drug could more safely be used subcutaneously.

Fourteen feeder steers were given intramuscular injections of procaine penicillin G and dihydrostreptomycin in fixed combination, either in four consecutive daily doses, or as a single massive dose. The mean concentration of penicillin in serum from steers given the single massive dose reached a peak (7.2 units/ml) 1 to 3 hours after dosing, with concentrations of 0.25 and 0.05 units/ml being present at 24 and 36 hours, respectively. During the repeated dose regimen, penicillin concentrations peaked at 2.24 units/ml serum at 1 hour after each dose. No residues of penicillin were found at 30, 60, or 90 days posttreatment via either dosage pattern.

The extent and comparison of protein binding between penicillin G and ampicillin were recently studied in the horse. It was found that in the therapeutic range of concentration, 6.8 to 8.0
percent of total ampicillin was bound to protein and 52 to 54 percent of penicillin G was bound to protein. The half-life of penicillin G in the horse was found to be $53.3\pm4.5$ minutes and ampicillin half-life was $98\pm7.0$ minutes\textsuperscript{13}.

The volume of distribution of penicillin G in the horse was found to be $3.6$ l/kg. This value exceeds the total water space of the body indicating that the drug is concentrated somewhere in the animal, either by active mechanisms or more probably, by binding to cell membranes, connective tissue and the like\textsuperscript{13}. 
CHAPTER III

MATERIALS AND METHODS

Two to three days prior to the initiation of the drug kinetic studies, the double isotope, single injection method for estimating normal renal function was determined for each group of pigs. It was mentioned earlier that withdrawal times might well be based on the animal with the poorest metabolic capabilities. The one single factor most often involved in alternations in drug excretion patterns is the malfunctioning kidney. Most drugs (particularly antibiotics) depend mainly on renal function as the primary route of elimination; therefore, one animal with inadequate renal function in a drug residue study could greatly influence the establishment of a proper withdrawal time. Thus, any pharmacokinetic or drug residue study should be preceded by a renal function test. The single intravenous injection technique using the radioiodine compounds $^{125}$I-iothalamate (Glofil-125) and $^{131}$I-iodohippurate (Hipputope) can be used to measure renal function. Clearance of Glofil and Hipputope estimate glomerular filtration rate (GFR) and effective renal plasma flow (ERPF), respectively. While

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$^{a}$Glofil $^{125}$I. Abbott Laboratories, North Chicago, Illinois.

$^{b}$Hipputope $^{131}$I Injection. Mallinckrodt. St. Louis, Missouri.
not thoroughly studied in food animals, the method has a great deal of appeal and was considered an essential part of the studies described herein.

Chloramphenicol

Eighteen nonfasted, 12 to 16 week old pigs from several litters were used. The weights ranged from 20.5 kg to 38.6 kg. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined for these animals prior to initiation of kinetic studies. The GFR for these pigs was found to be $6.07\pm0.89$ ml/min/kg; and ERPF was found to be $22.65\pm5.58$ ml/min/kg.

The study was divided into two phases, an IV kinetics phase and a tissue kinetics phase. The tissue kinetics phase was conducted 3 weeks following the IV kinetics phase. Nine of the pigs used in the IV kinetics phase were used in the tissue phase also. Thirteen additional pigs from different litters were also utilized (nine treated and four controls).

Plasma IV Kinetics

Each of the pigs was anesthetized to a level of light anesthesia using pentobarbital. A polyethylene cannula was introduced into the anterior vena cava via a 13 gauge needle. The needle was then removed leaving the cannula in place. A mattress suture was used

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*C Pentasol. A. J. Buck and Sons, Inc., Cockeysville, Maryland.

*Clay-Adams Intramedic Tubing; I.D. = 0.033, O.D. = 0.064, Becton-Dickinson and Co., New Jersey.
to secure the cannula to the skin. The cannula extended along the lateral surface of the neck to a point on the midline dorsal to the shoulder and was taped in place. A 20 gauge blunt needle was used as a cannula "hub".

Chloramphenicol\textsuperscript{e} was administered intravenously via the cannula at a rate of 22 mg/kg. The cannula was then flushed thoroughly with a warmed 3.5% solution of sodium citrate in lactated ringer's solution. Blood samples were collected via the cannula before and at 5, 10, 15, 30, 60, 90, 120, 180, 240, 360, 480, 720 and 1440 minutes after administration of the drug. On nine of the animals, 2.5 minute samples were also collected. The cannula was flushed with approximately 3 ml of citrated lactated ringer's solution following collection of each sample. The blood samples were put into 10 ml tubes with liquid di-sodium ethylenediaminetetraacetate (EDTA) added as anticoagulant and were mixed well. The cannulas on several pigs lost their patency during the experiment; thereafter the pigs were bled from the anterior vena cava using a hyperdermic needle and syringe, and the blood was injected into EDTA tubes. Samples were centrifuged to separate the plasma, which was then aspirated off and frozen. After all samples had been collected, the cannulas were removed.

\textsuperscript{e}TEVOCOCIN-oral solution 100 mg/cc. International Multifoods, Minneapolis, Minnesota 55402.
Analyses of plasma samples were carried out using an automated fluorometric analysis, which was modified from the procedure reported by Clarenburg, et al.\textsuperscript{9}. This procedure directly measures the fluorescence with a fluorometer, of fluorescamine (4-phenylspiro[furan-2(3-H), 1\(^1\)-phthalan]-3-3\(^1\)-dione) that has reacted with reduced chloramphenicol. This method measures total chloramphenicol. Nine milliliters of 4% trichloroacetic acid (TCA) was added to 1 ml of plasma. The sample was shaken and allowed to sit for 10 to 15 minutes. This allowed proteins to precipitate out of the solution. Approximately 500 mg of zinc dust was then added to the test tube containing the plasma-TCA solution. The tube was capped, shaken and placed in a water bath at 100°C for 30 minutes. Samples were cooled and filtered using Whatman No. 42 filter paper in 75 by 90 mm filter funnels. The filtrate was put on the Autoanalyzer\superscript{10} set up so that samples flow through orange-white standard tubing (0.23 ml/min), are mixed with a 1.0 M sodium acetate buffer (pH 4.8) flowing in through gray (0.88 ml/min) standard tubing, and the solution finally combines with Fluram\textsuperscript{TM} which was introduced into the system through orange-white (0.23 ml/min) silicone tubing. Measurement of the fluorescence was at the lowest sensitivity (1X) of the fluorometer\textsuperscript{g} with a 10-inch

\textsuperscript{f}Fluram\textsuperscript{TM} (Fluorescamine) - Roche 43023, Div. Hoffman LaRoche, Inc., Nutley, New Jersey 07110.

\textsuperscript{g}Turner Model III Fluorometer, G. K. Turner and Associates, 2524 Pulgas Avenue, Palo Alto, California 94303.
recorder^h set at a chart speed of 0.5 inch/min with a range of 100 millivolts.

**Tissue Kinetics Phase**

Nine of the animals previously used in the plasma IV phase were employed in the tissue kinetics phase. At the initiation of this phase, their weights ranged from 25.0 kg to 61.8 kg with a mean of 43.6 kg.

Chloramphenicol was administered intravenously utilizing a plastic syringe and 18 gauge 1 1/2 inch needle via the anterior vena cava at a rate of 22 mg/kg body weight. The sacrifice schedule was 5, 15, 45, 90, 180, 240, 360, 480 and 1440 minutes after dosing. A pair of animals was employed for each sacrifice period. Animals were sacrificed by electrocution. Following this, the jugular vein and/or carotid artery was transected and a sample was collected into a 10 ml tube containing liquid EDTA, which was stoppered and mixed well. Portal blood samples were collected by syringe from the portal vein and were put in a 10 ml liquid EDTA tube and mixed well. These samples were centrifuged, the plasma was aspirated off and frozen.

The urinary bladder was removed, total urine volume was measured in a graduated cylinder and samples were frozen and retained for analysis. The stomach was tied off and transected at the

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^h Beckman 10-inch recorder, Beckman Instrument, Inc., Fullerton, California 92634.
gastroesophageal junction and pyloric sphincter, the small intestine was tied and transected at the ileocolic junction, and the large intestine was tied and transected at the terminal colon. The sections were removed with the contents, the contents of each segment were removed and the volume was measured and recorded. The contents of each segment were then mixed well and samples of approximately 50 grams were retained and frozen. The stomach wall was rinsed, the glandular portion was blocked and the sections were mixed and sampled. The large and small intestinal walls were rinsed, chopped, mixed and representative 50 gram samples were retained. The liver, kidney, brain, spleen and lung were weighed, whole organ weights were recorded, then these organs were chopped, mixed and retained for analysis. Skeletal muscle, collected from the hams were chopped, mixed and a representative sample collected. Perirenal, omental and ventral abdominal fat was collected, mixed and sampled.

The experimental work area and all utensils were cleaned thoroughly after each pair of pigs to prevent contamination between sacrifice periods. All samples were frozen, then analyzed for chloramphenicol residues.

Plasma, both portal and venous, were analyzed by the same procedure used for the IV kinetics phase. The procedure for analyzing tissues was as follows: a 50 gram sample of tissue was blended with 50 ml distilled water in a Virtis\textsuperscript{1} blender at 10,000 rpm for

\textsuperscript{1}The Virtis Company, Gardner, New York 12525.
distribution kinetics of oxytetracycline HCl in body fluids and tissues. The weights of these pigs ranged from 27.2 kg to 36.3 kg. This group of pigs was dosed with a total of 1750 mg per pig or a range of 48 to 65 mg/kg body weight. An exaggerated dose was used in order that tissue and plasma concentrations would be sufficient to allow tissue/serum ratios to be clearly established. The animals in this study were all sacrificed at 2 and 4 hours after dosing.

The plasma and IV kinetics phase was conducted as described for chloramphenicol.

Oxytetracycline hydrochloride\(^j\) was administered intravenously via the cannula at a rate of 11 mg/kg. The cannula was then flushed thoroughly with a 3.0% solution of sodium citrate in lactated ringer's solution. Blood samples were collected via the cannula before and at 5, 15, 30, 60, 90, 120, 180, 240, 360, 480, 720, 1440, 1920 and 2880 minutes after administration of the drug. The cannula was flushed with approximately 3 ml of citrated lactated ringer's solution following collection of each sample. The blood samples were put in 5 ml heparinized tubes and mixed well. Samples were centrifuged to separate the plasma, which was aspirated off and frozen. Analysis of samples was carried out by the NCAIA\(^k\) by using a microbiological cyclinder plate assay procedure with *Bacillus cereus* var. *mycoides* (ATCC 11778)\(^{30}\).

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\(^j\) Liquimycin Injectable, 50 mg/cc Pfizer, Inc., New York, N.Y.

\(^k\) National Center for Antibiotic and Insulin Analysis, Food and Drug Admin., Dept. of Health, Education and Welfare, Washington, D.C.
After all samples had been collected, the cannulas were removed.

**Tissue Kinetics Phase**

Fifteen of the animals previously used in the plasma IV phase were employed in the tissue kinetics phase. At the initiation of this phase, their weights ranged from 24.1 kg to 52.3 kg, with a mean of 38.20 kg.

Oxytetracycline hydrochloride was administered intravenously utilizing a plastic syringe and 18 gauge 1 1/2 inch needle via the anterior vena cava at a rate of 11 mg/kg body weight.

The sacrifice schedule was 1/4, 1, 2, 4, 8, 12, 24 and 32 hours post-dosing. A pair of animals selectively matched by litter were employed for each sacrifice period. Animals were sacrificed by electrocution, and sample collections followed the same procedure as described for chloramphenicol.

**Oral Kinetics Phase**

At 2 and 4 hours after the oral dose, the pigs were electrocuted and the following samples collected immediately:

Liver - entire organ was weighed and then approximately half of the liver was blended in 4.5 pH phosphate buffer. Two 10 ml aliquots were taken from the blended material.

Kidney - both organs were removed, weighed and then approximately half of each kidney was blended in 4.5 pH phosphate buffer. Two 10 ml aliquots were taken from the blended material.

Stomach - entire stomach wall was weighed and then approximately half of the stomach wall was blended in 4.5 pH phosphate buffer. Two
10 ml aliquots were taken from the blended material.

Stomach content - stomach was tied off, total volume placed in a beaker and stomach flushed with 4.5 pH phosphate buffer. Two 10 ml aliquots were taken from obtained stomach content-buffer mixture.

Intestinal content (duodenum-jejunum) - intestine was tied off, total volume placed in a beaker, then flushed with 4.5 pH phosphate buffer. Two 10 ml aliquots were taken from the intestinal content-buffer mixture.

Urine - total urine was collected and measured. Two 10 ml (or equal) aliquots were taken from total urine.

Serum samples - blood samples were drawn at 0, 2, and 4 hours after oxytetracycline administration. Clotted whole blood was spun down and serum collected. Each serum sample was divided into two aliquots for analysis.

The experimental work area and all utensils were cleaned thoroughly after each pair of pigs and each experiment to prevent contamination between sacrifice periods.

All samples were frozen and subsequent analyses were performed within 3 to 5 days.

Penicillin G Methods

Eighteen nonfasted, 7 to 9 week old pigs from 3 litters were used. The weights ranged from 13.6 kg to 28.1 kg. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined for these animals prior to initiation of kinetics studies.
GFR was found to be 5.46±0.71 ml/min/kg and ERPF was found to be 16.50 ±2.73 ml/min/kg.

The study was divided into two phases, an IV kinetics phase and a tissue kinetics phase. The tissue kinetics phase was conducted three weeks following the IV kinetics phase, and involved both IV and oral routes of administration. Sixteen of the pigs used in the IV kinetics phase were also used in the tissue phase. Eight additional animals were also used (two controls, four treated orally, and two included in the IV group).

Each of the pigs was surgically cannulated as was described for the chloramphenicol study.

Potassium penicillin G\textsuperscript{1} in sodium chloride\textsuperscript{m} was administered intravenously via the cannula at a rate of 5,000 units/lb equivalent to approximately 3.47 mg/lb or 7.64 mg/kg. The cannula was then flushed thoroughly with a 3.5% solution of sodium citrate in lactated ringer's solution. Blood samples were collected via the cannula before and at 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 720 and 1440 minutes after administration of the drug. From 10 animals, 2.5 minute samples were also taken. The cannula was flushed with approximately 3 ml citrated lactated ringer's solution following collection of

\textsuperscript{1}Buffered Potassium Penicillin G Crystalline 5 million units/vial, Parke-Davis, Detroit, Michigan 48232.

\textsuperscript{m}Sodium Chloride Injection, U.S.P. Abbott Laboratories, North Chicago, Illinois 60064.
each sample. The blood samples were put into 10 ml tubes with liquid
EDTA added as anticoagulant and mixed well. Samples were centrifuged to
separate the plasma, which was then aspirated off and frozen. Analysis
was carried out by the NCAIA by use of a microbiological cylinder plate
bioassay procedure which utilized the organism Sarcinia lutea (ATCC
6633)\textsuperscript{30}. After all samples had been collected the cannulas were
removed.

\textbf{Tissue Kinetics Phase}

Sixteen of the animals previously used in the plasma IV phase
were employed in the tissue kinetics phase following IV dosing. Four
additional animals were orally dosed and processed the same as the IV
dosed animals. At the initiation of this phase, their weights ranged
from 16.8 kg to 42.2 kg with a mean of 27.5 kg.

Potassium penicillin G in sodium chloride was administered
intravenously via the anterior vena cava at a rate of 7.64 mg/kg body
weight.

Orally dosed animals were given size 1 gelatin capsules con-
taining a dose equivalent to 7.64 mg/kg potassium penicillin G. The
capsules were administered with the aid of a speculum and balling gun.

The sacrifice schedule for IV dosed animals was 5, 15, 45, 90,
180, 240, 360, 480, and 1440 minutes post-dosing. Orally dosed pigs
were sacrificed at 90 and 180 minutes post-dosing. A pair of animals
selected and matched according to litter were employed for each sacri-
fice period. Animals were sacrificed by electrocution. Following
this, the jugular vein and/or carotid artery was transected and a
sample was collected into a 10 ml tube containing liquid EDTA, which was stoppered and mixed well. Portal blood samples were collected by syringe from the portal vein and were put in a 10 ml liquid EDTA tube and mixed well. These samples were centrifuged, the plasma was aspirated off and frozen. The other tissue samples were collected as described for the chloramphenicol study.

Mathematical Models Used to Analyze the Data

The decline of antibiotic activity (or concentration) in serum with time was examined for applicability to a one- or two-compartment open model for kinetic analysis (Appendix Figure 1). According to a two-compartment model, the drug after being injected directly into the bloodstream, distributed reversibly between the central and peripheral (or tissue) compartments and was eliminated by excretion and/or metabolism solely from the central compartment. The one-compartment model may be used when the drug appears to distribute only in the central compartment. The compartments of the model are mathematical entities rather than physiological realities, but are most useful in describing the disposition kinetics of a drug. Semilogarithmic plots for all three drugs were shown to conform to the biexponential equation:

\[ C_p = Ae^{-\alpha t} + Be^{-\beta t} \]

Where \( C_p \) is the serum concentration of each antibiotic, \( t \) is time, and \( A, \alpha, B \) and \( \beta \) are constants describing each phase of the biphasic serum concentration-time profile. The application of the two-compartment model for chloramphenicol was debatable, and thus the data
were also fit to a one-compartment model for comparative purposes. In this instance, the exponential equation:

\[ C_p = B e^{-\beta t} \]

may be used to analyze the serum-concentration-time profile. Both methods have been adequately described in pertinent pharmacokinetic literature\(^2\),\(^{40}\). The constants were evaluated using graphic techniques, in which least square regression lines were calculated for the terminal (i.e., elimination) and "feathered" (i.e., distribution) portions of the log serum drug activity-time curves. Values for the various pharmacokinetic terms (Table 16) were computed for each individual animal by accepted techniques\(^{21},\)\(^{47}\). A mean value for each term and the standard deviation were calculated. An automated program for processing pharmacokinetic data was used for these analyses\(^{43}\).

The tissue kinetics were evaluated by the use of semi-logarithmic plots from the time of peak tissue concentration or equilibration to elimination. Linear regression analysis was performed on the mean tissue concentrations (two pigs at each time interval) and the slope of the elimination curves determined. These values should be considered only as estimates for elimination constants in tissues. F-values were then determined on the relationships of the plasma elimination versus tissue elimination slopes.
CHAPTER IV

RESULTS

Chloramphenicol

The peak plasma levels for the principal animals were 33.7±
6.0 µg/ml, and this concentration occurred at 2.5 minutes after the
IV dose. At 12 hours, plasma concentrations had decreased to 0.2
µg/ml which was essentially the sensitivity of the assay method\(^n\)
(Table 17). The pharmacokinetic evaluation of this drug in swine
proved to be a difficult task. The initial evaluation of the drug
indicated that the data should be fit to a two-compartment model, as
illustrated in Figure 9. In the example given in Table 2, the
character of the biexponential curve is given by:

\[
P = 46.44 e^{-0.3760 \tau} + 18.62 e^{-0.0057 \tau}
\]

where \( C_p \) is the concentration in plasma (µg/ml), and \( \tau \) is the time
(min). The semilogarithmic plot illustrating this analysis can be found
in Figure 1. However, a great deal of variability was observed during

\(^n\)Positive and negative residues are terms that may be used
throughout this text. Positive residues mean that values were equiva-
lent or greater than the sensitivity of the assay method (described for
each drug in each tissue in Tables of Results). Negative residues
mean values below the sensitivity of the analytical method.
the time span of 15 - 60 minutes after the dosing interval (Figure 3). The typical distribution or "α phase" appeared very short, and plasma concentrations were erratic (increasing and decreasing) in various pigs at various time intervals. Because of this erratic behavior during the 15 - 60 minute time interval, the rapid "α phase," and because the conventional two-compartment analysis did not easily fit the data, a further analysis utilizing the concepts of a single compartment was attempted. In this case, the character of the exponential curve could best be described by:

\[ C_p = 17.32 e^{-0.0055t} \]

where \( C_p \) was the concentration in plasma (\( \mu g/ml \)), and \( t \) was the time in minutes. The semilogarithmic plot of this analysis is illustrated in Figure 2. Neither of these models provided satisfactory fits during the 15 - 60 minute post-dosing time interval. In both cases, F-values were low, indicating that several data points during this phase were possible outliers to the remainder of the data points on the linear regression slope. Because of the discrepancies observed with either model, and because of the need to compare chloramphenicol with the other two drugs, it was decided that the emphasis should be placed upon chloramphenicol as a two-compartment drug.

The mean initial concentration in plasma (\( C_{ip}^{oc} \)) was found to be 66.7±41.7 \( \mu g/ml \) with a distribution half-life of 4.3±3.8 minutes. The elimination half-life was found to have a mean of 2.66±1.06 hours, and the volume of distribution was 1.39±0.32 l/kg. This compares to
the single compartment values of $C_p^0$ of $15.77 \pm 3.80$ µg/ml; an elimination half-life of $2.29 \pm 0.44$ hours, and a volume of distribution of $1.43 \pm 0.29$ l/kg (Table 1).

The body clearance of chloramphenicol was determined to be $6.70 \pm 2.16$ ml/min/kg in the two-compartment analysis and was found to be $7.32 \pm 1.48$ ml/min/kg in the one compartment analysis (Table 1). Because these pharmacokinetic parameters are not significantly different, the pharmacokinetic analysis employing a one-compartment model is adequate. Furthermore, the wide variability in the determination of $\alpha$ in the two-compartment model analysis, indicates that no meaningful interpretation of microconstants can be made.

The pharmacokinetic macro- and microconstants for the representative pigs treated with each drug are provided in Table 2. The estimations for $k_{12}$, which is the rate constant for tissue penetration of chloramphenicol, was found to be $0.2508$ min$^{-1}$, and the rate constant for drug leaving tissue ($k_{21}$) was $0.117$ min$^{-1}$ which was approximately one-half as rapid as tissue penetration ($k_{12}/k_{21}$ ratio = 2.25). However, as noted above, no meaningful interpretation of these parameters can be made.

The concentrations of chloramphenicol in a variety of tissues and body fluids are presented in Tables 3 and 4. It should be noted that in the major solid tissue organs (e.g., liver, lung, spleen, muscle and fat), peak concentrations of the drug were already
established at 5 minutes after the dose. In liver, the concentration at 5 minutes (92.42 µg/ml) was approximately three times that of plasma and did indeed indicate a very rapid and thorough penetration of this drug in liver tissue. In lung, the concentration at 5 minutes post-dose was found to be 78.52 µg/g, or about 2 1/2 times the plasma concentration. The rate of tissue penetration and quantity of drug in this organ could be an important asset to its therapeutic success in pneumonic conditions. In spleen, the concentration was 68.91 µg/g or 2 times the level in plasma. Concentrations in muscle closely paralleled the plasma concentration profile and may be reflective of the blood vascular supply to the muscle masses. Initial fat concentrations were about 35% that of plasma and were likely related to the sparse vascularity of fat tissue. It required approximately 90 minutes for chloramphenicol to reach an equilibrium status in brain tissue (7.64 µg/g); however, chloramphenicol had penetrated the blood-brain barrier in significant amounts (4.30 µg/g) at 5 minutes post-dose. The concentrations observed in kidney (85.03 µg/g) were high, but expected since this is apparently the primary organ of elimination for this drug.

Several important observations can be made regarding the rapid mobility of this drug into extracellular body fluids. At 5 minutes, portal blood had concentrations (46.19 µg/ml) which exceeded venous blood by 30%. It is granted that liver levels were high at 5 minutes, but it was never expected that chloramphenicol could pass the liver into bile and small intestine and be reabsorbed in these quantities in so short a period of time. These data may suggest an extremely efficient enterohepatic recycling system. The erratic behavior of
plasma concentrations as illustrated in Figure 3 may be indicative of the impact that recycling has upon this parameter. The greatest impact occurs between 15 to 60 minutes post-dosing, causing a distinct spike in plasma concentrations. This plasma spike could well be related to the concentrations observed in the small intestine (7.57, 10.70 and 17.22 µg/ml at 5, 15, and 45 minutes, respectively) during this time interval. In fact, during the first 6 hours after an IV dose of chloramphenicol, there were substantial concentrations in both the stomach and small intestine, which no doubt have a continuing, but subtle effect upon plasma concentrations. There was ample evidence that there was active elimination via liver and bile, as well as the kidney; but of major importance was the apparent reabsorption via the intestinal pathway. Good evidence for this was the level found in the gut walls at all time intervals after dosing. There was also good evidence that chloramphenicol was actively secreted or was passively diffusing into the stomach. Stomach content reached a peak concentration of 19.87 µg/ml within 90 minutes. The concentration did decrease to a total of 5 to 6 mgs at 24 hours (Table 20). There did not seem to be any particular pattern of elimination from the stomach contents.

The tissues and body fluids with persistent residue profiles at 24 hours following the IV dose may be ranked in the following order of descending concentrations (Table 20): stomach content, 5.33 mg; large intestine content, 2.59 mg; lung, 0.76 mg; urine, 0.42 mg; spleen, 0.19 mg. It was of interest that none of these tissues represented major edible organs.
One of the major objectives of these studies was to determine whether or not there were tissues or body fluids from pigs treated with chloramphenicol which could be used as monitoring or target tissue with predictive value regarding the residue profile. Obviously, non-edible tissues or organs which are easily accessible (e.g., blood, urine, stomach content, or intestinal content) would be desirable in lieu of edible tissues which have economic value. Thus, a series of correlations derived from the elimination kinetics of selected tissues were made with blood plasma, since it is generally the most accessible and desirable as a monitoring device for tissue levels. The slope of the elimination curve (beta) for plasma was compared to the estimated slope of the elimination curve for major edible tissues and related body fluids. F-values were calculated for each of the plasma-tissue pairs where definite plateau points could be established, and where subsequent elimination phases were linear for 3 or more points. These values are presented in Table 5.

The slopes of the elimination curves determined for liver, brain, muscle, spleen, lung, kidney, and small and large intestinal walls were found to be significantly similar to the elimination curve for chloramphenicol in plasma (P<.005). The concentrations of chloramphenicol in urine would be expected to be inversely related to the declining concentrations in plasma, and thus no significant similarities were observed. A plot to illustrate these highly correlated elimination profiles may be found in Figure 4.
Oxytetracycline

Peak plasma concentrations of oxytetracycline were found to be 93.82±45.04 μg/ml at 5 minutes. These concentrations dropped rapidly to 12.70±5.55 μg/ml at 15 minutes post-dose, then gradually declined to finite levels of 0.02 μg/ml at 32 hours (Table 18). At 5 to 15 minutes, a high degree of variation was observed; however, from 30 minutes until depletion, concentrations observed in these fourteen pigs were very consistent. During the injection process (one minute injection, 3 - 5 minute observation) all animals experienced pain and discomfort as evidenced by excessive struggling and squealing, and in the light-colored pigs, there was extensive erythema observed. This only lasted for the few minutes during and immediately after intravenous injection, with pigs quickly returning to normal behavior and appearance.

The regression lines which represent the distribution (α) and elimination (β) phases of oxytetracycline activity decline in serum of pig F are shown in Figure 5. This pig was representative of the group and individual kinetic parameters are provided in Table 2. The distribution and elimination curve can best be described by the two-compartment model (Figure 9) and the biexponential equation:

\[ C_p = 91.25 e^{-0.1036t} + 6.23 e^{-0.003t} \]

The distribution half-time and biologic half-life of pig F was 6.69 minutes and 3.71 hours, respectively. The mean kinetic values for the remainder of the pigs are presented in Table 1. There was a high degree of consistency in the behavior of this drug in pigs.
The mean volume of distribution was found to be 1.26±0.18 liters/kg.

The body clearance of oxytetracycline in pigs was estimated to be 3.82 ml/kg/min, which was less than the estimated glomerular filtration rate (GFR) of 4.44±0.51 ml/kg/min as determined immediately prior to this study. This suggests that oxytetracycline was being eliminated in the kidney by glomerular filtration; however, it was obvious that elimination was occurring via other mechanisms. Judging by the concentration of oxytetracycline in most portions of the intestinal tract, it was clear that excretion via liver, bile, and the gastrointestinal tract represents an important pathway for oxytetracycline elimination in pigs.

The tissue to serum ratio for a typical pig (Table 2) during the time of apparent distribution equilibrium \((k_{12}/(k_{21}-B))\) equalled 9.91, suggesting along with the volume of distribution parameter, that oxytetracycline was widely distributed in tissues, the extent of which will be discussed in a later section. Fifteen minutes following the intravenous injection of oxytetracycline in pigs, concentrations in portal plasma (9.90 µg/ml); stomach contents (20.5 µg/ml); small intestine content (36.45 µg/ml); large intestine content (33.10 µg/ml); and urine (154.40 µg/ml) exceeded that found in venous plasma (8.21 µg/ml), Table 6. These data provided clear evidence that a very effective enterohepatic recirculation of this drug occurs early and rapidly after IV injection. The pattern of appearance of oxytetracycline in the stomach contents would suggest a sporadic mechanism of passive diffusion or active secretion (Table 6). Levels were highly variable with time after dose.
However, it is likely that these variable levels may simply be reflecting sporadic gastric motility. The pattern of oxytetracycline concentrations in the small intestinal content indicated that there was a steady source of drug for recycling via portal plasma, liver and bile, and these concentrations remained rather constant during the first 4 to 6 hours. Concentrations then began to decline, only to spike again at 32 hours. The concentration profile in the large intestinal content indicated that increasing amounts pass to the lower tract (peak levels at 109.15 μg/ml at 12 hours, Table 6), and gradually decline to levels similar to those found in urine (16.20 versus 20.05 μg/ml at 32 hours) suggesting the potential for equivalent elimination via these two routes.

Peak concentrations of oxytetracycline occurred in liver (17.97 μg/g) at 60 minutes (Table 7), then gradually declined in a fashion closely resembling that of both venous and portal plasma. Peak concentrations occurred in lung (9.20 μg/g); spleen (9.15 μg/g); and fat (3.52 μg/g) within 15 minutes, then gradually declined with time in a similar fashion to venous plasma. Peak concentrations did not occur in muscle and kidney until 1 to 2 hours after the dose. Concentrations in muscle then declined in a pattern similar to venous plasma. Kidney levels were reflective of the elimination process during the first 3 to 4 hours post-dose. Detectable levels did not occur in brain at any time, thus confirming the similarity between swine and other species regarding the impermeability of the blood-brain barrier to the tetracyclines.
Table 21 illustrates the total drug component in various
tissues and body fluids at various time intervals after dosing. Con­siderable quantities of oxytetracycline (in milligrams) were available
in the gastrointestinal tract from 15 minutes post-dose to 32 hours.
A sum of total drug in the gastrointestinal tract at 15 minutes indi­cates that 81 mg (or approximately 31% of the total dose in smaller
pigs) was available for enterohepatic recycling. At 60 minutes, as
much as 409 mg (or approximately 72% of total dose in larger pigs) was
available for recycling. At 32 hours, 60 mg (or 23% of small pig dose)
was available. These amounts of drug in the enteric tract are no doubt
related and may provide a good explanation for the long half-life of
oxytetracycline (3.87 hours) in swine. Even so, the half-life of this
drug in swine was much shorter than that observed in other species
(4 to 19 hours)\(^5, \, 18\) thus, enterohepatic cycling in other species,
particularly the horse and cow with low glomerular filtration capacity,
may explain the tendency of the drug to behave as a three-compartment
drug\(^2\). It is also important to point out that the high volumes of
distribution and \(k_{12}/k_{21}\) ratios which have been observed with this drug,
and assumed to be indicative of extensive tissue penetration,\(^42\) may
be reflective of the extensive enterohepatic recycling process.

It has generally been assumed that there were significant
differences between oral and intravenous routes of administration.
For drugs that are rapidly eliminated via kidney, with little or no
enterohepatic recycling, there can be significant differences between
the two routes, not to mention formulation effects. However, in the
case with oxytetracycline, it may be difficult to differentiate the
route of administration (oral versus IV) after the first 1 to 2 hours after dosing. In Table 8, data were provided to compare the two routes of administration in swine. The oral dose was 4 to 6 times the IV dose, thus any differences should have been accentuated. It should be noted that the tissue-serum ratios, from both routes at both 2 and 4 hours were equipollent. During the first 2 hours, there were some differences in magnitude of concentration, particularly in the stomach and intestinal content, but much of this difference had diminished at 4 hours. It is reasonable to conclude that given time for equilibria to occur between dose and absorption rates, there may be few differences between orally and IV administered oxytetracycline.

Comparisons were made between the slope of the elimination curve for oxytetracycline in plasma versus the same parameter in several of the body tissues. These comparisons may be found in Table 9. The slope of the elimination curve for liver, muscle, spleen, lung, kidney, and stomach wall were found to be significantly (P<.005) related to the elimination slope of oxytetracycline in plasma. Fat was found to be significantly related to plasma at the P<.025 level. Urine would be expected to have an inverse relationship to drug concentrations in plasma and was thus found to have a nonsignificant relationship. Oxytetracycline concentrations in the small and large intestinal walls never did establish a clear elimination mode and thus could not be evaluated. The relationship between plasma concentrations of oxytetracycline and concentrations in several other body organs is illustrated in Figure 6.
The volume of distribution (Table 1) for penicillin G in pigs was found to be 0.53±0.12 l/kg, while the body clearance was found to be 19.06±5.06 ml/min/kg. The effective renal plasma flow (ERPF) in these animals was estimated to be 16.50±2.73 ml/min/kg and glomerular filtration rate was 5.46±0.71 ml/min/kg. Thus, one can readily see that the body clearance of penicillin G in swine approximates ERPF; indicating that penicillin is cleared by the kidney in a manner similar to p-aminohippuric acid. This is consistent with the renal clearance of penicillin in other species where it has been estimated that 80 to 90% is eliminated by tubular secretion and 10 to 20% by glomerular filtration.

Tables 10 and 11 provide data to illustrate the concentration-time profile of penicillin G in plasma and a variety of extravascular body fluids and tissues. One interesting observation involves the rate at which penicillin G appears in the portal vein. At 5 minutes, an estimated 25% of venous plasma concentrations were present, at 15 minutes about 45% and at 45 minutes, portal concentrations were in equilibrium with plasma concentration and maintained this equilibrium for the remainder of the elimination phase. The fact that there was a lack of measurable drug concentration in the gastrointestinal contents at 5 minutes, but measurable amounts in the stomach and intestinal walls provides an interesting basis for speculation regarding where much of this drug may go in its distribution phase. There are two principal routes that the drug could reach the portal plasma. One would be through the liver, bile, and small intestine,
from where it could be reabsorbed. The data do not indicate that this occurred. Judging by the lack of drug concentration in the liver, it can be assumed that the liver of swine plays a minor or no role in the elimination of penicillin. The second route and probably the one involved in this instance, may be the extensive capillary network and lymphatic system present in the walls and mesentery of the swine gastrointestinal tract. This aspect will be discussed in a later section.

It is also important to note that the tissue distribution pattern of oral penicillin G (Tables 10 and 11) at 90 and 180 minutes does not differ drastically from that observed from IV administration. The blood levels were lower (oral versus IV) at 90 minutes, but were equivalent at 180 minutes. Intestinal contents were similar for both routes at 90 minutes, but penicillin concentration was slightly higher in small intestine content at 180 minutes in the orally treated pigs. Organ concentrations were essentially equivalent at both 90 and 180 minutes.

Table 22 provides a profile of total drug concentrations in distinguishable fluid and tissue compartments. As noted earlier, there were substantial amounts of penicillin G in lung and kidney at 5 through 45 minutes. Concentrations diminish rapidly and as mentioned above, and as illustrated in Tables 10 and 11, these concentrations appear to be a function of the kinetics of the drug in plasma. It was obvious that the major compartments in terms of total drug were venous plasma and urine. At 45 to 90 minutes, it
appears that the plasma levels were purely a function of the rate of renal elimination.

A comparison between biological half-lives and elimination constants (β) of mean plasma concentrations of penicillin G and concentrations of penicillin in major organs and body fluids of swine treated with single intravenous bolus of the drug (dose = 7.64 mg/kg) is given in Table 12.
FIGURE 1

Semilogarithmic plot of chloramphenicol concentration in serum versus time after intravenous administration of a single dose of the drug (22 mg/kg). The circle dots (©) represent actual concentrations of drug in serum; the crosses (x) are the concentrations obtained by the feathering technique. Least square regression lines describe the α- and β- phases of the biexponential drug concentration-time profiles. A and B are the intercept values for pig No. 5.
Semilogarithmic plot of chloramphenicol concentrations in serum versus time after intravenous administration of a single dose of the drug (22 mg/kg). This plot illustrates the fitting of the single compartment model to the data from pig No. 10. The circle dots (©) represent actual concentrations of drug in serum.
Semilogarithmic plot of chloramphenicol concentration in serum versus time obtained after intravenous administration of a single dose of chloramphenicol (22 mg/kg). Plot represents a single animal (pig No. 3) which illustrates the enterohepatic recycling effect on plasma levels between 30 to 60 minutes post-dose as observed in 16 of 17 animals.
The concentration of chloramphenicol in pig tissues, plasma and urine at various times following the intravenous administration of chloramphenicol.
The concentration of oxytetracycline in pig tissues, plasma and urine at various times following the intravenous administration of oxytetracycline.
Semilogarithmic plot of penicillin G concentration in serum versus time obtained after administration of a single dose of the drug (7.4 mg/kg). The circle dots (O) represent the measured activity of antibiotic in serum; the open squares (□) are the concentrations obtained by the feathering technique. Least square regression lines describe the α- and β- phases of the biexponential drug concentration-time profile. A and B are the concentration at zero time (C₀ intercepts).
FIGURE 8

The concentration of penicillin G in pig tissues, plasma and urine at various times following the intravenous administration of the drug.
TABLE 1

Distribution and elimination kinetics of chloramphenicol, oxytetracycline, and penicillin G in pigs following a single intravenous injection.

<table>
<thead>
<tr>
<th>Kinetic parameter</th>
<th>Chloramphenicol(^a)</th>
<th>Oxytetracycline(^a)</th>
<th>Penicillin G(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose = 22 mg/kg</td>
<td>Dose = 11 mg/kg</td>
<td>Dose = 7.64 mg/kg</td>
</tr>
<tr>
<td></td>
<td>One compartment</td>
<td>Two compartment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N = 13</td>
<td>N = 17</td>
<td>N = 14</td>
</tr>
<tr>
<td>(C_0), (\mu g/ml)</td>
<td>15.77±3.8</td>
<td>66.70±41.79</td>
<td>106.92±58.26</td>
</tr>
<tr>
<td>(A), (\mu g/ml)</td>
<td>--</td>
<td>51.34±41.51</td>
<td>100.72±58.56</td>
</tr>
<tr>
<td>(t_1/2 (\alpha)), min</td>
<td>--</td>
<td>4.31±3.78</td>
<td>6.71±1.13</td>
</tr>
<tr>
<td>(B), (g/ml)</td>
<td>15.77±3.8</td>
<td>15.35±3.35</td>
<td>6.18±1.06</td>
</tr>
<tr>
<td>Beta ((\beta)), min(^{-1})</td>
<td>-0.0052±0.001</td>
<td>-0.0049±0.0020</td>
<td>-0.0031±0.0005</td>
</tr>
<tr>
<td>(t_1/2 (\beta)), min</td>
<td>137.62±26.9</td>
<td>159.62±63.87</td>
<td>232.23±37.92</td>
</tr>
<tr>
<td>(t_1/2, h)</td>
<td>2.29±0.44</td>
<td>2.66±1.06</td>
<td>3.87±0.62</td>
</tr>
<tr>
<td>(V_d) (area), liter/kg</td>
<td>1.43±0.29</td>
<td>1.39±0.32</td>
<td>1.26±0.18</td>
</tr>
<tr>
<td>(Cl), ml/kg/min</td>
<td>7.32±1.48</td>
<td>6.70±2.16</td>
<td>3.82±0.59</td>
</tr>
<tr>
<td>(k_{12}/k_{21})</td>
<td>--</td>
<td>2.79</td>
<td>6.19</td>
</tr>
<tr>
<td>(C_p) (12 hr), (\mu g/ml)</td>
<td>0.5±0.4</td>
<td>0.5±0.4</td>
<td>0.63±0.13</td>
</tr>
</tbody>
</table>

\(^a\)All values given as mean±standard deviation.

\(^b\)Penicillin values given as units/ml instead of \(\mu g/ml\).

\(^c\)Concentration in plasma at 2 hours instead of 12 hours.
TABLE 2

Disposition kinetics of chloramphenicol, oxytetracycline, and penicillin G in representative pigs following a single intravenous bolus.

<table>
<thead>
<tr>
<th>Parameter, units</th>
<th>Chloramphenicol Dose = 22 mg/kg Pig No. 8</th>
<th>Oxytetracycline Dose = 11 mg/kg Pig No. F</th>
<th>Penicillin G Dose = 7.6 mg/kg Pig No. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_0$, µg/ml</td>
<td>65.06</td>
<td>97.48</td>
<td>40.29</td>
</tr>
<tr>
<td>$A$, µg/ml</td>
<td>46.44</td>
<td>91.25</td>
<td>28.19</td>
</tr>
<tr>
<td>$\alpha$, min$^{-1}$</td>
<td>-0.3760</td>
<td>-0.1036</td>
<td>-0.1944</td>
</tr>
<tr>
<td>$t_{1/2}(\alpha)$, min</td>
<td>1.84</td>
<td>6.69</td>
<td>3.57</td>
</tr>
<tr>
<td>$B_p$, µg/ml</td>
<td>18.62</td>
<td>6.23</td>
<td>12.09</td>
</tr>
<tr>
<td>$\beta$, min$^{-1}$</td>
<td>-0.0057</td>
<td>-0.0031</td>
<td>-0.0320</td>
</tr>
<tr>
<td>$t_{1/2}(\beta)$, min</td>
<td>121.58</td>
<td>222.69</td>
<td>21.68</td>
</tr>
<tr>
<td>$k_{12}$, min$^{-1}$</td>
<td>0.2508</td>
<td>0.0634</td>
<td>0.0687</td>
</tr>
<tr>
<td>$k_{21}$, min$^{-1}$</td>
<td>0.1117</td>
<td>0.0095</td>
<td>0.0807</td>
</tr>
<tr>
<td>$k_{el}$, min$^{-1}$</td>
<td>0.0192</td>
<td>0.0338</td>
<td>0.0770</td>
</tr>
<tr>
<td>$V_a$ (area), liter/kg</td>
<td>1.14</td>
<td>1.23</td>
<td>0.46</td>
</tr>
<tr>
<td>$Cl_B$, ml/kg/min</td>
<td>6.49</td>
<td>3.82</td>
<td>14.60</td>
</tr>
<tr>
<td>$k_{12}/k_{21}$</td>
<td>2.25</td>
<td>6.67</td>
<td>0.85</td>
</tr>
</tbody>
</table>

*Penicillin G values are given in units/ml instead of µg/ml.*
TABLE 3
Concentrations of chloramphenicol in selected extracellular fluid compartments and associated organs from pigs treated intravenously with a single dose (22 mg/kg) of the drug.

<table>
<thead>
<tr>
<th>Sacrifice time (min)</th>
<th>Venousa plasma</th>
<th>Portala plasma</th>
<th>Stomacha contents</th>
<th>Smalla intestine content</th>
<th>Largea intestine content</th>
<th>Urinea</th>
<th>Stomachb wall</th>
<th>Smallb intestine wall</th>
<th>Largeb intestine wall</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>32.63</td>
<td>46.19</td>
<td>0.10</td>
<td>7.57</td>
<td>3.97</td>
<td>0.95</td>
<td>18.52</td>
<td>21.09</td>
<td>20.82</td>
</tr>
<tr>
<td>15</td>
<td>43.34</td>
<td>29.40</td>
<td>4.60</td>
<td>10.70</td>
<td>4.57</td>
<td>36.50</td>
<td>8.29</td>
<td>17.95</td>
<td>15.46</td>
</tr>
<tr>
<td>45</td>
<td>23.09</td>
<td>27.73</td>
<td>7.87</td>
<td>17.22</td>
<td>4.32</td>
<td>1245.12</td>
<td>3.90</td>
<td>10.01</td>
<td>10.86</td>
</tr>
<tr>
<td>90</td>
<td>16.67</td>
<td>20.98</td>
<td>19.87</td>
<td>5.27</td>
<td>6.02</td>
<td>1789.70</td>
<td>10.09</td>
<td>8.76</td>
<td>10.69</td>
</tr>
<tr>
<td>180</td>
<td>5.65</td>
<td>7.81</td>
<td>8.72</td>
<td>20.55</td>
<td>4.52</td>
<td>1879.97</td>
<td>-0-</td>
<td>4.15</td>
<td>3.67</td>
</tr>
<tr>
<td>240</td>
<td>6.35</td>
<td>6.18</td>
<td>5.22</td>
<td>10.77</td>
<td>5.25</td>
<td>1090.22</td>
<td>0.81</td>
<td>2.69</td>
<td>8.71</td>
</tr>
<tr>
<td>360</td>
<td>9.22</td>
<td>3.36</td>
<td>4.57</td>
<td>18.25</td>
<td>4.75</td>
<td>1707.17</td>
<td>-0-</td>
<td>2.52</td>
<td>2.82</td>
</tr>
<tr>
<td>480</td>
<td>0.44</td>
<td>0.84</td>
<td>1.92</td>
<td>4.00</td>
<td>11.07</td>
<td>252.92</td>
<td>-0-</td>
<td>0.24</td>
<td>2.32</td>
</tr>
<tr>
<td>1440</td>
<td>0.23</td>
<td>-0-</td>
<td>6.35</td>
<td>-0-</td>
<td>3.10</td>
<td>2.80</td>
<td>-0-</td>
<td>17.10</td>
<td>0.44</td>
</tr>
</tbody>
</table>

a All values represent average of two pigs; units = \( \mu g/ml \) for fluid compartments.

b Units = \( \mu g/g \) for solid tissues; sensitivity of assay = 0.2 \( \mu g/ml \).
TABLE 5

Comparison between elimination constants and biological half-lifes of plasma versus other organs and body fluids from animals dosed with 22 mg/kg chloramphenicol intravenously.

<table>
<thead>
<tr>
<th>Organ or body fluid</th>
<th>Beta (β) min$^{-1}$</th>
<th>T 1/2 hours</th>
<th>F-values</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous plasma (mean - 17 pigs)</td>
<td>0.0049</td>
<td>2.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venous plasma (at time of sacrifice)</td>
<td>0.0052</td>
<td>2.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.0065</td>
<td>1.77</td>
<td>97.07</td>
<td>P&lt;.005</td>
</tr>
<tr>
<td>Brain</td>
<td>0.0031</td>
<td>3.71</td>
<td>181.23</td>
<td>P&lt;.005</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.0022</td>
<td>5.24</td>
<td>21.39</td>
<td>P&lt;.005</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.0020</td>
<td>5.64</td>
<td>114.71</td>
<td>P&lt;.005</td>
</tr>
<tr>
<td>Lung</td>
<td>0.0025</td>
<td>4.67</td>
<td>117.28</td>
<td>P&lt;.005</td>
</tr>
<tr>
<td>Fat</td>
<td>0.0020</td>
<td>5.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>0.0092</td>
<td>1.25</td>
<td>75.23</td>
<td>P&lt;.005</td>
</tr>
<tr>
<td>Urine</td>
<td>0.0052</td>
<td>2.21</td>
<td>12.66</td>
<td>N.S.</td>
</tr>
<tr>
<td>Small intestine wall</td>
<td>0.0029</td>
<td>4.01</td>
<td>270.13</td>
<td>P&lt;.005</td>
</tr>
<tr>
<td>Large intestine wall</td>
<td>0.0024</td>
<td>4.81</td>
<td>29.47</td>
<td>P&lt;.005</td>
</tr>
</tbody>
</table>

$^a$ Sequential plasma levels determined on 2 pigs per slaughter interval through 24 hours post-dosing.
$^b$ The non-significant (N.S.) level was established at P>.01.
$^c$ Insufficient values to calculate F-value.
TABLE 6

Concentrations of oxytetracycline in selected extracellular fluid compartments and associated organs from pigs treated intravenously with a single dose (11 mg/kg) of the drug.

<table>
<thead>
<tr>
<th>Sacrifice time (min)</th>
<th>Venous plasma</th>
<th>Portal plasma</th>
<th>Stomach contents</th>
<th>Small intestine content</th>
<th>Large intestine content</th>
<th>Urine</th>
<th>Stomach wall</th>
<th>Intestine wall</th>
<th>Intestine wall</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>8.21</td>
<td>9.80</td>
<td>20.50</td>
<td>36.45</td>
<td>33.10</td>
<td>154.40</td>
<td>4.37</td>
<td>7.07</td>
<td>4.37</td>
</tr>
<tr>
<td>60</td>
<td>8.93</td>
<td>10.70</td>
<td>2.50</td>
<td>78.90</td>
<td>86.90</td>
<td>519.40</td>
<td>4.82</td>
<td>10.65</td>
<td>5.18</td>
</tr>
<tr>
<td>120</td>
<td>9.51</td>
<td>7.30</td>
<td>16.70</td>
<td>39.10</td>
<td>46.40</td>
<td>594.40</td>
<td>4.01</td>
<td>3.48</td>
<td>4.35</td>
</tr>
<tr>
<td>240</td>
<td>6.21</td>
<td>5.53</td>
<td>31.75</td>
<td>53.70</td>
<td>34.65</td>
<td>658.90</td>
<td>2.42</td>
<td>4.27</td>
<td>3.89</td>
</tr>
<tr>
<td>480</td>
<td>3.72</td>
<td>2.10</td>
<td>-0-</td>
<td>16.90</td>
<td>33.35</td>
<td>485.90</td>
<td>0.12</td>
<td>1.19</td>
<td>1.79</td>
</tr>
<tr>
<td>720</td>
<td>0.67</td>
<td>1.02</td>
<td>14.30</td>
<td>4.00</td>
<td>109.15</td>
<td>68.85</td>
<td>-0-</td>
<td>0.44</td>
<td>2.66</td>
</tr>
<tr>
<td>1440</td>
<td>2.30</td>
<td>0.51</td>
<td>-0-</td>
<td>-0-</td>
<td>68.25</td>
<td>31.40</td>
<td>-0-</td>
<td>-0-</td>
<td>2.14</td>
</tr>
<tr>
<td>1920</td>
<td>0.41</td>
<td>0.42</td>
<td>9.30</td>
<td>27.80</td>
<td>16.20</td>
<td>20.05</td>
<td>0.18</td>
<td>0.59</td>
<td>-0-</td>
</tr>
</tbody>
</table>

*All values represent average of two pigs; units=ug/ml for fluid components.

*Units = ug/g for solid tissues; sensitivity of assay = 0.15 ug/ml.
### TABLE 7

Concentration of oxytetracycline in selected organs of pigs treated intravenously with a single dose (11 mg/kg) of the drug.

<table>
<thead>
<tr>
<th>Sacrifice time (min)</th>
<th>Liver</th>
<th>Lung</th>
<th>Spleen</th>
<th>Brain</th>
<th>Muscle</th>
<th>Kidney</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>12.00</td>
<td>9.20</td>
<td>9.15</td>
<td>-0-</td>
<td>5.96</td>
<td>55.10</td>
<td>3.52</td>
</tr>
<tr>
<td>60</td>
<td>17.97</td>
<td>6.87</td>
<td>7.43</td>
<td>-0-</td>
<td>8.92</td>
<td>64.00</td>
<td>2.94</td>
</tr>
<tr>
<td>120</td>
<td>14.27</td>
<td>6.52</td>
<td>4.87</td>
<td>-0-</td>
<td>9.29</td>
<td>58.60</td>
<td>1.61</td>
</tr>
<tr>
<td>240</td>
<td>11.07</td>
<td>3.85</td>
<td>3.61</td>
<td>-0-</td>
<td>7.36</td>
<td>41.40</td>
<td>4.03</td>
</tr>
<tr>
<td>480</td>
<td>4.61</td>
<td>1.52</td>
<td>1.74</td>
<td>-0-</td>
<td>4.19</td>
<td>17.60</td>
<td>1.00</td>
</tr>
<tr>
<td>720</td>
<td>2.77</td>
<td>0.99</td>
<td>1.32</td>
<td>-0-</td>
<td>2.67</td>
<td>13.35</td>
<td>0.65</td>
</tr>
<tr>
<td>1440</td>
<td>0.77</td>
<td>0.69</td>
<td>0.73</td>
<td>-0-</td>
<td>0.95</td>
<td>4.80</td>
<td>0.21</td>
</tr>
<tr>
<td>1920</td>
<td>0.84</td>
<td>0.48</td>
<td>0.37</td>
<td>-0-</td>
<td>0.82</td>
<td>3.85</td>
<td>0.28</td>
</tr>
</tbody>
</table>

*a Units are µg/g; assay sensitivity = 0.15 µg/ml.

*b Values represent averages from two pigs at each sacrifice interval.
## TABLE 9

Comparisons of biological half-lives and elimination constants (beta) between oxytetracycline plasma concentrations and concentrations in major organs and body fluids of swine treated with a single intravenous bolus of the drug (dose = 11 mg/kg)

<table>
<thead>
<tr>
<th>Organ or body fluid</th>
<th>Beta_1 (β) min</th>
<th>T 1/2 hours</th>
<th>F-value</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous plasma (mean - 15 pigs)</td>
<td>-0.0031</td>
<td>3.87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venous plasma(^a) (at time of slaughter)</td>
<td>-0.0014</td>
<td>8.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>-0.0018</td>
<td>5.25</td>
<td>50.31</td>
<td>P&lt;.005</td>
</tr>
<tr>
<td>Muscle</td>
<td>-0.0014</td>
<td>8.27</td>
<td>48.90</td>
<td>P&lt;.005</td>
</tr>
<tr>
<td>Lung</td>
<td>-0.0015</td>
<td>7.67</td>
<td>31.99</td>
<td>P&lt;.005</td>
</tr>
<tr>
<td>Fat</td>
<td>-0.0015</td>
<td>7.87</td>
<td>8.36</td>
<td>P&lt;.025</td>
</tr>
<tr>
<td>Kidney</td>
<td>-0.0016</td>
<td>7.40</td>
<td>66.47</td>
<td>P&lt;.005</td>
</tr>
<tr>
<td>Urine</td>
<td>-0.0021</td>
<td>5.48</td>
<td>5.75</td>
<td>N.S.(^b)</td>
</tr>
<tr>
<td>Stomach wall</td>
<td>-0.0016</td>
<td>7.21</td>
<td>68.90</td>
<td>P&lt;.005</td>
</tr>
</tbody>
</table>

\(^a\)Sequential plasma levels determined on two pigs per slaughter interval through 32 hours post-dosing.

\(^b\)N.S. = non-significance at the P>.05 level.
Concentrations of penicillin G in selected extracellular fluid compartments and associated organs from pigs treated intravenously and orally with a single dose (7.64 mg/kg) of the drug.

<table>
<thead>
<tr>
<th>Sacrifice time minutes</th>
<th>Venous plasma</th>
<th>Portal plasma</th>
<th>Stomach contents</th>
<th>Small intestine content</th>
<th>Large intestine content</th>
<th>Urine</th>
<th>Stomach wall</th>
<th>Small intestine wall</th>
<th>Large intestine wall</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>45.35</td>
<td>12.80</td>
<td>-0-</td>
<td>-0-</td>
<td>0.64</td>
<td>1.61</td>
<td>1.03</td>
<td>-0-</td>
<td>-0-</td>
</tr>
<tr>
<td>15</td>
<td>22.70</td>
<td>0.55</td>
<td>-0-</td>
<td>0.03</td>
<td>797.38</td>
<td>0.23</td>
<td>0.43</td>
<td>-0-</td>
<td>-0-</td>
</tr>
<tr>
<td>45</td>
<td>5.11</td>
<td>5.49</td>
<td>-0-</td>
<td>0.02</td>
<td>1008.5</td>
<td>-0-</td>
<td>0.15</td>
<td>-0-</td>
<td>-0-</td>
</tr>
<tr>
<td>90</td>
<td>1.36</td>
<td>1.38</td>
<td>0.20</td>
<td>0.16</td>
<td>686.82</td>
<td>-0-</td>
<td>0.02</td>
<td>-0-</td>
<td>-0-</td>
</tr>
<tr>
<td>Oral-90</td>
<td>0.05</td>
<td>0.09</td>
<td>0.04</td>
<td>0.35</td>
<td>28.45</td>
<td>-0-</td>
<td>0.60</td>
<td>-0-</td>
<td>-0-</td>
</tr>
<tr>
<td>180</td>
<td>0.09</td>
<td>0.12</td>
<td>-0-</td>
<td>0.05</td>
<td>3591.5</td>
<td>-0-</td>
<td>-0-</td>
<td>-0-</td>
<td>-0-</td>
</tr>
<tr>
<td>Oral-180</td>
<td>0.01</td>
<td>0.01</td>
<td>-0-</td>
<td>1.43</td>
<td>38.85</td>
<td>-0-</td>
<td>0.50</td>
<td>-0-</td>
<td>-0-</td>
</tr>
<tr>
<td>240</td>
<td>0.02</td>
<td>0.03</td>
<td>-0-</td>
<td>-0-</td>
<td>17.46</td>
<td>-0-</td>
<td>-0-</td>
<td>-0-</td>
<td>-0-</td>
</tr>
<tr>
<td>360</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>4.01</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>480</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>0.57</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>1440</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>0.07</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
</tbody>
</table>

* All values represent average of two pigs; units = units/ml for fluid components.

* Units = units/g for solid tissues; sensitivity of assay = 0.05 units/ml
TABLE 11

Concentration of penicillin G in selected organs of pigs treated intravenously and orally with a single dose (7.64 mg/kg) of the drug.

<table>
<thead>
<tr>
<th>Sacrifice time (minutes)</th>
<th>Liver</th>
<th>Lung</th>
<th>Spleen</th>
<th>Brain</th>
<th>Muscle</th>
<th>Kidney</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.09</td>
<td>5.88</td>
<td>2.38</td>
<td>0.09</td>
<td>4.15</td>
<td>5.92</td>
<td>0.80</td>
</tr>
<tr>
<td>15</td>
<td>Neg</td>
<td>4.25</td>
<td>1.30</td>
<td>0.04</td>
<td>3.40</td>
<td>5.21</td>
<td>1.50</td>
</tr>
<tr>
<td>45</td>
<td>Neg</td>
<td>1.93</td>
<td>0.29</td>
<td>0.02</td>
<td>0.26</td>
<td>3.46</td>
<td>0.19</td>
</tr>
<tr>
<td>90</td>
<td>Neg</td>
<td>0.22</td>
<td>0.09</td>
<td>Neg</td>
<td>0.24</td>
<td>0.89</td>
<td>0.07</td>
</tr>
<tr>
<td>Oral-90</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>180</td>
<td>Neg</td>
<td>0.03</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>Oral-180</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>240</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>360</td>
<td>Neg</td>
<td>Neg</td>
<td>0.12</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>480</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
</tbody>
</table>

^Units are units/g; assay sensitivity = 0.05 units/ml.

bValues represent averages from two pigs at each sacrifice interval.
TABLE 12

Comparison between biological half-lives and elimination constants ($\beta$) of mean plasma concentrations of penicillin G and concentrations in major organs and body fluids of swine treated with single intravenous bolus of the drug (dose = 7.64 mg/kg).

<table>
<thead>
<tr>
<th>Organ or body fluid</th>
<th>$\beta_{1}$ ($\beta$) min</th>
<th>$T_{1/2}$ minutes</th>
<th>F-values</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous plasma</td>
<td>0.0310</td>
<td>22.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mean - 18 pigs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venous plasma$^a$</td>
<td>0.0320</td>
<td>21.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(at time of sacrifice)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain$^b$</td>
<td>0.0343</td>
<td>20.23</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.0362</td>
<td>19.08</td>
<td>42.57</td>
<td>$P&lt;.005$</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.0063</td>
<td>109.48</td>
<td>575.12</td>
<td>$P&lt;.005$</td>
</tr>
<tr>
<td>Lung</td>
<td>0.0310</td>
<td>22.34</td>
<td>9.99</td>
<td>$P&lt;.025$</td>
</tr>
<tr>
<td>Fat</td>
<td>0.0394</td>
<td>17.59</td>
<td>108.45</td>
<td>$P&lt;.005$</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.0213</td>
<td>32.5</td>
<td>6.32</td>
<td>N.S.$^c$</td>
</tr>
<tr>
<td>Urine</td>
<td>0.006</td>
<td>115.12</td>
<td>0.109$^d$</td>
<td>N.S.</td>
</tr>
<tr>
<td>Small intestine wall</td>
<td>0.0442</td>
<td>15.66</td>
<td>3.88</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

$^a$ Sequential plasma levels determined on two pigs per slaughter interval through 24 hours post-dosing.

$^b$ Insufficient data points for statistical analysis.

$^c$ Level of non-significance = $P>.05$.

$^d$ This regression line does not fit the data, therefore no conclusions should be drawn from it.
CHAPTER V

DISCUSSION AND CONCLUSIONS

Previous literature has indicated that chloramphenicol in swine\textsuperscript{10,11} had a half-life of 1.3 hours and a volume of distribution of 1.05 l/kg. Both of these values are less than the results reported in this study. The half-life of chloramphenicol was determined to be 2.66 hours and a volume of distribution of 1.39 l/kg. However, Davis, et al.\textsuperscript{10} found that after correction for protein binding, the volume of distribution in swine was 1.61 l/kg. The extent of protein binding was not determined in these studies; however, a rough estimation of 40% binding to plasma protein would result in a volume of distribution of approximately 1.90 l/kg. Thus, the volume of distribution reported in this study is in close agreement with the value reported in the earlier study of Davis et al.\textsuperscript{10}.

The biological half-life reported in these studies was approximately twice as long as that previously reported\textsuperscript{10}. The most probable explanation for this difference may be the analytical procedure. The method used in the current studies measured total chloramphenicol and its metabolites, whereas the previous studies used an alternate assay method for chloramphenicol which required the reduction of chloramphenicol by titanous chloride and diazotation of the resulting amine. This method essentially measured only the free chloramphenicol and few, if any, metabolites\textsuperscript{22}. 

83
Previous work had indicated that chloramphenicol was found to attain a distribution equilibrium between blood and tissues in less than 30 minutes\(^{10}\). Results from the current work would substantiate this concept, further defining the distribution equilibrium to occur in most tissues in less than 5 minutes. In the major organs, e.g., liver, lung, spleen, muscle, and kidney, the concentrations attained in this 5-minute period were 1 to 3 times the plasma concentration.

There is conflicting data in the literature regarding the concept that tissue levels parallel the decline in plasma levels of chloramphenicol. One author suggested\(^{14}\) that in the horse, chloramphenicol quickly leaves the blood, enters extracellular and intracellular locations and remains there after blood levels have fallen to zero. Another investigator\(^{22}\) suggested that in one dog he observed a simultaneous fall in tissue and blood levels. The work in swine clearly supports the concept that tissue and blood levels decline approximately parallel to each other.

In the cow, it has been reported\(^{53}\) that there is a recycling effect observed as periodic rises in blood levels at approximately 90-minute intervals following the IV dosing of chloramphenicol. The authors concluded that since almost all of the drug (or metabolites) were excreted via bile in the cow, that enterohepatic cycling was affecting chloramphenicol blood levels. This same effect was observed in the current studies, except that the effect on blood concentrations was noted as early as 15 minutes and continued to occur in a sporadic fashion for up to 60 minutes, thereafter having no further
likely that in their studies they also experienced erratic blood concentrations during the distribution phase of the curve. Their results of tissue elimination were found to parallel the decay of drug concentration in serum. This aspect of their work has been confirmed with the currently reported studies.

The ability of the tetracyclines to penetrate nonfat body tissues has been related to the chloroform/water partition coefficient. The tetracycline with the highest partition coefficient attained the highest muscle concentration\(^{50, 51}\). In a study with radiolabeled tetracycline, it was stated that all nonfat tissues are penetrated by the antibiotics within 4.5 hours after injection\(^{29}\). Oxytetracycline has one of the lower chloroform/water partition coefficients, yet it clearly demonstrates an ability to penetrate most tissues of swine at levels equivalent to blood concentrations, and tissue penetration occurred within 15 minutes of the IV dose.

While it has long been recognized that penicillin G is mainly eliminated from the body by the kidneys, it has been reported that a major site of extra renal disposition for penicillin G was the liver. Biliary excretion has been considered at least a minor pathway for penicillin elimination\(^{23, 27}\). It appears that in swine, with potassium penicillin G, the liver and biliary excretion play essentially no role in penicillin elimination. Perhaps a more intriguing question regarding these reported studies was the mechanism by which penicillin G was able to reach the portal plasma at concentrations that were 25 to 28% that observed in venous plasma within 5 minutes, yet showed no demonstrable
levels in liver or intestinal contents. The data do not support a very effective enterohepatic recycling pattern for penicillin in swine, yet there were measurable quantities of penicillin G in the gut walls within 5 minutes after injection. These data suggest that there may be considerable direct transfer of drug in the extensive capillary and lymphatic networks located in the mesentery and the intestinal walls of the swine enteric tract.

The concentrations of both chloramphenicol and oxytetracycline in stomach contents following parenteral administration were of interest. Oxytetracycline first appeared in stomach content in substantial concentrations (20.50 μg/ml) at 15 minutes post-dosing. There was a decline at 60 minutes, a resurging concentration at 2 to 4 hours, another decline to zero concentration at 8 hours, an elevation again at 12 hours, a decline to zero at 24 hours, and a recurrence of substantial concentrations (9.30 μg/ml) at 32 hours (Table 6). If one examined both the venous and portal plasma concentrations, there was generally a progressive depletion of drug levels through 32 hours. Also, concentrations exceeded stomach content concentrations only at the zero or "valley" points in the cycling pattern of stomach contents. One cannot assume that the mechanism by which oxytetracycline was entering the stomach contents was via passive diffusion although there is no current knowledge of an active secretory process in the stomach mucosa for the tetracycline drugs. There does appear to be a pattern of accumulation and depletion of drug in stomach content. It is common knowledge that the rate of stomach emptying (e.g., stomach motility) is one of the
principle factors influencing rate of absorption of orally administered drugs\textsuperscript{32}. In fact, this effect was observed in the oral phase of the current studies. Some pigs retained high levels of drug in the stomach, while others had greatly reduced content. Thus, one cannot explain this erratic or cycling effect seen with oxytetracycline as due solely to the sporadic emptying pattern of the stomach contents since this effect was not also observed with chloramphenicol where stomach contents were low at 5 minutes, gradually increased over the first 1 1/2 hours, then became rather constant at 2-6 µg/ml for the duration of the study. The rationale used in explaining the oxytetracycline behavior should be applicable to chloramphenicol unless some other mechanisms are involved.

There are at least two possible explanations for the chloramphenicol behavior, neither of which were substantiated by these studies. Since the analytical method used also measured certain metabolites and glucuronide conjugated drug, it is possible that there were bound metabolites and/or free drug transported from blood into stomach contents; however, there is a second explanation that there was substantial drug metabolized in stomach and intestinal walls (which includes the mucosa and mucosal cells) since the intestinal wall content of chloramphenicol remained high for the entire duration of the study (Table 3). If it is assumed that the primary site of the glucuronide conjugation process is the hepatocyte, then the water soluble chloramphenicol glucuronide may be dumped into the intestinal contents in significant quantities in the early stages of elimination.
The smaller the value of beta, then the greater the half-life. As mentioned earlier, the current studies have defined a longer biological half-life for chloramphenicol in swine. In addition, other pharmacokinetic parameters would be affected. For instance, when elimination decreases, the area under the curve increases, and if the method for determining volume of distribution involves area under the curve values, an increase in area would ultimately result in a decrease in volume of distribution. In comparing the present data with data in the literature, there do not appear to be significant differences in volumes of distribution in pigs, regardless of methods used. Another factor that would be influenced is body clearance since total body clearance calculations depend upon values of beta and volumes of distribution and would be less if either beta or \( V_d \) were decreased. However, pharmacokinetic analyses cannot be applied when both drug and metabolites are measured since each species has its own distinct pharmacokinetic profile. There is no question that from a tissue residue standpoint, it is much more appropriate to use a method that measures total drug, including metabolites, although a pharmacokinetic analysis is precluded.

The key pharmacokinetic parameters which have been predicted to provide the most valuable information to a drug residue profile were the overall elimination rate constants, the biological half-life, apparent volume of distribution, the \( k_{12}/k_{21} \) ratio, and the body clearance rates. While most of these elements can be derived from an analysis of drug concentrations in serum or urine, the key element of
missing data to justify the value of pharmacokinetics in evaluating a residue profile has been the actual determination of drug kinetics in major tissues and body fluids.

The overall elimination rate constant and the biological half-life parameters are interrelated and probably represent the single-most important elements in the study of a drug residue profile. Although a half-life for chloramphenicol could not be accurately determined as explained earlier, the calculated value gives the half-life of the most persistent species. Hence, if drug withdrawal times are based on this value, one would not underestimate withdrawal times. The elimination rate constants were determined to be: chloramphenicol, \(-0.0049 \pm 0.0016 \text{ min}^{-1}\); oxytetracycline, \(-0.0031 \pm 0.0005 \text{ min}^{-1}\); and penicillin G, \(-0.0359 \pm 0.0032 \text{ min}^{-1}\), with respective biological half-lives of 2.66 hours, 3.87 hours and 0.32 hours. On the basis of these data, one would predict that the order of elimination from the tissues of the body would be penicillin first, chloramphenicol second, and oxytetracycline last. Examples of the actual elimination in a major edible tissue, e.g., muscle, shows that in fact penicillin G was eliminated from muscle within 90 minutes of dosing, whereas chloramphenicol and oxytetracycline were still present in muscle at low concentrations up to 24 and 32 hours, respectively, with depletion occurring in a log-linear fashion. The liver was also an interesting tissue for comparative purposes. Again, the expected rate of elimination, on the basis of the elimination constants determined in plasma would be penicillin G > chloramphenicol > oxytetracycline. The
actual drug elimination in liver indicated that penicillin G was absent after 5 minutes post-dose, chloramphenicol was eliminated from liver by 24 hours (half-life = 1.77 hours), and oxytetracycline, while still detectable at 32 hours, was declining at a rate compatible with the half-life determined in liver to be 5.25 hours. Thus, on the basis of the elimination rate constants and biological half-lives in plasma for these three drugs, the relative tissue residue elimination order could be projected as follows: penicillin G>chloramphenicol>oxytetracycline. This was the actual order observed in these studies.

The apparent volumes of distribution were determined to be: chloramphenicol, 1.39±0.32 l/kg; oxytetracycline, 1.26±0.18 l/kg; and penicillin G, 0.53±0.12 l/kg; within 5 minutes of the time of dosing, chloramphenicol concentrations in liver were approximately 3 times that in plasma; in lung were approximately 2 times plasma; in spleen were approximately 3 times plasma; with equivalent concentrations in muscle tissue. At 15 minutes post-dose with oxytetracycline, concentrations in liver were 30% greater than plasma, and were essentially equivalent in lung, spleen and muscle. With penicillin G, at 5 minutes post-dose, there were no tissues which contained concentrations greater than 13% of the plasma concentrations. Thus, in a relative sense, it was clear that the calculation of the apparent volumes of distribution was generally reflective of tissue penetration, and could be of value in estimating the potential degree of drug residues which might occur in tissues. However, the physiological meaning of volume of distribution still was not clear. If we examine the generally recognized values²,3
for the compartments of the body that contain water, we find that total body water is equivalent to approximately 58 to 60% of total body weight, with intracellular fluid accounting for 41%, extracellular fluid accounting for 17%, with plasma volume and red blood cells contributing 4% each to the respective extracellular fluid pool. The volume of distribution for chloramphenicol was 1.39 l/kg. This represents two times the total body water which is 0.60 l/kg.

The volume of distribution for oxytetracycline was 1.21 l/kg which is again about two times the total body (total of 2.2 liters). The volume of distribution for penicillin was calculated to be 0.53 l/kg which is only slightly less than total body water. It should be emphasized that pharmacokinetic volumes of distribution bear no relationship to actual anatomic volumes except in certain circumstances such as antipyrene and Evan's blue.

We do, however, have factual data as to where the majority of these drugs were actually distributed in the animal body. These data can be found in Tables 17, 18, and 19. If we examine the percentage of dose in various tissue locations at specific time intervals we find the following: For chloramphenicol, the liver, venous plasma, and lung concentrations were the major sites of distribution and accounted for 30% of the dose at 5 minutes. The muscle mass has been estimated to represent 48% total body weight, thus 48% of 43.6 kg body weight = 20.9 kg muscle mass which contained at 5 minutes a concentration of 28.54 µg/g chloramphenicol. This calculates out to be an estimated 567 mg of drug in muscle tissue. This value represents 59% of the
of the total dose. Thus, the major tissues of distribution for chloramphenicol were muscle, liver, venous plasma, and lung, and those organs at 5 minutes post-dose contained approximately 90% of the total dose.

For oxytetracycline, the large intestine content, the small intestine content, venous plasma, and stomach content accounted for approximately 24% of the total dose at 15 minutes. The muscle mass of a 38.20 kg pig is equivalent to 18.33 kg of muscle, which contained a concentration of 5.96 µg/g oxytetracycline for a total amount of 109 mg of drug. This value represents 25% of the total dose. Thus, at 15 minutes after the dose, only 49% of the drug can be accounted for in the major sites of distribution, e.g., intestinal content, venous plasma, and muscle. However, at 60 minutes after the dose, over 90% of oxytetracycline can be accounted for in the intestinal contents.

Penicillin appears to act very simply. It goes from venous plasma to urine; however, it is difficult to account for a total volume of distribution which exceeds all of the extracellular fluids by three times. The observation that penicillin reaches the portal plasma very rapidly without going through the liver and intestinal tract may be a possible clue as to its distribution. Swine mesentery and intestinal walls have an extensive network of capillaries and lymphatics. It is tempting to speculate that penicillin may rapidly and thoroughly diffuse into the abdominal lymphatic system, and/or kidney, thereby possibly accounting for its apparent distribution into compartments greater than the extracellular body water.
The body clearance rates were estimated to be: chloramphenicol was 6.70±2.16 ml/kg/min; oxytetracycline was 3.82±0.59 ml/kg/min; and penicillin was 19.06±5.06 ml/kg/min. Chloramphenicol, particularly the glucuronide conjugate, is primarily excreted by the renal tubules, whereas the free drug may be excreted by glomerular filtration.

The studies that have been reported in the current communication have dealt with the intravenous dosing of the drug. Obviously, all drugs used in food animals are not administered intravenously. A recent literature report has provided a detailed classification scheme with examples of drugs which demonstrate that the absorption phase of any drug given by a route other than intravenously can have a definite impact upon its sojourn in the body. The impact of injection site depots following intramuscular administration has been previously discussed. Oral dosing of certain drugs, aside from an early absorption phase and after a plasma plateau is reached, may behave similarly to the intravenously administered drug. This has been clearly demonstrated with oxytetracycline. Dosage forms and formulations specifically designed to increase the time-concentration of a drug in the food animal will most definitely affect the pharmacokinetics and the residue profile. The major point should be that the pharmacokinetic characterization of a drug intended for use in food animals greatly expands the data base currently developed by conventional residue methods; allows for some type of classification scheme which could prevent all drugs designed for use in food animals from being regulated in an equivocal manner; and would provide an important data base for the calculation of effective therapeutic regimens.
In recent years, the emphasis on improved tissue distribution of antimicrobial agents has increased. It is now generally accepted that "bacteria germinate more frequently in tissues than in blood" and that "bacteria are more common in other tissues than in blood." One investigator has even proposed that dosage regimens for chloramphenicol may need to be established upon the basis of tissue concentrations. Tissue concentrations are difficult to obtain and most experimental situations will only allow for point analysis. In order to estimate dosage of a drug on the basis of its concentration in a specific tissue, one must have information about the kinetics of the drug in that tissue. The alternative, of course, is to rely upon the kinetics in blood plasma. The work reported herein has examined the tissue kinetics of three drugs, as well as the recent progress on two sulfonamide drugs.

For many years, and even now, a very large amount of resources are expended in collecting tissue samples from slaughter animals. These tissues undergo exhaustive analytical procedures for a large variety of drugs and chemicals. This is a highly inefficient process because sample numbers are necessarily small, when drug residue-laden carcasses are found, they must by necessity be destroyed and often by the time the analytical results are available, the contaminated carcass (or carcasses, since most animals today are slaughtered in lots) would have already entered the food chain. It was believed that if it could be experimentally confirmed that changes in drug concentrations in plasma or urine accurately paralleled changes in concentrations in tissues, then the emphasis in methodology development could be
concentrated upon blood plasma or urine, which are much simpler and faster than body tissues to collect and assay. The data currently available have experimentally confirmed relationships for changes in concentrations of sulfamethazine and sulfathiazole between tissues and plasma in two species\(^6,7\), and the results reported herein for penicillin G, chloramphenicol and oxytetracycline, have shown a high level of significance between plasma and tissue elimination rates in pigs.

The experimental data for the sulfonamides have since been tested under field conditions to validate the hypothesis that blood plasma could be used instead of body tissues in a drug residue surveillance program.

In March 1977, a field screening survey was instituted at a large midwestern abattoir\(^19\). Blood and kidney samples were collected from 243 swine during the slaughtering process. All samples were identified as to animal of origin. The animals had been previously identified as to farm of origin. The animals had been previously paint branded so that the samples could be obtained from at least five animals in each herd slaughtered. Plasma samples (240) were analyzed by a thin-layer chromatographic method\(^6\). Sulfamethazine was the only sulfonamide detected in plasma and concentrations ranged from 0 to 4.6 ppm. Of the plasma samples analyzed, 50 (20.83\%) contained at least 0.3 ppm sulfamethazine and 18 (7.5\%) contained sulfamethazine concentrations in excess of 1.0 ppm. Upon completion of the plasma assays, kidney samples were selected from animals having 0 to 4.6 ppm sulfamethazine in plasma. Sixty kidney tissues selected in this
APPENDIX
The two-compartment open model consists of a central (1) and a peripheral (or tissue, 2) compartment. The constants $k_{12}$ and $k_{21}$ are the first-order rate constants for the passage of the drug between the two compartments; $k_{el}$ is the first-order rate constant for elimination of the drug from the central compartment. A single compartment open model consists of the central (1) compartment only.
TABLE 13

Linear regression analysis of plasma concentration of SMZ (mg/100 ml) and amount of SMZ to be excreted (percent of dose) versus the concentration of the drug (ppm) in various tissues following IV administration of sulfamethazine (107.25 mg/kg/bwt).

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Plasma conc.</th>
<th>Amount to be excreted</th>
<th>Concentration in tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kidney</td>
</tr>
<tr>
<td>6</td>
<td>17.4</td>
<td>48.7</td>
<td>124.2</td>
</tr>
<tr>
<td>12</td>
<td>9.0</td>
<td>25.9</td>
<td>63.4</td>
</tr>
<tr>
<td>24</td>
<td>2.4</td>
<td>5.2</td>
<td>18.3</td>
</tr>
<tr>
<td>36</td>
<td>0.4</td>
<td>0.5</td>
<td>5.2</td>
</tr>
<tr>
<td>48</td>
<td>0.1</td>
<td>--</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Correlation coefficients between plasma concentration and tissue concentration

.9998   .9987   .9826   .9939   .9984   .9764

Correlation coefficients between amount to be excreted and tissue concentration

.9989   .9996   .9773   .9916   .9886   .9698

From Ref. No. 52.
Linear regression analysis of average plasma concentration of sulfathiazole and rate of excretion of unchanged sulfathiazole versus the concentration of sulfathiazole in various tissues following intravenous administration of sodium sulfathiazole (72 mg/kg) to swine.

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Average Plasma conc. (mg/100ml)</th>
<th>Average rate of excretion (% dose/hr)</th>
<th>Concentration in tissue (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kidney</td>
</tr>
<tr>
<td>2</td>
<td>4.49</td>
<td>9.37</td>
<td>146.5</td>
</tr>
<tr>
<td>4</td>
<td>1.89</td>
<td>4.58</td>
<td>46.5</td>
</tr>
<tr>
<td>8</td>
<td>0.44</td>
<td>1.14</td>
<td>7.7</td>
</tr>
<tr>
<td>16</td>
<td>0.08</td>
<td>0.11</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Correlation coefficient between plasma and tissue data

|        | .994  | 1.000 | 1.000 | .996  | .940  |

Correlation coefficient between urine and tissue data

|        | .985  | .997  | .997  | .986  | .961  |

From Ref. No. 8.
Linear regression analysis of average plasma concentration of sulfathiazole and rate of excretion of unchanged sulfathiazole versus the concentration of sulfathiazole in various tissues following intravenous administration of sulfathiazole (72 mg/kg).

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Average plasma conc. (mg/100ml)</th>
<th>Average rate of excretion (%dose/hr)</th>
<th>Concentration in tissue</th>
<th>Body fat</th>
<th>Omental fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td>Kidney</td>
<td>Liver</td>
<td>Heart</td>
</tr>
<tr>
<td>2.0</td>
<td>4.7</td>
<td>13.4</td>
<td>308</td>
<td>40</td>
<td>34</td>
</tr>
<tr>
<td>4.0</td>
<td>1.2</td>
<td>4.3</td>
<td>55</td>
<td>9.4</td>
<td>9.3</td>
</tr>
<tr>
<td>8.0</td>
<td>.1</td>
<td>.7</td>
<td>2.3</td>
<td>.7</td>
<td>.3</td>
</tr>
</tbody>
</table>

Correlation coefficient between plasma concentration and tissue concentration

1.000 1.000 1.000 .999 .998 .998

Correlation coefficient between rate of excretion and tissue concentration.

.990 .996 .998 .995 1.000 .992

From Ref. No. 7.
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_p^O$</td>
<td>Concentration of drug in serum immediately after intravenous injection ($C_p^O = A + B$).</td>
</tr>
<tr>
<td>A, B</td>
<td>Zero-time intercepts of serum drug concentration, obtained by extrapolation of linear least square regression lines.</td>
</tr>
<tr>
<td>$\alpha, \beta$</td>
<td>Hybrid rate constants dependent on all three specific rate constants of two-compartment open model. Values of $\alpha$ and $\beta$ are related to the slopes of distribution and elimination phases, respectively, of the biexponential serum drug concentration versus time profile.</td>
</tr>
<tr>
<td>$t^{1/2}$</td>
<td>Biological (or serum) half-life of drug ($t^{1/2} = 0.693/\beta$).</td>
</tr>
</tbody>
</table>
| $k_{12}/k_{21}$ | First-order rate constants for distribution of the drug between the central and peripheral compartments.  

\[
k_{12} = A^1B^1(\beta \alpha)^2/k_{21}; \quad k_{21} = A^1\beta + B^1\alpha.
\]

A$^1 = A/C_p^O$, $B^1 = B/C_p^O$.  

| $k_{el}$ | First-order rate constant for elimination of the drug from the central compartment.  

\[
k_{el} = 1/(A^1/\alpha + B^1/\beta)
\]

| $V_d(\text{area})$ | Apparent volume of distribution based on total area under linear drug concentration in serum-time plot from $t = 0$ to $t = \infty$ after administration of a single dose [$V_d(\text{area}) = \text{dose/area} \cdot \beta$]. |

| $Cl_B$ | Total body clearance ($Cl_B = \beta \cdot V_d$); $\beta$ is the overall elimination rate constant and $V_d$ is the apparent volume of distribution of the drug. |
TABLE 18

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>Time (min)</th>
<th>0.5</th>
<th>15.0</th>
<th>30.0</th>
<th>60.0</th>
<th>90.0</th>
<th>120.0</th>
<th>180.0</th>
<th>240.0</th>
<th>360.0</th>
<th>480.0</th>
<th>720.0</th>
<th>1440.0</th>
<th>1920.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>178.00a</td>
<td>10.60</td>
<td>7.10</td>
<td>6.10</td>
<td>4.40</td>
<td>3.90</td>
<td>1.56</td>
<td>1.34</td>
<td>1.21</td>
<td>.87</td>
<td>.43</td>
<td>.14</td>
<td>.14</td>
<td></td>
</tr>
<tr>
<td>D</td>
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a Units = μg/ml; assay sensitivity = 0.06 μg/ml.
b N. S. = No sample.
c All samples at 2880 minutes (48 hrs) were negative.
d Pig Nos. A, C, J (and Q at 30 min) lost patency in cannulas.
### TABLE 19

Plasma concentrations of penicillin G in swine dosed intravenously with 5,000 units/lb or equivalent to 7.64 mg/kg body weight.

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| S DEV         | 6.3          | 6.92  | 1.82  | 2.31 | 1.73 | 1.63 | .59  | .22  | .12  | .02   | .00   |       |
| AEM           | 2.12         | 1.73  | .45   | .56  | .42  | .54  | .14  | .05  | .03  | .01   | .00   |       |
| N             | 8            | 16    | 16    | 17   | 17   | 9    | 17   | 16   | 13   | 1     |       |

* NS = No Sample

b Units/ml; assay sensitivity equivalent to 0.01 units/ml; activity identified as penicillin with penicillinase.

c Samples taken at 6, 8, 12 and 24 hours were all negative.
TABLE 20

Total tissue and body fluid levels of chloramphenicol in pigs\textsuperscript{a} at selected intervals following a single intravenous dose (22 mg/kg)\textsuperscript{b}. Total in milligrams (mg), and percent of dose.

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\textsuperscript{a}Average of 2 pigs per slaughter interval.

\textsuperscript{b}Pig weight ranged from 25.0 to 61.8 kg (mean 43.6 kg). Thus total dose range was 550 milligrams to 1359 milligrams.

\textsuperscript{c}Values in parentheses ( ) represent percent of total dose.
TABLE 21

Total tissue and body fluid levels of oxytetracycline in pigs at selected intervals following a single intravenous dose (11 mg/kg)\(^a\). Total in milligrams (mg) and percent of dose.

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<th>Stomach content</th>
<th>Small intestine content</th>
<th>Large intestine content</th>
<th>Liver</th>
<th>Lung</th>
<th>Brain</th>
<th>Spleen</th>
<th>Kidney</th>
<th>Urine</th>
<th>Venous plasma</th>
</tr>
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<tbody>
<tr>
<td>15</td>
<td>15.38 (^{b})</td>
<td>23.76</td>
<td>41.55</td>
<td>13.84</td>
<td>3.57</td>
<td>-0-</td>
<td>0.61</td>
<td>11.31</td>
<td>6.54</td>
<td>23.60</td>
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<tr>
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<td>(3.89) (^{b})</td>
<td>(5.65)</td>
<td>(8.92)</td>
<td>(3.03)</td>
<td>(.78)</td>
<td>(.13)</td>
<td>(2.48)</td>
<td>(1.43)</td>
<td>(5.22)</td>
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<td>324.73</td>
<td>83.06</td>
<td>17.14</td>
<td>2.86</td>
<td>-0-</td>
<td>0.40</td>
<td>10.77</td>
<td>51.98</td>
<td>20.62</td>
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<tr>
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<td>(.24) (^{b})</td>
<td>(75.97)</td>
<td>(23.76)</td>
<td>(4.82)</td>
<td>(.79)</td>
<td>(.11)</td>
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<td>(14.57)</td>
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<td>7.19</td>
<td>24.23</td>
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<td>14.87</td>
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<td>-0-</td>
<td>0.37</td>
<td>10.96</td>
<td>69.25</td>
<td>25.59</td>
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<td>(5.57)</td>
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<td>(.58)</td>
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<td>(.35)</td>
<td>(.06)</td>
<td>(1.52)</td>
<td>(24.04)</td>
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<tr>
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<td>-0-</td>
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<td>30.01</td>
<td>4.63</td>
<td>0.69</td>
<td>-0-</td>
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<td>(.11)</td>
<td>(.02)</td>
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<td>(.12)</td>
<td>(.43)</td>
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<td>-0-</td>
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<tr>
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<td>(.16)</td>
<td>(.13)</td>
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</tbody>
</table>

\(^a\)Pig weights ranged from 24 to 52 kg, thus total dose range was 264 to 572 milligrams.

\(^b\)Values in parentheses ( ) represent percent of total dose.
TABLE 22

Total tissue and body fluid levels of penicillin G in pigs at selected intervals following a single intravenous or oral dose (7.64 mg/kg). Total in units and percent total dose.

<table>
<thead>
<tr>
<th>Sacrifice time (min)</th>
<th>Stomach content</th>
<th>Small intestine content</th>
<th>Large intestine content</th>
<th>Liver</th>
<th>Lung</th>
<th>Brain</th>
<th>Spleen</th>
<th>Kidney</th>
<th>Urine</th>
<th>Venous plasma</th>
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<td>Neg</td>
<td>91</td>
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<td>889</td>
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<td>(.02)</td>
<td>(.51)</td>
<td>(0)</td>
<td>(.04)</td>
<td>(.23)</td>
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<td>(28.87)</td>
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<td>35</td>
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<td>1506</td>
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<td>97</td>
<td>695</td>
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<td>(.02)</td>
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<td>Neg</td>
<td>736</td>
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<td>515</td>
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<td>Neg</td>
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<td>Neg</td>
<td>44</td>
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<tr>
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<td>(1.06)</td>
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<td>(.01)</td>
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<td>(1.06)</td>
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<td>(0)</td>
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<tr>
<td>8 hrs</td>
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<td>Neg</td>
<td>Neg</td>
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<td>Neg</td>
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<td>20</td>
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<td>Neg</td>
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</tr>
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</table>

^a Pig weights ranged from 16.8 to 42.4 kg (mean 27.5 kg), thus average dose was 302.5x10^3 units or 210.23 mg.

^b Only two slaughter intervals were available for oral dosing, e.g., at 90 and 180 minutes.

^c Values in parentheses ( ) represent percent of total dose.


