STUDIES ON
THE RATE OF DISAPPEARANCE OF CHLORAMPHENICOL FROM THE BLOOD OF GOATS AND CALVES

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THESIS

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C E R T I F I C A T E

Certified that this thesis is a record of research work done independently by Shri A.D.Joy under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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C O N T E N T S

I N T R O D U C T I O N

INTRODUCTION

The advances in Chemotherapy have been along three main lines, namely, Replacement therapy with hormones, Replacement therapy with vitamins and Chemotherapy. None of these three lines of advance in curative therapy is new; all three have their roots in the past. The replacement therapy with hormones was clearly demonstrated by Berthold in 1849. Curative or replacement therapy, with what we now call a vitamin, is even older than with hormone. James Lind's historic trial of the value of orgnges and lemons in controlling Scurvy occured in 1747. The third line of advance in curative therapy, and the one with which we are more concerned is Chemotherapy.

Cinchona bark and ipecac, discovered accidently for the treatment of malaria and dysentry by Jesuits, can be considered as the first few chemotherapeutic agents of plant origin to be used in medicine (Marshall, 1964). Paul Ehrlich the undisputed "Father of chemotherapy" postulated that it should be possible to successfully treat diseases by specific drugs, and defined chemotherapy as the treatment of a systemic parasitic disease with a chemical of known constitution.

Ehrlich with his expert organic chemist Bertheim prepared and tested a large number of organic arsenic compounds and discovered "Salvarsan" (a term meaning saviour of man kind) in 1910. This chemical, the first man made drug to be effective in a human parasitic disease, was found highly effective not only for Trypanosome infection but also in rabbit syphilis. Ehrlich's discovery made a new epoch in the history of Therapeutics. He concentrated his attention towards a fundamental principle that drugs should have minimum possible toxicity for the animal host and the maximum parasiticidal action and thus laid down a very sound basis for further work in Chemotherapeutic research.

Further, great strides were made to discover chemical agents effective against protozoa and worms; however no real progress was made against bacterial infection until many years when sulphonamides were introduced into medicine. Sulphonamide was first synthesised by Gelmo in 1906 in Technische Hochschule of Vienna, in the course of investigation of azo dyes and his historic paper was published in 1908. Prior to 1935 systemic bacterial infections could not be effectively treated with druge. There were many antiseptics and disinfectants that could eradicate infections when applied topically, but their

systemic use was precluded by their unfavourable therapeutic index. In 1935, Gerhard Domagk a research director of the I.G. Farbenindustrie of Germany discovered Prontosil, the first member of sulphonamide group of drugs and he also observed that mice infected with streptococci could be protected by this drug.

Further advances in bacterial chemotherapy were made in the past two decades, with the introduction of antibiotics. The application of antibiotic therapy, without recognition of it as such, and the concept to use substances derived from one living organism to kill another (Antibiosis) are considerably older. 2500 years ago, the Chinese were aware of the therapeutic properties of moldy curd of soyabean applied to carbuncles, boiles and similar infections and used this material as a standard treatment in such disorders. The fact has been known for many years that some bacteria and molds interfere the growth of other micro-organisms and as long ago as 1877 Pasteur and Joubert, in describing experiments on the growth of anthrax bacilli, suggested that this antagonism might be of value for therapeutic purposes.

A completely different field of chemotherapy was opened up when the first effective antibiotic penicillin was reported by Fleming in 1929. Fleming found that

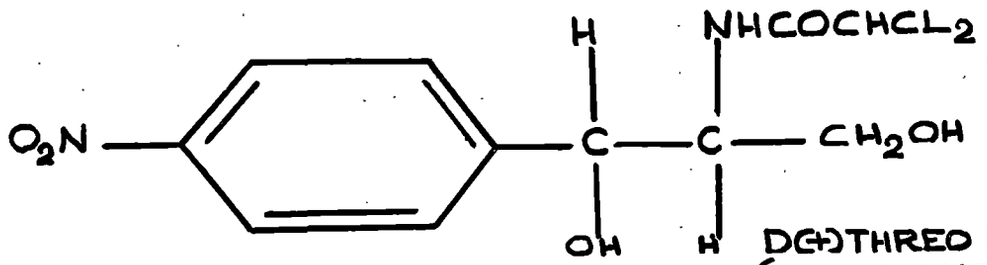
a mold which had accidently contaminated a culture of staphylococci prevented the growth of bacteria in the regions where mold was growing and he isolated the mold and named the active principle, Penicillin. Since Penicillin was effective against bacteria which are gram positive and not in general against gram negative organisms, it was natural for investigators to attempt to findout the counter part of Penicillin which would act on gram negative organisms.

The next important development was the so called broad spectrum antibiotics, those effective against both gram negative and gram positive bacteria. Chloramphenicol was the first of these broad spectrum antibiotics to be discovered. Other broad spectrum antibiotics in clinical use are the three tetracycline sisters--Tetracycline, Chlortetracycline and Oxytetracycline. The development of chloramphenicol opens a new field of antibiotic therapy and represents an epochal achievement in the field of medicine. Because of its rapid action on both gram negative and gram positive bacteria, rickettsiae and certain of larger viruses, it has a wide range of usefulness for the veterinarian. Chloramphenicol was isolated independently by Ehrlich et al.(1947) from a streptomycete (S.venesuelae) an organism isolated by Burkholder in 1947 from soil in

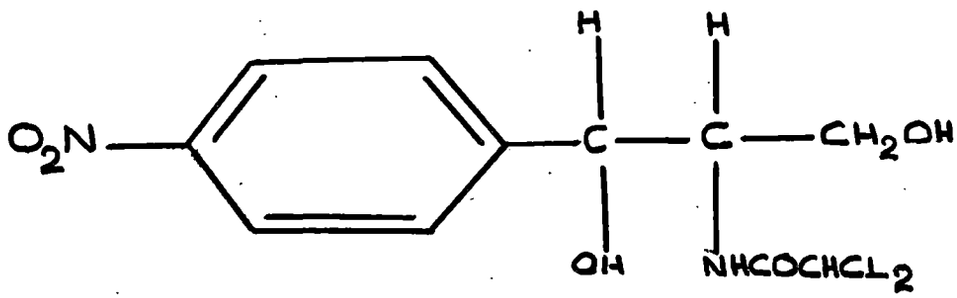
Venezuela and by Carter et al.(1948) from a similar organism found in a sample of soil from a compost heap in Illinois. Filtrates of liquid cultures of the organisms were found to possess marked effectiveness against several gram negative bacteria and also to exhibit antirickettsial and antiviral activity (Ehrlich et al., 1948). A crystalline antibiotic substance was isolated (Bartz, 1948) and named Chloromycetin because it contained chlorine and was obtained from an actinomycete. Chloramphenicol is the first antibiotic to be produced synthetically and the chemical structure was determined and confirmed (Controulis et al., 1949). It is an aromatic compound D-(-)-threo-2-dichloroacetamido-1-p-nitrophenyl-1-3-propanediol. The propanediol moiety of the molecule is critical for activity since alterations of this compound results in loss of activity. Four isomers of chloramphenicol have been synthesised, but neither its isomers nor the many structurally related compounds which have been synthesized have greater activity than natural D-(-)-threoisomer of chloramphenicol (Maxwell and Nickel, 1954).

Chloramphenicol consists of yellowish white crystals with an intensely bitter taste. It is only slightly soluble in water (1:400). The biologically active form is Levorotatory. The antibiotic is extremely

CHLORAMPHENICOL AND ITS ISOMERS



D(-)THREO ISOMER
(CHLORAMPHENICOL)



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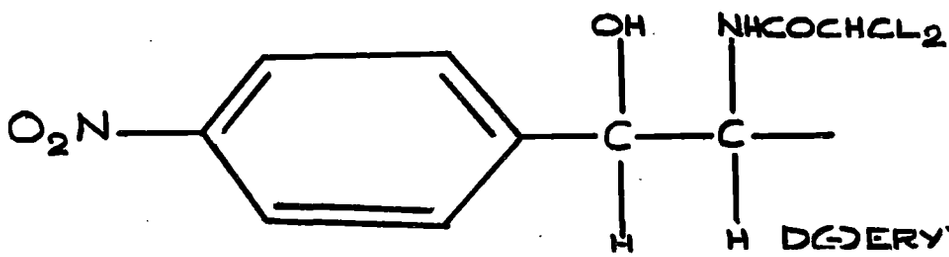

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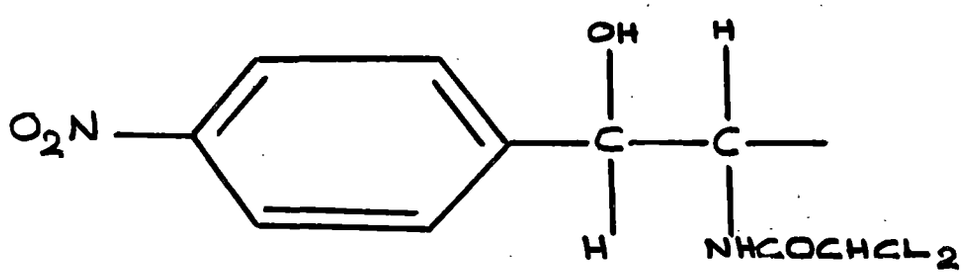


L(+)THREO ISOMER

stable, unaffected over the pH range from 2.0 to 9.0 and resists boiling in water. Aquous solutions have a pH of about 5.5 and are extremely stable and they keep indefinitely at ordinary room temperature if protected from light.

Chloramphenicol can be administered orally, intramuscularly, intravenously, intraperitoneally, subcutaneously and applied locally. Oral and intramuscular route is commonly used. In small animals like dogs and non-ruminating calves the drug is better absorbed and peak levels are achieved rapidly (Watson, 1972). Theodorides et al.(1968) indicate that chloramphenicol was rapidly degraded by ruminal contents, which precludes effective oral therapy. Simple suspensions of finely ground chloramphenicol are available which are suitable only for intramuscular injection. Various salts of chloramphenicol are used in medicine as chloramphenicol palmitate, chloramphenicol succinate etc. which are freely soluble and undergo hydrolysis in tissues with the liberation of chloramphenicol. The intravenous route has the advantage of producing immediate high blood concentration, but has the disadvantage of having a shorter duration of action. The intraperitoneal route is used in animals like swine in which intravenous route is difficult.

Chloramphenicol possesses a fairly wide spectrum of antimicrobial activity. It is primarily bacteriostatic, although it may be bactericidal to certain species under some conditions. Inspite of intensive study by numerous workers, the mechanism of action of chloramphenicol remains obscure. The most significant findings have been that chloramphenicol inhibits protein synthesis in bacteria and in cell free systems. It acts primarily on 50s ribosomes and specifically prevents binding of m-RNA to ribosomes in micro-organisms. The activity of peptidal transferase, which directly forms a peptide bond is suppressed (Carter and McCarty, 1966).

Chloramphenicol is a broad spectrum antibiotic and is widely used in veterinary practice. Clinical trials of Eastman et al.(1952) have demonstrated the effectiveness of chloramphenicol against several disease conditions in both large and small domestic animals. Barnes (1955) recommended chloramphenicol as a drug of choice against Pasturellosis (shipping fever) in cattle. It has been found to have specific therapeutic value in Pasturellosis (shipping fever), Fusiformis infections (calf diphtheria), Salmonella infections (calf scours), Rickettsial infections (infectious bovine keratitis), Contageous caprine Pleuropneumonia, Infectious feline panleukopenia and in the

secondary infections of Canine distemper (Schirmer et al., 1951; Pugh, 1956 and Manjrekar, 1955).

Continued treatment with chloramphenicol may produce toxicity in animals. Toxicity produced may be acute or chronic. Acute toxicity occurs from intravenous injection of large doses with symptoms of acute respiratory depression, fall of blood pressure and anoxia. Chronic toxicity may occur by erythropoietic depression of the bone marrow and characterised by haematological disorders.

Chloramphenicol in the blood undergoes various chemical changes. About 60 per cent of the chloramphenicol in the blood is bound to protein. Studies of organ distribution have shown the diminishing order of concentration to be kidney, liver, lung, heart, spleen, muscle and brain. Free diffusion occurs into serous effusions, spinal fluid and foetal circulation (Glazko et al., 1949 a).

Before excretion, most of chloramphenicol in the body is inactivated either by conjugation with glucuronic acid or by reduction to inactive aryl amines, and the main site of these process is the liver. The inactivation of chloramphenicol by conjugation with glucuronic acid occurs by glucuronyl transferase and reduction to inactive aryl amines by reductase.

Excretion of chloramphenicol is mainly renal; 90 per cent of the dose can be detected in the urine but only about 10 per cent of this amount is unaltered antibiotic (Glazko et al., 1949 a). Chloramphenicol itself is excreted by the glomeruli, but excretion of its inactive derivations is by active tubular secretion. About 3 per cent of the administered dose is excreted in the bile but only about 1 per cent appears in the faeces and that mostly in inactive form. Chloramphenicol is also found in the cerebrospinal fluid, bile, milk and semen (Glazko et al., 1949 a, 1950).

The discovery of methods for the estimation of chloramphenicol in the body fluids was an important landmark in the development of studies on chloramphenicol. It is now a regular practice in chemotherapeutic research that as soon as a new compound is discovered steps are immediately taken to elaborate methods for its estimation in biological fluids. The basic procedure for the colorimetric determination of aromatic nitro compounds is described by Glazko et al. (1949 b). The analytical methods described in this paper involve reduction of the aromatic nitro group and diazotization of resulting amine, and coupling with the Bratton-Marshall reagent (Bratton and Marshall, 1939). Various modifications of colorimetric procedures have been

mentioned (Levine and Fischbach, 1951 and Shah et al., 1968). The chemical method is not specific for active antibiotic. Reliable estimates of active chloramphenicol are best made by microbiological assay (Joslyn and Galbraith, 1950 and Mercer et al., 1971). The microbiological method is direct but not convenient. The colorimetric method can be made more specific for the active antibiotic by using the solvent extraction procedure.

Present study

Inspite of dynamic developments on scientific aspects of livestock rearing, the goats and calves still remain a neglected group among the farm animals. The goats and calves in Kerala are not properly housed and most of them are kept in the thatched extension of the human dwellings. Due to lack of enough ventilation and high humidity, respiratory diseases are very common in goats and calves. The humid climate of west coast helps only to aggravate this state of affair. Compared to other domestic animals goats and calves are highly susceptible to most of the infectious diseases. Chloramphenicol being broad spectrum antibiotic and specific for certain diseases in calves, like infectious diarrhoea (White scour), Haemorrhagic septicemia (shipping fever), Calf diphtheria etc. and also in goats for Mastitis, infectious diarrhoea

etc., the veterinarians have a very important role to play with chloramphenicol in the treatment of bacterial, rickettsial and certain viral diseases of goats and calves.

From the literature available it can be seen that not much work has been done in respect of the chloramphenicol blood level, excretion and toxicity in goats and calves. The present investigation was taken up with the intention to study in detail some aspects of chloramphenicol concentration occuring in blood, plasma and urine of goats and calves after the experimental intramuscular administration of chloramphenicol. An attempt was also made to assess the toxicity of chloramphenicol in calves. Information on the above aspects of chloramphenicol therapy in goats and calves is not available in the literature eventhough extensive studies have been made in other species of domestic animals. An investigation to evaluate the persistance of the drug in blood and urine will be helpful for a judicious and rational use of the drug in clinical practice.

The chloramphenicol commonly available in the market and the one which is widely used in veterinary practice is selected for the study. The preparation chosen for this study is Vetycetine T.C.F.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The level of chloramphenicol in blood is one of the important factors influencing the antibacterial activity of the drug in most of the infections. Eads et al. (1952) recommended a working dosage schedule for administration of chloramphenicol and it was observed that concentrations of the antibiotic in blood was dose related. Studies of Smith et al. (1948) established that survival rate of infected mice was directly proportional to blood concentration of the drug and duration of treatment. Davies et al. (1972) demonstrated considerable species variations in the persistence of chloramphenicol in animal body. Hence periodic determination of the plasma level of the drug is highly essential for the successful therapy. In field condition, it is not economical and feasible to ascertain plasma level of the drug and it is therefore highly desirable to have a precise idea of the dosage schedule required to maintain adequate blood level of chloramphenicol in various species of animals. Schirmer et al. (1951), Eastman et al. (1952) and Eads et al. (1952) opened a new field of antibiotic therapy in veterinary medicine with the introduction of chloramphenicol in animals. Barns (1955) also reported excellent clinical results when chloramphenicol was used

intramuscularly in the treatment of two hundred Hereford calves suffering from shipping fever.

Blood and plasma concentrations of chloramphenicol

Eads et al. (1952) suggested that 5 to 10 mcg per ml of blood as the effective therapeutic level of chloramphenicol in most infections. McLean et al. (1949) have reported that while most chloramphenicol sensitive bacteria are susceptible to concentrations of 10 mcg per ml, the majority are susceptible to concentrations as low as 2.5 mcg per ml.

Smith et al. (1948) found that the binding of chloramphenicol is relatively little affected by variations in drug concentrations, but it varies with amount of protein present and it was estimated that 60 per cent binding with albumin occurs in blood. Since chloramphenicol is partly bound to serum proteins, a figure of 5 to 10 mcg per ml of serum probably represents a clinically effective blood serum level (Glazko et al., 1949 a).

Eads et al. (1952) observed the blood serum levels of chloramphenicol in dogs following the administration by mouth, at rates of 50, 100, 150 and 400 mg per kg of body weight in divided doses. The levels were roughly proportional to dosage and therapeutic blood levels of

5 to 10 mcg per ml were maintained over most of the period between doses by administration of 150 mg per kg body weight over a 24 hour period, divided into three or four doses. They have also noted no adverse reactions in any of the experimental animals including those receiving 400 mg per kg body weight.

Borgman (1957) reported that a variety of infectious diseases in dogs and cats responded satisfactorily to intramuscular chloramphenicol administration and had the advantages of assuring a blood level in animals in cases when oral administration was unfeasible. It also required less frequent administration due to the repository nature of the intramuscular form. English and Withy (1959) determined the serum, urine and tissue levels of chloramphenicol may diffuse from blood into the tissues, whence it is released back into blood stream in low, undetectable amounts. Brook and Paris (1956) administered chloramphenicol to horses by various routes and found that intramuscular injections in normal saline at dosage rates 4.5 and 16.5 mg per kg body weight have no serum levels.

A study of the plasma and tissue concentration of chloramphenicol in the pig by English and Seawright (1961) indicated that since certain tissue concentrations are higher than plasma levels, it is more difficult to

assess the relative merits of oral and intramuscular dosage which produce lower plasma levels.

Glazko et al. (1968) compared the absorption characteristics of four different chloramphenicol preparations in normal adult volunteers by means of blood level measurements and urinary excretion. They found that the absorption characteristics are varied for different chloramphenicol preparations containing identical amounts of drugs.

Theodorides et al. (1968) observed that when chloramphenicol administered intraruminally at the dose level of 50 mg per kg of body weight to adult sheep, produced no detectable serum levels, where as in two week old lambs a peak level of 15 mcg per ml of serum was obtained within 2 hours. When administered intra-abomasally to adult sheep, it produced high blood levels 1 hour after administration. They also concluded that chloramphenicol was rapidly degraded by ruminal contents which precludes oral therapy.

In a study conducted on serum levels of chloramphenicol in children, Rhesus monkeys and cats after administration of chloramphenicol palmitate suspension it is observed that absorption is species specific (Banerjee et al., 1971).

Oh-Ishi (1968) determined blood concentration in horses after intramuscular or oral administration. He found that intramuscular administration of chloramphenicol failed to elevate the blood level over 5 mcg per ml and Hyaluronidase was not effective to increase the level. Mercer et al. (1971) conducted multiple dose studies on the administration of chloramphenicol to dogs to compare the concentrations in blood obtained from several pharmaceutical dosage forms of different manufacturers by several routes of administration and indicated that presently recommended dosages may not be adequate. Studies on the pharmacokinetics of chloramphenicol in domestic animals by Davis et al. (1972) indicated that chloramphenicol was rapidly absorbed after the intramuscular injection of chloramphenicol sodium succinate, but the dose (22 mg/kg) was insufficient to maintain therapeutic blood concentrations of drug. They have also noted that chloramphenicol was not detected in plasma of goats receiving the drug by the oral route. Pauli and English (1971) compared the blood levels of chloramphenicol after the parenteral administration in the dog and found that the duration of blood levels was not prolonged even after intramuscular injection of chloramphenicol sodium succinate.

Lakshamana et al.(1973) studied absorption kinetics of four different brands of chloramphenicol in

rabbits using both colorimetric and turbidimetric method. The colorimetric method revealed that all four brands are almost equally absorbed but turbidimetric method showed slight lower and variable results. They also suggested that both colorimetric and microbiological methods should be employed for assay of chloramphenicol till such time that a specific chemical method is available. A pharmacologic study conducted by Sisodia et al. (1973 a) in cattle reported that in calves intramuscular administration of chloramphenicol at 10 mg per kg body weight failed to produce therapeutic concentration in blood, but doses of 20 and 30 mg per kg produced and maintained therapeutic concentration between 2nd and 8th hour and between 2nd and 12th hour. They also observed that when given orally chloramphenicol was poorly absorbed from the gastrointestinal tract. Sisodia et al. (1973 b) noticed that an intravenous dose of chloramphenicol (30 mg/kg) in male white tail deer (O.cervine) produced therapeutic levels in blood for five hours and biological half life of chloramphenicol in deer was calculated to be 90 minutes. In cows the therapeutic levels of chloramphenicol were maintained in blood for 4 to 5 hours following intravenous dose of 11 mg per kg. However, the same dose when given intramuscularly produced blood levels which are likely to be therapeutically ineffective (Sisodia et al., 1973 c).

It was also reported that chloramphenicol is recirculated via enterohepatic cycle in cattle.

Watson (1974) observed that there were marked variations within and between dogs in the drug levels produced by the oral administration of chloramphenicol.

Excretion of chloramphenicol

The biochemical studies of Glazko et al. (1949 a) on chloramphenicol found that in man, major route of excretion is by way of the kidneys and in lower animals lesser amounts are excreted in urine. The bulk of the excreted drug is in the form of an inactive nitrocompound, and less than 10 per cent of the administered dose is excreted, unchanged. They have also observed that chloramphenicol is largely excreted by glomerular filtration, while the inactive metabolic products appear to be excreted mainly by tubular secretion.

Glazko et al. (1950) found that following the administration of chloramphenicol the principal nitrocompounds in the urine of rat, dog and man are (i) unchanged chloramphenicol (ii) a hydrolysis product of chloramphenicol and (iii) a conjugate of chloramphenicol with glucuronic acid. It is interesting that in rat the pattern of elimination is different from that in humans

(Glazko et al., 1949 a , 1950). In rat bulk of antibiotic is excreted as glucuronide into the intestine with bile where the becteria hydrolyse the glucuronide ester regenerating the active antibiotic. Thus in rat active antibiotic appears in the faeces.

Ley et al. (1948) recorded the observations on three volunteers who were treated with chloramphenicol orally and found that the antibiotic is absorbed from the gastrointestinal tract and excreted rapidly. It was also suggested that in order to maintain appreciable levels in blood, frequent administration of the drug is required. English and Withy (1959) observed that after intramuscular injection of chloramphenicol in horses (20 and 30 mg/kg) urine concentrations were higher and persisted 24 to 30 hours.

Kunin et al. (1959) discovered that microbially active chloramphenicol disappears from the blood of patients with anuria at about the same rate as in subjects with normally functioning kidneys. Metabolic products (glucuronide and arylamine derivatives) are retained in the circulating blood. They concluded that for this antibiotic the loss of antibacterial effect was not related to urinary excretion. Lindberg et al. (1966) established that the excretion of biologically active chloramphenicol in

urine diminishes linearly with decreasing renal function. They have also found that in elderly patients with decreased renal filtration there is the risk for development of drug resistant microorganisms and exalted toxicity.

Shrotriya and Sharma (1970) observed unmetabolised chloramphenicol excreted in urine of five human subjects.

Sisodia et al. (1973 a) detected highest concentration of chloramphenicol in urine of calves at 2 hours after intravenous injection of 20 mg per kg and the next highest in bile. Chloramphenicol readily passes through blood-brain barrier, placental barrier, intestinal barrier, milk barrier and serous membrances (Brander and Pugh, 1971). When chloramphenicol was given 11 mg per kg both intravenously and intramuscularly the chloramphenicol levels were lower in milk. However, an intravenous dose of chloramphenicol (22 mg/kg) provided therapeutic levels in milk between two to eight hours after medication (Sisodia et al., 1973 c).

Toxicity of chloramphenicol

Extensive scientific literature is available about the toxicological studies on chloramphenicol. In contrast to the observations in man, there have been very

few published reports on the toxic reactions arising from the use of chloramphenicol in domestic animals.

Smith et al. (1948) and Gruhzit et al. (1949) reported that the LD50 for chloramphenicol in mice is 245 mg per kg intravenously, 1320 mg per kg intraperitoneally and 2640 mg per kg orally. In rats, LD50 of chloramphenicol was reported to be 171 to 278 mg per kg intravenously while in rabbits as 117 mg per kg intravenously. In dogs LD50 was found to be 150 mg per kg when given intravenously and 300 mg per kg orally.

Zelkowitz et al. (1968) observed that chloramphenicol in a concentration of 10 mcg per ml causes complete inhibition of protein synthesis in sensitive bacteria, while concentrations exceeding 100 mcg per ml are needed to exert a similar effect in mammalian cells. They also concluded that the response of the protein synthesizing machinery of mammalian cells to chloramphenicol is different from that of bacteria.

Gruhzit et al. (1949) reported that acute toxicity of chloramphenicol from large doses usually occurs within a few hours with symptoms of acute respiratory depression, fall of blood pressure and anoxia. The pathologic symptoms resemble a "Shock like" effect of the antibiotic.

Smiley et al. (1952), Rheingold et al. (1952), Wilson et al. (1952), Claudon and Holbrook (1952) described cases of fatal aplastic anaemia in patients that followed prolonged intermittant therapy with chloramphenicol. They suggested periodic haematological examination while treatment with chloramphenicol. Volini et al. (1950) observed that in man receiving chloramphenicol in therapeutic doses, a severe, reversible granulopenia appeared in the blood and granulocytic hypoplasia with both erythroid and granulocytic maturation arrest in bone marrow. Best (1967) reported chloramphenicol associated blood dyscrasia and suggested that occasional monitoring of blood counts may be worthwhile in deciding when the drug should be and should not be used. Various studies conducted by Reutner and Eads (1952) on a total of 16 dogs revealed that chloramphenicol when given either orally or intravenously in doses of 50 to 200 mg per kg twice daily for one month or longer were without evidence of any blood dyscrasia.

Barnes (1955) observed no signs of irritation, discomfort or toxicity in any of 200 calves receiving intramuscular injections of chloramphenicol in doses of 1 to 2 g per day for 2 to 8 days.

Leitman (1964) reported that physicians have given very large doses or treated thousands of patients without encountering haematological abnormalities.

Penny et al. (1967) observed changes in peripheral blood and bone marrow of four cats given chloramphenicol for 21 days in a daily dose of 50 mg per kg body weight by intramuscular route. Bone marrow examination revealed vacuolation of many of the early members of both the myeloid and erythroid series of cells in chloramphenicol treated cats and in peripheral blood, a marked downward trend in the total white cell count and percentage of erythroblasts.

Saidi et al. (1961) administered chloramphenicol to twenty two patients with infections, to six anaemic patients and to seven normal individuals and found that ten of the patients with infections, all of the anaemic subjects, but none of the normal individuals developed numerous vacuoles in the primitive bone marrow erythroblasts. Normal marrow and blood values became re-established when chloramphenicol was discontinued.

Studies of Yunis et al. (1970) suggested that the reversible dose related depressant effect of chloramphenicol on haemopoises is one result of this inhibition of mitochondrial protein synthesis. Beard et al. (1969) explained the inhibitory effect of chloramphenicol on protein synthesis by an interferance with the function of mRNA. Chloramphenicol acts only during the earliest stages of mRNA - ribosomal interactions and unlike bacteria,

once the polyribosomal complex is formed, mammalian cells are no longer accessible to inhibition by chloramphenicol.

Rubin et al. (1960) and Suhrland and Weiseberger (1962) found that a rise in plasma iron content and an increase in saturation of the iron binding globulin can serve as a reliable and sensitive index of early erythropoietic toxicity. It is also suggested that routine serial determinations of plasma iron content be performed in conjunction with the administration of drugs with potential haematologic toxicity in an attempt to avoid serious injury to the marrow. They also found that the nitrobenzene moiety of chloramphenicol may be of importance in haematologic toxicity. Chloramphenicol is a phenylalanine analogue, and impared intestinal transport of phenylalanine is most likely explanation for chloramphenicol toxicity (Weksler et al., 1968).

Chloramphenicol has been shown to retard the biotransformation of tolubutamide, diphenylhydantoin and dicoumarol in man (Christensen and Skovsted, 1969). Dixon and Fouts (1962) reported that chloramphenicol decrease the rate of metabolic transformation of hexobarbitone, acetanilide, codeine and aminopyrene in mice. They have also suggested that this antibiotic should be given with

a great deal of caution to patients who are treated with other medicaments and its use should be restricted as much as possible.

Caulfield and Burke (1971) noticed that intravenous administration of chloramphenicol caused a distinct diminution in the amount of granulation tissue and rate of wound closure.

Levine et al. (1970) reported the appearance of chloramphenicol associated encephalopathy after oral but not after parenteral medication. They also noticed that such disfunction may depend on inhibition of synthesis of hepatic enzymes involved in biochemical process necessary for normal brain function or an alterative possibility is a direct effect on brain cells.

An intensive study has been made of the toxicity of chloramphenicol to new born infants (Best, 1967). It is reported that repeated high doses of chloramphenicol to new born infants have produced severe reactions that have been called "grey syndrome". In new born infants the conjugation mechanisms of the body are deficient and renal function may be below normal.

Leitman et al. (1964) observed cases of optic neuritis followed by prolonged chloramphenicol therapy.

M A T E R I A L S A N D M E T H O D S

MATERIALS AND METHODS

Adult female Malabari-saanan cross bred goats aged 2 to 3 years and Jersey-Sindhi cross bred calves of age ranging from 8 months to 1 year were selected for the study. The investigation consisted of two separate series of experiments. In the first series the whole blood, plasma and urine levels of chloramphenicol were studied after intramuscular administration.

In the second series the toxic effects of chloramphenicol, intravenously at high dose level were observed. The effects of drug on some of the haematological constituents were noted. Postmortem examination was also conducted at the end of the trial to note the histopathological changes of the internal organs.

Experiment I

Ten apparently healthy adult goats of Malabari-saanan cross and ten Jersey-sindhi cross calves maintained at the Livestock Farm, Mannuthy were selected for the study.

The body weight of the goats varied from 13 to 23 kg with an average of 18 kg and of the calves varied

from 50 to 95 kg with an average of 73 kg. They were under close observation for a period of 10 days prior to the experiment. Blood, urine and faeces were examined for pathological constituents. Except during the course of experiment, the animals were allowed to be grazed and fed with the flock in the farm. During the experimental period the animals were kept in separate but adjacent houses. All the experimental animals were maintained under identical conditions. The houses and premises were kept clean. The animals were sent once daily in the fore noon for grazing about three hours. During the entire experimental period the animals were maintained on an adequate diet consisting of a concentrate part and a roughage part. The concentrate mixture used for feeding was the Livestock and Poultry feed manufactured by the state owned feed factory at Malampuzha, Kerala. The roughage part for goats consisted of jack leaves and for calves straw and grass. Drinking water was provided ad libitum. A second or subsequent course of medication was resorted to on previously used animals only after at least a period of 30 days had elapsed after the first administration. Control blood and urine samples were collected from each animals prior to the administration of chloramphenicol.

Technique of administration of drug

The animals were weighed just before the administration of the drug. The chloramphenicol (Vetycetine T.C.F.) was administered in three different intramuscular doses of 10 mg per kg, 20 mg per kg and 30 mg per kg body weight. The recommended intramuscular dose of chloramphenicol is 5 to 15 mg per pound of body weight in small animals and 2 mg per pound body weight in large animals (Jones, 1966).

The intramuscular injection was given into the gluteal muscle in standing posture. The area was clipped and disinfected with 70 per cent spiritus methylatus. An eighteen guage hypodermic needle was used for injection. The site was well massaged after injection.

Collection of materials for chloramphenicol estimation

Blood and urine samples were collected from each animal simultaneously at the time interval of one hour, two hour, four hour, eight hour, twelve hour, sixteen hour and subsequently at eight hour interval until the drug disappeared from blood. Urine samples were collected only upto 48 hours. All samples collected were refrigerated immediately following collection and chloramphenicol estimation was made within 12 hours of collection. Blood sample was

collected using reagent grade EDTA (Ethylene diamine tetra-cetic acid) as the anticoagulant. 2 mg of this was used to keep 5 to 10 ml of blood without clotting. Blood was drawn from the Jugular vein using a sterilised 17 guage needle. 7 to 10 ml of blood was drawn directly into a clean dry test tube containing the required amount of EDTA. The blood was thoroughly mixed with EDTA in the tube. Urine samples were collected simultaneously with blood. A sterile rubber catheter was used for this purpose and 5 ml of urine was collected into a clean dry test tube. A portion of the whole blood was kept separate for analyses.

Preparation of sample

a) Preparation of plasma

Plasma was separated within one hour after collection. The blood was centrifuged at 3000 r.p.m. for 30 minutes in an electric centrifuge.

One ml of plasma was pipetted into a 50 ml Ehrlenmeyer flask containing 15 ml of water. Four ml of 15 per cent trichloroacetic acid was added with vigorous shaking, and the mixture was allowed to stand for 20 minutes. The contents of the flask were then filtered through Whatman No.50 paper to remove the protein precipitate. This gives a 1:20 dilution of plasma in 3 per cent trichloroacetic acid.

b) Preparation of whole blood

One ml of whole blood pipetted into the flask and handled in the same manner as for plasma gives 1:20 dilution of whole blood in 3 per cent trichloroacetic acid.

c) Preparation of urine

Due to high concentration of nitrocompounds found in urine following administration of chloramphenicol, aquous dilutions of 1:50 and 1:250 may be needed.

Estimation of chloramphenicol

The technique described by Glazko et al. (1949 b) was adopted for estimation of chloramphenicol in whole blood, plasma and urine.

Principle

This involves reduction of the nitro group at room temperature with titanous chloride, removal of excess reagent by precipitation at pH 9 to 10, diazotization of the resulting amine, and coupling with the Bratton-Marshall reagent.

Reagents

1. Trichloroacetic acid (TCA) : 15% solution in water.

2. Titanous chloride: (20% solution) 45 ml is added to 300 ml of concentrated hydrochloric acid, heated to boilding in a hood, and cooled to room temperature. It is then poured into 3600 ml of previously boiled and cooled water in a storage bottle. Air is displaced with hydrogen from a Kipp generator and the contents are thoroughly mixed.

3. Sodium hydroxide:

a) 1.7 N - for use with 3% TCA filters;

b) 1.0 N - for use in the absence of TCA.

4. Hydrochloric acid: 0.25 N and 0.5 N solutions.

5. Sodium nitrite: 0.1% aquous solution prepared fresh daily.

6. Ammonium sulfamate: 0.5% aquous solution stored in refrigerator.

7. Bratten-Marshall coupling reagent: 0.1% aquous solutions of N-(1-naphthyl) ethylenediamine dihydrochloride, stored in a brown bottle under refrigeration.

8. Chloramphenicol standard solution: Pure crystalline chloramphenicol received from M/s. Parke Davis and Co. was used for this purpose.

a) Preparation of stock solution: 10.0 mg of pure chloramphenicol was dissolved in 100 ml of distilled water (100 mcg/ml) and stored in a refrigerator.

b) Working standard: When TCA was used with the samples, the working standards were also prepared using TCA. The stock solution (100 mcg/ml) was diluted 1:5 with water to get a concentration of 20 mcg per ml. 0, 1, 2, 3 and 4 ml were pipetted into flasks, and sufficient water was added to bring the volumes to 16 ml. 4 ml of 15 per cent TCA was then added to prepare final standards, which were equivalent to 0, 20, 40, 60 and 80 mcg per ml of plasma or whole blood or urine. The standards were diluted with the same reagents and handled as the unknowns.

Colorimetric procedure:

Reduction

Three ml of filtrate or sample dilutions were pipetted into 15x125 mm test tubes and 1 ml of titanous chloride reagent was added. The mixtures were shaken briefly, and then 1 ml of sodium hydroxide was added, with additional shaking. With filtrates containing TCA, the NaOH used was about 1.7N, where no TCA had been added the NaOH used was 1.0N. The final pH was 9 to 10 at which maximum precipitation occurs. All tubes were then centrifuged for a few minutes to remove the precipitate. Three ml of the supernatant solution was transferred by pipette to 18x150 mm cuvettes containing 2 ml of 0.5 N Hcl. These samples were ready for diazotization and coupling.

Aryl amine determination

A parellel set of analyses were made on all samples for aryl amines. One ml of each filtrate or dilution was transferred by pipette to 18x150 mm cuvettes containing 4 ml of 0.25 N Hcl. These samples were ready for diazotization and coupling without reduction.

Diazotization and coupling

To each cuvette was added 0.5 ml of sodium nitrite reagent. The contents were mixed and allowed to stand for 5 minutes. Then 0.5 ml of ammonium sulfamate reagent was added to decompose the excess nitrous acid. After standing for 3 minutes with occasional shaking, 0.5 ml of the Bratton-Marshall coupling reagent was added. The contents were thoroughly mixed by shaking, and all tubes were placed in a waterbath at 38°C for one hour. At the end of this time, the tubes were removed, wiped dry and the absorbance was measured at 555 microns with "Spectronic 20" (Junior spectrophotometer) against a reagent blank.

Free chloramphenicol

Free chloramphenicol was extracted with ethyl acetate to separate it from its metabolites. One ml of the sample was transferred to a glass stoppered test tube containing 2 ml of 0.2 M phosphate buffer at pH 6. Six ml of

ethyl acetate was added and the tubes were placed in a mechanical shaker for 10 minutes. The tubes were then centrifuged and the aquous phase was aspirated off. The organic layer was washed several times with pH 6 buffer saturated with ethyl acetate. After separation of the two phases 5 ml of ethyl acetate layer was transferred to a beaker. The solvent was evaporated to dryness on a steam bath. The residue was dissolved in 3 ml of water, analysed by the titanous chloride reduction and estimated by colorimetric method.

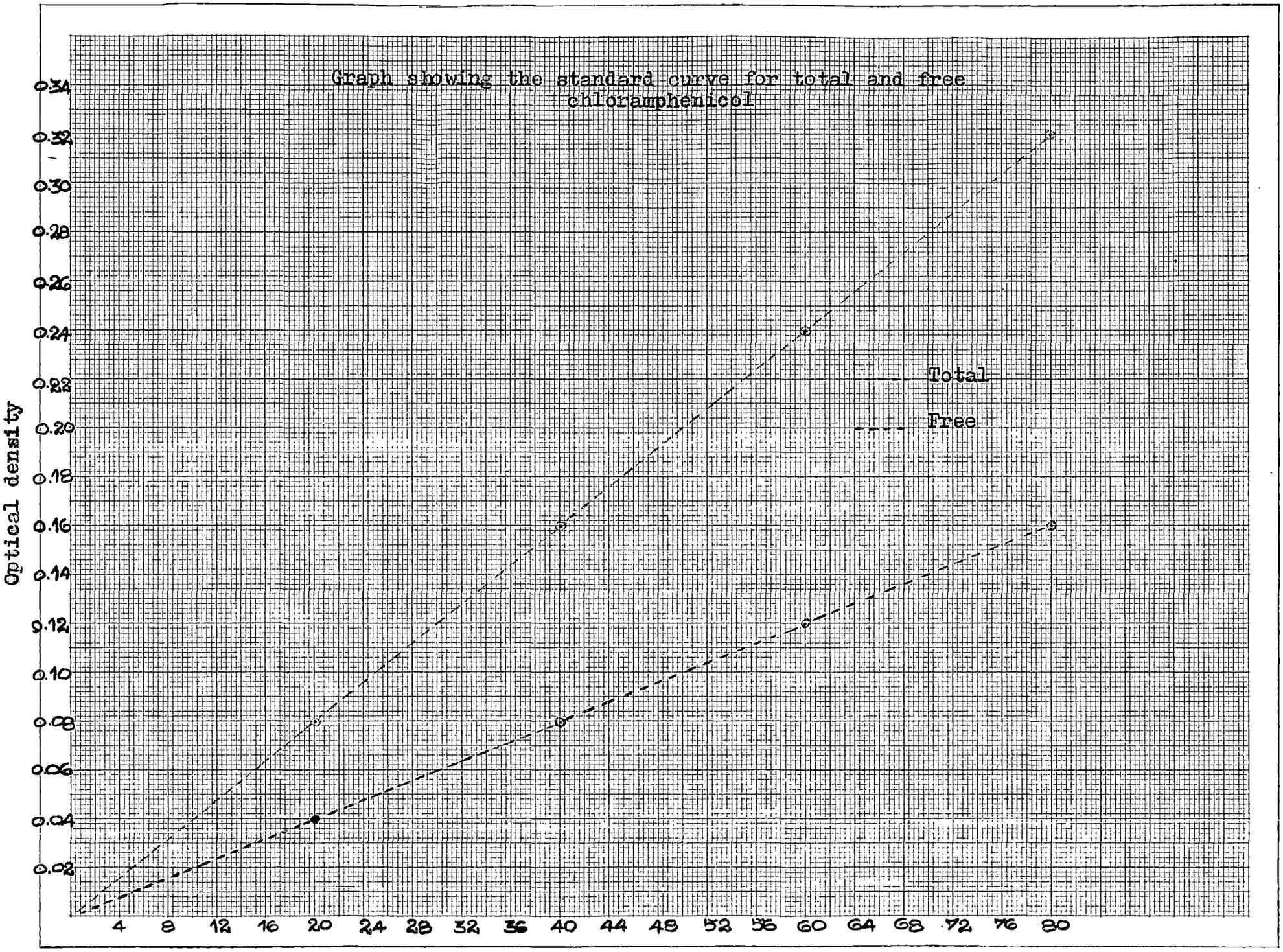
Calculation of results

A simple graphic method was used for calculation. A graph was plotted using the optical densities of different known amounts of chloramphenicol ie., 0, 20, 40, 60 and 80 mcg per ml from the standard solution prepared. Unknown solutions were handled in the same manner as for the standard and from the optical density of unknown the concentration of chloramphenicol was directly read from the standard graph prepared.

Experiment II

Toxicity of chloramphenicol in calves

Four apparently healthy male calves (Tattoo Nos. C56, 419, 408, 362) of 1 to 2 years of age were used. They


Graph showing the standard curve for total and free chloramphenicol
Optical density
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
Total
Free
4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80
mcg/ml

were maintained on a uniform ration as the animals in experiment I described earlier. The calves were kept in separate houses. Drinking water was provided ad libitum. They were under a close observation to detect any gross abnormalities for a period of 10 days prior to the experiment. The body weight, temperature, pulse and respiration were recorded for every third day. Blood samples were collected every third day from each animal and the erythrocyte count, erythrocyte sedimentation rate, packed cell volume, percentage of haemoglobin, total white blood cell count, differential count and blood urea were determined. The mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were also determined. Urine collected from each animal was examined for the presence of albumen, bile salts, bile pigments, blood and sediments.

After recording the normal data, the calves were divided into two groups ie., the experimental group and the control group consisting of two animals in each group. The animals in the experimental group received 100 mg per kg of chloramphenicol (vetycetine T.C.F.) intravenously for 30 days.

Administration of the drug

The animals were weighed and the total quantity of the drug to be given at the rate of 100 mg per kg

body weight ascertained and drawn into a record syringe. The calf was secured on its side. The area at the middle of the neck on the jugular vein was clipped closely and disinfected with 70 per cent spiritus methylatus. Injection was given slowly by introducing a 17 guage hypodermic needle into the jugular vein. The injections were given every day at 10 A.M. for 30 days continuously. Blood and urine sample from each calf was collected just before administration of the drug on every third day. In collecting the blood, care was taken to see that only the minimum quantity of blood required for the estimation was taken. 24 hours after the administration of last dose of chloramphenicol one of the animals in the experimental group was sacrificed and postmortem examination was conducted. Since the animals in the experimental group did not show much gross toxicity symptoms, the other animal was not sacrificed.

Blood

Erythrocyte count

The technique described by Schalm (1965) was adopted.

Principle

The blood diluted accurately with an isotonic fluid having anticoagulant properties, is placed in a counting chamber and the number of cells in circumscribed area enumerated under the microscope.

Reagent

Hayem's solution 0.5 g of mercuric chloride, 2.2 g of anhydrous sodium sulfate and 1.0 g of sodium chloride were dissolved in distilled water and diluted to 200 ml.

Procedure

The blood was drawn upto the 0.5 mark in the red cell pipette, excess blood wiped off from the tip of the pipette, diluted to the 101 mark with Hayem's solution and well mixed by shaking for 3 minutes. The first few drops were discarded to remove the cell free fluid from the stem. The Levy ultraplane haemocytometer counting chamber, with improved Neubauer ruling was filled with the diluted blood. The erythrocytes in four big squares in the four corners and one big square in the centre (each consisting of 10 small squares) of the one sq mm ruled area in the middle of the counting chamber, were counted.

Calculation

Area of each small square	= 1/400 sq mm
Depth of the counting chamber	= 1/10 mm
Volume of diluted blood in one small square	= 1/4000 cm
Volume of diluted blood in the 80 small squares counted	= 80/4000 cm
Dilution	= 1 in 200.

If 'n' be the number of cells in 80/4000 cmm of diluted blood, the red cell count in 1 cmm of undiluted blood = $\frac{nx4000x200}{80}$ = nx10,000.

Erythrocyte sedimentation rate (ESR)

The technique of Wintrobe and Landsberg (1935) was followed.

Principle

In blood samples - prevented from clotting by addition of an anticoagulant - the cells settle down to the bottom on standing. The distance the corpuscles have fallen in an arbitrary period of time is recorded.

Procedure

Wintrobe haematocrit tubes were filled upto the '0' mark (at the left of the scale) with the blood and noted the time. The tubes were placed vertically in the Wintrobe sedimentation rack and left undisturbed. The reading were taken at the end of one hour.

Packed cell volume (PCV)

The method of Wintrobe (1961) was followed.

Principle

When a sample of blood to which an anticoagulent has been added is centrifuged, complete packing of corpuscels

occurs without distortion or expulsion of their contents and the blood separates into three distinct compartments from which the relative volume of blood cells and plasma are found out.

Procedure

Wintrobe haemotocrit tubes were filled to the '0' mark with blood by means of the capillary pipette, centrifuged for 30 minutes at 3000 r.p.m. and the packed cell volume read. Completeness of packing of the red cells was checked by recentrifugation.

Determination of haemoglobin

The haemoglobin content of the blood was estimated by Wong's method as given by Hawk, Oser and Summerson (1954).

Principle

The iron from the haemoglobin molecule is liberated in the cold, by treating blood with concentrated sulphuric acid in the presence of potassium persulphate. The protein are precipitated off tungstic acid and their filtrate treated with potassium thiocyanate. The light red colour produced due to the formation of ferric thiocynate is estimated colorimetrically. The amount of haemoglobin is calculated assuming that haemoglobin contains 0.34 per cent iron.

Reagents

(1) Concentrated sulphuric acid (iron free).

(2) Saturated potassium persulphate solution. 8 g of reagent grade, iron free, potassium persulphate was shaken with 100 ml of distilled water in a glass stoppered bottle.

(3) 10 per cent sodium tungstate solution. 100 g of reagent grade, iron free sodium tungstate dissolved in distilled water and diluted to 1 litre.

(4) Standard iron solution. Dissolved 0.702 g of reagent grade crystalline ferrous ammonium sulphate in 100 ml of distilled water. Added 5 ml of concentrated sulphuric acid, warmed and then added concentrated potassium permanganate solution, drop by drop until a permanganate pink colour was produced. The solution was transferred to one litre volumetric flask with rinsings and diluted to the mark. This solution contained 0.1 mg of ferric iron per ml.

(5) 3 N potassium thiocynate solution. 146 g of reagent grade potassium thiocynate dissolved in distilled water, diluted to 500 ml and mixed with 20 ml of pure acetone.

Procedure

0.5 ml of the oxalated blood and 2 ml of iron free concentrated sulphuric acid were transferred to a 50 ml

= haemoglobin in g per 100 ml of the blood. Where 0.25 was the iron content in the standard and 0.5 the volume of blood used. 3.4 represent the mg of iron per g of haemoglobin.

Calculation of the erythrocytic indices

MCV (Mean corpuscular volume),

MCH (Mean corpuscular haemoglobin) and MCHC (Mean Corpuscular haemoglobin content) were calculated from the red cell count, packed cell volume and haemoglobin content as follows:-

$$\text{MCV in cubic microns} = \frac{\text{Volume of packed red cells in ml per 100 ml of blood}}{\text{Red cell count in millions per cmm of blood}}$$

$$\text{MCH in micro-micrograms} = \frac{\text{Haemoglobin in g per 1000 ml of blood}}{\text{Red cell counts in million per cmm of blood}}$$

$$\text{MCHC in per cent} = \frac{\text{Haemoglobin in g per 100 ml of blood}}{\text{Volume of packed red cells in ml per 100 ml of blood}} \times 100$$

Leukocyte count

The technique described by Schalm (1965) was followed.

Principle

A measured quantity of blood is accurately diluted 20 times with Thoma's fluid. Acetic acid present in the solution causes lysis of the erythrocytes while gentian violet in the reagent stains the leukocytes.

Reagents

Thoma's fluid. Dissolved 0.025 g of powdered gentian violet in a little quantity of water added 2 ml of glacial acetic acid and diluted to 100 ml with water.

Procedure

Blood was drawn into the white cell pipette upto the 0.5 ml mark and then the Thoma's fluid drawn upto the mark 11. The blood was thoroughly mixed with the Thoma's fluid in the bulbs. After discarding the fluid in the stem of the pipette, the Levy haemocytometer counting chamber with improved Neubauer ruling was charged with diluted blood. The number of leukocyte in the four corner squares, each one having an area of 1 sq mm was counted.

Calculation

Area of the four corner squares	= 4 sq mm
Depth of the counting chamber	= 1/10 cmm
Volume of diluted blood in the four squares counted	= 4/10 cmm
Dilution	= 1 in 20

If 'n' be number of cells in 4/10 cmm of diluted blood, leukocyte count in 1 cmm of undiluted blood = $\frac{n \times 10 \times 20}{4}$

= nx50.

Differential leukocyte count

The method described by Schalm (1965) was adopted.

Principle

A thin smear of blood is stained with Wright's stain and different types of white cells counted under an oil immersion lens of high power microscope.

Reagents

1. Wright's stain. 0.1 g of Wright's stain powder, ground with 60 ml of absolute methyl alcohol (acetic acid free) was transferred to a bottle tightly stoppered, stored in the dark for two weeks and filtered before use.

2. Phosphate buffer solution pH 6.6. Dissolved 3.80 g of disodium hydrogen phosphate and 5.47 g of monopotassium phosphate in water and diluted to 1000 ml with water.

Procedure

Thin smears prepared directly from fresh samples of blood on clean grease free slides were dried, kept on a

rack and flooded with 20 drops of Wright's stain. After one minute, 20 drops phosphate buffer solution were added and mixed thoroughly by blowing until a metallic film appeared on the surface of the stain. After another four minutes the scum and the precipitated stain were washed off with tap water. The stained smears were dried and examined under the oil immersion objective of a microscope. A differential count of 300 leukocytes in each smear was made adopting the battlement method. The different types of leukocytes were identified based on the characteristics as described by Coffin (1945).

Determination of urea

The urea nitrogen in the protein free blood filtrate was determined by the method of Folin and Svedberg as given by Hawk et al. (1954).

Principle

The urea in the protein free blood filtrate is treated with the enzyme urease which converts the nitrogen in the former to ammonia. The ammonia formed is determined, distilled off and determined colorimetrically after direct nesslerization.

Reagents

1) Acetate buffer solution. Dissolved 15 g of crystalline sodium acetate in 50 ml water in a 100 ml

volumetric flask. Added 1 ml of glacial acetic acid, diluted to volume and mixed.

2) Urease solution. 0.5 g of jack bean meal was shaken with 20 ml of 30% alcohol for 10 minutes and then filtered. This was prepared immediately before use.

3) Antifoaming oil mixture. Mixed one volume of crude fuel oil with 10 volume of toluene.

4) Saturated borax solution. 40 g of reagent grade tetraborate was shaken with a litre of boiling water and allowed to cool. The undissolved borax settled to the bottom.

5) 0.1 N hydrochloric acid. 9 ml of concentrated hydrochloric acid was diluted to one litre with distilled water.

6) Standard ammonium sulphate solution. A stock standard solution was prepared by dissolving 0.944 mg of dry, reagent grade ammonium sulphate in water, adding a few drops of concentrated sulphuric acid diluting to a litre with distilled water. To prepare the dilute standard, 5 ml of the above stock standard was diluted to 100 ml. This solution contained 0.1 mg of nitrogen in 10 ml.

7) Nessler's reagent. Always prepared just before use. 100 g of mercuric iodide and 70 g of potassium

iodide were dissolved in 400 ml water in a litre flask. 100 g of sodium hydroxide were dissolved separately in 500 ml of water, cooled, added the above solution and diluted upto the mark with distilled water.

Procedure

Five ml of the protein free filtrate was pipetted into a tube of 30 ml capacity and added two drops of acetate buffer solution and one ml of urease solution. The tube was corked and allowed to stand for 25 minutes at room temperature. After the period, an antibumping tube was introduced and two drops of antifoaming oil mixture and two ml of saturated borax solution were added. A delivery tube was connected to the 30 ml tube and the contents distilled over a microburner. The distillate was collected for a period of 4 minutes in a test tube graduated at 25 ml containing one ml each of 0.1 N hydrochloric acid and water. At the end of distillation, the distillate was diluted to about 20 ml.

A standard was prepared by taking in another test tube having a graduation at 25 ml. 10 ml of standard ammonium sulphate solution containing 0.1 ml of nitrogen and one ml of 0.1 N hydrochloric acid and dilute it to about 20 ml.

A blank was prepared in a similar manner omitting the standard ammonium sulphate solution.

To each of the above three tubes, 2.5 ml of Nessler's reagent was added and diluted to 25 ml mark with distilled water. Reading of the standard and unknown were taken after 10 minutes and within the next 10 minutes in an Eel photoelectric colorimeter, using green filter, after setting the instrument at zero with the blank.

Calculation

$$\frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 0.1 \times \frac{100}{0.05}$$

= mg urea nitrogen per 100 ml of blood, where 0.1 was the mg of nitrogen in 10 ml of the ammonium sulphate solution taken and 0.5 the volume of blood used.

Urine

Qualitative determination of albumin in urine

Heller's nitric acid test. The method given by Miller (1955) was adopted. Pipetted 3 ml of concentrated nitric acid into a test tube. With another pipette urine was allowed to run slowly down the side of the inclined tube so as to form two layers of fluid. A white precipitate or ring at the junction of the two layers denoted the presence of the albumin in the urine. It was best developed in about one minute.

= haemoglobin in g per 100 ml of the blood. Where 0.25 was the iron content in the standard and 0.5 the volume of blood used. 3.4 represent the mg of iron per g of haemoglobin.

Calculation of the erythrocytic indices

MCV (Mean corpuscular volume),

MCH (Mean corpuscular haemoglobin) and MCHC (Mean Corpuscular haemoglobin content) were calculated from the red cell count, packed cell volume and haemoglobin content as follows:-

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$$\text{MCHC in per cent} = \frac{\text{Haemoglobin in g per 100 ml of blood}}{\text{Volume of packed red cells in ml per 100 ml of blood}} \times 10$$

Leukocyte count

The technique described by Schalm (1965) was followed.

Determination of blood in urine

Guaiac test described by Miller (1955) was adopted. To 5 ml of urine in a test tube few drops of glacial acetic acid were added. Fresh alcoholic solution of gum guaiac was dropped into the urine until the solution turned slightly turbid. Added a few millilitre of 3 per cent hydrogen peroxide solution. Development of a blue colour indicated the presence of haemoglobin in the urine.

Determination of bile pigment in urine

Gmelin's test given by Miller (1955) was adopted. To 5 ml of urine in a test tube 3 ml of concentrated nitric acid was added in such a way that two layers are formed. The formation of a play of colours with green predominent at the junction of the layers showed the presence of bile pigments.

Detection of bile salts in urine

Hay's test described by Miller (1955) was adopted. Flowers of sulphur were sprinkled over the surface of cold urine. The particles of sulphur came to the bottom if bile salts were present. This is due to reduction in the surface tension.

Microscopic examination of urine sediments

Agitated the urine to stir up any sediments that

may have settled to the bottom. Placed approximately 10 ml of urine in a conical tipped centrifuge tube and centrifuged for 10 minutes at a speed of 1400 r.p.m. in an electric centrifuge. Poured out all the urine in the tube and placed the tube in an upright position so that the fluid remained on the sides of the tube flowed to the bottom to dilute the sediment. Shaking the sediment well, placed a drop on a clean glass slide and covered it with a cover slip. The slide was examined under microscope with the low and high power objectives with minimum light.

Postmortem examination

One of the animal (C56) was killed by jugular bleeding after stunning with a Captive-bolt pistol. A thorough postmortem examination was conducted and the following tissues were collected for histopathological slides, such as lung, heart, liver, spleen, kidney and bone marrow.

Preparation of tissues for microscopic examination

Tissues were preserved in 10 per cent formalin and paraffin embodied sections of 5 microns thickness were taken and stained with Haemotoxiline and eosin stain (Harri's method) for histopathological examination.

RESULTS AND DISCUSSION

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Experiment I

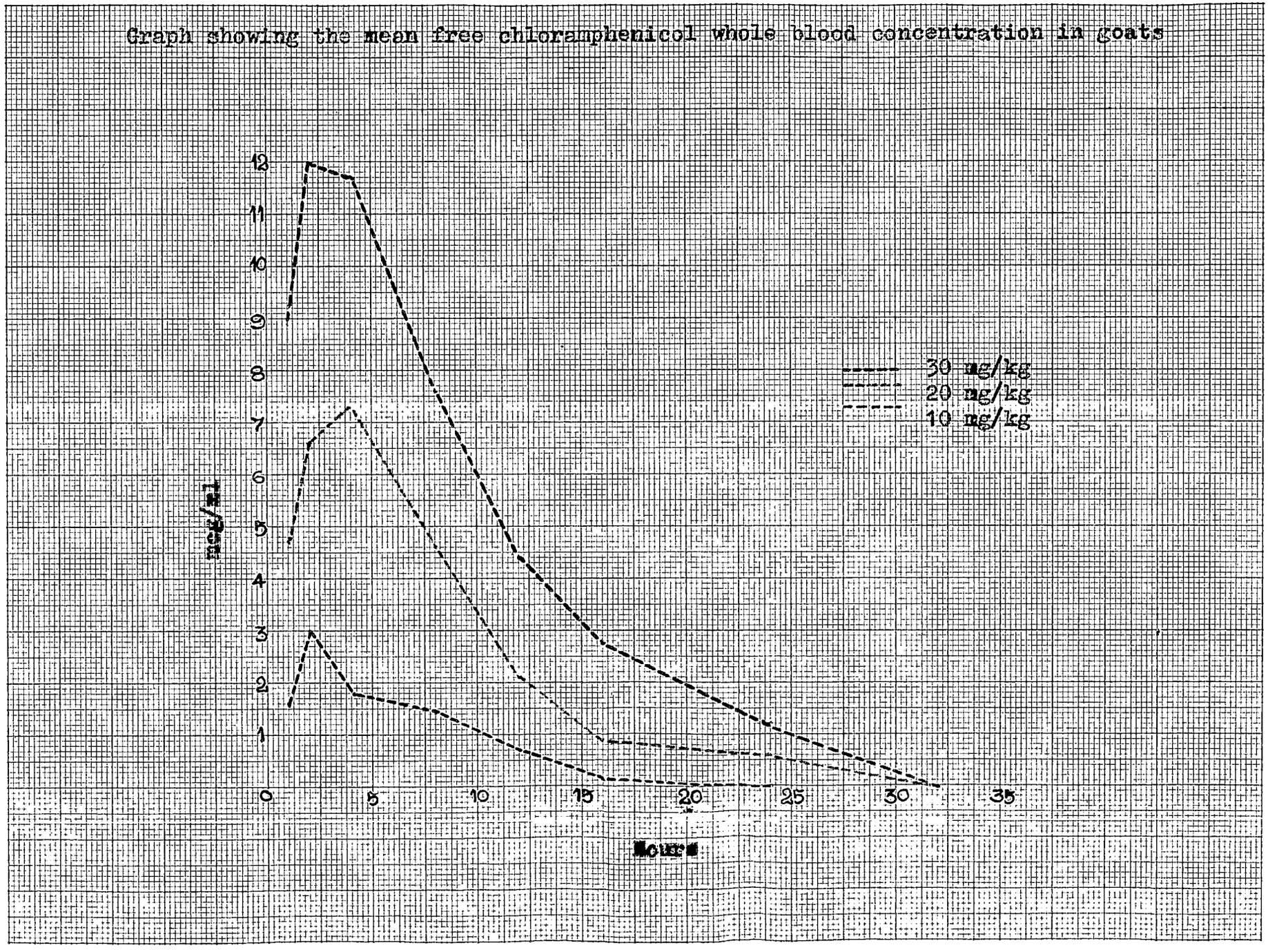
Chloramphenicol is recommended for sheep, goats, pigs, foals and calves in a dose ranging from 4 to 10 mg per kg body weight daily administered intramuscularly (Brander and Pugh, 1971).

Chloramphenicol concentration in whole blood and plasma

Table I a to III a and Fig. 1 gives the free and total chloramphenicol concentration in whole blood of six goats at different time intervals after the intramuscular administration of 10 mg per kg, 20 mg per kg and 30 mg per kg body weight of chloramphenicol. Statistical evaluation of the experimental data was undertaken, to ascertain the effect of different dose rates, on whole blood, plasma and urine concentration (Snedecor, 1957).

The intramuscular administration of 10 mg per kg body weight of chloramphenicol resulted in a maximum mean free chloramphenicol whole blood concentration of 3 mcg per ml at 2 hours 34 minutes and total concentration of 4.1 mcg per ml at 2 hours 34 minutes. The drug persisted in the blood for about 24 hours. The minimum therapeutic concentration of 5 mcg per ml of blood was not achieved. The mean concentration of free drug per ml of whole blood

Fig. 1


Graph showing the mean free chloramphenicol whole blood concentration in goats
30 mg/kg
20 mg/kg
10 mg/kg
µg/ml
Hours
0
1
2
3
4
5
6
7
8
9
10
11
12
5
10
15
20
25
30
35

tested at various time intervals were 1.5 mcg at first hour, 3.0 mcg at second hour, 1.8 mcg at fourth hour, 1.4 mcg at eighth hour, 0.7 mcg at twelfth hour and 0.16 mcg at sixteenth hours of post administration.

The intramuscular administration of 20 mg per kg body weight of chloramphenicol resulted in a maximum mean free chloramphenicol whole blood concentration of 7.3 mcg per ml at 2 hours 12 minutes and total chloramphenicol concentration of 9.4 mcg per ml at 2 hours 55 minutes. The drug persisted in blood for about 32 hours. The mean concentration of free drug per ml of whole blood tested at various time interval were 4.7 mcg at first hour, 6.6 mcg at second hour, 7.3 mcg at fourth hour, 4.6 mcg at eighth hour, 2.1 mcg at twelfth hour, 0.9 mcg at sixteenth hour and 0.6 mcg at twenty fourth hour. The minimum therapeutic concentration of 5 mcg per ml was maintained for 7 hours 30 minutes.

The intramuscular administration of 30 mg per kg body weight of chloramphenicol resulted in a maximum mean free chloramphenicol whole blood concentration of 12.0 mcg per ml at 2 hours 56 minutes and total chloramphenicol concentration of 13.8 mcg per ml at 1 hour 43 minutes. The drug persisted in blood for about 32 hours. The mean concentration of free drug per ml of whole blood tested

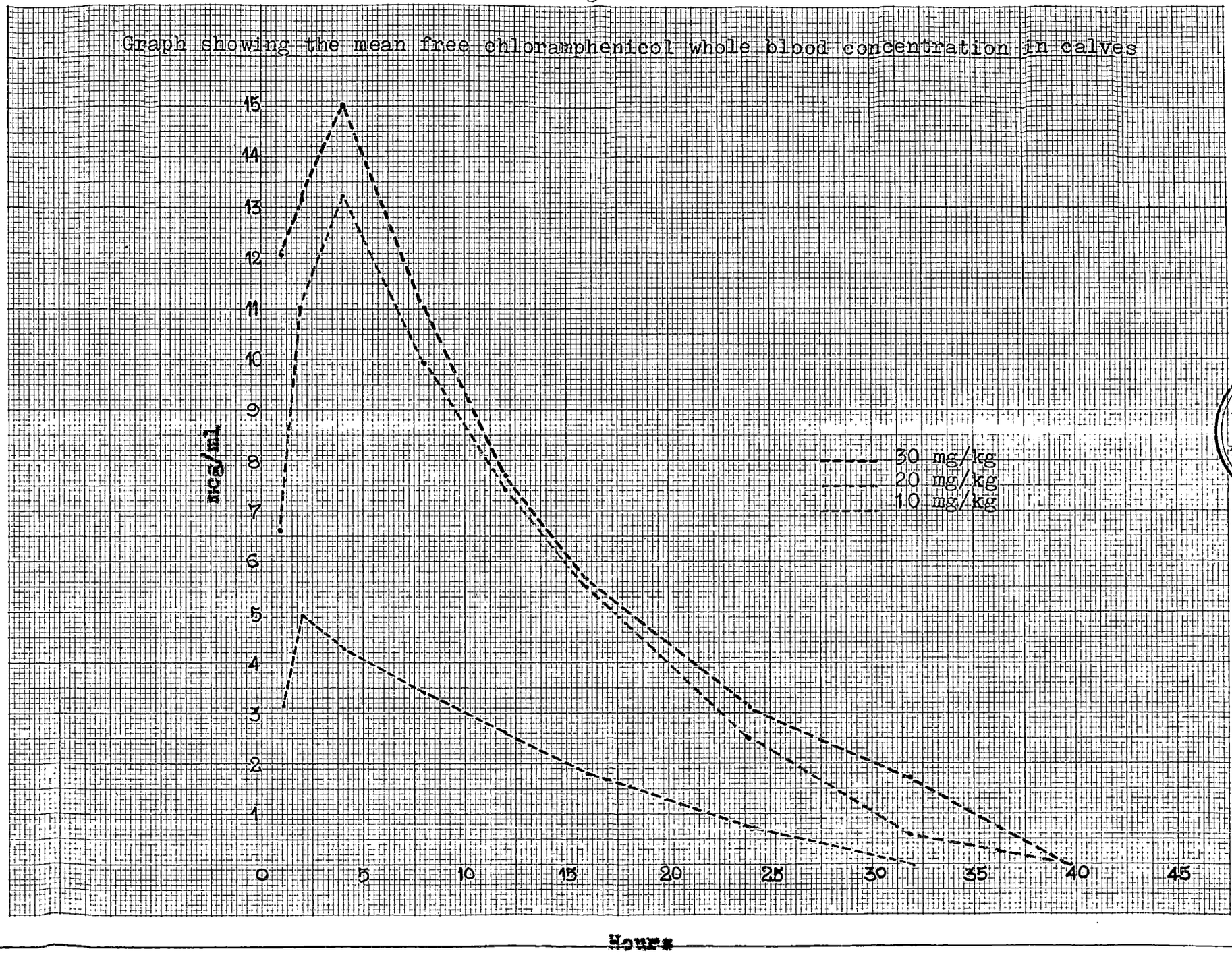
at various time intervals were 8.9 mcg, 12.0 mcg, 11.7 mcg, 7.6 mcg, 4.4 mcg, 2.8 mcg and 1.2 mcg respectively at 1, 2, 4, 8, 12, 16 and 24 hours post administration. The minimum therapeutic concentration of 5 mcg per ml was maintained for 11 hours.

Table I b to III b and Fig. 2 gives the free and total chloramphenicol concentrations in whole blood of six calves at different time intervals after intramuscular administration of 10 mg, 20 mg and 30 mg per kg body weight of chloramphenicol.

The intramuscular administration of 10 mg per kg body weight of chloramphenicol resulted in a maximum mean free chloramphenicol whole blood concentration of 4.9 mcg per ml at 2 hours 48 minutes and total chloramphenicol concentration of 6.0 mcg per ml at 2 hours 36 minutes. The drug persisted in the blood for about 32 hours. The mean concentration of the free drug per ml of whole blood tested at various time intervals were 3.1 mcg, 4.9 mcg, 4.2 mcg, 3.4 mcg, 2.5 mcg, 1.8 mcg and 0.7 mcg respectively at 1, 2, 4, 8, 12, 16 and 24 hours post administration. The therapeutic concentration of 5 mcg was not achieved.

The intramuscular administration of 20 mg per kg body weight of chloramphenicol resulted in a maximum mean free chloramphenicol whole blood concentration of 13.2 mcg

Fig. 2



per ml at 2 hours 22 minutes and total chloramphenicol concentration of 14.9 mcg per ml at 2 hours 26 minutes. The drug persisted in blood for about 40 hours. The minimum therapeutic concentration of 5 mcg per ml was maintained for 17 hours 30 minutes. The mean concentration of free drug per ml of whole blood tested at various time intervals were 6.6 mcg at first hour, 11.0 mcg at second hour, 13.2 mcg at fourth hour, 9.9 mcg at eighth hour, 7.4 mcg at twelfth hour, 5.5 mcg at sixteenth hour, 2.5 mcg at twenty fourth hour and 0.6 mcg at thirty second hour.

The intramuscular administration of 30 mg per kg body weight of chloramphenicol resulted in a maximum mean free chloramphenicol concentration of 15 mcg per ml at 2 hours 14 minutes and total concentration of 16.3 mcg per ml at 2 hours 30 minutes. The drug persisted in blood for about 40 hours. The minimum therapeutic concentration of 5 mcg per ml was maintained for 18 hours 30 minutes. The mean concentration of free drug per ml of whole blood tested at various time intervals were 12.0 mcg at first hour, 13.1 mcg at second hour, 15.0 mcg at fourth hour, 11.0 mcg at eighth hour, 7.7 mcg at twelfth hour, 5.6 mcg at sixteenth hour, 3.1 mcg at twenty fourth hour and 1.7 mcg at thirty second hour.

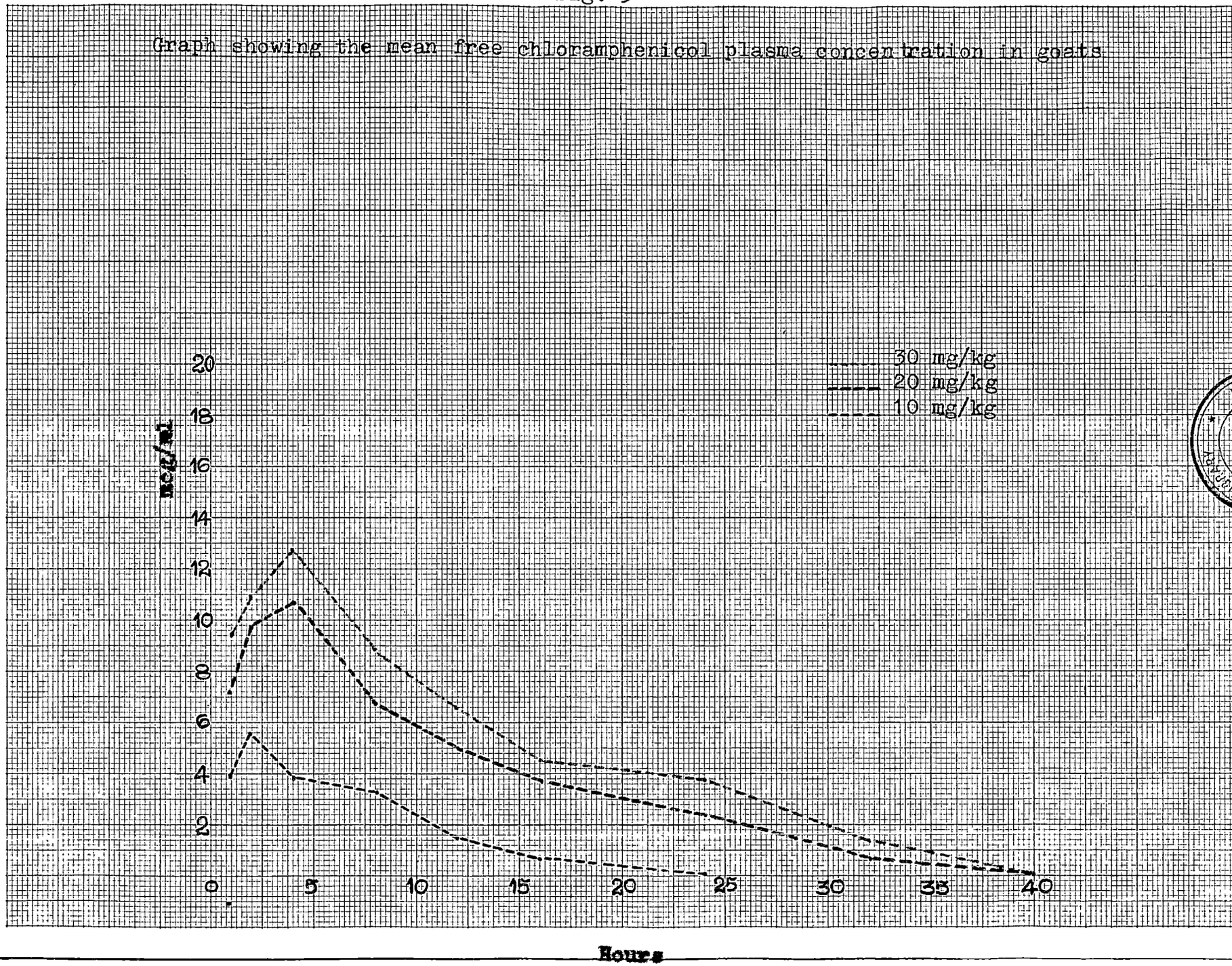
Chloramphenicol concentration in plasma

Table IV a to VI a and Fig. 3 gives the free and total chloramphenicol concentrations in plasma of six goats at different time intervals after the intramuscular administration of 10 mg per kg, 20 mg per kg and 30 mg per kg body weight of chloramphenicol.

The intramuscular administration of 10 mg per kg body weight of chloramphenicol resulted in a maximum mean free chloramphenicol plasma concentration of 5.4 mcg per ml at 2 hours 28 minutes and total chloramphenicol concentration of 7.0 mcg per ml at 2 hours 25 minutes. The drug persisted in the plasma for about 24 hours. The minimum therapeutic concentration of 5 mcg was maintained for 2 hours. The mean concentration of free drug per ml of plasma tested at various time intervals were 3.9 mcg, 5.4 mcg, 3.8 mcg, 3.1 mcg, 1.4 mcg and 0.5 mcg respectively at 1, 2, 4, 8, 12, 16 and 24 hours post administration.

The intramuscular administration of 20 mg per kg body weight of chloramphenicol resulted in a maximum mean free chloramphenicol plasma concentration of 10.5 mcg per ml at 1 hour 50 minutes and total concentration of 12.6 mcg per ml at 1 hour 53 minutes. The drug persisted in plasma for about 40 hours. The mean concentration of free

Fig. 3



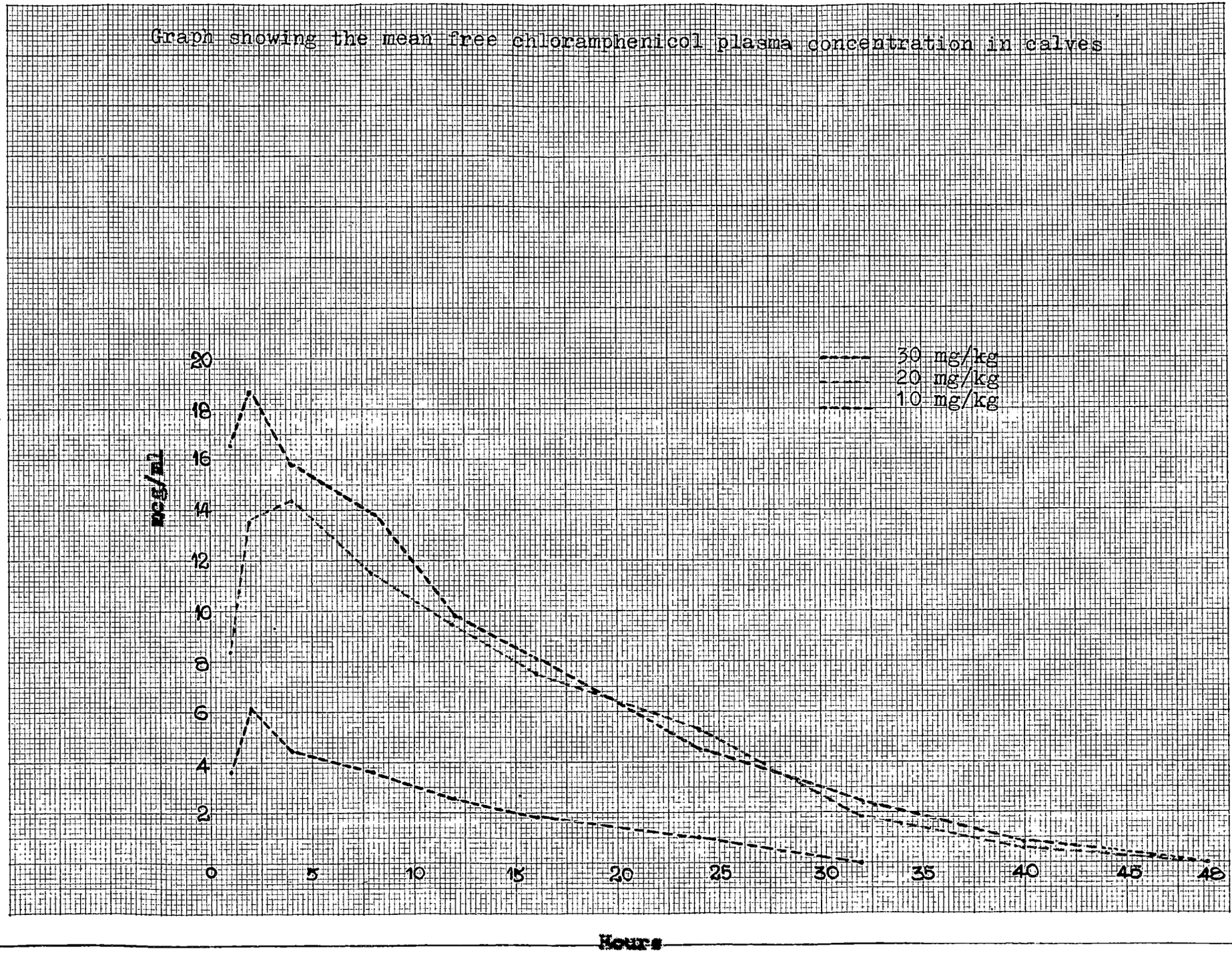
drug in plasma tested at various time intervals were 7.0 mcg, 9.7 mcg, 10.5 mcg, 6.6 mcg, 5.1 mcg, 3.6 mcg, 2.2 mcg and 0.5 mcg respectively at 1, 2, 4, 8, 12, 16, 24 and 32 hours post administration. The minimum therapeutic concentration of 5 mcg per ml was maintained for 11 hours.

The intramuscular administration of 30 mg per kg body weight of chloramphenicol resulted in a maximum mean free chloramphenicol plasma concentration of 12.6 mcg per ml at 2 hours 14 minutes and total chloramphenicol concentration of 15.9 mcg per ml at 2 hours 6 minutes. The drug persisted in blood for about 40 hours. The mean concentration of free drug per ml of plasma tested at various time intervals were 9.4 mcg, 10.7 mcg, 12.6 mcg, 8.7 mcg, 6.4 mcg, 4.3 mcg, 3.6 mcg and 1.3 mcg respectively at 1, 2, 4, 8, 12, 16, 24 and 32 hours of post administration. The minimum therapeutic concentration of 5 mcg per ml was maintained for 15 hours.

Table IV b to VI b and Fig. 4 gives the free and total chloramphenicol concentrations in plasma of 6 calves at different time intervals after the intramuscular administration of 10 mg per kg, 20 mg per kg and 30 mg per kg body weight of chloramphenicol.

The intramuscular administration of 10 mg per kg body weight of chloramphenicol resulted in a maximum mean

Fig. 4


Graph showing the mean free chloramphenicol plasma concentration in calves
30 mg/kg
20 mg/kg
10 mg/kg
mcg/ml
20
18
16
14
12
10
8
6
4
2
0
5
10
15
20
25
30
35
40
45
48
Hours

free chloramphenicol concentration of 6.1 mcg per ml at 2 hours 39 minutes and total chloramphenicol concentration of 7.3 mcg per ml at 2 hours 38 minutes. The drug persisted in the plasma for about 32 hours. The minimum therapeutic concentration of 5 mcg per ml was maintained for 3 hours. The mean concentration of the free drug per ml of plasma tested at various time intervals were 3.6 mcg, 6.1 mcg, 4.5 mcg, 3.6 mcg, 2.6 mcg, 1.9 mcg and 1.0 mcg respectively at 1, 2, 4, 8, 12, 16 and 24 hours post administration.

The intramuscular administration of 20 mg per kg body weight of chloramphenicol resulted in a maximum mean free chloramphenicol plasma concentration of 14.3 mcg per ml at 2 hours 31 minutes and total concentration of 17.0 mcg per ml at 2 hours 21 minutes. The drug persisted in plasma for about 48 hours. The mean concentration of free drug per ml of plasma tested at various time invervals were 8.4 mcg, 11.5 mcg, 14.3 mcg, 11.6 mcg, 9.5 mcg, 7.5 mcg, 5.1 mcg, 1.9 mcg and 0.5 mcg respectively at 1, 2, 4, 8, 12, 16, 24 and 40 hours post administration. The minimum therapeutic concentration of 5 mcg per ml was maintained for 24 hours 30 minutes.

The intramuscular administration of 30 mg per kg body weight of chloramphenicol resulted in a maximum mean free chloramphenicol plasma concentration of 18.6 mcg per ml

at 2 hours 25 minutes and total concentration of 20.4 mcg per ml at 2 hours 28 minutes. The drug persisted in blood for about 48 hours. The mean concentration of free drug per ml of plasma tested at various time intervals were 16.4 mcg, 18.6 mcg, 15.9 mcg, 13.8 mcg, 9.9 mcg, 8.0 mcg, 4.4 mcg, 2.4 mcg and 0.6 mcg respectively at 1, 2, 4, 8, 12, 16, 24, 32 and 40 hours post administration. The minimum therapeutic concentration of 5 mcg per ml was maintained for 24 hours 30 minutes.

In the present study the dose of 10 mg per kg body weight failed to produce therapeutic concentration (5 mcg/ml) in blood of goats and calves. These observations are in agreement with the reports of Eads and Van Hocker (1955) and Sisodia et al. (1973a, 1973c). However, the recommended dosage given is 4 to 10 mg per kg (Brander and Pugh, 1971).

Oh-Ishi (1968) reported that in horses intramuscular administration of 50 mg per kg body weight of chloramphenicol failed to elevate the blood level over 5 mcg per ml. This may be due to the influence of species variation as observed by Banerjee et al. (1971) and Davis et al. (1972).

In the present study, it was observed that the calves maintained higher blood level for a longer duration when compared to goats. Davis et al.(1972) observed that

total duration of persistance of chloramphenicol succinate in blood, after intramuscular injection was longer in dogs and cats and it was shorter in goats. These differences in the rates of elimination of chloramphenicol were most likely to be related to differences in rates of biotransformation existing among various species. Species differences were observed in the rates of absorption and in the magnitude of drug concentrations after intramuscular administration of different salts of chloramphenicol. Moreover, the absorption characteristics vary for different chloramphenicol preparations containing the identical amounts of drugs (Glazko et al., 1968 and Mercer et al., 1971).

Peak concentration in blood, were attained in between second and third hour both in calves and goats, indicating fairly rapid absorption of chloramphenicol. High blood levels, however, were not sustained for 24 hours. Nevertheless, high initial blood levels of a drug are often desirable in antibiotic therapy.

Chloramphenicol given intramuscularly in single doses of 10 mg, 20 mg and 30 mg per kg body weight produced blood levels that were sustained for periods of atleast 24 hours after injection. This indicates that the drug is probably absorbed slowly over a long period of time and may,

in part, account for the excellent therapeutic effect (Eads and Van Nocker, 1955 and Barnes, 1955).

McLean et al. (1949) have shown that while most chloramphenicol sensitive bacteria are succeptible to concentrations of 10 mcg per ml, majority are susceptible to concentrations as low as 2.5 mcg per ml.

After an intramuscular dose of 20 mg per kg body weight therapeutic concentration of the antibiotic were readily produced and maintained in blood between second and seventh hour in goats, second and seventeenth hour in calves. After an intramuscular dose of 30 mg per kg body weight, therapeutic concentrations were produced and maintained in blood for 2 to 11 hours in goats and 2 to 19 hours in calves. Therapeutic concentrations were produced and maintained in plasma of goats between second and eleventh hour in goats and second and twenty fourth hour in calves after an intramuscular dose of 20 mg per kg body weight. When given an intramuscular dose of 30 mg per kg body weight of chloramphenicol, the therapeutic concentrations in plasma were produced and maintained for 2 to 15 hours in goats and 2 to 14 hours in calves.

In the present study on calves slightly higher and prolonged blood levels could be obtained over that of the findings of Sisodia et al. (1973 a).

Sisodia et al. (1973 c) reported that periodic rises in blood levels were observed in cattle due to entero-hepatic cycle. But in the present study such elevation in blood levels were not observed both in goats and calves.

After a dose of 30 mg per kg body weight chloramphenicol residues fell below detectable levels in blood by 40 hours after medication in goats and 48 hours after medication in calves. The present observation agrees with the reports of Eads et al. (1955) and Sisodia et al. (1973 c). Eads et al. (1952) reported that the levels were roughly proportional to dosage.

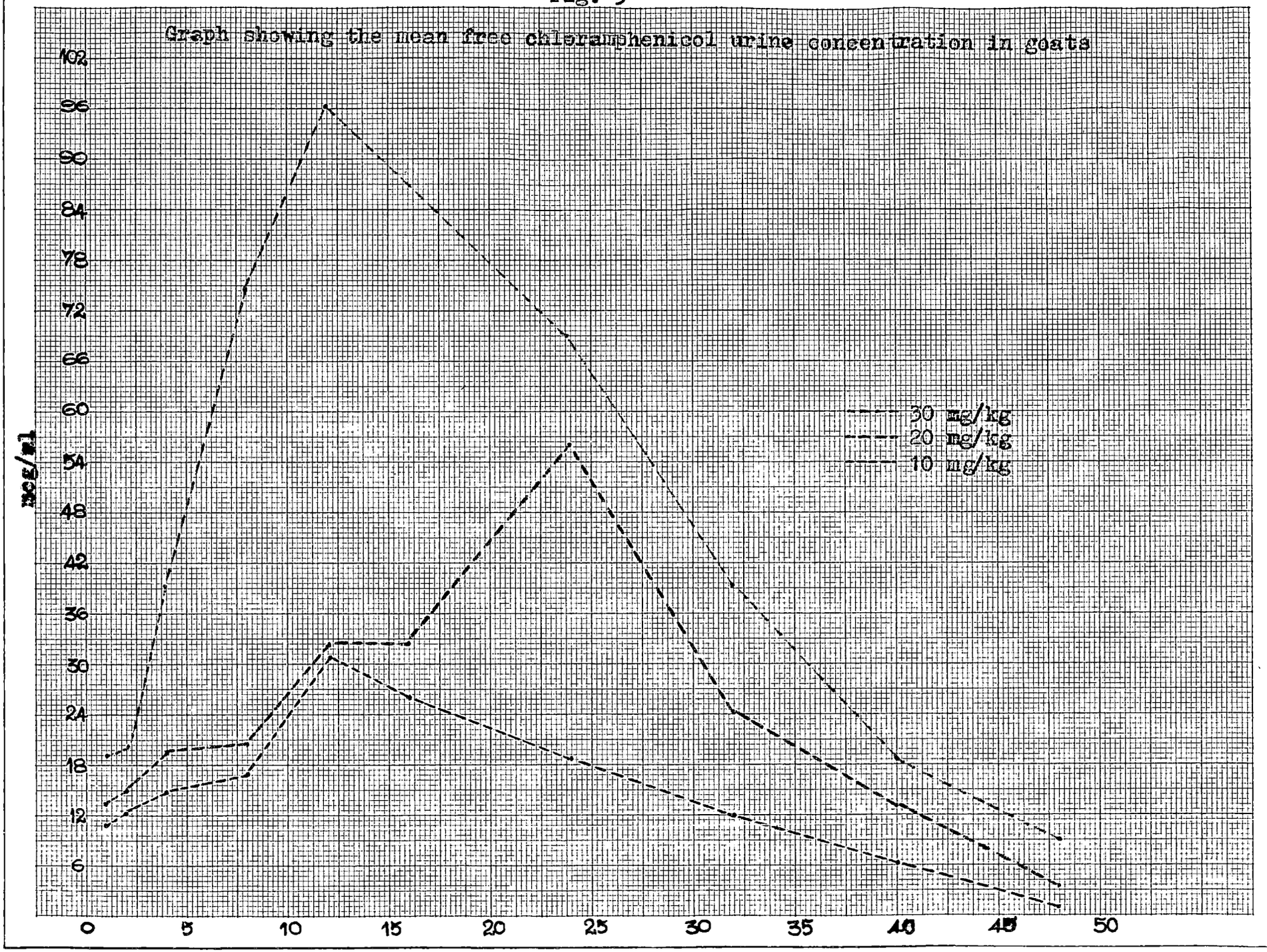
Excretion of chloramphenicol

Table VII a to IX a and Fig. 5 gives the free and total chloramphenicol concentration in urine of 6 goats at different time intervals after the intramuscular administration of 10 mg per kg, 20 mg per kg and 30 mg per kg body weight of chloramphenicol.

The intramuscular administration of 10 mg per kg body weight of chloramphenicol resulted in a maximum mean free chloramphenicol concentration of 31.9 mcg per ml of urine and total concentration of 294.1 mcg per ml.

The intramuscular administration of 20 mg per kg body weight of chloramphenicol resulted in a maximum mean

Fig. 5


Graph showing the mean free chloramphenicol urine concentration in goats
mcg/ml
102
96
90
84
78
72
66
60
54
48
42
36
30
24
18
12
6
0
5
10
15
20
25
30
35
40
45
50
Hours
30 mg/kg
20 mg/kg
10 mg/kg

free chloramphenicol urine concentration of 56.0 mcg and total concentration of 542.4 mcg per ml.

The intramuscular injection of 30 mg per kg body weight of chloramphenicol resulted in a maximum mean free urine concentration of 97.0 mcg per ml and total concentration of 919.8 mcg per ml. The drug persisted in urine for more than 48 hours and the therapeutic concentration was maintained more than 24 hours at all the three dose levels.

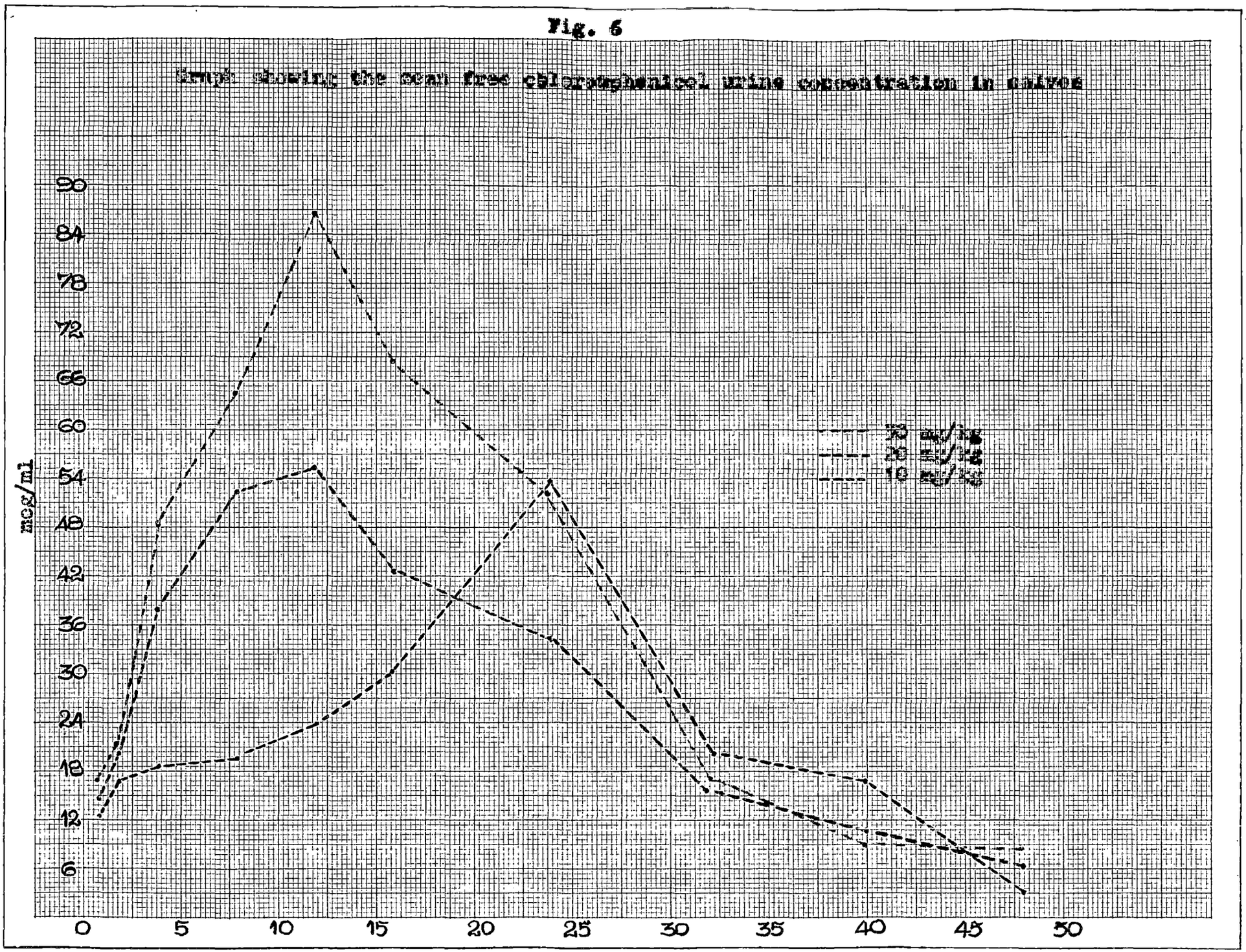
Table VII b to IX b and Fig. 6 gives the free and total chloramphenicol concentration in the urine of 6 calves at different time intervals after the intramuscular administration of 10 mg, 20 mg and 30 mg per kg body weight of chloramphenicol.

The intramuscular administration of 10 mg per kg body weight of chloramphenicol resulted in a maximum mean free chloramphenicol urine concentration of 59.3 mcg per ml and total chloramphenicol concentration of 613.3 mcg per ml.

The intramuscular administration of 20 mg per kg body weight of chloramphenicol resulted in a maximum mean free chloramphenicol urine concentration of 55.1 mcg per ml and total concentration of 568.5 mcg per ml.

The intramuscular administration of 30 mg per kg body weight of chloramphenicol resulted in a maximum mean

Fig. 6


Graph showing the oral free chloramphenicol urine concentration in calves
90
84
78
72
66
60
54
48
42
36
30
24
18
12
6
mcg/ml
0
5
10
15
20
25
30
35
40
45
50
Hours

free chloramphenicol concentration of 87.1 mcg and total concentration of 876.8 mcg per ml.

The drug persisted in urine for more than 48 hours and therapeutic concentration was maintained for more than 24 hours at all the three dose levels.

High concentrations of chloramphenicol in urine have been reported in man, dog and swine, and in this respect, cattle seem to resemble these species for the excretion of chloramphenicol (English and Seawright, 1961; Glazko et al., 1949 a and Sisodia et al., 1973 a).

From the results obtained, it is found that the concentrations of the drug in urine were higher and persisted for more than 48 hours in all the three doses both in goats and calves. The observations of English and Withy (1959) shows that the urine concentration in horses were higher and it persisted only for 24 to 30 hours after intramuscular injection of chloramphenicol. These differences in the rate of elimination of chloramphenicol were most likely related to differences in rates of biotransformation existing among the various species.

Findings of Glazko et al. (1949 a) and Shrotriya and Sharma (1970) established that bulk of the drug is excreted unchanged. The present study is also in full agreement with above findings.

Experiment II

Toxicity of chloramphenicol

Effects of 100 mg per kg body weight of chloramphenicol given intravenously at 24 hours intervals for 30 days.

Table X and XI give the pretreatment and post-treatment values for rectal temperature, pulse, respiration and blood constituents of calf numbers 056 and 419 respectively.

The calves showed acute "shock-like" symptoms immediately after the intravenous injection of chloramphenicol. The rate of respiration became very rapid and laboured. The pulse rate also increased and gradually returned to normal in about 10 minutes. The calves were unable to stand voluntarily for about 5 to 10 minutes after the injection. Further the animals evinced staggering gait and were unable to walk normally. After a lapse of 15 minutes they were found quite normal. The results of the examination of urine for pathological constituents and the estimated free and total chloramphenicol levels in blood are also tabulated (table X and XI).

Perusal of the data collected and tabulated on the haematological parameters such as the erythrocyte count,

differential leucocyte count, erythrocyte sedimentation rate, packed cell volume and haemoglobin percentage of blood revealed that they were within the normal range for the species.

The urea content of blood remained within the normal range for the species throughout the period of 30 days of observations.

Examination of urine did not reveal the presence of any pathologic constituents for the entire period of 30 days under observation.

Autopsy lesions

Postmortem conducted on one calf had the following lesions:-

Gross lesions: Examination of the heart revealed hydropericardium. The pericardium contained 350 ml of pale, yellow fluid. Gall bladder distended with thin, pale yellow bile. Slight ventricular dilatation was noticed on the left side of the heart.

There were few tape worms in the intenstine and setaria in the peritoneal cavity.

Histopathologic lesions: Liver showed focal areas of granular degeneration, necrosis and hepatocytomegaly. In isolated areas there were regenerating hepatic cells (Plate I).

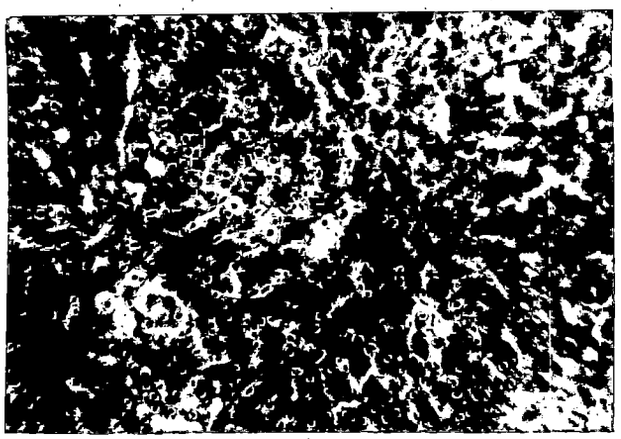
There were focal areas of tubular degeneration and desquamation of epithelial lining. Eosinophilic granulations were noticed filling the renal tubules (Plate I).

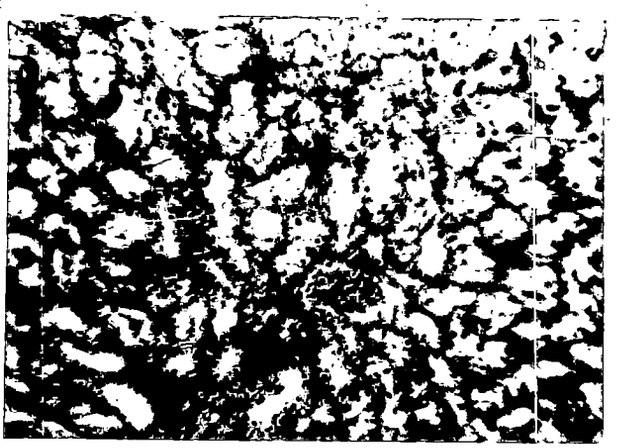
The myocardium showed focal granular degeneration. There were moderate interstitial oedema (Plate I).

Extensive pathological and toxicological studies have shown chloramphenicol to be relatively non toxic and well tolerated in domestic animals. The toxic symptoms observed after the intravenous administration of chloramphenicol in this experiment is almost identical to that reported by Gruhzit et al.(1949) in dog. The immediate toxic symptoms as evidenced by laboured respiration and elevated pulse rate might be due to a "shock like" effect induced by the drug.

In man, toxicity of chloramphenicol has been mainly aplastic anaemia induced by prolonged administration of the drug (Rheingold et al., 1952; Wilson et al., 1952; Claudon and Holdbrook, 1952; Volini et al., 1950 and Best, 1967). However, the present study on calves did not suggest the occurrence of aplastic anaemia. Reutner and eads (1952) administered chloramphenicol to dogs either orally or intravenously in doses of 50 to 200 mg per kg body weight twice daily for one month or longer without any evidence of blood dyscrasia.

PLATE I





Following exaggerated oral doses of chloramphenicol in monkeys and baboons, there were no apparent drug-related changes in the peripheral blood (Glazko et al., 1949 a).

Penny et al. (1967) observed changes in peripheral blood and bone marrow of cats given chloramphenicol for 21 days in a daily dose of 50 mg per kg body weight intramuscularly.

Barnes (1955) reported that there were no signs of irritation, discomfort or toxicity in any of the 200 calves receiving intramuscular injections of chloremphenicol in doses of 1 to 2 g per day for 2 to 8 days.

In recommended doses, no dogs receiving chloramphenicol orally had significant changes in total white blood cell or differential counts (Smith et al., 1948).

With the limited toxicity study conducted in calves, it can only be stated that 100 mg per kg body weight of chloramphenicol given intravenously for 30 days did not give rise to acute toxicity eventhough kidney, liver, and heart showed moderate lesions. Thus, it appears that further detailed study would be necessary to completely assess the chronic toxicity of chloramphenicol in domestic animals.

S U M M A R Y

S U M M A R Y

I. Intramuscular injections of chloramphenicol (Vetycetine T.C.F.) were given at 10 mg per kg, 20 mg per kg and 30 mg per kg body weight in both goats and calves. Single dose of chloramphenicol was given to six animals and the whole blood and plasma levels of free and total chloramphenicol were estimated by the method of Bratton and Marshall (1939), at time intervals of first hour, second hour, fourth hour, eighth hour, twelfth hour, sixteenth hour, twenty fourth hour, thirty second hour, fortieth hour and forty eighth hour after administration. The free and total chloramphenicol levels in urine were also estimated at intervals mentioned above for 48 hours. The data obtained revealed that:

(1) The dose of 10 mg per kg body weight when given intramuscularly failed to produce therapeutic concentrations (5 mcg/ml) in whole blood of goats and calves. However, therapeutic concentrations were detected in plasma of both goats and calves. After a dose of 20 mg per kg body weight intramuscularly, the therapeutic concentrations were readily produced and maintained in blood between second and seventh hour in

goats, second and seventeenth hour in calves. At 30 mg per kg body weight the therapeutic concentration in whole blood persisted between second and eleventh hour in goats and second and nineteenth hour in calves. The therapeutic concentration in plasma at 20 mg per kg intramuscularly were maintained between second and twelfth hour in goats, second and twenty fifth hour in calves. At 30 mg per kg body weight intramuscularly the therapeutic concentrations in plasma were obtained between second and fourteenth hour in goats and second and twenty fourth hour in calves.

(2) Peak concentrations were obtained both in goats and calves, between second and third hour after the administration of chloramphenicol in all the three dose levels.

(3) In goats and calves, no detectable levels of chloramphenicol in blood could be found at the forty eighth hour after medication.

(4) The concentrations were higher in urine than in plasma and a therapeutic concentration of 5 mcg per ml was found to persist for 24 hours in all the three dose levels.

(5) Residues of chloramphenicol continued to persist in urine for more than 48 hours.

II. In a limited attempt made to study the toxicity of chloramphenicol in two calves, chloramphenicol (vetycetine T.C.F.) was given in a dose of 100 mg per kg body weight daily for 30 days. The blood and urine samples were collected at 3 day intervals and examined for pathological changes. Postmortem examination at the end of the experiment was also conducted in one calf. Following observations were made.

(1) The intravenous injection of 100 mg per kg body weight of chloramphenicol did not produce acute toxicity.

(2) Transient "shock like" symptoms for 10 to 15 minutes were noticed soon after intravenous administration of the drug.

(3) Alterations in the blood constituents were found to be well within the normal range for calves.

(4) Liver, kidney and heart showed moderate lesions.

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T A B L E S

TABLE I a

Chloramphenicol 10 mg per kg body weight intramuscular

Whole blood concentration of chloramphenicol in goats

(values expressed in mcg/ml)

Goat No.		Time post administration (hours)									
		1	2	4	8	12	16	24	32	40	48
1	Total	2.8	4.6	2.8	2.6	1.8	1.6	1.0	0.0	0.0	0.0
	Free	2.0	3.2	2.2	1.6	1.0	0.0	0.0	0.0	0.0	0.0
2	Total	3.2	3.8	3.0	2.8	2.4	1.8	1.2	0.0	0.0	0.0
	Free	2.2	3.0	2.6	1.8	1.2	0.0	0.0	0.0	0.0	0.0
3	Total	2.8	4.0	2.0	2.0	1.8	1.4	0.0	0.0	0.0	0.0
	Free	1.2	3.4	1.6	1.4	1.0	1.0	0.0	0.0	0.0	0.0
4	Total	2.0	4.6	2.2	2.2	1.6	1.2	0.0	0.0	0.0	0.0
	Free	1.2	3.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
5	Total	1.8	3.4	2.6	2.4	1.8	1.2	1.0	0.0	0.0	0.0
	Free	1.0	2.6	1.8	1.4	1.0	0.0	0.0	0.0	0.0	0.0
6	Total	2.6	4.2	2.8	2.6	1.8	1.2	1.2	0.0	0.0	0.0
	Free	1.8	2.8	1.6	1.4	0.0	0.0	0.0	0.0	0.0	0.0
Average											
	Total	2.53	4.10	2.56	2.43	1.86	1.40	0.73	0.00	0.00	0.00
	Free	1.56	3.00	1.80	1.43	0.70	0.16	0.00	0.00	0.00	0.00

TABLE II a

Chloramphenicol 20 mg per kg body weight intramuscular

Whole blood concentration of chloramphenicol in goats

(values expressed in mcg/ml)

Goat No.		Time post administration (hours)									
		1	2	4	8	12	16	24	32	40	48
7	Total	7.8	10.4	7.8	3.8	2.6	2.0	1.6	1.0	0.0	0.0
	Free	5.8	7.6	6.6	2.0	1.8	1.2	1.0	0.0	0.0	0.0
8	Total	6.0	9.6	10.2	6.8	3.2	1.8	1.0	0.0	0.0	0.0
	Free	4.6	6.8	7.6	3.8	2.0	1.0	0.0	0.0	0.0	0.0
9	Total	7.6	10.0	10.4	8.0	4.2	2.6	1.2	0.0	0.0	0.0
	Free	5.0	7.8	6.6	7.2	3.2	1.2	1.0	0.0	0.0	0.0
10	Total	5.8	9.6	10.0	7.6	3.8	2.0	1.4	0.0	0.0	0.0
	Free	4.6	6.0	7.0	6.2	1.8	1.2	1.0	0.0	0.0	0.0
1	Total	6.6	9.6	8.4	6.4	3.0	1.8	1.0	0.0	0.0	0.0
	Free	4.4	6.2	6.8	4.8	2.2	0.0	0.0	0.0	0.0	0.0
2	Total	5.8	7.6	8.2	6.4	3.2	2.2	1.4	0.0	0.0	0.0
	Free	4.0	5.2	7.2	4.0	1.8	1.2	1.0	0.0	0.0	0.0
Average	Total	6.60	9.46	9.16	6.46	3.33	2.06	1.26	0.16	0.00	0.00
	Free	4.73	6.60	7.30	4.63	2.13	0.96	0.66	0.00	0.00	0.00

TABLE III a

Chloramphenicol 30 mg per kg body weight intramuscular

Whole blood concentration of chloramphenicol in goats

(values expressed in mcg/ml)

Goat No.		Time post administration (hours)									
		1	2	4	8	12	16	24	32	40	48
3	Total	12.8	15.2	15.6	10.8	6.4	4.6	3.0	1.0	0.0	0.0
	Free	9.4	11.6	11.8	7.6	5.6	3.0	1.8	0.0	0.0	0.0
4	Total	10.0	13.4	14.2	11.0	7.6	3.8	2.6	0.0	0.0	0.0
	Free	7.6	12.4	11.6	8.0	6.0	2.8	1.0	0.0	0.0	0.0
5	Total	12.8	15.0	14.8	11.4	5.8	4.0	2.8	1.2	0.0	0.0
	Free	11.4	13.0	12.0	9.2	4.8	2.8	1.2	0.0	0.0	0.0
6	Total	9.6	13.2	13.2	10.4	5.8	3.6	1.8	1.0	0.0	0.0
	Free	8.0	12.6	11.8	8.0	4.0	2.0	0.0	0.0	0.0	0.0
7	Total	10.8	12.6	13.0	10.8	4.8	4.8	2.0	0.0	0.0	0.0
	Free	7.6	11.4	11.8	6.8	3.2	3.2	1.8	0.0	0.0	0.0
8	Total	11.6	11.8	12.0	9.8	7.0	3.8	3.2	1.8	0.0	0.0
	Free	9.8	10.8	11.6	6.0	3.0	3.2	1.6	0.0	0.0	0.0
Average	Total	11.26	13.53	13.80	10.70	6.23	4.10	2.56	0.83	0.00	0.00
	Free	8.96	12.00	11.76	7.60	4.43	2.80	1.23	0.00	0.00	0.00

TABLE I b

Chloramphenicol 10 mg per kg body weight intramuscular

Whole blood concentration of chloramphenicol in calves

(values expressed in mcg/ml)

Calf No.		Time post administration (hours)									
		1	2	4	8	12	16	24	32	40	48
1	Total	3.8	6.2	5.0	4.2	3.6	2.3	1.8	0.0	0.0	0.0
	Free	3.0	4.8	4.8	3.6	3.0	2.0	1.0	0.0	0.0	0.0
2	Total	4.2	6.2	5.4	4.6	3.0	2.6	1.6	0.0	0.0	0.0
	Free	3.4	4.6	4.6	3.4	2.8	2.0	1.0	0.0	0.0	0.0
3	Total	3.4	5.4	4.8	4.0	3.2	2.8	1.8	0.0	0.0	0.0
	Free	2.8	4.6	4.0	3.6	2.6	2.0	1.2	0.0	0.0	0.0
4	Total	3.6	6.8	4.2	4.0	3.2	2.2	1.0	0.0	0.0	0.0
	Free	3.2	6.0	3.8	3.6	2.6	1.8	0.0	0.0	0.0	0.0
5	Total	3.0	5.4	4.8	3.8	2.4	2.0	1.2	0.0	0.0	0.0
	Free	2.8	4.4	4.2	3.0	1.8	1.4	1.0	0.0	0.0	0.0
6	Total	4.0	6.2	5.2	4.2	3.4	2.6	1.0	0.0	0.0	0.0
	Free	3.6	5.0	4.2	3.6	2.6	1.6	0.0	0.0	0.0	0.0
Average											
	Total	3.66	6.03	4.30	4.13	3.13	2.50	1.40	0.00	0.00	0.00
	Free	3.13	4.90	4.26	3.46	2.56	1.80	0.70	0.00	0.00	0.00

TABLE II b

Chloramphenicol 20 mg per kg body weight intramuscular

Whole blood concentration of chloramphenicol in calves

(values expressed in mcg/ml)

Calf No.		Time post administration (hours)									
		1	2	4	8	12	16	24	32	40	48
7	Total	7.8	13.2	15.8	12.0	10.2	8.6	5.4	2.4	1.0	0.0
	Free	6.0	10.6	13.8	10.6	7.6	5.6	3.8	1.8	0.0	0.0
8	Total	6.8	12.6	16.0	13.2	11.0	7.8	4.4	1.8	0.0	0.0
	Free	5.2	11.0	14.2	9.8	6.8	7.0	2.6	0.0	0.0	0.0
9	Total	7.6	13.0	14.8	12.4	9.6	6.8	4.8	2.6	1.0	0.0
	Free	7.4	11.4	12.6	8.4	7.0	5.2	2.6	1.2	0.0	0.0
10	Total	8.6	14.0	15.8	13.2	8.6	7.4	4.2	1.8	0.0	0.0
	Free	7.0	12.6	14.0	9.8	7.8	5.2	2.0	0.0	0.0	0.0
1	Total	8.6	11.6	13.4	12.2	9.6	6.8	4.0	2.6	1.0	0.0
	Free	6.8	9.6	12.0	10.0	6.4	4.8	1.8	1.0	0.0	0.0
2	Total	9.2	13.6	14.8	12.6	11.2	7.6	5.2	1.2	1.0	0.0
	Free	7.4	11.2	13.0	11.2	9.0	5.2	2.6	0.0	0.0	0.0
Average											
	Total	8.00	13.00	14.93	12.60	10.03	7.50	4.66	2.06	0.66	0.00
	Free	6.63	11.06	13.26	9.96	7.43	5.50	2.56	0.66	0.00	0.00

TABLE III b

Chloramphenicol 30 mg per kg body weight intramuscular

Whole blood concentration of chloramphenicol in calves

(values expressed in mcg/ml)

Calf No.		Time post administration (hours)									
		1	2	4	8	12	16	24	32	40	48
3	Total	13.6	15.2	16.0	12.8	10.2	6.8	4.8	3.2	1.8	0.0
	Free	12.6	14.8	15.6	9.6	7.0	4.6	3.4	2.0	0.0	0.0
4	Total	12.2	14.6	15.8	13.6	9.6	7.0	5.0	2.8	1.0	0.0
	Free	11.8	12.0	15.0	10.8	6.8	5.2	3.2	1.2	0.0	0.0
5	Total	14.8	16.2	17.2	14.2	10.8	8.0	4.4	2.6	1.2	0.0
	Free	13.2	14.0	15.6	11.0	8.2	7.2	3.0	1.4	0.0	0.0
6	Total	13.6	14.2	16.8	13.8	11.0	6.2	4.2	3.0	1.6	0.0
	Free	11.8	13.0	14.8	10.6	8.6	5.8	2.8	2.2	0.0	0.0
7	Total	12.0	13.8	17.0	16.0	11.6	7.2	4.8	2.4	1.0	0.0
	Free	10.8	12.2	14.6	13.6	8.4	6.0	3.0	1.6	0.0	0.0
8	Total	13.2	14.0	15.2	14.2	9.8	6.8	5.0	3.2	1.8	0.0
	Free	12.0	12.8	14.4	10.8	7.6	5.2	3.2	2.0	0.0	0.0
Average	Total	13.23	14.66	16.33	14.10	10.50	7.00	4.70	2.86	1.40	0.00
	Free	12.00	13.13	15.00	11.06	7.76	5.66	3.10	1.73	0.00	0.00

TABLE IV a

Chloramphenicol 10 mg per kg body weight intramuscular

Plasma concentration of chloramphenicol in goats

(values expressed in mcg/ml)

Goat No.		Time post administration (hours)									
		1	2	4	8	12	16	24	32	40	48
1	Total	5.8	7.6	5.2	5.2	2.8	2.4	1.8	0.0	0.0	0.0
	Free	4.6	6.8	4.0	3.8	1.2	1.0	0.0	0.0	0.0	0.0
2	Total	4.6	7.8	5.6	5.4	2.0	1.8	1.0	0.0	0.0	0.0
	Free	3.8	6.6	4.2	3.6	1.8	0.0	0.0	0.0	0.0	0.0
3	Total	6.2	7.6	5.2	4.8	2.2	1.0	1.0	0.0	0.0	0.0
	Free	4.2	5.8	4.4	3.0	1.2	0.0	0.0	0.0	0.0	0.0
4	Total	5.8	6.8	4.6	4.4	2.2	1.4	1.2	0.0	0.0	0.0
	Free	3.6	4.2	3.4	2.4	1.2	0.0	0.0	0.0	0.0	0.0
5	Total	6.0	6.4	5.6	5.0	2.6	2.0	1.2	0.0	0.0	0.0
	Free	4.2	5.0	3.8	3.2	1.4	1.0	0.0	0.0	0.0	0.0
6	Total	5.0	6.2	4.8	4.2	2.8	2.6	1.0	0.0	0.0	0.0
	Free	3.4	4.2	3.2	2.8	1.6	1.2	0.0	0.0	0.0	0.0
Average											
	Total	5.56	7.06	5.20	4.86	2.43	1.86	1.20	0.00	0.00	0.00
	Free	3.96	5.43	3.83	3.13	1.40	0.53	0.00	0.00	0.00	0.00

TABLE V a

Chloramphenicol 20 mg per kg body weight intramuscular

Plasma concentration of chloramphenicol in goats

(values expressed in mcg/ml)

Goat No.		Time post administration (hours)									
		1	2	4	8	12	16	24	32	40	48
7	Total	8.2	12.8	10.8	7.6	7.6	6.8	4.2	1.8	0.0	0.0
	Free	6.8	9.0	7.6	5.4	5.0	5.2	3.2	1.0	0.0	0.0
8	Total	9.6	12.2	15.2	10.0	6.2	5.2	3.6	2.0	0.0	0.0
	Free	7.6	11.0	12.8	7.6	4.8	3.4	2.8	1.0	0.0	0.0
9	Total	7.8	11.0	12.6	9.8	7.0	4.2	2.6	1.4	0.0	0.0
	Free	6.7	8.2	11.0	6.7	5.2	3.2	1.8	0.0	0.0	0.0
10	Total	9.8	11.8	11.8	8.6	6.4	3.8	2.0	1.6	0.0	0.0
	Free	7.0	9.6	9.8	6.0	4.8	2.6	1.2	0.0	0.0	0.0
1	Total	10.6	12.6	12.4	7.0	6.6	6.2	4.0	2.6	0.0	0.0
	Free	7.6	10.2	9.0	6.8	6.0	4.8	2.8	1.0	0.0	0.0
2	Total	8.6	11.4	13.2	8.4	6.2	3.8	3.0	1.8	0.0	0.0
	Free	6.8	10.6	12.8	7.6	5.0	2.6	1.8	0.0	0.0	0.0
Average	Total	9.10	11.96	12.66	8.56	6.66	5.00	3.23	1.86	0.00	0.00
	Free	7.08	9.76	10.50	6.68	5.13	3.63	2.26	0.50	0.00	0.00

TABLE VI a

Chloramphenicol 30 mg per kg body weight intramuscular

Plasma concentration of chloramphenicol in goats

(values expressed in mcg/ml)

Goat No.		Time post administration (hours)									
		1	2	4	8	12	16	24	32	40	48
3	Total	12.8	15.6	17.6	11.6	8.6	5.2	4.8	2.4	0.0	0.0
	Free	8.6	10.2	12.4	8.2	6.8	4.0	3.2	1.8	0.0	0.0
4	Total	11.6	14.2	15.2	11.4	8.0	5.6	3.4	2.0	0.0	0.0
	Free	8.2	9.6	11.2	8.4	7.2	3.8	2.9	1.0	0.0	0.0
5	Total	13.6	15.6	16.2	12.4	7.8	6.6	5.2	3.6	0.0	0.0
	Free	9.2	10.8	13.4	9.6	6.4	4.4	4.2	2.0	0.0	0.0
6	Total	12.8	14.2	14.4	10.6	8.6	5.4	5.2	2.8	0.0	0.0
	Free	10.2	11.6	12.0	8.2	6.0	4.2	4.0	1.0	0.0	0.0
7	Total	11.4	13.6	14.8	12.2	9.2	6.8	5.2	2.6	0.0	0.0
	Free	10.0	11.6	12.4	9.4	6.2	5.4	3.8	1.2	0.0	0.0
8	Total	12.2	13.6	17.6	10.8	8.8	5.8	4.6	1.8	0.0	0.0
	Free	10.4	10.8	14.8	8.4	6.0	4.2	3.8	1.0	0.0	0.0
Average											
	Total	12.40	14.46	15.96	11.46	8.50	5.90	4.73	2.53	0.00	0.00
	Free	9.43	10.76	12.66	8.70	6.43	4.33	3.65	1.33	0.00	0.00

TABLE IV b

Chloramphenicol 10 mg per kg body weight intramuscular

Plasma concentration of chloramphenicol in calves

(values expressed in mcg/ml)

Calf No.		Time post administration (hours)									
		1	2	4	8	12	16	24	32	40	48
1	Total	4.8	7.8	5.2	4.8	3.6	3.0	2.6	1.2	0.0	0.0
	Free	4.6	6.6	4.8	3.2	2.8	2.2	2.0	0.0	0.0	0.0
2	Total	4.2	7.4	5.8	4.4	3.0	2.4	1.8	0.0	0.0	0.0
	Free	3.8	6.0	4.6	3.8	2.4	2.0	1.2	0.0	0.0	0.0
3	Total	4.8	7.6	5.0	4.8	3.6	2.8	2.0	1.0	0.0	0.0
	Free	4.2	6.8	4.2	4.0	2.8	2.2	1.0	0.0	0.0	0.0
4	Total	3.8	7.0	5.2	4.0	3.2	2.4	1.6	0.0	0.0	0.0
	Free	3.2	6.2	5.0	3.2	2.4	1.8	1.0	0.0	0.0	0.0
5	Total	4.0	7.2	5.6	4.8	3.4	2.0	1.8	1.0	0.0	0.0
	Free	3.4	6.4	3.8	3.8	2.4	1.6	1.2	0.0	0.0	0.0
6	Total	3.6	6.8	5.4	4.2	3.6	2.0	1.0	0.0	0.0	0.0
	Free	2.8	5.0	4.8	3.8	3.2	2.0	0.0	0.0	0.0	0.0
Average											
	Total	4.20	7.30	5.36	4.50	3.40	2.43	1.80	0.53	0.00	0.00
	Free	3.66	6.16	4.53	3.63	2.66	1.96	1.06	0.00	0.00	0.00

TABLE V b

Chloramphenicol 20 mg per kg body weight intramuscular

Plasma concentration of chloramphenicol in calves

(values expressed in mcg/ml)

Calf No.		Time post administration (hours)									
		1	2	4	8	12	16	24	32	40	48
7	Total	10.2	15.6	17.6	12.8	12.0	11.6	10.2	4.8	2.2	0.0
	Free	6.8	9.6	11.4	10.6	9.6	8.2	7.2	3.0	1.0	0.0
8	Total	11.8	17.6	20.2	15.2	13.6	10.8	7.6	3.4	1.8	0.0
	Free	8.4	12.4	17.6	14.0	11.0	9.6	5.8	2.0	1.0	0.0
9	Total	8.4	12.8	16.4	13.2	12.2	9.6	6.0	2.8	1.0	0.0
	Free	8.0	10.4	14.6	11.4	12.0	7.8	5.6	1.0	0.0	0.0
10	Total	10.4	11.8	14.8	12.6	11.8	9.8	5.4	3.2	2.0	0.0
	Free	9.4	10.0	13.6	11.0	9.8	7.0	4.8	2.6	1.0	0.0
1	Total	11.0	14.6	14.8	12.8	10.6	8.8	4.2	1.8	1.0	0.0
	Free	10.4	13.8	13.8	12.0	10.8	6.6	3.4	1.0	0.0	0.0
2	Total	10.8	15.6	18.2	13.8	9.8	7.6	5.6	2.8	1.2	0.0
	Free	7.6	12.8	14.8	11.0	7.2	5.8	4.0	2.0	0.0	0.0
Average	Total	10.43	14.66	17.00	13.40	11.66	9.70	6.50	3.13	1.53	0.00
	Free	8.43	11.50	14.30	11.66	9.56	7.50	5.13	1.93	0.50	0.00

TABLE VI b

Chloramphenicol 30 mg per kg body weight intramuscular

Plasma concentration of chloramphenicol in calves

(values expressed in mcg/ml)

Calf No.		Time post administration (hours)									
		1	2	4	8	12	16	24	32	40	48
3	Total	17.6	19.8	16.6	14.8	11.6	9.6	5.6	3.8	1.8	0.0
	Free	16.8	18.6	15.2	13.2	9.6	7.2	5.0	2.6	1.0	0.0
4	Total	18.2	21.2	18.6	17.4	13.2	10.2	6.8	3.2	1.6	0.0
	Free	17.0	19.2	16.0	15.2	10.4	8.0	5.0	2.0	0.0	0.0
5	Total	16.2	20.2	18.2	16.2	14.6	9.8	7.6	4.8	2.6	1.0
	Free	16.8	18.8	17.2	14.8	10.8	7.2	4.0	3.2	1.8	0.0
6	Total	17.2	19.4	16.0	15.8	13.6	7.8	5.4	4.0	1.8	0.0
	Free	16.2	17.8	15.6	13.6	11.0	5.2	4.2	2.8	1.0	0.0
7	Total	17.6	21.2	17.0	14.8	13.0	9.2	5.4	2.8	1.0	0.0
	Free	15.8	17.2	16.2	13.2	9.2	7.0	4.6	2.2	0.0	0.0
8	Total	19.0	21.0	17.6	13.8	12.6	9.0	5.0	3.0	1.2	0.0
	Free	17.2	20.0	15.2	12.8	8.8	8.2	3.8	1.8	0.0	0.0
Average											
	Total	17.63	20.46	17.33	15.46	13.10	9.26	5.96	3.60	1.66	0.00
	Free	16.46	18.60	15.90	13.80	9.96	8.06	4.43	2.43	0.63	0.00

TABLE VII a

Chloramphenicol 10 mg per kg body weight intramuscular

Urine concentration of chloramphenicol in goats

(values expressed in mcg/ml)

Goat No.		Time post administration (hours)									
		1	2	4	8	12	16	24	32	40	48
1	Total	113.6	124.0	160.2	178.4	321.0	262.4	212.4	123.2	68.4	22.0
	Free	12.4	13.6	18.8	21.6	32.0	28.4	24.2	13.2	6.4	1.8
2	Total	106.2	112.4	150.8	164.2	308.0	240.0	202.2	112.8	72.0	21.0
	Free	11.8	16.4	17.2	18.8	34.6	24.8	18.2	11.8	9.6	1.6
3	Total	98.2	126.0	145.8	156.8	302.4	246.6	203.8	120.6	64.8	17.2
	Free	9.6	14.0	14.8	16.6	31.2	28.6	18.0	12.0	6.8	1.0
4	Total	108.4	124.2	128.4	145.8	284.6	252.2	196.0	120.4	58.2	20.0
	Free	12.8	13.2	13.4	17.2	32.6	27.2	16.8	12.0	5.8	2.6
5	Total	106.0	114.4	132.0	148.6	264.2	238.4	206.4	118.2	62.8	16.2
	Free	10.6	11.4	14.2	16.8	30.0	24.2	19.4	13.2	6.6	1.2
6	Total	110.0	122.4	133.8	164.0	284.8	264.2	198.4	106.8	56.2	18.2
	Free	9.6	14.2	14.0	16.4	31.2	24.2	21.2	11.6	6.0	1.0
Average	Total	107.06	120.56	141.83	159.63	294.10	250.63	203.20	117.00	63.73	19.10
	Free	11.13	13.80	15.40	17.90	31.93	26.23	19.60	12.30	6.86	1.53

TABLE VIII a

Chloramphenicol 20 mg per kg body weight intramuscular

Urine concentration of chloramphenicol in goats

(values expressed in mcg/ml)

Goat No.		Time post administration (hours)									
		1	2	4	8	12	16	24	32	40	48
7	Total	124.2	147.4	160.8	197.2	272.6	310.2	563.2	264.2	116.0	22.0
	Free	14.8	16.8	21.2	23.8	32.6	38.2	58.0	29.4	13.6	2.6
8	Total	138.0	157.4	169.0	178.4	237.2	284.8	538.4	217.0	122.8	42.6
	Free	16.6	17.6	19.0	21.2	38.4	29.6	56.6	24.6	13.2	3.8
9	Total	117.6	142.2	173.4	176.0	228.4	280.0	524.6	206.4	128.6	37.0
	Free	10.0	14.8	22.6	21.0	32.6	28.2	53.8	23.2	15.2	4.0
10	Total	126.4	130.8	164.2	182.4	245.2	268.6	542.0	188.8	134.2	28.2
	Free	13.8	14.0	19.8	20.8	29.4	29.6	52.0	20.6	13.2	2.8
1	Total	141.2	152.8	182.4	198.0	261.2	282.2	559.0	231.0	122.0	29.0
	Free	16.2	16.8	19.0	22.6	32.0	32.2	58.2	24.6	13.8	3.2
2	Total	128.2	137.8	164.2	188.8	246.2	289.0	527.6	232.2	160.0	47.6
	Free	12.8	15.2	18.8	21.8	28.6	34.6	57.6	26.2	17.8	4.4
Average											
	Total	129.26	144.73	169.00	186.80	248.46	285.80	542.46	223.26	130.60	34.40
	Free	14.03	15.86	20.00	21.86	32.26	32.06	56.03	24.76	14.46	3.46

TABLE IX a

Chloramphenicol 30 mg per kg body weight intramuscular

Urine concentration of chloramphenicol in goats

(values expressed in mcg/ml)

Goat No.		Time post administration (hours)									
		1	2	4	8	12	16	24	32	40	48
3	Total	178.6	192.6	482.6	825.0	925.6	964.6	801.4	410.2	185.2	68.4
	Free	22.2	21.2	45.4	89.2	101.8	102.2	81.6	44.0	24.4	12.8
4	Total	154.2	183.4	395.0	745.2	945.0	895.2	760.2	460.6	195.2	85.6
	Free	19.8	28.2	31.8	73.4	92.6	92.4	78.0	46.8	22.2	9.6
5	Total	186.4	199.8	420.2	765.0	867.2	803.2	734.2	395.8	165.4	120.0
	Free	18.4	19.6	48.0	66.6	91.6	89.8	74.2	38.6	18.6	12.2
6	Total	176.2	192.6	465.2	783.2	933.0	865.6	760.2	398.0	178.4	99.6
	Free	24.2	24.6	34.8	68.2	99.4	86.4	81.6	41.2	17.0	9.2
7	Total	142.8	176.2	402.4	738.4	923.8	800.6	540.8	365.0	164.2	78.6
	Free	21.0	22.4	38.2	71.6	102.4	87.2	63.6	46.8	14.2	6.0
8	Total	182.4	194.2	420.2	724.0	924.2	769.8	420.8	392.6	175.8	69.4
	Free	17.1	19.2	45.6	83.0	94.6	70.4	40.2	21.0	18.8	8.4
Average	Total	170.10	189.80	430.93	763.46	919.80	863.16	677.93	403.70	177.36	86.93
	Free	20.45	22.53	40.63	75.33	97.06	88.06	69.86	39.73	19.20	9.70

TABLE VII b

Chloramphenicol 10 mg per kg body weight intramuscular

Urine concentration of chloramphenicol in calves

(values expressed in mcg/ml)

Calf No.		Time post administration (hours)									
		1	2	4	8	12	16	24	32	40	48
1	Total	146.6	174.2	192.4	201.4	262.2	324.0	640.2	220.4	126.8	32.8
	Free	15.2	18.0	21.4	22.0	24.6	34.8	60.2	21.6	19.4	3.6
2	Total	152.4	168.4	184.2	197.2	248.6	297.8	624.4	201.8	84.2	28.2
	Free	14.6	21.6	20.6	21.8	25.2	29.6	61.4	19.8	17.6	1.8
3	Total	127.6	151.8	174.2	210.8	238.4	310.6	601.8	208.4	96.8	48.6
	Free	12.2	17.4	16.6	17.6	21.8	34.8	58.4	18.0	17.0	4.8
4	Total	128.0	164.0	187.8	209.4	247.4	311.2	597.4	232.4	90.4	57.2
	Free	10.4	13.6	19.2	19.4	26.6	29.6	50.2	21.8	16.6	5.6
5	Total	131.2	149.6	166.4	199.2	264.0	308.4	604.4	200.6	82.4	41.8
	Free	13.2	15.8	21.6	19.8	27.2	30.8	59.4	24.0	17.6	3.8
6	Total	129.8	152.6	161.6	179.8	227.6	301.4	611.8	217.8	104.6	44.4
	Free	13.0	14.2	18.8	16.6	20.0	31.2	66.4	21.0	14.8	2.2
Average											
	Total	135.93	160.10	177.76	199.60	248.06	308.93	613.33	213.56	97.56	42.16
	Free	13.10	16.76	19.36	19.53	24.23	31.80	59.33	21.03	17.16	3.63

TABLE VIII b

Chloramphenicol 20 mg per kg body weight intramuscular

Urine concentration of chloramphenicol in calves

(values expressed in mcg/ml)

Calf No.		Time post administration (hours)									
		1	2	4	8	12	16	24	32	40	48
7	Total	165.6	204.8	402.6	584.2	563.0	462.4	361.6	160.4	110.2	62.8
	Free	13.4	18.6	44.4	56.2	54.2	41.8	36.0	17.2	11.2	9.5
8	Total	145.0	221.2	410.4	482.6	540.2	432.0	374.6	158.8	118.2	72.4
	Free	17.0	24.8	39.0	40.0	53.2	39.0	32.2	14.8	10.0	7.6
9	Total	162.6	200.8	390.6	520.4	584.8	414.4	382.8	172.0	100.0	70.8
	Free	14.0	18.8	36.0	58.4	54.8	48.4	39.0	16.6	11.6	6.8
10	Total	135.6	210.6	398.6	560.0	594.4	428.8	394.6	174.0	120.0	69.4
	Free	14.6	22.0	38.8	55.8	54.6	46.6	37.8	16.8	12.4	7.2
1	Total	154.8	248.0	413.6	576.6	568.6	433.2	380.4	167.8	98.2	62.4
	Free	16.4	22.2	38.8	52.8	59.2	41.0	37.6	17.6	9.6	6.2
2	Total	152.6	212.6	400.8	529.0	559.6	424.4	352.4	158.8	110.6	59.6
	Free	16.2	21.0	36.4	56.2	54.6	44.4	32.8	15.8	11.6	5.8
Average											
	Total	152.70	216.33	402.76	542.13	568.50	432.60	374.46	165.30	109.56	66.23
	Free	15.23	21.23	38.90	53.23	55.10	43.53	35.90	16.46	11.13	7.18

TABLE IX b

Chloramphenicol 30 mg per kg body weight intramuscular

Urine concentration of chloramphenicol in calves

(values expressed in mcg/ml)

Calf No.		Time post administration (hours)									
		1	2	4	8	12	16	24	32	40	48
3	Total	185.6	201.2	480.6	625.8	853.8	628.4	508.4	189.2	125.6	62.4
	Free	18.4	22.6	47.2	63.4	83.4	63.6	51.6	21.4	12.6	6.8
4	Total	178.4	232.8	508.4	641.6	908.4	721.8	524.6	148.8	89.6	48.2
	Free	16.4	24.2	49.8	64.8	89.6	71.2	53.4	17.0	8.9	4.2
5	Total	164.4	228.4	516.4	608.6	893.4	745.0	521.4	121.6	96.0	70.6
	Free	14.8	23.6	50.4	79.4	87.2	73.0	56.8	14.6	14.4	6.0
6	Total	191.8	192.6	499.8	624.4	876.2	783.4	498.4	138.2	121.4	84.4
	Free	17.8	21.2	47.6	67.6	86.2	81.8	49.4	14.6	12.6	4.2
7	Total	172.6	221.4	514.6	617.2	901.4	629.6	506.2	196.0	98.2	91.0
	Free	14.8	20.2	54.8	57.8	94.6	64.6	51.8	17.4	8.0	7.6
8	Total	177.8	227.0	492.4	632.0	827.8	649.0	546.4	168.6	101.8	68.2
	Free	16.0	18.4	44.6	61.4	81.6	62.8	57.6	19.4	11.6	6.0
Average											
	Total	178.43	217.26	502.03	625.00	876.83	692.83	517.56	160.46	105.43	70.80
	Free	16.36	21.70	49.06	65.73	87.13	69.50	53.43	17.60	11.38	5.80

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A B S T R A C T

ABSTRACT OF "STUDIES ON THE RATE OF DISAPPEARANCE OF CHLORAMPHENICOL FROM THE BLOOD OF GOATS AND CALVES".

Studies were conducted in goats and calves on the blood level of chloramphenicol at three dose levels of 10 mg, 20 mg and 30 mg per kg body weight intramuscularly. The data obtained revealed that:

Single dose of 10 mg per kg body weight, failed to produce minimum therapeutic concentration (5 mcg/ml) of the drug in blood of both goats and calves. At 20 mg per kg body weight, the minimum therapeutic concentration persisted between second and seventh hour in goats, and second and seventeenth hour in calves. At 30 mg per kg body weight, the minimum therapeutic concentration maintained between second and eleventh hour in goats and second and nineteenth hour in calves.

Peak concentrations of the drug were obtained both in goats and calves between second and third hour after the administration of chloramphenicol.

The drug concentration was higher in urine than in blood and minimum therapeutic concentration was found to persist for 24 hours in all the three doses.

170004

In a limited attempt made to study the toxicity of chloramphenicol in calves, 100 mg per kg body weight, administered daily, intravenously for 30 days, did not produce acute toxicity.

Transient "shock like" symptoms were noticed soon after injection and persisted for 10 to 15 minutes.

No alterations in blood constituents could be detected for the 30 days duration.

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