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# DOSAGE REGIMEN FOR CHLORAMPHENICOL IN CALVES BASED UPON KINETIC DATA

by

TAIWO A. COLE

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Daniel Gibson

Major Professor

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# INTRODUCTION

Chloramphenicol is a broad-spectrum antibiotic which is being widely used in veterinary medical practice. Following several reports of toxicity accompanying the use of chloramphenicol in man, several countries have put restrictions on its use especially in food-producing animals. However veterinary practitioners have found the antibiotic useful for the treatment of enteric and respiratory infections in these animals, notably calves and baby pigs.

The pharmacokinetic disposition of chloramphenicol has been studied in several animals, but there are relatively few such studies done in calves, most of these studies have involved single route of administration, usually oral or intramuscular. In the field, however, the subcutaneous and intramuscular routes are commonly used for the administration of chloramphenicol.

The minimal therapeutic blood concentration usually suggested is 5 ug/ml, but there is inconsistency in the recommended dosages that would produce and maintain this level in calves.

The objectives of the present study were: (a) to determine the absorption into and the disappearance of chloramphenicol base from the plasma of calves after single-dose intramuscular, subcutaneous, and intravenous

administrations of the antibiotic; (b) to gather similar data after single dose subcutaneous administration of chloramphenicol sodium succinate; (c) to determine the kinetic parameters - plasma $t_{\frac{1}{2}}$ (half-life), and $V_d$ (volume of distribution) - of chloramphenicol base following each single dose administration; and (d) to test and recommend a three day dosage regimen in calves for chloramphenicol based upon the data obtained.

# LITERATURE REVIEW

## HISTORY

The discovery of the therapeutic properties of penicillin at Oxford in 1940 stimulated interest in the ability of micro-organisms to produce antibacterial substances.[1] CHLORAMPHENICOL is the generic name of an antibiotic produced by Streptomyces venezuelae, an organism first isolated by Burkholder in 1947 from a soil sample collected in a mulched field near Caracas, Venezuela.[2,3] When the organism was grown in liquid media, filtrates of these cultures proved to possess marked antibacterial activity in broth-dilution assays against several gram-negative bacteria,[2] and also indications of antirickettsial and antiviral activity.[4]

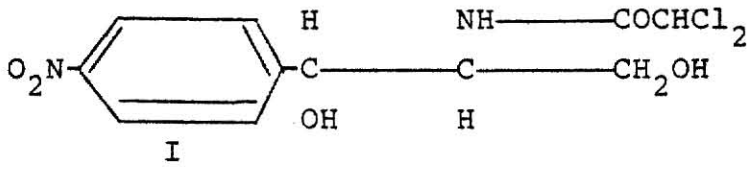
A crystalline antibiotic substance was then isolated by Bartz[5] and given the trade name CHLOROMYCETIN because it contained chlorine and was obtained from an actinomycete.

In 1949, the chemical structure and the chemical synthesis were announced along with manufacturing methods,[6,7,8] making chloramphenicol the first antibiotic to be manufactured synthetically on a commercial scale.[9]

The antibiotic was increasingly put to general clinical use but by 1950 it became evident that it could cause serious and fatal blood dyscrasias in man.[3]

CHEMISTRY AND FORMS

Degradation studies have shown[10] chloramphenicol to have the structure I, D-(-)-threo-2-dichloroacetamido-1-p-nitrophenyl-1,3-propanediol.



I

The antibiotic is unique among natural compounds in that it contains a nitrobenzene moeity and is a derivative of dichloroacetic acid. There are four stereoisomers of chloramphenicol but only the D-threo form has antibacterial activity, so that in the formation of derivatives such as the palmitate ester the stereochemical configuration of the propanediol side chain must be retained.[9]

Chloramphenicol is a neutral compound which has the molecular formula $C_{11}H_{12}Cl_2N_2O_5$.[1] It is a white, but sometimes slightly grey or yellow, fine crystalline powder with a bitter taste and considerable heat stability. It is only slightly soluble in water (1:400)[3,9] but is soluble in most organic solvents such as propylene glycol.[11]

Chemical dosage forms of chloramphenicol have been described.[12,13] They include chloramphenicol, available as the base and referred to by that name, as well as such esters as chloramphenicol palmitate, succinate, glycinate

and undecylenate. Chloramphenicol (base) is relatively insoluble in water and is mostly used clinically in topical formulations or orally. It is soluble in and used in a number of organic solvents, such as dimethylacetamide and propylene glycol, for intramuscular use.

Chloramphenicol palmitate contains 57.5% chloramphenicol[13] and is usually given orally. Since it is tasteless, it is more easily consumed in food or water by animals than the bitter base. It requires enzymatic hydrolysis to chloramphenicol before absorption can take place.

Chloramphenicol sodium succinate contains 65% chloramphenicol.[13] It is sufficiently soluble to be used in solutions that are absorbed more rapidly than suspensions. It is usually marketed as a sterile lyophilized stable product ready for aseptic reconstitution for intramuscular or intravenous use. It is hydrolyzed in tissues to therapeutically active chloramphenicol.

Chloramphenicol undecylenate contains 65.7% chloramphenicol. The antifungal undecylenate fraction gives this form an oral and topical advantage.[13]

## MECHANISM OF ACTION

Chloramphenicol is a potent inhibitor of bacterial protein synthesis.[14] It acts primarily on the 50S ribosomal subunit.[3] The activity of peptidyl transferase, which

catalyzes peptide bond formation, is suppressed, although ribosomes may still bind to and move along strands of mRNA. Ribosomal translocation thus appears to be uncoupled from peptide bond synthesis.

The effects on mammalian cells, including some of the hematological abnormalities observed in man, are also thought to be related primarily to inhibition of protein synthesis.[3,15]

Chloramphenicol is usually classed as a "bacteriostatic" agent because in vitro it arrests the multiplication of bacteria, but does not reduce the number of living organisms. However, chloramphenicol in high concentrations may be "cidal" to some organisms.[15]

It has been suggested[16] that the p-nitro group in the chloramphenicol molecule is directly or indirectly responsible for the distinct metabolic properties of the drug, e.g. rapid influx across cell membrane, capacity of inhibition of DNA synthesis, and marked intracellular covalent binding.

## CLINICAL USES AND DOSAGE

Chloramphenicol is a broad-spectrum antibiotic and is widely used in veterinary practice in spite of varying regulations in a number of countries concerning its administration to meat, milk and egg producing animals for human consumption. These regulations have been placed because

data on the persistence of chloramphenicol residues are inadequate.[13]

Chloramphenicol has been of value in treating pasteurellosis (shipping fever), calf scours, Salmonella infections, various secondary invaders in virus diseases, kennel cough, infectious bovine keratitis, and footrot in sheep. It has also been used in conjunction with many other drugs in intramammary injections.[9,11,17]

Many clinicians consider chloramphenicol to be the drug of choice for certain ocular infections because it penetrates ocular tissues better than many other antibiotics. It can be administered parenterally, topically, orally, or by direct injection into the aqueous or vitreous humor, or subconjunctivally.[17]

The antibiotic has been useful for treating urinary tract infections caused by sensitive Aerobacter aerogenes and Escherichia coli species.[11]

Bacterial resistance is related to dosage, and outbreaks of enteritis in calves, swine and other animals have been caused by resistant salmonellae.[17] In a recent outbreak of Salmonella typhimurium var copenhagen infection in which the organisms were resistant to chloramphenicol, the resistance occurred suddenly and the bacteria were also found resistant to tetracyclines and sulfonamides.[18]

The minimum therapeutic blood concentration of chloramphenicol usually suggested is 5 ug/ml,[11] but this is only an average value since microorganisms vary in their susceptibility to chloramphenicol.[17]

Although oral administration of chloramphenicol is feasible in monogastric animals, it is not, in adult ruminants because the drug is rapidly destroyed by rumen microflora.[19,20] Prior to the development of rumen microflora in young ruminants the rapid anatomical development of the still physiologically afunctional rumen results in an increasing ruminal volume and delayed reticulo-rumen emptying. This is the major obstacle to drug absorption after oral administration of chloramphenicol.[21]

It has been suggested that an oral loading dose of 100 mg/kg followed by 50 mg/kg at 8 hour intervals should maintain effective plasma values in all species except ruminants.[22] Although it has been recommended that ruminant and equine animals be treated by the intramuscular route because of the rapid elimination of chloramphenicol following intravenous administration,[22] 10 g of the drug given intravenously for two consecutive days, successfully treated pasteurellosis in a bull.[9]

Calf diptheria has been successfully treated with single daily intramuscular injections given at the rate of 4.4 mg/kg body weight for 2 to 3 days.[9] However,

intramuscular doses needed to produce and maintain therapeutic blood concentrations in calves were suggested to be 20 to 30 mg/kg body weight administered twice each day for five days.[20]

Dosages in some animal species, according to Rossoff[13] are as follows:

| | | |
|---|---|---|
| Cattle | 10 g | IV - repeat in 18 hours - for 2-3 days |
| Heifers | 5 g | IV - BID for 2-3 days |
| Cattle | 10-30 mg/kg | IM - D |
| Calves | 1-2 g | IM - D |
| 2 weeks old | 20 mg/kg | IM - BID for 5 days after a similar IV dose |
| 2 weeks old | 500 mg | Oral - TID |
| Horses | 50 mg/kg | IM or oral - once D |
| Sheep | 10% ointments | Topically - in footrot |
| Lambs | 250 mg | Oral - BID |
| Swine | 25-35 mg/kg | Oral or IP |

## TOXICITY

Chloramphenicol produces serious toxic effects in man,[3,23] although there have been several reports of toxicity in other animals when large doses of the drug are given, similar effects have not been reported in cattle.

The toxic effects of chloramphenicol have been divided into 6 categories as follows: depression and anorexia; bone

marrow depression; interference with antibody formation; prolongation of anesthesia; blocking of neuromuscular responses; and miscellaneous.[24]

The depression and anorexia may occur any time after chloramphenicol therapy is initiated. When 4 cats were given 50 mg/kg/day intramuscularly for 21 days, 3 became anorectic on the 6th day and the other one decreased its food consumption on the 8th day.[25] In another study involving 32 cats, chloramphenicol or chloramphenicol palmitate was given orally.[26] The cats were given either 50 or 100 mg/kg BID for 5 out of 7 days for 30 days (22 treatments). None of the cats given 50 mg/kg developed any signs of toxicity. In contrast, 4 of the 8 cats given 100 mg/kg/day exhibited either depression, anorexia, diarrhea, emesis, or combinations of these signs. These cats displayed fewer signs though given larger doses than cats in the previous experiment, possibly because they were given chloramphenicol on only 5 of every 7 days. The fact that they were given the drug orally rather than intramuscularly should make little difference because studies have shown that approximately the same blood concentrations are reached following either oral or parenteral administration.[24]

Chloramphenicol is more toxic to cats than dogs, since cats are unable to conjugate drugs with glucuronic acid.[27]

Depression and anorexia appear to precede more serious effects of chloramphenicol on the hemopoietic system. Chloramphenicol interferes with heme formation, hematopoiesis, and the availability of energy to cells. The bone marrow depression and resulting anemia have been divided into 2 types: a reversible, dose-related anemia and an irreversible aplastic anemia that is not dose-related.[24] It has been estimated that 1 of every 40,000 persons treated with chloramphenicol may develop this aplastic anemia,[3] which has not been reported in animals. One case report[28] suggests the distinction between the two types of anemia may not be as clear as previously believed, since a fatal aplastic anemia resulted following an apparent dose-related chloramphenicol toxicity.

A reversible, dose-related anemia occurs in dogs, cats, ducks, man and other animals.[24,29] An adequate dose of chloramphenicol will first depress the erythroid bone marrow elements and if high enough doses are given, the myeloid elements are also depressed. Because of the long life of erythrocytes, this type of anemia will not become evident in animals treated for less than 1 or 2 weeks.

There is some evidence that chloramphenicol will suppress the formation of antibodies. This has been demonstrated in rabbits in which chloramphenicol suppressed the development of antibodies against foreign globulins and

skin grafts.[30,31] The dosage required to suppress antibody formation (500-600 mg/kg) was very large and just under the lethal dose. It has also been observed that the response to a booster injection of tetanus toxoid in man was inhibited by normal therapeutic doses of chloramphenicol.[32]

When given in conjunction with barbiturate anesthetics, chloramphenicol will increase the duration of both the surgical anesthesia and total time required for recovery. This effect has been demonstrated in mice, rats, cats, dogs, and monkeys.[33-35] The total increase in duration varies from insignificant to 35 times that of control animals. In cats and dogs the duration of anesthesia usually doubles. In studies on cats given 30-35 mg/kg of chloramphenicol 10 to 15 minutes prior to administration of pentobarbital, the duration of anesthesia increased 2.4 to 3.6 times that of controls.[34] The depth of anesthesia does not increase; only the time required for recovery is changed.[33,34] This effect occurs when chloramphenicol is given intramuscularly, intravenously or orally.[35] Chloramphenicol prolongs the effect of anesthesia by inhibiting the drug-metabolizing capabilities of liver microsomal enzymes.[36]

When given intravenously in large doses, some antibiotics, such as the aminoglycosides, produce a curare-like effect at the myoneural junction. Cats have been tested

for this effect with chloramphenicol, and doses up to 500 mg/kg did not cause neuromuscular blockade.[37]

The effect of chloramphenicol on wound healing has been studied in rats. Oral and intramuscular administration of chloramphenicol caused no effect, but intravenous administration of the drug resulted in a marked dimunition in the amount of granulation tissue and in the rate of wound closure.[38]

Studies have been performed on rats to determine the effect of oral or intraperitoneal administration of chloramphenicol on the intestinal epithelium.[39] The rats were given daily doses of 10 to 200 mg/kg of chloramphenicol for up to 6 days and killed at varying time intervals. Damage to the intestinal epithelium was characterized mainly by villous atrophy.

## BIOTRANSFORMATION AND KINETICS

Chloramphenicol is transformed in the body by deacylation and glucuronide synthesis, yielding the 3-glucuronide of chloramphenicol. This is the major excretory product in the urine of man[40] and the bovine species.[41] However, biliary excretion into the intestinal tract accounts for the elimination of as much as 75% of an administered dose in the dog, guinea pig and rat.[42]

In addition to the glucuronide derivative of chloramphenicol, a number of aromatic amines have been detected as products of chloramphenicol metabolism. The formation of amines seems to vary considerably in different species of animals and apparently is associated with the excretion of chloramphenicol metabolites in the bile.[43] Only small amounts of diazotizable amines were found in human urine after administration of chloramphenicol, whereas large amounts consistently appeared in dog and in rat urine.[42]

High concentrations of chloramphenicol and its metabolites are excreted in bile, but very little appears in feces.[9] This suggests that almost all of the drug (or metabolites) excreted via bile is reabsorbed from the intestine into the blood (enterohepatic cycle). Direct evidence of this cycle has been recorded in cattle.[20] In this study, periodic rises of the blood level of chloramphenicol suggested re-entry of the drug into the blood through a recirculation cycle of approximately every 90 minutes.[20]

The differences in elimination of chloramphenicol between animal species are related to biotransformation because there are no such differences for the rate of elimination of tetraethylammonium (a drug which is not metabolized in the body).[44]

Chloramphenicol diffuses into many body tissues and readily penetrates pleural and ascitic fluids. It also crosses the placenta.[45,46] Unlike many other antibiotics, it penetrates well into all parts of the eye, and into the cerebrospinal fluid, even in the absence of meningitis.[15]

It is evident from available data that after a given dose plasma concentrations of chloramphenicol vary widely in different animals,[19] and also in the same animal. For example, a dog was given the same dose of chloramphenicol orally on 4 different occasions. The maximum plasma values at 45, 90, and 180 minutes varied from 1.4 to 2 times the minimum value.[12] Timecourses of drug concentrations were studied in dogs, cats, pigs, goats, and ponies given intramuscular injections of chloramphenicol sodium succinate (22 mg/kg).[19] Absorption was rapid, with peak concentrations occurring at 15 or 30 minutes in all species except the dog (1 hour). Plasma half-life values varied from 0.9 hour in ponies to 5.1 hours in cats. The apparent volume of distribution ($V_d$) varied from 1.02 L/kg in ponies to 2.36 L/kg in cats.

Studies in cows revealed that plasma half-life values for chloramphenicol ranged from 3.5 to 7.8 hours depending on the product used,[47,48] while the Vd was 1.6 L/kg.[48] Such Vd values greater than unity are consistent with drugs, such

as chloramphenicol, not uniformly distributed and a fluid of lower concentration is used to determine the Vd.[20,49]

Following oral administration of chloramphenicol to adult ruminants, the antibiotic did not reach detectable values in the plasma.[19,50] However, the antibiotic was readily detected in blood samples collected from calves following oral administration. It was suggested that absorption in the young ruminant may be related to the non-functioning of the rumen during the first three months of life.[21,51]

When 4 calves were given (intramuscular administration) chloramphenicol, a dose of 10 mg/kg of body weight failed to produce therapeutic blood concentrations ($\geq$5 ug/ml). However, doses of 20 and 30 mg/kg produced and maintained therapeutic concentrations between the 2nd and 8th hours and between the 2nd and 12th hours respectively.[20] In a study in cows,[52] therapeutic concentrations of chloramphenicol in blood were maintained for 4 to 5 hours following intravenous administration, but were not achieved after intramuscular administration of 11 mg/kg. By either route, therapeutic concentrations were not attained in milk. However, at a dose of 22 mg/kg, therapeutic concentrations were maintained for up to 6 hours in blood, and for between 2 to 8 hours in milk.

One trial[53] showed that the concentrations of chloramphenicol in milk were on the average 50% of the serum concentrations. The dose used was 50 mg/kg (intramuscular administration). It has been suggested[47] that a dose of 50 mg/kg be administered intramuscularly at 2 sites when adult ruminants are treated with chloramphenicol. Such injections prolonged the duration of serum drug concentrations.

The data on plasma protein binding of the antibiotic in cattle indicate that at therapeutic concentrations the bound fraction is 50 to 60% of the total.[20,48] It was lower - 30 to 46% - in dogs, cats, swine, goats, and ponies.[19]

In a comparative study in calves,[54] serum chloramphenicol concentrations were found to rise less rapidly but they persisted longer with subcutaneous injection than with intramuscular injection. Serum concentrations of the drug were significantly lower from 2 to 3 hours post-treatment and significantly higher at 12 hours post-treatment.

Recently published data[55] suggest that chloramphenicol is much more readily absorbed from the lungs of calves than from intramuscular or subcutaneous injection sites. The rapid disappearance of the antibiotic from serum after intratracheal administration was attributed to a high excretion

rate associated with the rapid achievement of peak serum concentrations, as well as with the lack of drug reservoir at the site of administration.

## ASSAY METHODS

Several techniques have been developed for the assay of chloramphenicol in biological fluids and animal tissues.

A colorimetric method[56] was among the first used for the determination of the antibiotic in body fluids. The procedure depends on reduction of the nitro group to am amino group using titanous chloride[56] or stannous chloride,[57] followed by diazotization with acidic nitrous acid and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride. The procedure measures only aromatic nitro compounds that are products of the biotransformation of chloramphenicol, so that inactive metabolites as well as free chloramphenicol are measured.

In a modification of the colorimetric method by Kakemi et al,[58] only the free chloramphenicol extracted into isoamyl acetate is measured. The assay is unaffected by the presence of other nitro-or-amino group-containing compounds.[59]

Following the discovery that serum bilirubin interferes with the colorimetric method of Kakemi et al, it was recommended[60] that small amounts of activated charcoal be added to the isoamyl extraction step. This eliminated the bilirubin interference.

Microbiological procedures have been developed for the assay of chloramphenicol. These assays are slow and difficult, if not impossible when two antibiotics are used simultaneously.[61,62] Among the test organisms that have been used are Clostridium perfringens,[61] Sarcina lutea,[63] and Bacillus subtilis.[64]

In one trial[53] serum chloramphenicol concentrations determined by a colorimetric method were 50 to 60% higher than the concentrations determined microbiologically. Another study[47] showed a difference of 20 to 40%.

Recently several groups have published methods for the determination of chloramphenicol by high-performance liquid chromatography.[65-70] In one method,[68] the drug was extracted from biological samples with methanol and separated by reverse-phase high-pressure liquid chromatography. Detection and subsequent quantitation were performed by ultra-violet spectrophotometry. Comparison with a microbiological assay showed good correlation (correlation coefficient 0.98), but the bioassay gave slightly higher values. This suggested that a metabolite possessed some biological activity. These methods are specific for chloramphenicol.

A gas-liquid chromatographic method for chloramphenicol in serum has been described which uses the electron capture detector for quantitative measurement.[71] In this procedure

chloramphenicol was extracted from the serum with isoamyl acetate and the ditrimethylsilyl ether was formed and chromatographed.

Enzymatic procedures for the assay of chloramphenicol have been described.[72-74] One such procedure[73] was based on the enzymological acetylation of chloramphenicol catalyzed by an R factor-mediated enzyme. $^{14}$C-acetyl coenzyme A served as the donor of the labelled acetyl group, and the product, $^{14}$C-acetoxychloramphenicol, was separated from the labelled precursor by utilizing its preferential extraction into benzene. The product was then quantified by liquid scintillation counting. A simplified modification of this method[75] allowed the direct extraction of $^{14}$C-acetoxychloramphenicol into scintillation fluid and circumvented the cumbersome extraction procedures.

A fluorometric method to assay chloramphenicol involved reduction of the nitro group by heating with zinc dust in a hydrochloric acid medium.[76] A primary amine was formed and was reacted with fluorescamine which formed intensely fluorescent fluorophors with primary amines.[77] The fluorescence produced was then measured.

# MATERIALS AND METHODS

Animals. Four healthy yearling calves having an average weight of 202 kg (range: 168-242 kg) were used. They were maintained on nonmedicated feed and water throughout the study period. Each calf was given 0.2 kg of grain daily and allowed to feed on hay ad libitum.

Chloramphenicol preparations. Two chloramphenicol products - chloramphenicol base in propylene glycol, and the water soluble sodium succinate ester of chloramphenicol - were available (Table 1).

Treatments. Chloramphenicol was administered by intramuscular, subcutaneous, and intravenous routes at various calculated doses. For intramuscular or subcutaneous administrations, injections were made deep into the gluteal muscles or the cervical subcutaneous tissues, a maximum volume of 20 mls being injected into any single subcutaneous site. Intravenous injections were made within 2 to 4 minutes into the jugular vein.

The experiments were carried out according to the following sequence:

| EXPERIMENT NO. | CHLORAM-PHENICOL FORM | DOSE (mg/kg) | ROUTE OF ADMINISTRATION | NUMBER OF CALVES (n) |
|---|---|---|---|---|
| I | Base | 20 | I.M. | 4 |
| II | Base | 20 | S.C. | 4 |
| III | Base | 40 | S.C. | 4 |
| IV | Succinate ester | 40 | S.C. | 3 |
| V | Base | 40 | I.V. | 4 |
| VI[a] | Base | 30 | S.C. | 2 |
| VI[b] | Base | 40 | S.C. | 2 |

TABLE 1. CHLORAMPHENICOL FORMULATIONS USED IN STUDY

| PRODUCT | MANUFACTURER | COMPOSITION | SOLVENT |
|---|---|---|---|
| TEVCOCIN® | INTERNATIONAL MULTIFOODS, MINNEAPOLIS | CHLORAMPHENICOL BASE, 10% | PROPYLENE GLYCOL |
| MYCHEL-S® | RACHELLE LABORATORIES, INC. LONG BEACH | CHLORAMPHENICOL SODIUM SUCCINATE POWDER RECONSTITUTED TO 10% | BOILED, COOLED, TAP WATER |

The doses were single doses except in experiments VI[a] and VI[b] in which the doses were administered once a day for three consecutive days.

A time lapse of at least 2 weeks was allowed between experiments.

Sample Collection and Assay. 9 ml samples of blood were collected into heparinized tubes (50 units heparin sodium being contained in each 10 ml test tube) from the jugular vein prior to drug administration (zero hour sample) and at the following hours after drug administration:

| EXPERIMENT NO. | HOURS POST ADMINISTRATION |
|---|---|
| I | 1/4, 1/2, 3/4, 1, 2, 3, 4, 6, 12, 24, 36, 48 |
| II | 1/4, 1/2, 3/4, 1, 2, 3, 4, 6, 8, 10, 12, 18, 24 |
| III & IV | 1/4, 1/2, 3/4, 1, 2, 3, 4, 6, 8, 10, 12, 18, 24, 30, 36 |
| V | 1/4, 1/2, 3/4, 1, 1½, 2, 2½, 3, 3½, 4, 6, 8, 10, 12, 24 |
| VI[a] & VI[b] | 2, 4, 6, 12, 18, 24 |

With experiments VI[a] and VI[b], 36 and 48 hour samples were also collected after the administration of the final dose.

A fluorometric method was used to assay for chloramphenicol and metabolites contained in the samples.[76] The samples were centrifuged immediately for 15 min. at 1,344 g to obtain plasma which was then homogenized and deproteinized. The deproteinized samples were kept at -20° C until the following day when they were reduced (the nitro group being reduced with zinc in acid medium to p-amino-aminodiol).[56] The reduced samples were diluted (1:9) with 0.5 M sodium acetate buffer and fluorescence read before and after adding fluorescamine.[a] A Turner fluorometer, model 110, with no. 7-60 primary filter and no. 4 secondary filter was used. All the fluorometric readings were carried out at the IX instrument range.

Standards. Bovine plasma containing 1, 3, 5, 10 and 20 ug/ml chloramphenicol plasma were prepared the day before each experiment and similarly treated as the samples. A reliable lower sensitivity limit for the fluorometric detection of chloramphenicol in bovine plasma (1.0 ug/ml) was established prior to the start of the study.

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[a]Sigma Chemical Company, St. Louis, Mo. 63178.

Kinetic parameters. Plasma half-life values ($T_{\frac{1}{2}}$) and elimination rate constants were derived using a WANG 700 Series Advanced Programming Calculator. The areas under the plasma concentration-to-time curve (AUC) were determined by the weighing method.[78]

# RESULTS

Chloramphenicol concentrations produced in the plasma of calves after intramuscular, subcutaneous, and intravenous administrations of various doses of the antibiotic are shown in Tables 2 and 3 and Figs. 1 and 2. Chloramphenicol base in propylene glycol was administered intramuscularly and subcutaneously to four calves at a dose of 20 mg/kg (Table 2, Fig. 1). Plasma chloramphenicol concentrations reached peak levels sooner after intramuscular administration (2 hours) than after subcutaneous administration (10 hours). The peak concentration obtained with intramuscular administration was higher (6.2 $\pm$ 2.9 ug/ml) than with subcutaneous administration (3.6 $\pm$ 1.4 ug/ml). However, the antibiotic was still measurable in plasma 18 hours after subcutaneous administration while only traces were present 12 hours after intramuscular administration.

The calculated AUC for each of these two administrations were very similar; 4289.8 and 4317.1 mg min/L (Table 4). The plasma half-life of the drug (7 hours) was also similar. The volume of distribution was slightly higher (0.83 L/kg) after subcutaneous administration than after intramuscular administration (0.61 L/kg), but the elimination rate constant was lower (0.00556/min. and 0.00761/min; Table 4).

TABLE 2. PLASMA CONCENTRATION AFTER A SINGLE ADMINISTRATION OF 2 CHLORAMPHENICOL PREPARATIONS IN CALVES.

| PRODUCT | Base | Base | Base | Succinate | Base |
|---|---|---|---|---|---|
| MODE OF ADMINISTRATION | I.M. | S.C. | S.C. | S.C. | I.V. |
| DOSE mg/kg | 20 | 20 | 40 | 40 | 40 |
| No. of Calves | 4 | 4 | 4 | 3 | 4 |
| Time in Hours | Concentration, ug/ml | | | Mean (standard deviation) | |
| 0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 1/4 | 0.0 | T | T | 11.4 (1.6) | 37.9 (1.6) |
| 1/2 | 1.5 (2.4) | T | 1.1 (0.2) | 8.7 (1.1) | 33.1 (2.4) |
| 3/4 | 1.6 (1.7) | T | 0.8 (0.5) | 8.5 (1.9) | 32.6 (5.6) |
| 1 | 3.4 (3.5) | T | 1.7 (0.4) | 8.9 (1.6) | 30.7 (4.2) |
| 1 1/2 | X | X | X | X | 25.7 (5.4) |
| 2 | 6.2 (2.9) | 1.5 (1.6) | 4.5 (1.3) | 8.4 (0.7) | 27.8 (2.5) |
| 2 1/2 | X | X | X | X | 23.3 (4.7) |
| 3 | 5.3 (2.8) | 2.4 (2.6) | 6.5 (3.3) | 7.3 (0.6) | 29.5 (2.5) |
| 3 1/2 | X | X | X | X | 23.2 (6.1) |
| 4 | 4.9 (2.7) | 3.3 (3.0) | 7.5 (3.8) | 7.8 (0.3) | 21.3 (3.2) |
| 6 | 4.9 (1.5) | 3.3 (1.7) | 9.4 (3.2) | 6.8 (0.4) | 16.2 (2.2) |
| 8 | X | 2.7 (1.1) | 8.8 (2.4) | 4.9 (0.5) | 12.7 (1.9) |
| 10 | X | 3.6 (1.4) | 8.1 (1.7) | 4.3 (0.1) | 11.8 (1.3) |
| 12 | T | 3.1 (1.5) | 7.3 (1.7) | 3.7 (0.4) | 7.7 (1.5) |
| 18 | X | 1.8 (0.8) | 5.4 (0.9) | 1.7 (0.2) | X |
| 24 | 0.0 | T | 2.8 (0.4) | T | 1.3 (0.9) |
| 30 | X | X | T | T | X |
| 36 | 0.0 | X | T | 0.0 | X |
| 48 | 0.0 | X | X | X | X |

X = not determined

T = Trace

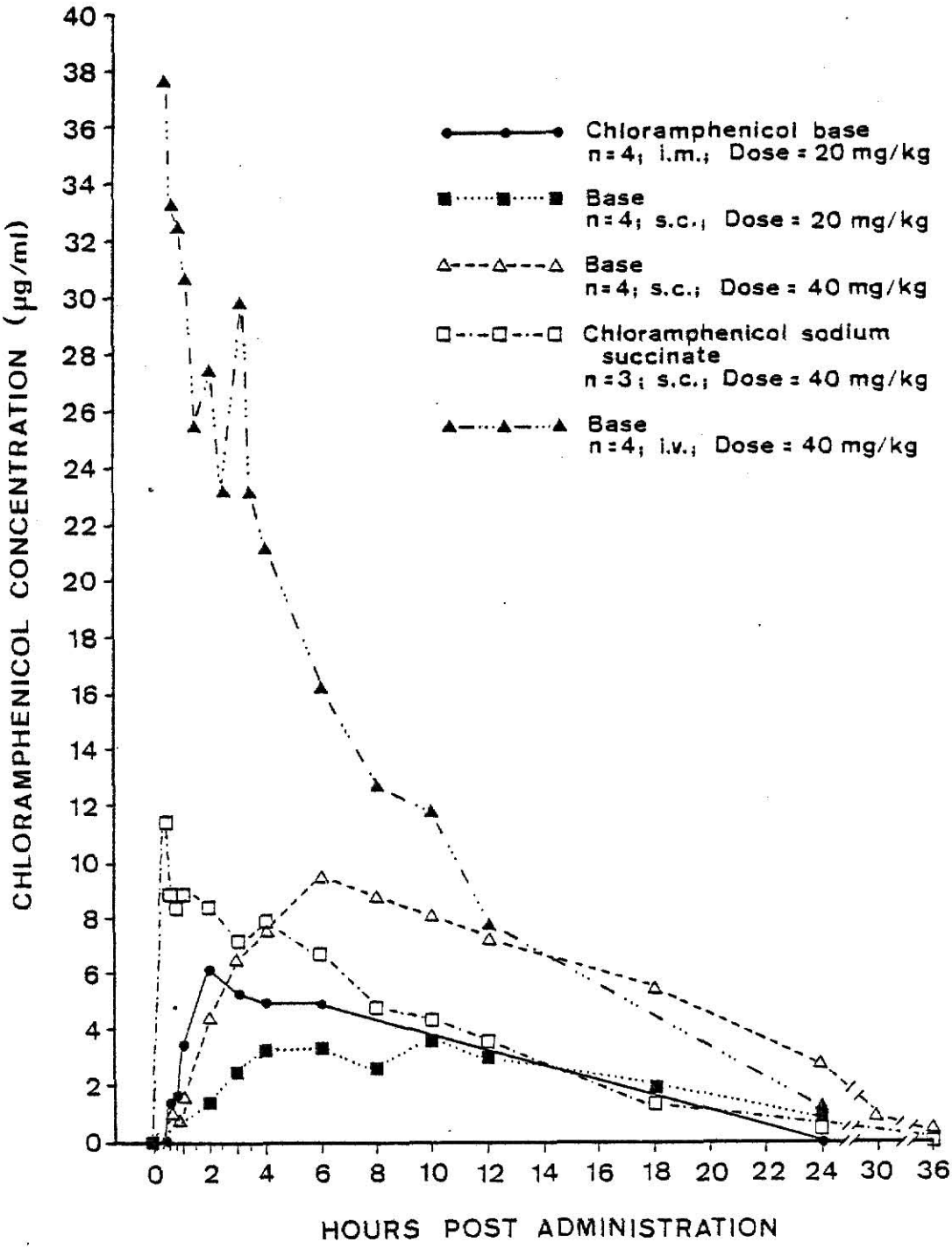
i.e., Chloramphenicol concentration qualitatively detectable but below the reliable lower sensitivity limit (1.0 ug/ml) of the procedure used.

TABLE 3. PLASMA CONCENTRATION AFTER THE SUBCUTANEOUS ADMINISTRATION OF CHLORAMPHENICOL BASE DAILY FOR THREE CONSECUTIVE DAYS IN 2 CALVES

| DOSE mg/kg<br>POST ADMINISTRATION | | 30 | 40 |
|---|---|---|---|
| | Time (hours) | Concentration (ug/ml)<br>Mean (standard deviation) | |
| Day 1: | 0 | 0.0 | 0.0 |
| | 2 | 2.4 (0.8) | 4.4 (0.4) |
| | 4 | 3.8 (0.7) | 5.5 (0.8) |
| | 6 | 3.6 (0.3) | 9.0 (1.0) |
| | 12 | 4.9 (1.0) | 7.0 (1.0) |
| | 18 | 3.1 (1.2) | 6.2 (2.2) |
| | 24 | 1.2 (0.7) | 2.4 (0.6) |
| Day 2: | 2 | 3.2 (0.1) | 6.1 (0.9) |
| | 4 | 5.4 (0.3) | 9.8 (0.1) |
| | 6 | 6.2 (0.6) | 10.2 (0.7) |
| | 12 | 5.9 (0.8) | 9.8 (1.8) |
| | 18 | 3.8 (0.7) | 7.4 (1.4) |
| | 24 | 2.0 (0.6) | 5.3 (1.1) |
| Day 3: | 2 | 5.9 (1.3) | 9.9 (0.2) |
| | 4 | 11.2 (2.1) | 14.1 (1.9) |
| | 6 | 9.6 (0.0) | 11.9 (0.9) |
| | 12 | 7.4 (0.3) | 11.7 (0.7) |
| | 18 | 4.4 (0.5) | 7.4 (1.7) |
| | 24 | 2.7 (0.1) | 4.6 (1.3) |
| | 36 | T | 1.9 (1.2) |
| | 48 | T | T |

T = Trace, i.e., chloramphenicol concentration qualitatively detectable but below the reliable lower sensitivity limit (1.0 ug/ml) of the procedure used.

Fig. 1. Mean plasma chloramphenicol concentrations in calves after the administration of two products.



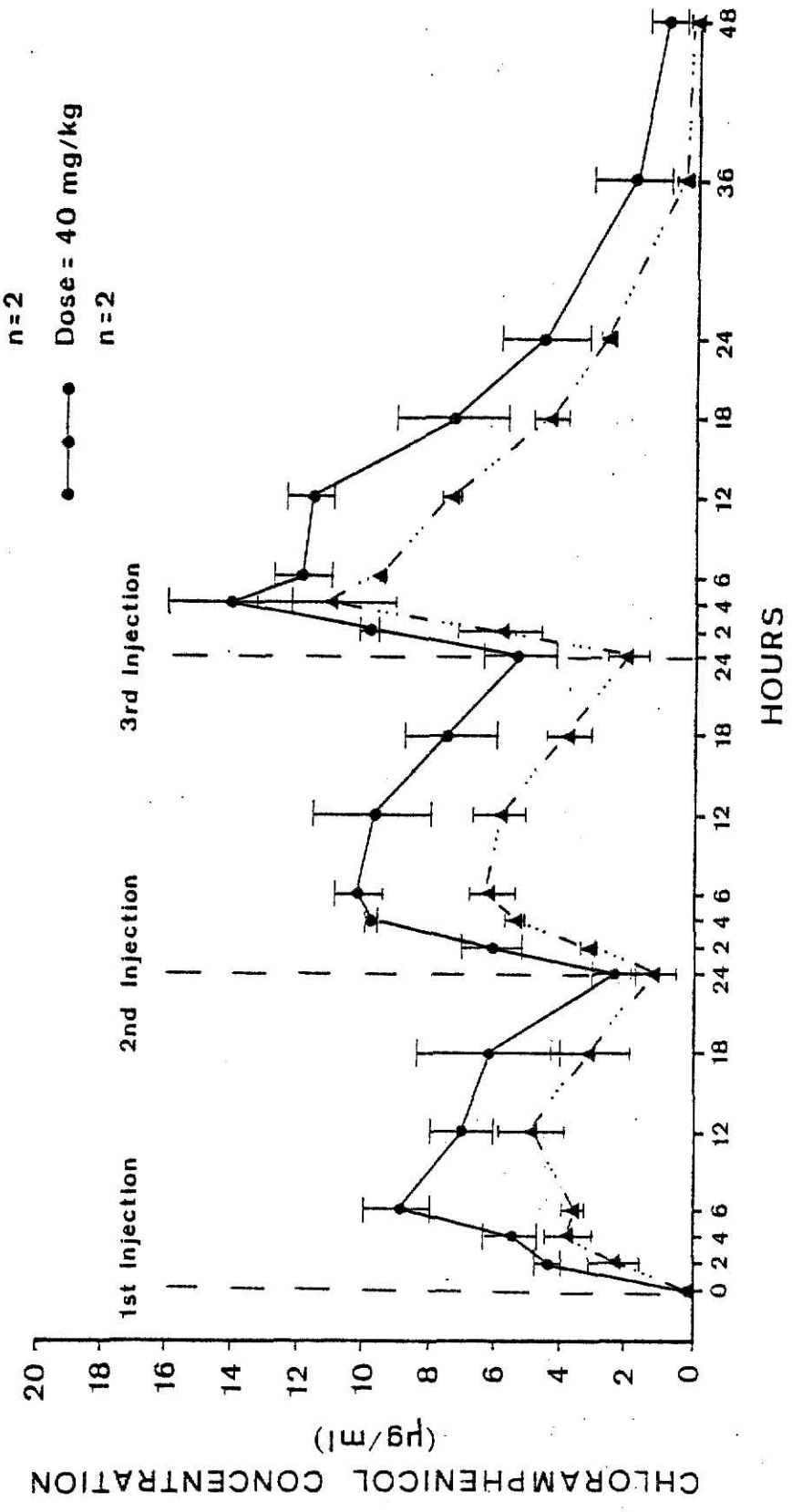



Fig. 2. Drug concentration in plasma of calves after subcutaneous administration of chloramphenicol base once a day for three consecutive days.

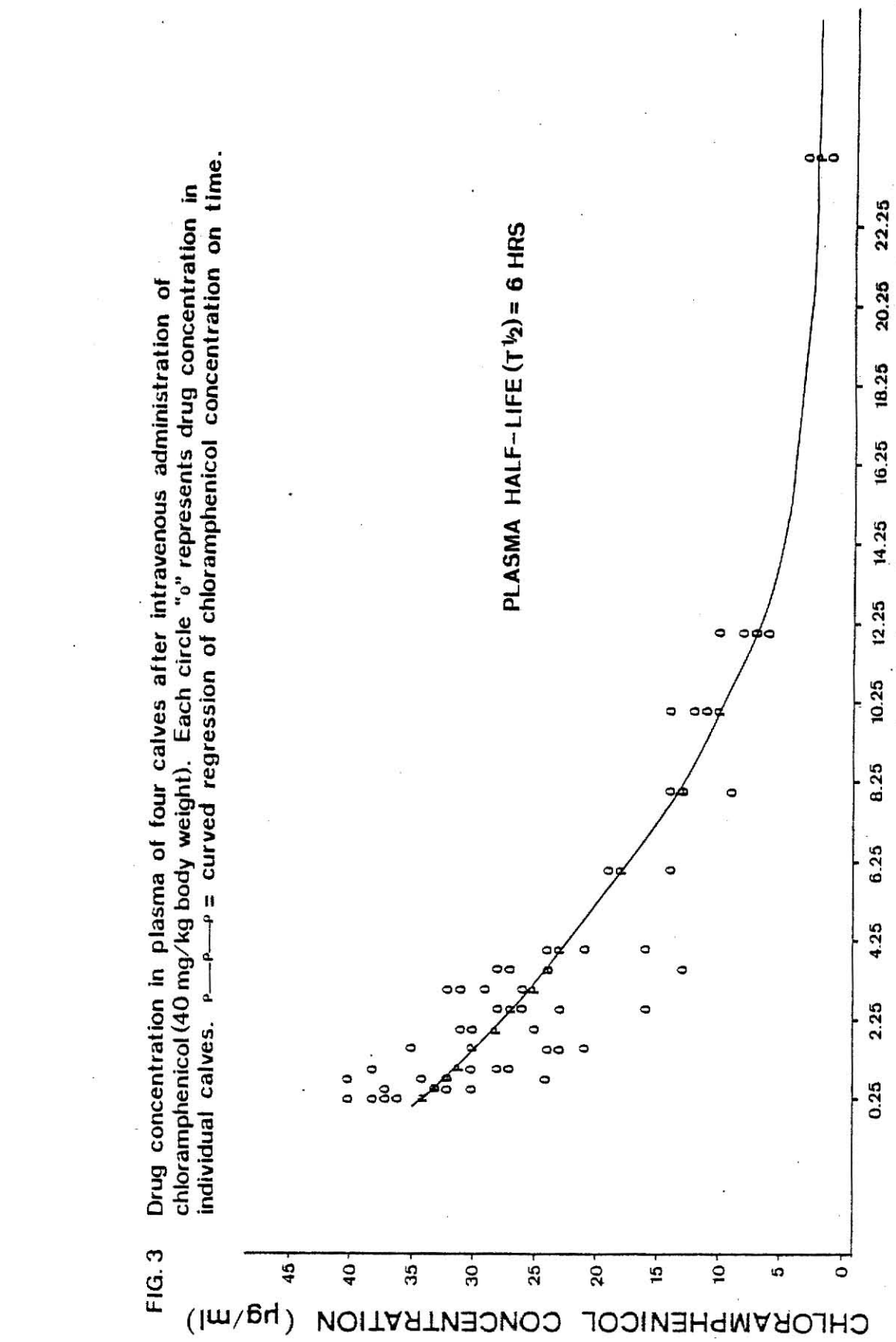



FIG. 3 Drug concentration in plasma of four calves after intravenous administration of chloramphenicol (40 mg/kg body weight). Each circle "o" represents drug concentration in individual calves. P—P—P = curved regression of chloramphenicol concentration on time.

TABLE 4. MEAN KINETIC PARAMETERS FOR THE DISAPPEARANCE OF CHLORAMPHENICOL FROM THE PLASMA OF CALVES.

| PRODUCT | Base | Base | Base | Succinate Base | |
|---|---|---|---|---|---|
| MODE OF ADMINISTRATION | i.m. | s.c. | s.c. | s.c. | i.v. |
| No. of Calves | 4 | 4 | 4 | 3 | 4 |
| DOSE mg/kg | 20 | 20 | 40 | 40 | 40 |
| KINETIC PARAMETERS | | | | | |
| $T_{½}$ (hr.) | 7 | 7 | 8 | 7 | 5 |
| $\beta$ ($min.^{-1}$) | 0.00761 | 0.00556 | 0.00804 | 0.00963 | 0.0513 |
| $V_d$ (L/kg) | 0.61 | 0.83 | 0.57 | 0.69 | 0.93 |
| AUC ($mg.min.L^{-1}$) | 4289.8 | 4317.1 | 8788.3 | 5977.4 | 16992.0 |

$T_{½}$ = plasma half-life.

$\beta$ = elimination rate constant.

$V_d$ = volume of distribution.

AUC = area under the plasma drug concentration versus time curve.

Chloramphenicol base in propylene glycol was injected subcutaneously (40 mg/kg) and peak drug concentrations (9.4 ± 3.2 ug/ml) were obtained 6 hours post-administration (Table 2, Fig. 1). The AUC was approximately double the value obtained when half this dose was given either subcutaneously or intramuscularly, but the half-life (8 hours) was very similar (Table 4). The elimination rate constant (0.00804/min) was higher than the value obtained (0.00556/min) after the antibiotic was administered at the dosage of 20 mg/kg subcutaneously.

Peak plasma chloramphenicol levels (11.4 ± 1.6 ug/ml) were attained rapidly (15 minutes) after 3 calves were given chloramphenicol sodium succinate subcutaneously at a dose of 40 mg/kg (Table 2, Fig. 1). The plasma half-life of the drug was 7 hours, but the AUC (5977.4 mg.min/L) was considerably lower than that obtained when the base was given at this same dose (40 mg/kg) subcutaneously (Table 4). The largest AUC (16992.0 mg.min/L) occurred after the intravenous administration of chloramphenicol base at a dose of 40 mg/kg to four calves; peak plasma concentrations of 37.9 ± 1.6 ug/ml were obtained. The elimination rate constant by this route was more than six times the value obtained when the same dose of the base was administered subcutaneously (0.0513 and 0.00804 mg.min/L; Table 4).

Three subcutaneous injections of chloramphenicol base (30 mg/kg or 40 mg/kg), given 24 hours apart, resulted in progressively increasing plasma drug levels (Fig. 2). Mean peak plasma concentrations were found 12 hours after the first injection of 30 mg/kg, while the mean peak plasma concentration occurred at 6 hours after the first injection of 40 mg/kg (Table 3). In both groups this peak plasma concentration occurred at 4 hours post-administration following the third treatment.

# DISCUSSION

The minimum inhibitory concentration of chloramphenicol suggested for most pathogens is 5 ug/ml.[9,11,49,79] One of the objectives of this study was to monitor plasma concentrations of chloramphenicol after the intramuscular, subcutaneous and intravenous administrations of the antibiotic at different dosages. Since this was not a tissue residue study, sampling periods were not extended over 48 hours post administration in any of the trials.

Chloramphenicol base was rapidly absorbed by the calves given the drug via intramuscular injection (20 mg/kg). Chloramphenicol was detectable in plasma 30 minutes after administration and therapeutic plasma levels were attained 2 hours after administration, but were maintained for barely 4 hours thereafter (Table 2). Sisodia, et al[20] used this dose in calves and reported therapeutic concentrations of chloramphenicol between 2 and 8 hours after administration. The large individual variations (Table 2) seen after intramuscular treatment have been reported elsewhere[47] and reflect the erratic absorptive nature of intramuscularly administered chloramphenicol. Davis, et al[19] has shown that an intramuscular dose of chloramphenicol sodium succinate (22 mg/kg) was insufficient to maintain therapeutic blood concentrations in pigs longer than 1½ hours. In this same study,[19]

chloramphenicol was not detected in the plasma of pigs given chloramphenicol dissolved in N,N-dimethylacetamide by the intramuscular route. This shows that the choice of vehicle is important with the intramuscular administration of chloramphenicol.

It is clear that the dose of 20 mg/kg given subcutaneously did not produce therapeutic plasma levels. Absorption was probably delayed because of the poorer blood supply of that region compared to muscle.[80] Palpable subcutaneous swellings developed at the injection sites and persisted for over one week, but did not appear to cause any discomfort. From a practical standpoint, neither the intramuscular or subcutaneous dose of 20 mg/kg should be used in treating calves.

Chloramphenicol base injected subcutaneously (40 mg/kg) produced and maintained therapeutic concentrations between 3 and 18 hours after administration (Table 2). The antibiotic was still detectable in plasma 24 hours after administration (Table 2, Fig. 1). Subcutaneous swellings developed but persisted longer, disappearing in all but one calf before the animals were used again two weeks later. Absorption was faster when the same dose of chloramphenicol sodium succinate rather than the base was administered subcutaneously, peak plasma levels being attained 15 minutes after administration (Table 2, Fig. 1). Therapeutic concentrations produced were

maintained through the sixth hour after administration. There were no swellings at the injection sites. It is probable that the swellings observed after the subcutaneous administration of the base were caused by the propylene glycol solvent.

When administered in equal doses, chloramphenicol and its sodium succinate ester exhibit different pharmacokinetic patterns in the same animal species. It has been shown that in infants and children,[81] a 50 mg/kg intramuscular dose of the ester produced peak serum levels at least twice as high as those observed after treatment with chloramphenicol base. Mercer, et al[82] treated dogs intramuscularly at the rate of 120 mg/kg/day in equally divided doses. Mean peak serum levels were 4.1 ug/ml after administration of chloramphenicol and 19.5 ug/ml with the ester. In calves, Hjerpe[55] showed that at a similar dose (20 mg/lb. intramuscular) the ester produced peak serum levels three times as high as the peak produced by chloramphenicol. Ziv, et al[53] reported that after a single intramuscular injection of chloramphenicol or its ester given in equivalent doses (50 mg/kg), different mean peak serum levels were produced in cows; 3.8 ug/ml at 3-4 hours for chloramphenicol and 25.0 ug/ml at 1 hour for the ester. Our data complement these findings.

We studied the disappearance of intravenously injected chloramphenicol base (40 mg/kg; Table 2, Fig. 1). The

initial rapid phase described for piglets[83] was not observed in the calves. Its absence in calves might be due to rapid distribution in the body since steady state concentrations of metabolites build up rapidly and also because of the high lipid solubility of chloramphenicol.[48] Plasma chloramphenicol concentrations were high (37.9 $\pm$ 1.6 ug/ml) 15 minutes after administration and were still above therapeutic concentrations (Table 2) 12 hours after administration. However, since all samples collected from 15 minutes to 8 hours after administration showed extensive hemolysis, the treatment of calves with intravenous chloramphenicol base in propylene glycol is not feasible, especially if the patient is critically ill. The hemolysis observed was probably due to the solvent, propylene glycol, rather than the drug. Slight hemolysis in blood samples have been reported[48] in cows after the administration of chloramphenicol base in an unspecified organic solvent at a dose of 20 mg/kg. Gross, et al[84] has shown that propylene glycol was responsible for adverse cardiovascular effects following the intravenous administration of commercially available oxytetracycline in propylene glycol. The intravenous injection of aqueous oxytetracycline preparations did not produce any adverse effects.

When chloramphenicol dissolved in a 50% aqueous solution of N,N-dimethylacetamide was given intravenously at a dosage

of 22 mg/kg, plasma levels dropped below detectable limits within 6 hours in the swine and pony, but at later times in the goat, cat and dog.[19]

The finding of transient elevations of plasma chloramphenicol levels 2 and 3 hours after intravenous administration of chloramphenicol base (Fig. 1) corroborate the report of others[47,52] and are considered to result from the enterohepatic circulation of chloramphenicol. Kinetic parameters (Table 4) confirm that the plasma half-life is independent of the dose and route of administration.[85,86] A half-life value of 5 hours (Table 4) was determined from a semilogarithmic plot[85] of chloramphenicol concentration against time when the antibiotic was given intravenously. However, it was found that a quadratic equation[87] fitted the data more satisfactorily, and the half-life of 6 hours thus obtained (Fig. 3) corresponded better with values calculated for intramuscular and subcutaneous administrations (Table 4). Plasma half-life values for chloramphenicol depend to a large extent on the animal species and on the formulation of the antibiotic. When dogs, cats, swine, goats, and ponies were given a single intravenous dose of chloramphenicol (22 mg/kg), plasma half-life values varied from 0.9 hour in ponies to 5.1 hours in cats.[19] A plasma half-life of 3.5 hours was reported[48] in cattle when chloramphenicol dissolved in an unspecified organic solvent was injected intravenously

(20 mg/kg). Nouws and Ziv,[47] using several parenteral formulations of chloramphenicol reported half-life values ranging from 4 to 7 hours.

Since the AUC reflects the total absorption of drug,[78] the highest value for this parameter was calculated for the intravenous administration (Table 4). The AUC was higher when chloramphenicol base was injected subcutaneously at the dosage of 40 mg/kg than when the ester was injected at the same dose. The initial rapid rise in plasma drug concentrations and the exponential fall in concentrations during the 8 hours after peak concentrations were reached (Fig. 1) suggest that the ester undergoes rapid hydrolysis.[88]

The calculated values for the volume of distribution (Table 4) are lower than those reported elsewhere,[48,49] and reflect upon the assay method used[76] that detect the unchanged chloramphenicol and its metabolites being measured. Nevertheless, the calculated values (Table 4) indicate widespread distribution of chloramphenicol in the body.[49,89,90]

From the data obtained after the chloramphenicol base was administered subcutaneously at 20 mg/kg and 40 mg/kg (Table 2), it was decided to design and test a dosage regimen for chloramphenicol in calves. The lower dosage (30 mg/kg) did not produce therapeutic plasma levels of chloramphenicol during the first day of treatment (Table 3, Fig. 2), but plasma levels increased steadily to therapeutic proportions

during the second and third days of treatment. The higher dose (40 mg/kg) produced therapeutic concentrations of the drug all three treatment days (Table 3).

It is recommended that to treat infections in calves susceptible to chloramphenicol, an initial dosage of 40 mg/kg of body weight be given subcutaneously. Doses ranging from 30 to 40 mg/kg (depending on the severity of the infection) may then be administered subcutaneously once every 24 hours to maintain therapeutic blood concentrations ($\geq$5 ug/ml). From Table 3 and Fig. 2 it is obvious that if needed treatment must continue beyond three days since plasma concentrations of the antibiotic had dropped below therapeutic levels 24 hours after the third (last) injection.

## LITERATURE CITED

1. Abraham EP: New Antibiotics; Chloramphenicol, Aureomycin, Tetramycin, and Neomycin. J Pharm and Pharmacol 3:257-270, 1951.

2. Ehrlich J, Bartz QR, Smith RM, Joslyn DA: Chloromycetin, a new antibiotic from a soil actinomycete. Science 106:417, 1947.

3. Fingl E, Woodbury DM: Chloramphenicol. In: The Pharmacological Basis of Therapeutics, 5th ed. L. S. Goodman and A. Gilman, eds. Macmillan Co; New York:1194-1200, 1975.

4. Smadel JE, Jackson EB: Chloromycetin, an antibiotic with chemotherapeutic activity in experimental rickettsial and viral infections. Science 106:418-419, 1947.

5. Bartz QR: Isolation and characterization of chloromycetin. J Biol Chem 172:445-450, 1948.

6. Controulis J, Rebstock MC, Crooks Jr. HM: Chloramphenicol (Chloromycetin). V. Synthesis J Am Chem Soc 71:2463-2468, 1949.

7. Long LM, Troutman HD: Chloramphenicol (Chloromycetin) VI. A synthetic approach. J Am Chem Soc 71:2469-2472, 1949.

8. Long LM, Troutman HD: Chloramphenicol (Chloromycetin) VII. Synthesis through p-Nitroacetophenone. J Am Chem Soc 71:2473-2475, 1949.

9. Brander GC, Pugh DM: Antibiotics of miscellaneous structure. In: Veterinary Applied Pharmacology and Therapeutics. 2nd ed. Balliere Tindall. London:359-363, 1971.

10. Rebstock MC, Crooks Jr. HM, Controulis J, Bartz QR: Chloramphenicol (Chloromycetin) IV. Chemical Studies. J Am Chem Soc 71:2458-2462, 1949.

11. Huber WG: Chloramphenicol and other antibacterial substances. In: Veterinary Pharmacology and Therapeutics. 4th ed. L. M. Jones, N. H. Booth and L. E. McDonald, eds. Iowa State University Press, Ames, Iowa:946-949, 1977.

12. Clark CH: Chloramphenicol dosage. Modern Vet Practice 59(10):749-754, 1978.

13. Rossoff IS: Handbook of veterinary drugs. A compendium for research and clinical use. Springer Pub. Co., N.Y.:96-100, 1974.

14. Goldberg IH: Mode of action of antibiotics. II. Drugs affecting nucleic acid and protein synthesis. Amer J Med 39:722, 1965.

15. Kucers A: The use of antibiotics. A comprehensive review with clinical emphasis. Philadelphia J. B. Lippincott:164-185, 1972.

16. McLeod TF, Manyan DR, Yunis AA: The cellular transport of chloramphenicol and thiamphenicol. J Lab Clin Med 90(2):347-353, 1977.

17. Clark CH: Clinical uses of chloramphenicol. Modern Vet Practice 59(12):889-894, 1978.

18. Sato G, Furuta Y, Kodama H, Iwao T, Oka M: Enzootic occurrence of chloramphenicol-resistant Salmonella typhimurium var copenhagen in a calf population. Am J Vet Res 36(6):839-841, 1975.

19. Davis LE, Neff CA, Baggot JD, Powers TE: Pharmacokinetics of chloramphenicol in domesticated animals. Am J Vet Res 33(11):2259-2266, 1972.

20. Sisodia CS, Dunlop RH, Gupta VS, Taksas L: A pharmacologic study of chloramphenicol in cattle. Am J Vet Res 34(9):1147-1151, 1973.

21. DeBacker P, Debackere M: Comparative study of chloramphenicol absorption in calves after oral, intra-ruminal and intra-abomasal administration. J Vet Pharmacol Therap 2:195-202, 1979.

22. Davis LE, Davis CN: Dosage regimens of chloramphenicol in domesticated animals. J S Afr Vet Ass 45:77-79, 1974.

23. Haile CA: Chloramphenicol toxicity. Southern Med J 70(4):479-480, 1977.

24. Clark CH: Metabolic effects and toxicities of chloramphenicol. Modern Vet Practice 59(9):663-671, 1978.

25. Penny RHC, Carlisle CH, Prescott CW, Davidson HA: Effects of chloramphenicol on the hemopoietic system of the cat. Brit Vet J 123(4):145-153, 1967.

26. Teske RH, Mercer HD: Subchronic effects of chloramphenicol on the hemopoietic system of cats. Can Vet Jour 17(1):19-23, 1976.

27. Watson ADJ, Middleton DJ: Chloramphenicol Toxicosis in cats. Am J Vet Res 39(7):1199-1203, 1978.

28. Daum RS, Cohen DL, Smith AL: Fatal aplastic anemia following apparent "dose-related" chloramphenicol toxicity. Journal of Pediatrics 94(3):403-406, 1979.

29. Oski FA: Hematologic consequences of chloramphenicol therapy. Journal of Pediatrics 94(3):515-516, 1979.

30. Weisberger AS, Daniel TM, Hoffman A: Suppression of antibody synthesis and prolongation of homograft survival by chloramphenicol. J Exp Med 120:183-196, 1964.

31. Weisberger AS, Daniel TM: Suppression of antibody synthesis by chloramphenicol analogs. Proc Soc Exp Biol Med 131:570-575, 1969.

32. Daniel TM, Suhrland MD, Weisberger AS: Suppression of the anamnestic response to tetanus toxoid in man by chloramphenicol. New Eng J Med 273(7):367-369, 1965.

33. Adams HR: Prolongation of barbiturate anesthesia by chloramphenicol in laboratory animals. JAVMA 157(11): 1908-1913, 1970.

34. Adams HR, Dixit BN: Prolongation of barbiturate anesthesia by chloramphenicol in dogs and cats. JAVMA 156(7): 902-905, 1970.

35. Teske RH, Carter GG: Effect of chloramphenicol on pentobarbital-induced anesthesia in dogs. JAVMA 159(6): 777-780, 1971.

36. Adams HR, Isaacson EL, Siler-Masters BS: Inhibition of hepatic microsomal enzymes by chloramphenicol. J Pharm and Exp Therap 203(2):388-396, 1977.

37. Adams HR, Rossner SD: Lack of neuromuscular blocking effect of chloramphenicol. Am J Vet Res 33(5):1083-1086, 1972.

38. Caulfield JB, Burke JF: Inhibition of wound healing by chloramphenicol. Arch Path 92(2):119-125, 1971.

39. Al-Hussainy TM, Al-Dahan MI, Al-Zubaidy AJ, Abou YE: Chloramphenicol-induced pathological and biochemical alterations in the intestinal epithelium. Pharm Research Comm 11(3):199-209, 1979.

40. Glazko AJ, DiU WA, Rebstock MC: Biochemical studies on chloramphenicol (Chloromycetin) III. Isolation and identification of metabolic products in urine. J Biol Chem 183:679-691, 1950.

41. Nouws JFM, Ziv G: A study of chloramphenicol distribution and residues in dairy cows. Tijdschr Diergeneesk 103(14):725-735, 1978.

42. Glazko AJ, Wolf LM, Dill WA, Bratton Jr. AC: Biochemical studies on chloramphenicol (Chloromycetin) II. Tissue distribution and excretion studies. J Pharmac Exper Ther 96:445-459, 1947.

43. Glazko AJ: Identification of chloramphenicol metabolites and some factors affecting metabolic disposition. Antimic Agents and Chemother:655-665, 1966.

44. Neff CA, Davis LE, Baggot JD: Pharmacokinetics of tetraethylammonium in dogs, cats and goats. Am J Vet Res 34(3):425-426, 1973.

45. Ross S, Burke FG, Sites J, Rice EC, Washington JA: Placental transmission of chloramphenicol (Chloromycetin). JAMA 142:1361, 1950.

46. Scott WC, Warner RF: Placental transfer of chloramphenicol (chloromycetin). JAMA 142:1331-1332, 1950.

47. Nouws JFM, Ziv G: Serum chloramphenicol levels and the intramuscular bioavailability of several parenteral formulations of chloramphenicol in ruminants. Tijdschrift voor diergeneeskunde 104(2):47-58, 1979.

48. Pilloud M: Pharmacokinetics, plasma protein binding and dosage of chloramphenicol in cattle and horses. Res Vet Sci 15:231-238, 1973.

49. Burrows GE: Clinical Pharmacology of Systemic Antibiotics; Determination of Dosage. Oklahoma Veterinarian OVMA XXXII(1):2-4, 1980.

50. DeCorte-Baeten K, Debackere M: The non-absorption of chloramphenicol after oral administration in adult ruminants: a tentative explanation from in vitro studies: J Vet Pharmacol Therap 1:129-134, 1978.

51. DeBacker P, Debackere M, DeCorte-Baeten K: Plasma levels of chloramphenicol after oral administration in calves during the first weeks of life. J Vet Pharmacol Therap 1:135-140, 1978.

52. Sisodia CS, Gupta VS, Dunlop RH, Radostits VM: Chloramphenicol concentrations in blood and milk of cows following parenteral administration. Can Vet Jour 14(9): 217-220, 1973.

53. Ziv G, Bogin E, Sulman FG: Blood and milk levels of chloramphenicol in normal and mastitic cows and ewes after intramuscular administration of chloramphenicol and chloramphenicol sodium succinate. Zbl Vet Med A 20:801-811, 1973.

54. Hjerpe CA, Routen TA: Practical and theoretical considerations concerning treatment of bacterial pneumonia in feedlot cattle with special reference to antimicrobic therapy. Proceedings Am Assn Bovine Practitioners:97, 124, 126, 138, 1976.

55. Hjerpe CA: A comparison of serum antibiotic concentrations achieved in calves with intratracheal administration of procaine penicillin G, ampicillin trihydrate, tylosin, oxytetracycline hydrochloride, chloramphenicol, chloramphenicol sodium succinate, dihydrostreptomycin sulfate, and neomycin sulfate with those achieved with intravenous, intramuscular and subcutaneous administration. Bovine Practitioner 14:18-26, 1979.

56. Glazko AJ, Wolf LM, Dill WA: Biochemical studies on chloramphenicol (Chloromycetin) I. Colorimetric methods for the determination of chloramphenicol and related nitro compounds. Arch Biochem 23:411-418, 1949.

57. Bessman SP, Stevens S: A colorimetric method for the determination of chloromycetin in serum or plasma. J Lab Clin Med 35:129, 1949.

58. Kakemi KT, Arita T, Ohashi S: Absorption and excretion of drugs. IV. Determination of chloramphenicol in blood. Yakugaku Zasshi 82:342-345, 1962.

59. Nylan WL: Measurement of concentrations of chloramphenicol in the blood. J Pediat 67:1174-1175, 1965.

60. Mason EO, Kaplan SL, Baker CJ, Ritter DA: Modification of the colorimetric assay for chloramphenicol in the presence of bilirubin. Antimicrobial Agents and Chemotherapy 15:544-546, 1979.

61. Louie TJ, Tally FP, Bartlett JG, Gorbach SL: Rapid microbiological assay for chloramphenicol and tetracyclines. Antimicrobial Agents and Chemotherapy 9(6):874-878, 1976.

62. Szulczewski D, Eng F: Chloramphenicol In: Analytical profiles of drug substances. K. Florey, ed. Academic Press:72-90, 1975.

63. Randall WA, Kirshbaum A, Nielsen JK, Wintermere D: Diffusion plate assay for chloramphenicol and aureomycin. J Clin Investigation 28:940-942, 1949.

64. Smith DG, Landers CB, Forgacs J: A cylinder-plate assay for chloromycetin in body fluids and tissue extracts. J Lab and Clin Med 36:154-157, 1950.

65. Ali SL: Separation and determination of the hydrolysis products of chloramphenicol in pharmaceutical preparations by high-performance liquid chromatography. Journal of Chromatography 154:103-105, 1978.

66. Thies RL, Fischer LJ: High performance liquid chromatographic assay for chloramphenicol in biological fluids. Clin Chem 24(5):778-781, 1978.

67. Koup JR, Brodsky B, Lau A, Beam Jr. TR: High-performance liquid chromatographic assay of chloramphenicol in serum. Antimicrobial Agents Chemotherapy 14(3):439-443, 1978.

68. Nilsson-Ehle I, Kahlmeter G, Nilsson-Ehle P: Determination of chloramphenicol in serum and cerebrospinal fluid with high-pressure liquid chromatography. Journal of Antimicrobial Chemotherapy 4:169-176, 1978.

69. Petersdorf SH, Raisys VA, Opheim KE: Microscale method for liquid-chromatographic determination of chloramphenicol in serum. Clinical Chemistry 25(7):1300-1302, 1979.

70. Sample RHB, Glick MR, Kleiman MB, Smith JW, Oei TO: High-pressure liquid chromatographic assay of chloramphenicol in biological fluids. Antimicrobial Agents Chemotherapy 15:491-493, 1979.

71. Resnick GL, Corbin D, Sandberg DH: Determination of serum chloramphenicol utilizing gas-liquid chromatography and electron capture spectrometry. Analytical Chemistry 38(4):582-585, 1966.

72. Daigneault R, Guitard M: An enzymatic assay for chloramphenicol with partially purified chloramphenicol acetyltransferase. Journal of Infectious Diseases 103(5): 515-522, 1976.

73. Lietman PS, White TJ, Shaw WV: Chloramphenicol: an enzymological microassay. Antimicrobial Agents Chemotherapy 10(2):347-353, 1976.

74. Smith AL, Smith DH: Improved enzymatic assay of chloramphenicol. Clin Chem 24(9):1452-1457, 1978.

75. Robison LR, Seligsohn R, Lerner SA: Simplified radioenzymatic assay for chloramphenicol. Antimicrobial Agents and Chemotherapy 13(1):25-29, 1978.

76. Clarenburg R, Rao VR: A fluorometric method to assay chloramphenicol. Drug Metabolism and Disposition: The Biological Fate of Chemicals.

77. Udenfriend S, Stein S, Bohlen P, Dairman W, Leimgruber W, Weigele M: Fluorescamine: A reagent for assay of amino acids, peptides, proteins, and primary amines in the picomole range. Science 178:871-872, 1972.

78. Niazi S: Textbook of Biopharmaceutics and Clinical Pharmacokinetics. Appleton-Century-Crofts N.Y.:60-62, 1979.

79. Welch H: Principles and practice of antibiotic therapy. Med Encyc Inc. New York 205, 1954.

80. Gibaldi M: Biopharmaceutics and clinical pharmacokinetics. Lea and Febiger. Philadelphia 68-72, 1977.

81. Ross S, Puig JR, Zaremba EA: Chloramphenicol acid succinate (sodium salt): Some preliminary clinical and laboratory observations in infants and children. Antibiotics Annual 803-820, 1957-1958.

82. Mercer HD, Gelata JN, Kramer J and Carter G: Chloramphenicol blood concentration studies in dogs. JAVMA 158:47-52, 1971.

83. Rao VR, Clarenburg R: Pharmacokinetics of intravenously injected chloramphenicol in baby pigs. Drug Metabolism and Disposition: The Biological Fate of Chemicals.

84. Gross DR, Kitzman JV, Adams HR: Cardiovascular effects of intravenous administration of propylene glycol and of oxytetracycline in propylene glycol in calves. Am J Vet Res 40(6):783-791, 1979.

85. Baggot JD: Some aspects of clinical pharmacokinetics in veterinary medicine I. J Vet Pharmacol Therap 1:5-18, 1978.

86. Notari RE: Biopharmaceutics and pharmacokinetics. An Introduction. Marcel Dekker, Inc., New York 152-165, 181-185, 1971.

87. Snedecor GW, Cochran WG: Statistical methods. 6th ed. The Iowa State Printing Press, Ames, Iowa 447-456, 1976.

88. Glazko AJ, Carnes HE, Kazenko A, Wolf LM, Reutzer TF: Succinic acid ester of chloramphenicol. Antibiotics Annual 792-802, 1957-1958.

89. Baggot JD: Principles of drug disposition in domestic animals. The basis of veterinary clinical pharmacology. WB Saunders Co. Philadelphia 160-172, 1977.

90. Baggot JD: Some aspects of clinical pharmacokinetics in veterinary medicine II. J Vet Pharmacol Therap I:111-118, 1978.

# APPENDIX A

## Typical Trial Protocol

### The day before the trial

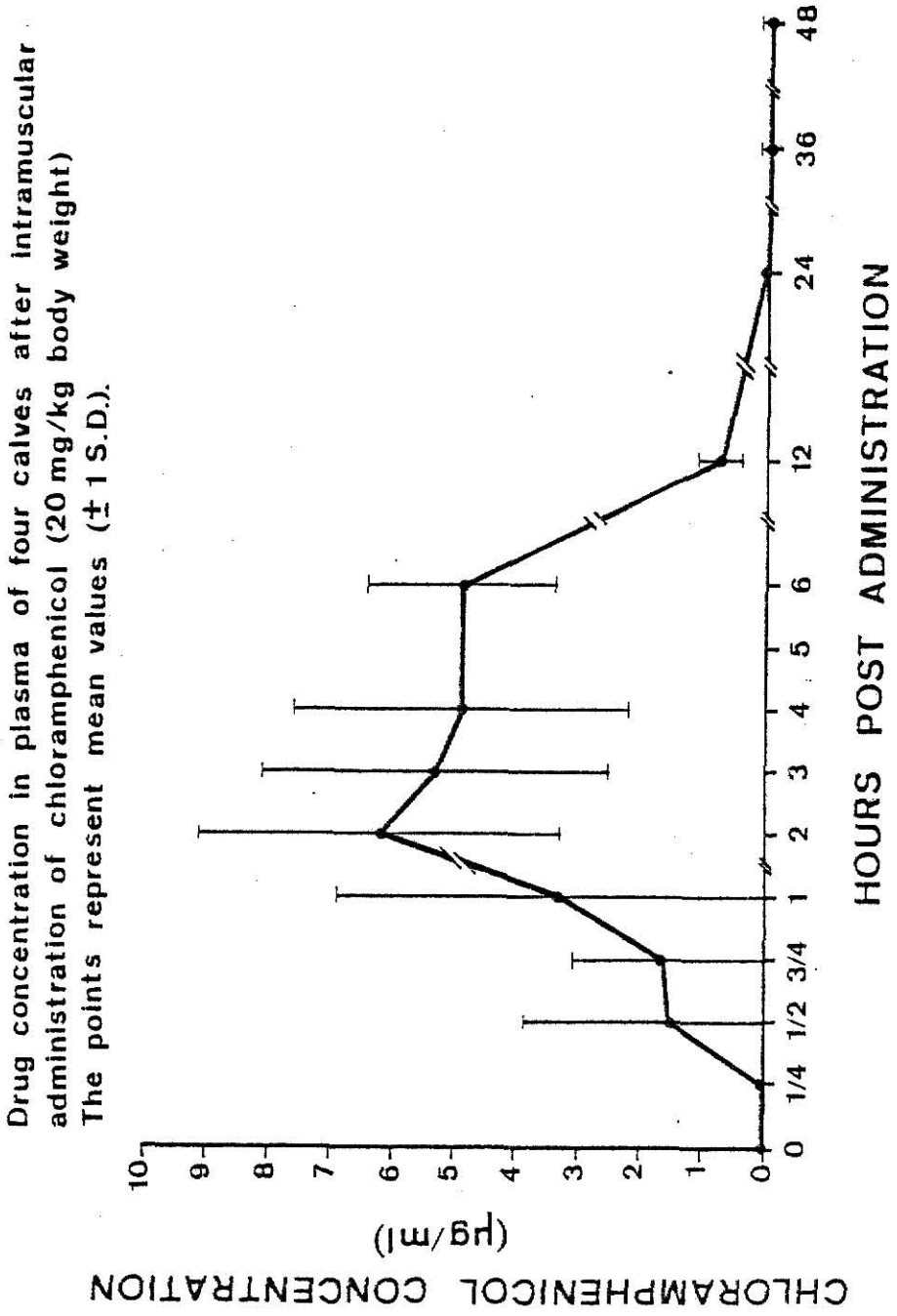
1. Weigh each calf. Record weights.
2. Prepare solutions:
   (a) For homogenizing, deproteinization, reduction and analysis.
   (b) For standards: to contain 0, 1, 3, 5, 10 and 20 ug chloramphenicol/ml of plasma.
3. Calculate trial dose on weight basis.
4. Label 10 ml test tubes to be used for blood collection.

### The day of the trial

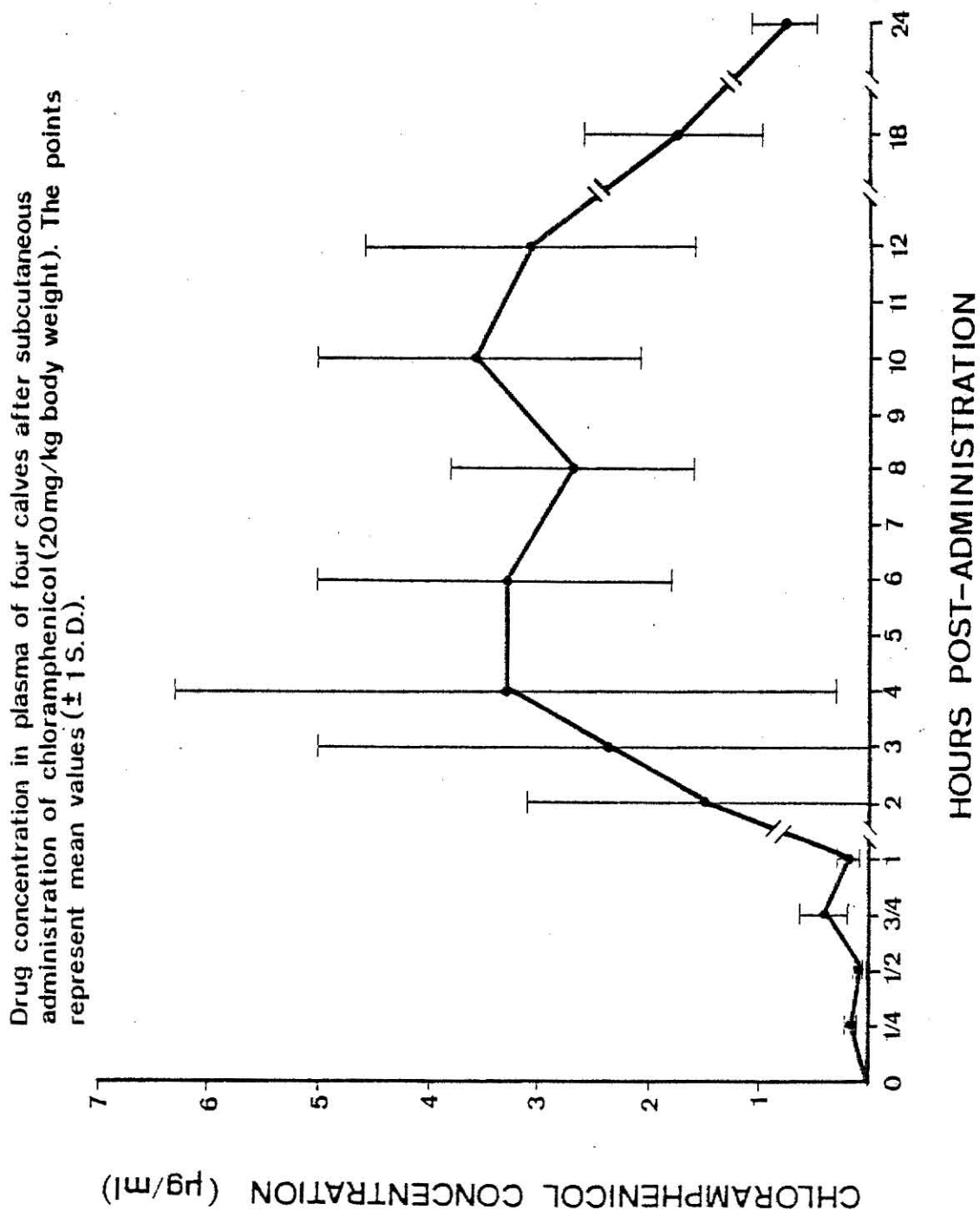
1. Add 50 units heparin sodium to each labelled test tube.
2. Prepare chloramphenicol solution: base in propylene glycol or sodium succinate ester in boiled and cooled tap water.
3. Prepare calves; each calf restrained in its stall.
4. Draw a blood sample from the jugular vein (zero hour sample).
5. Inject calculated amount of chloramphenicol.
6. Draw blood samples at specified post-administration periods.

7. Centrifuge samples immediately to yield plasma. During sampling intervals, label further sets of test tubes for:
   (a) plasma
   (b) homogenizing and deproteinizing plasma
   (c) reducing deproteinized samples, and
   (d) diluting reduced samples (using 0.5M sodium acetate buffer)
8. Homogenize and deproteinize the samples immediately after collecting them. Freeze at -20$^{o}$ C and store. Freeze and store the remaining plasma.
9. Carry out the reduction step the next day and read fluorescence.

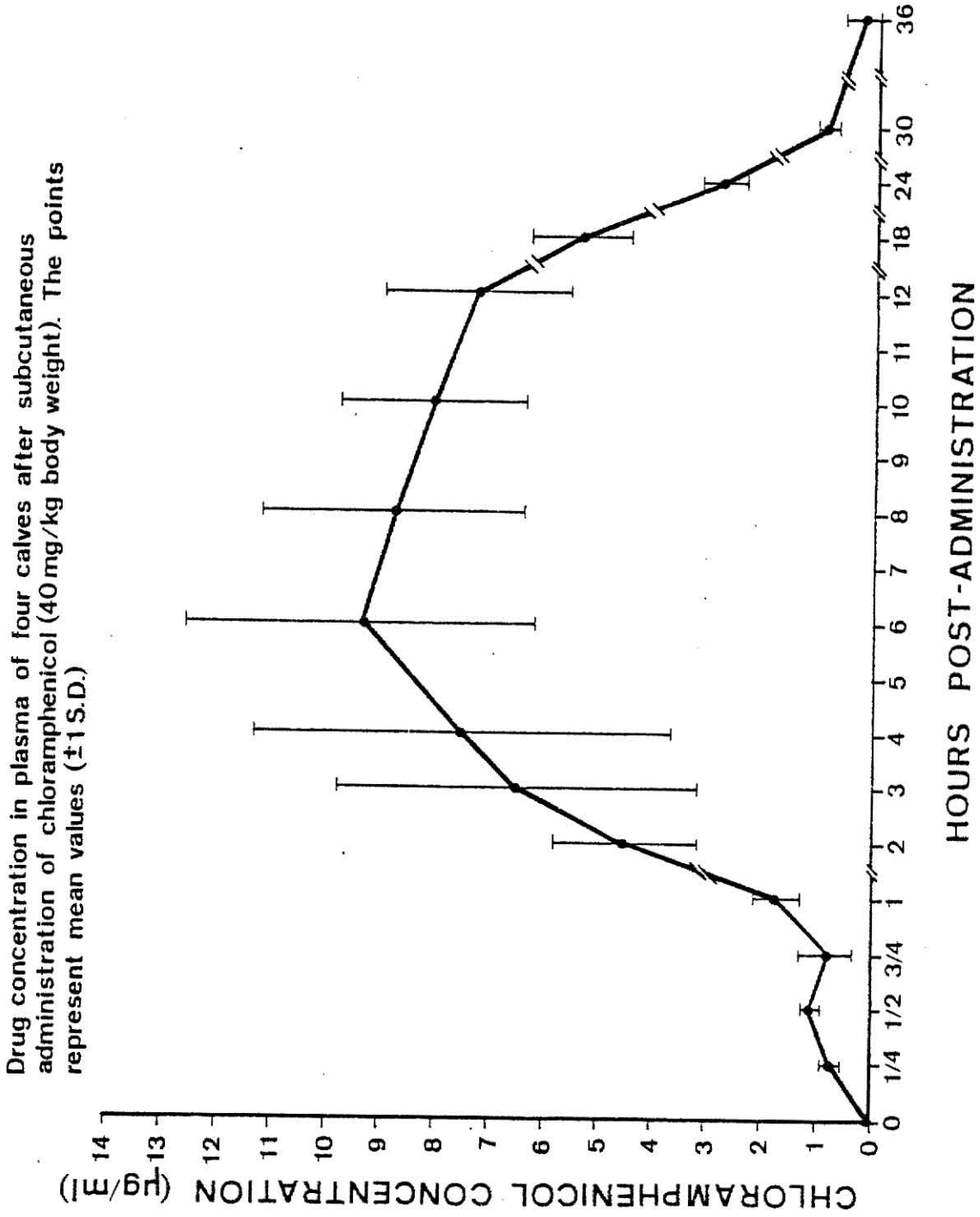
APPENDIX B


Drug concentration in plasma of four calves after intramuscular administration of chloramphenicol (20 mg/kg body weight)
The points represent mean values (± 1 S.D.).
CHLORAMPHENICOL CONCENTRATION
(μg/ml)
0
1
2
3
4
5
6
7
8
9
10
0
1/4
1/2
3/4
1
2
3
4
5
6
12
24
36
48
HOURS POST ADMINISTRATION

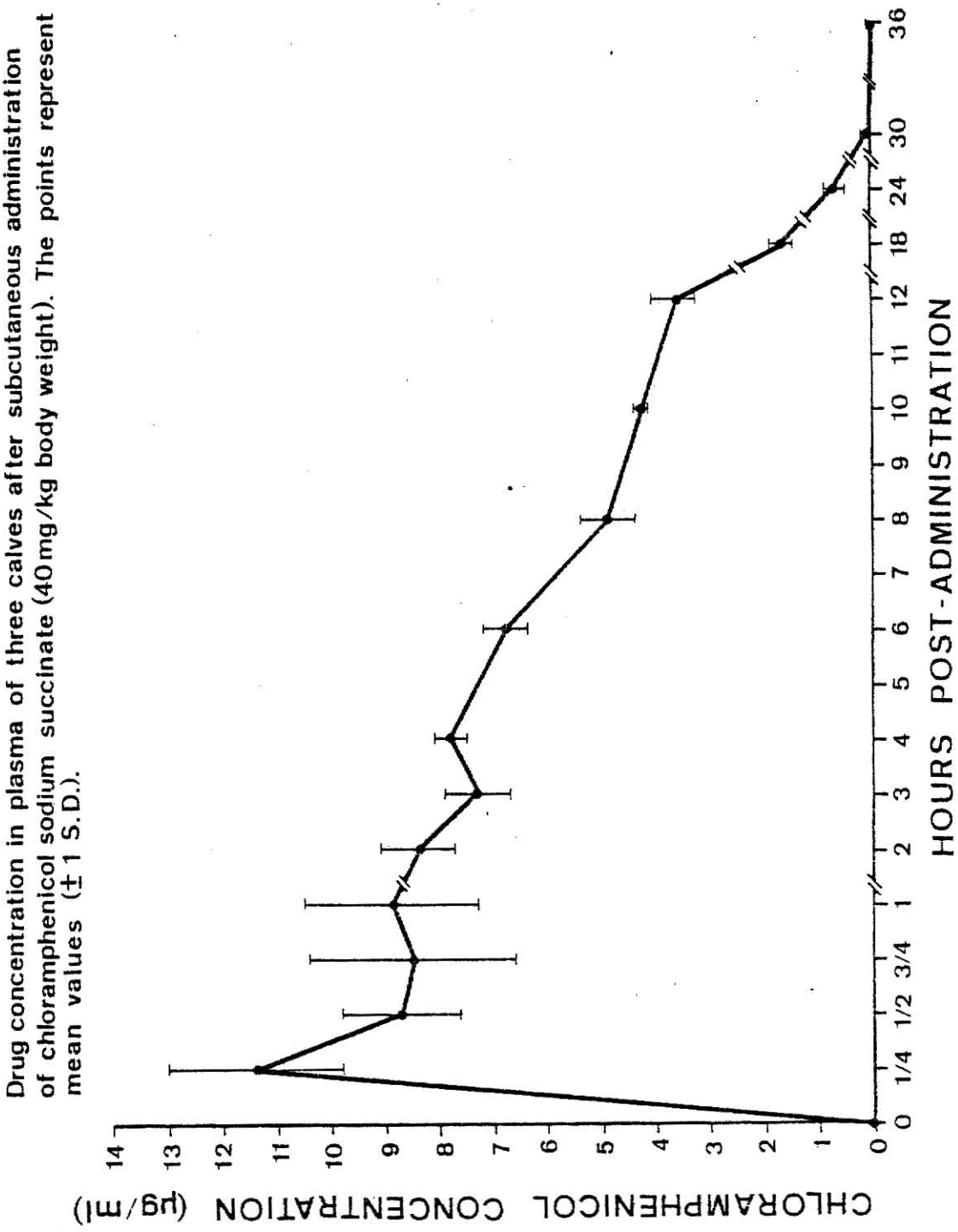
APPENDIX C


Drug concentration in plasma of four calves after subcutaneous administration of chloramphenicol (20mg/kg body weight). The points represent mean values (± 1 S.D.).
CHLORAMPHENICOL CONCENTRATION (µg/ml)
HOURS POST-ADMINISTRATION
0
1/4
1/2
3/4
1
2
3
4
5
6
7
8
9
10
11
12
18
24
1
2
3
4
5
6
7

APPENDIX D

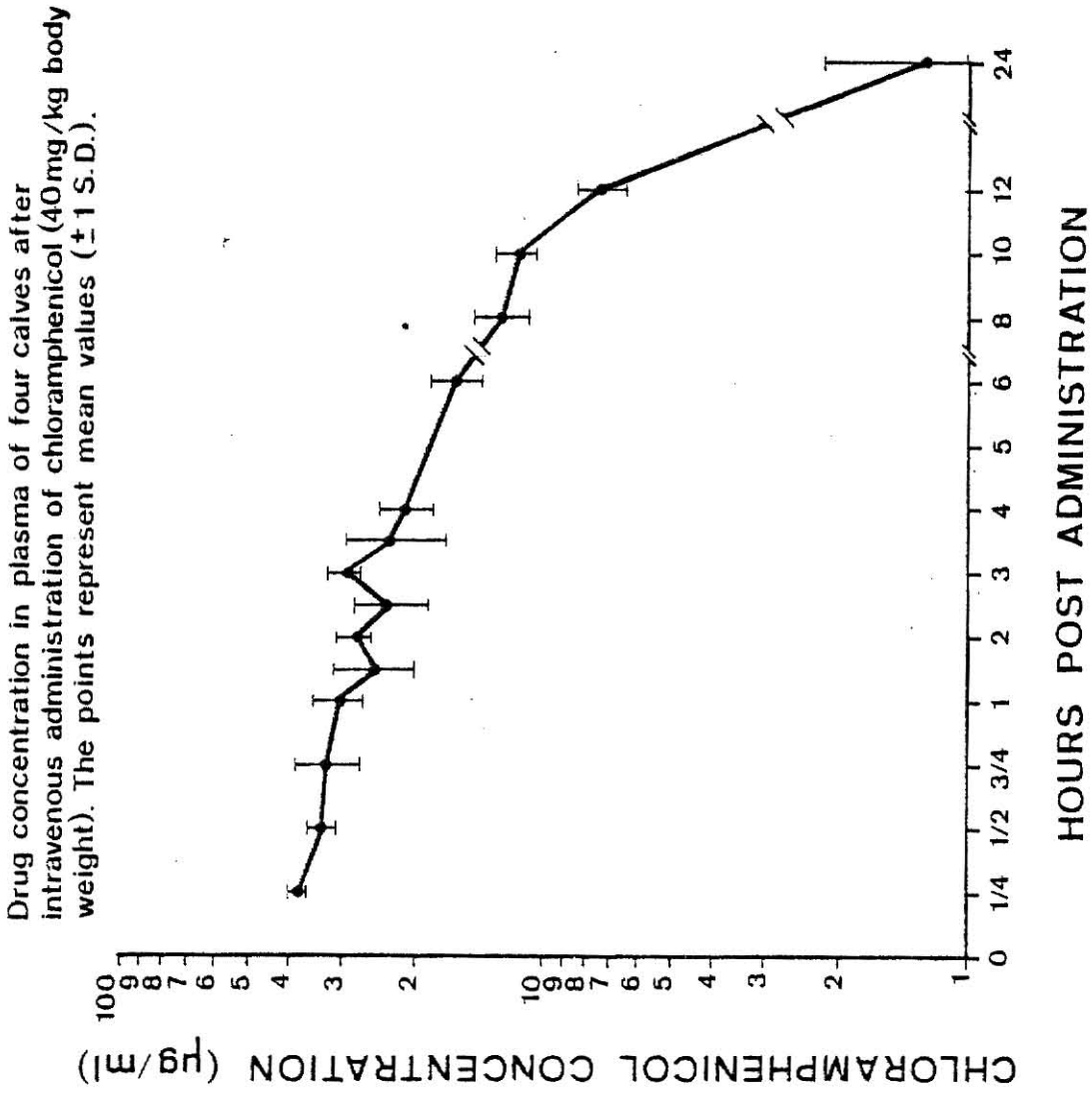

Drug concentration in plasma of four calves after subcutaneous administration of chloramphenicol (40 mg/kg body weight). The points represent mean values (±1 S.D.)
CHLORAMPHENICOL CONCENTRATION (µg/ml)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
0
1/4
1/2
3/4
1
2
3
4
5
6
7
8
9
10
11
12
18
24
30
36
HOURS POST-ADMINISTRATION

APPENDIX E




Drug concentration in plasma of three calves after subcutaneous administration of chloramphenicol sodium succinate (40 mg/kg body weight). The points represent mean values (±1 S.D.).

APPENDIX F


Drug concentration in plasma of four calves after intravenous administration of chloramphenicol (40mg/kg body weight). The points represent mean values (± 1 S.D.).
CHLORAMPHENICOL CONCENTRATION (µg/ml)
100
9
8
7
6
5
4
3
2
10
9
8
7
6
5
4
3
2
1
0 1/4 1/2 3/4 1 2 3 4 5 6 8 10 12 24
HOURS POST ADMINISTRATION

# DOSAGE REGIMEN FOR CHLORAMPHENICOL IN CALVES BASED UPON KINETIC DATA

by

TAIWO A. COLE

D.V.M., University of Ibadan, Nigeria, 1974

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

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Its broad spectrum of effectiveness and wide tissue distribution make chloramphenicol a very attractive drug in clinical practice. The antibiotic is therefore used to treat infections in animals such as calves and pigs, in spite of the restrictions placed on its used in food producing animals in the United States and other countries. Although there are dosage regimens designed for the intramuscular and intravenous use of the antibiotic in calves, no studies have been carried out for the subcutaneous use of the drug, in spite of the increasing popularity of this route among practitioners.

Our studies aimed at developing a dosage regimen for subcutaneously injected chloramphenicol in calves based upon kinetic data. Four calves were used in six experiments conducted with two weeks between each experiment. The calves were given single doses of chloramphenicol base in propylene glycol intramuscularly (20 mg/kg), subcutaneously (20 mg/kg and 40 mg/kg), and intravenously (40 mg/kg). Three of the calves were also tested with a single subcutaneous dose of chloramphenicol sodium succinate (40 mg/kg).

For the regimen study, the calves were randomly divided into two groups. One group received chloramphenicol base at a dose of 30 mg/kg subcutaneously, while for the other group the antibiotic was administered by the same route at

a dose of 40 mg/kg. Each calf was treated once a day for three days.

Minimal therapeutic concentrations (5 ug/ml) were attained two hours after the single intramuscular dose but were barely maintained for four hours. At no time were therapeutic concentrations attained after the single subcutaneous administration of the antibiotic at the lower dose (20 mg/kg). However when the higher dose (40 mg/kg) was used plasma chloramphenicol levels were above therapeutic concentrations between 3 and 18 hours after administration. Peak plasma chloramphenicol values (11.4 ug/ml) were reached rapidly (15 minutes) after the sodium succinate ester of the drug was administered as a single dose (40 mg/kg), but plasma levels dropped below therapeutic concentrations 8 hours after administration.

Although the single intravenous administration of the base (40 mg/kg) gave high plasma levels through 12 hours after administration, this route of delivering the drug is not feasible in critically ill animals, since the solvent, propylene glycol, caused extensive blood hemolysis for up to 8 hours after administration.

Kinetic parameters following single dose administration showed that the plasma half-life of chloramphenicol was independent of the dose or route of administration.

It is recommended that to treat those infections in calves which are susceptible to chloramphenicol, an initial dose of 40 mg/kg be administered subcutaneously. Doses ranging from 30 to 40 mg/kg may be administered once every 24 hours after the initial dose to maintain therapeutic blood concentrations.