PHARMACOKINETICS OF SULFAMOXOLE AND NITROFURANTOIN IN BUFFALO-CALVES

Thesis

Submitted to the Faculty of Veterinary Science,

Rajendra Agricultural University, Bihar

in partial fulfilment of the requirements

for the award of degree of

Master of Science (Veterinary)

IN

Pharmacology

BY

K. K. Sharan, B. V. Sc. & A. H.

BIHAR VETERINARY COLLEGE

PATNA

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C E R T I F I C A T E

Certified that the research work incorporated in this Thesis has not been published in part or in full in any other journal.

K. K. Sharan

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Dated, the 30th December, 1977.

This is to certify that the work embodied in this Thesis entitled "PHARMACO-KINETICS OF SULFAMOXOLE AND NITROFURANTOIN IN BUFFALO CALVES" is the bonafide work of Dr. K.K. Sharan and was carried out under my guidance and supervision.

N.C. Banerjee

(N.C. BANERJEE)

DEDICATED

TO

MY PARENTS

A C K N O W L E D G E M E N T

I am deeply indebted to my guide Dr. N.C.Banerjee, M.S., Ph.D., Professor and Head of the Department of Pharmacology, Bihar Veterinary College, Patna for his wise counsel, apt guidance and constant encouragement at each step of this research work.

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I appreciate the untiring assistance given to me by Sri Kailash Ram during the work.

Finally, my deep appreciation goes to my wife "LEELA" for her patience and understanding.

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C O N T E N T S

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INTRODUCTION

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

The evolution of chemotherapy can be traced through three distinct periods; a pre Ehrlich era before 1891, the period of Paul-Ehrlich and the period after 1935 which was highlightened by the break through introduction of sulfonamides followed by antibiotics and nitrated furan derivatives.

The pioneer work of Ehrlich established the importance of cellular chemistry and also opened the gateway for the synthesis of life saving antimicrobials. However, inspite of easy availability of effective antibiotics, clinical usages of furan derivatives and sulfonamides are very common, both in human and veterinary medicine. In veterinary medicine, especially the cost factor and comparatively low superinfection problem has kept the use of sulfonamides in an envious position even now.

Further, the reason for the rapid popularity of nitrofurans lies in the fact that besides their remarkable antibacterial action, the resistance and cross-resistance problem was deemed to be less than the antibiotics.

With the wide spread use of such antimicrobials, it has been imperative to identify the different pharmacokinetic parameters involved with these antimicrobials in

different species of animals.

Stewart and Paris (1962) reported the influence of species variation in regard to the concentration and duration of action of sulfonamides following oral and intravenous administration.

With the wide use of artificial insemination practices as also under the stall fed management of dairy herd including the high yielding buffaloes, the probability of urinary tract infection has increased.

The two broad spectrum antimicrobials, nitrofurantoin and sulfamoxole are widely used for treating cases of urinary tract infection in small animals and man. It seems that no comprehensive effort has been made so far to explore the physiological disposition of these two compounds in buffaloes as also in cattle. In view of the above, the present study was envisaged so as to identify the extent of passage of these two compounds in blood and also their excretion through the urinary tract. The result obtained are expected to equip the veterinarians with sufficient pharmacokinetic background so as to help them to evaluate the comprehensive clinical value of these compounds in buffaloes.

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REVIEW OF LITERATURE

REVIEW OF LITERATURE

DISTRIBUTION OF SULFONAMIDES.

Plasma :

The importance of analytical control of blood concentration of sulfonamides in respect to their antimicrobial action was studied by Marshal (1939).

Maclay and Slavin (1947) reported that a blood concentration of 5 mg per cent may be considered as the minimum effective therapeutic level of sulfonamide in the domestic animals. The same observation has also been made by Stowe *et al*. (1956) and Brander and Pugh (1971). Scheidy and Tillson (1947) studied the blood concentrations of sulfadiazine, sufamerazine and sulfamethazine in cattle following oral and intravenous administration. They observed that sulfamethazine gave highest blood level while sulfadiazine the lowest. They further studied the rate of absorption of these drugs from the digestive tract and found that sulfamethazine and sulfamerazine were more absorbed than sulfadiazine.

Stowe *et al*. (1956) investigated the blood levels attained by various sulfonamides in dairy cattle. They showed that sulfamethazine had longer duration and

sulfathiazole shorter duration in blood while sulfapyridine, sulfamerazine, sulfadiazine and sulfadimethoxine showed blood levels intermediate between sulfathiazole and sulfamethazine.

Stowe et al. (1958) further reported that in cattle an oral dose of sulfabromomethazine at the rate of 214 mg per kg body weight gave therapeutic concentration for about 40 - 48 hours. The drug diffused into the C.S.F. and milk to the extent of about 20 per cent of the corresponding blood level.

Stewart and Paris (1962) and Francis (1949) reported that species variation influenced in the attainment and persistance of sulfonamide blood level after oral and intravenous administration. Francis (1949) studied the blood level of different sulfonamides in mouse, rabbit, cat, dog, fox, man, sheep, calf, cow, horse, pig and chicken. He observed that in man the level attained by different sulfonamides were much higher than in other species of animals. In chicken, the levels were lowest and a higher dose of the drug was required for optimal therapeutic concentration.

In sheep, sulfanilamide gave the highest blood level followed by sulfamerazine, sulfamethazine and sulfadiazine while sulfathiazole and sulfapyridine gave insignificant blood levels. In calf, sulfamerazine showed the highest blood level followed by sulfamethazine, sulfapyridine and sulfanilamide. In cow, the blood level was highest for sulfamethazine followed by sulfamerazine, sulfanilamide,

sulfapyridine. In horse, the blood level was highest with sulfamethazine followed by sulfamerazine, sulfadiazine, sulfapyridine, sulfanilamide and sulfathiazole.

Stewart and Paris (1962) studied the blood concentration of sulfamethoxypyridine in horse, dog, sheep, calves and cows following oral administration of 110 mg of the drug per kg body weight and in calves and cows following oral administration of 220 mg per kg body weight. Species differences in the peak blood levels and the duration of therapeutic concentration were observed. The rate of absorption appeared to be more rapid in the species having simple stomach; and in cattle, the efficiency of absorption appeared to be comparatively poorer than that of sheep. The highest peak blood level and the longest duration of therapeutic concentration was observed in sheep and dog.

Stowe and Sisodia (1963) observed that maximum average sulfadimethoxine concentration in plasma after oral administration of 214 mg per kg in cattle was 11.9 to 13.5 mg per cent between 12 to 24 hours post administration. They further observed that the same dose of drug when given by intravenous route, produced a maximum blood concentration of 32.9 mg per cent at one hour post administration and therapeutic concentration was maintained till 12 hours. They reported that biological half-life of the compound was 5 hours and volume distribution of the drug was 50 per cent of the body weight.

Ullrich (1963) reported that intravenous injection of sulfadimethoxine in horses at the rate of 100 mg per kg body weight yielded therapeutic blood concentration of at least 5 mg per cent for 27 hours. After intravenous injection of 100 mg per kg into dogs, the drug persisted for 26 hours. In pigs, however, when the same dose was given intraperitoneally the same therapeutic concentration persisted for 43 hours.

Linkenheimer and Stolzenberg (1965) reported that a single oral dose administration of sulfadimethoxine at the rate of 50 mg per lb body weight in swine produced maximum blood concentration of 9.6 mg per cent at 6 hours. The therapeutic concentration was maintained from 2 to 15 hours post administration.

Rehm and Rieder (1965) reported that trials with sulfadimethoxine in 550 cattle after intravenous dose of 25-40 mg per kg gave a blood level of 2.84 mg per cent for about 11 hours. They further observed that a dose of 100 - 200 mg per kg of sulfadimethoxine gave a blood level of 5 mg per cent.

Silvestri et al. (1967) studied the distribution of sulfamethazine, sulfaethoxypyridazine, sulfamethoxydiazine, sulfadimethoxine, sulfamethylphenazole and sulfaphenazole in heifer and reported that all drugs attained peak concentration in blood and plasma between 6 to 9 hours.

Banerjee (1971) investigated the plasma level of spanbolets, sulfamethazine, sulfapyridine, sulfaphenazole,

sulfadiazine and sulfanilamide in goats. He reported that sulfamethazine showed maximum plasma level while other sulfonamides in order of their plasma concentrations were spanbolets, sulfapyridine, sulfaphenazole, sulfadiazine and sulfanilamide.

Singh (1974) reported that in buffalo calves the maximum plasma level of unchanged drug was attained by sulfamethazine, followed by sulfaphenazole, sulfapyridine, sulfadiazine and sulfanilamide.

Banerjee et al. (1977) investigated the blood levels and tissue dispersion of sulfamoxole in poultry. The mean blood level at 2, 6, 12 and 24 hours were 6.82 ± 0.57, 4.05 ± 0.27, 3.58 ± 0.40 and 1.13 ± 0.20 mg per cent respectively. The drug residual concentration in the kidney, lung, liver, spleen and yolk were comparatively higher than those in the brain and muscle. A mean concentration of 1.81 mg per cent was obtained in the kidney. The observation revealed that the drug could not attain the appreciable therapeutic concentrations at any time. This was in contrast to the observation in relation to sulfaquinoxaline as reported by Banerjee et al. (1974).

Jha et al. (1977) studied the blood levels of sulfamoxole and its biotransformation in goat. The study revealed that the highest free drug concentration was 6.23 mg per cent at 6 hours after a single oral administration of drug at the rate of 286 mg per kg body weight. The acetylated derivative was detected at 6 hours post administration of the drug and the highest per cent acetylation (34.3 per cent) was observed at 48 hours.

Urinary excretion :

Stowe (1966) and Shepherd (1970) reported variation in the reabsorption of non-acetylated sulfonamides in dog as follows : Sulfathiazole 13 per cent, sulfadiazine 69 per cent, sulfapyridine 72 per cent, sulfanilamide 75 per cent, sulfaphenazole 75 per cent, sulfamethazine 85 per cent and sulfamerazine 80 per cent.

Baggot (1968, 1970) observed that tubular reabsorption of sulfonamides in dogs, calves and pigs was dependent on pH-pka rationship.

Walker (1970) investigated the metabolism and excretion of sulfadimethoxine, sulfasomidine and sulfadimethoxypyrimidine in man; and Osbaldiston and Walker (1972) observed that at least 75 per cent of sulfisoxazole was recovered in the urine.

Bergan and Brodwall (1972) investigated the kidney

transport of sulfamethoxazole and trimethoprim in man. They concluded that trimethoprim is subjected to passive non-ionic tubular diffusion in the kidney at a urinary pH value below 6.2. There is generally net tubular secretion whereas there is net tubular reabsorption above this point. The re-absorption of active sulfamethoxazole was slightly influenced by urinary pH.

Singh (1974) studied the urinary excretion of certain sulfonamides namely sulfanilamide, sulfamethazine, sulfaphenazole, sulfapyridine and sulfadiazine in buffalo calves after a single oral dose of 200 mg per kg body weight. He observed that excretion of sulfonamides in urine started within one hour of their administration and concentration of free form of the drugs remained higher than 5 mg per cent from 1 - 48 hours. The overall mean percentage of conjugated form were found to be 59.44 for sulfanilamide, 27.06 for sulfamethazine, 26.97 for sulfapyridine and 22.07 for sulfa-diazine in urine.

Detoxication :

The main metabolic pathway for sulfonamides in domestic animal is acetylation, which mainly occur in the liver but spleen of cat, leucocytes and erythrocytes can also acetylate these compounds (VanWinkle and Cutting, 1940; Stowe, 1966 and Mandel, 1971).

Stowe et al. (1956) studied the rate of acetylation of sulfanilamide, sulfapyridine, sulfadiazine and sulfamethazine in cattle; and they reported 37.8, 19.5,15.1 and 6.7 per cent acetylation in blood and 72.7, 48.2, 36.2, and 26.00 per cent in urine of cattle.

Williams (1959) observed that acetylated derivatives are therapeutically inactive and may cause renal damage as they are less water soluble than the parent drug excepting the sulfapyrimidine.

Williams and Parke (1964) confirmed that acetylation is the major route of metabolism of sulfonamides and may vary with the individual, species and type of sulfonamide.

The acetylation of sulfonamides have been observed in most of the species such as fish, toad, chicken, mouse, rat, guineapig, rabbit, cat, pig, cow,buffalo,sheep, goat, horse and man (Marshall and Litchfield,1939; Northey, 1948; Marshall, 1954; William, 1971; Banerjee, 1971;and Singh, 1974). Acetylation does not occur in dog and turtle (Stowe, 1966; William, 1967).

Silvestri et al. (1967) found 20 per cent acetylation of sulfaphenazole and 9.62 per cent of sulfamezathine in the plasma of heifer.

Banerjee (1971) has reported the acetylation of sulfanilamide, sulfadiazine, sulfapyridine and sulfamethazine,

spanbolets, sulfaphenazole and trinamide after oral administration of each of the above drug at the rate of 130 mg per lb body weight in goat plasma and milk.

Singh (1974) studied the per cent acetylation of sulfanilamide, sulfamethazine, sulfaphenazole, sulfapyridine and sulfadiazine in buffalo calves after single oral dose of 200 mg per kg body weight. He observed that overall mean percentage of conjugated form of sulfonamides were found to be 16.20, 59.44 for sulfanilamide, 8.34 and 27.06 for sulfamethazine, 9.64 and 54.80 for sulfaphenazole, 9.37 and 26.97 for sulfapyridine and 8.37 and 22.07 for sulfadiazine in plasma and urine respectively.

NITROFURANTOIN.

Distribution :

Paul and Bender (1946) studied the urinary excretion of nitrofuran NF^{67} in albino rats at oral dosages of 1, 2, 3, 5, 10 and 20 mg per animal. The spectrophotometric examination of the urine samples indicated that the compound was rapidly excreted during four hour period in concentration proportional to the dosages used.

Paul *et al*. (1949) observed the urinary excretion of various furan derivatives in laboratory animals. The compound which were found to be metabolized to nitrofuroic

acid showed little or no *in vivo* activity.

Richards *et al*. (1955) studied the nitrofurantoin level in the blood and urine after oral administration, in clinical cases and laboratory animals. Therapeutically effective concentration of the compound was excreted in urine but no useful level in blood could be recorded.

Buzzard *et al*. (1956) determined colorimetrically the concentrations of nitrofurazone, nitrofurantoin and furazolidone in the plasma of rat. The rats were administered orally, various dos-es level of the drug. They observed that the drug did not appear in the plasma after an oral administration at the rate of 10 mg per kg body weight upto 8th hours. However, a dose of 100 mg per kg body weight produced a significant plasma level. The plasma level of these compounds were incorporated in the table below.

Plasma level

Compound	Dose mg/kg	Plasma level/µg/ml 4 hour	8 hour	16 hour	24 hour
Nitrofurantoin	10	0	0		
	100	2.6	1.6		
	500	2.6	2.4	4.00	8.3
Nitrofurazone	10	0	0		
	100	4.5	1.5		
	500	5.7	10.0	14.5	16.2
Furazolidone	10	0	0		
	100	2.5	1.0		
	500	3.3	3.6	1.3	1.6

Paul et al. (1959) investigated the renal clearance of nitrofurantoin in albino rats at various plasma levels. They reported a recovery of 30 - 40 per cent of the dose administered in the urine of rats, after either oral or intravenous administration. The low plasma concentration following intravenous administration indicated rapid distribution of the drug. Low blood level and high urinary concentration have been observed uniformly after nitrofurantoin administration, both in animal and human being. They found that 30 - 50 per cent of the orally administered drug is excreted through the urine which suggests that both in rat and dog the drug is well absorbed after oral administration.

Paul et al. (1960) determined the blood and urine concentration and plasma protein binding values of nitrofurantoin in dog and rat at different dosage level, wherein, nitrofurantoin appeared at a concentration of about 2 to 5 mg/litre after the usual oral dosage of 100 or 200 mg when given 4 times daily. In rat, the blood levels of nitrofurantoin have been reported to be 2.6 mg/litre when the drug was given orally at the rate of 100 mg/kg body weight. They further noted that the excretion through urine in case of nitrofurantoin was 40 to 50 per cent. Nitrofurantoin was found to be excreted to a similar extent by rat, dog or man.

Buzzard et al. (1961) studied the absorption, distribution and elimination of nitrofurantoin in the rat.

They found that rapid absorption of drug from small intestine of rat takes place and 50 per cent of total administered drug was recoverable in its urine.

Conklin and Hollifield (1965) studied the urinary concentration in rat, dog and human urine following an oral administration of drug at a rate of 10 mg/kg body weight. Urine samples of rat revealed a concentration of 400, 400, 200 and 113 mg per litre at 0-2, 2-4, 4-6 and 6-8 hours respectively whereas in dog urine 970, 630, 284 and 143 mg/litre; and in human urine 145, 49, 5 and 0 mg/litre of the drug concentration was recorded at above time intervals.

Miura and Rockendorf (1967) studied structure activity relationship, blood levels, urinary excretion, plasma protein binding and chemotherapeutic efficacy of nitrofurans. They reported low plasma concentration of nitrofurantoin after oral administration in rats. In the urine of rats and man 30 to 40 per cent of the administered dosage of the drug was excreted. The inhibitory effect on the growth rate of bacteria was further studied. The antibacterial activity of certain non-ionized nitrofurans (nitrofurazone and furazolidone) was not affected by pH of the medium, while ionized nitrofurans (nitrofurantoin) was affected to some degree.

Craine et al. (1972) studied the metabolites of furazolidone in urine of chicken. The authors reported

that clostomized chicken were medicated with single oral dose of the antibacterial furazolidone. An average 7.5 ± 3.00 per cent of the dose was excreted in the urine within 12 hours. Chromatographic analysis detected four metabolites containing a furan ring. Only one metabolite reacted to form 5-nitrofurfural phenyl-hydrozone. Elimination of the metabolites with a furan ring was complete in 12 hours. Only traces of furazolidone were detected by chromatographic procedure.

Pandey (1975) studied the blood level and urinary excretion of nitrofurantoin and furazolidone in goat. He observed that no significant concentration of drug was found in the plasma of goat after oral administration of drug at the rate of 10 mg per kg body weight. The peak concentration was found 2.61 ± 0.09 ug/ml and 1.86 ± 0.83 ug/ml for furazolidone and nitrofurantoin respectively, at 4 hours post administration of the drug. In urine the peak concentration of nitrofurantoin and furazolidone was found to be 169.00 ± 2.64 and 8.25 ± 0.50 ug/ml. 45 per cent of nitrofurantoin administered, excreted through urine whereas only 2 per cent of furazolidone administered was detected in the urine of goat.

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MATERIALS AND METHODS

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SULFAMOXOLE AND NITROFURATOIN.

In the present investigation, five and six normal healthy deshi buffalo calves between the age group of 6 months to one year were used for sulfamoxole and nitrofurantoin respectively. The buffalo calves were maintained on green grass and concentrates (Korai, Chokar and Dana) under stall feeding, during the period of experiment. Water was given ad-lib.

Collection of control samples.

Blood :

For collection of the blood samples, the hairs around the jugular veins of each experimental animals were shaved and the site was cleaned with ether. The blood samples were always collected in test tubes containing sodium oxalate crystals. Before administration of the drug, 30 ml of blood was collected from one of the jugular vein from a 18 gauge needle, for use as control and for prepairing the standards. The blood sample was centrifuged at 5000 r.p.m. for 15 minutes to separate the plasma. The plasma thus

obtained was stored in refrigerator for subsequent use .

Urine :

To enable proper collection of urine samples and total volume of urine, each animal was immobilised in a steel crate for 96 hours. Before administration of the drug, urine sample was collected from each animal for use as control and for the preparation of standards.

Administration.

Sulfamoxole :

For the determination of blood level and urinary excretion of sulfamoxole (Sulfuno^{R}), the drug was given orally at the rate of 200 mg/kg body weight to each of the five buffalo calves. The drugs were powdered and given with mollasses. Care was taken that no drug was lost during administration.

For intravenous administration, the drug was powdered and dissolved in normal saline solution. To make the drug dissolved completely, a few ml of 1 per cent NaoH solution was added and slightly warmed over water bath.When the drug dissolved completely, it was injected slowly into jugular vein at the rate of 50 mg/kg body weight. The same set of animals were only used for the second time after a period of four weeks.

Nitrofurantoin :

The finally powdered drug (Furadantin^{R}) was given with mollasses to each buffalo calves orally at the rate 10 mg/kg body weight.

Collection of experimental samples.

Blood :

After administration of drug, the animal was immobilised in a steel crate. The blood samples were collected in test tubes containing sodium oxalate crystals at 1,3, 6, 9, 12, 18, 24, 48, 72 and 96 hour intervals from a jugular vein punctured by 18 gauge needle. The blood samples were centrifuged at 5000 r.p.m. for 15 minutes and plasma separated. The plasma samples were then subjectedto chemical determination at the earliest time. In the animals which were given the drug intravenously, the jugular vein which was used for the administration of drug was not used for the collection of blood samples.

Urine :

Along with blood samples, urine samples were taken in test tubes from the urine collected between 0-1, 1-3, 3-6, 6-9, 9-12, 12-24, 24-48, 48-72 and 72-96 hour interval. The

volume of the urine samples was measured each time and quantity noted. However, in some specific hours, urine samples could not be collected as the animals did not micturate. The urine samples were then subjected to chemical determination.

Biological half life and volume distribution :

For determining the biological half life and volume distribution of sulfamoxole, the drug was injected intravenously in five buffalo calves, at the rate of 50 mg per kg body weight.

Blood samples were collected at 30 minutes, 1, 2, 4, 8, 24 and 48 hours and the drug concentration in each sample was determined. The drug concentration thus obtainedd was plotted against their time of concentration on semilog scale and extrapolated to zero time. The zero time concentration thus obtained, the biological half life of the drug was determined.

Apparent volume distribution of the drug was determined as follows : -

$$\text{Apparent volume distribution (\%)} = \frac{\text{total dose given in mg}}{\text{zero time plasma concentration of free drug x weight of animal.}} \times 100$$

CHEMICAL METHOD.

Determination of sulfamoxole :

The concentration of sulfamoxole (free and total) was determined by Bratton and Marshall technique (1939). For determination of drug concentration in the urine, the experimental urine samples were at first diluted 25 times. In such cases where these dilutions did not give colour reaction, urine was diluted 10 times or undiluted urine was used.

Free sulfonamide (Sulfamoxole) :

Concentration of free sulfonamides were determined by diazotisation reaction before hydrolysis as described by Bratton and Marshall (1939).

In this procedure, 1 ml of experimental sample was taken. Then 14 ml of distilled water was added to each tube and mixed thoroughly. After this, to each tube 5 ml of 15 per cent trichloro-acetic acid was added. The contents in the test tube were thoroughly mixed and shaken. The mixtures were allowed to stand for 10 minutes for complete deproteinisation. Then the contents were filtered and 5 ml of the filtrate was taken out. To this filtrate 0.5 ml of 0.05 per cent solution of sodium nitrate was added. This procedure formed diazo compound.

At this stage, the filtrate was thoroughly shaken and sodium nitrate was allowed to react for exactly 5 minutes. This reaction was stopped by adding to it 0.5 ml of 0.5 per cent ammonium sulfamate solution. The ammonium sulfamate was allowed to act exactly for 2 minutes. After this, 0.5 ml of 0.5 per cent solution of the coupling agent N-1-naphthyl-ethylene-diamine-dihydrochloride was added. The time interval in this analytical procedure being critical, each step was followed very carefully to maintain the proper time reaction.

After adding each of the reagents, thorough shaking and mixing of the mixture was done.

The resultant colour density was then measured colorimetrically at 624 milli micron (mu). The readings of the control samples were substracted from the unknown samples and the final colorimeter reading of the unknown samples were obtained. These final readings were then compared with the standard reading plotted in a graph and the plasma and urine concentration in mg per cent were noted.

Total sulfonamide :

Total sulfonamide are concentrations of sulfonamides which produced Bratton-Marshall colour reaction after hydrolysis.

The procedure for determining the concentration

of total sulfonamide was similar to the determination of free sulfonamide procedure upto the stage of deproteinisation.

Then 5 ml of the filtrate obtained from the precipitated solution was taken in a dry and clean pyrex test tube and 0.5 ml of 4N-HCl was added. The test tubes were then kept in boiling water both for 1 hour. Care was taken that all the test tubes were exposed to uniform and equal heating. After the process was completed, the test tubes were taken out and allowed to cool. When cooling was complete, the 5 ml volume was restored with distilled water. After this, for diazotisation, the same technique was adopted as for free sulfonamide estimation and calorimetric readings were taken. Reading of control samples were substrated from the reading of unknown samples.

A set of standards and control samples of plasma and urine were run along with the unknown samples adopting similar procedure and the concentration of total sulfonamide of respective unknown samples were obtained.

Preparation of standard curve :

Drug solution of 1.562, 3.125, 6.25, 12.5 and 25.00 mg per cent concentrations were prepared in plasma and urine (dilution 1 in 25). These solutions were run in the same way as in case of free and total sulfonamide estimation. The results were expressed as optical density

versus concentration. The optical densities were plotted against concentrations and standard curves obtained separately for plasma and urine.

Calculations :

$$\text{Conjugation per cent} = \frac{\text{Total drug - Free drug}}{\text{Total drug concentration}} \times 100.$$

$$\text{Total excretion (urine)} = \frac{\text{Total excretion per 100 ml x volume of urine.}}{100}$$

$$\text{Apparent volume distribution (\%)} = \frac{\text{total dose given in mg}}{\text{zero time plasma concentration of free drug x weight of animal.}} \times 100$$

NITROFURANTOIN.

The concentrations of nitrofurantoin in plasma and urine were determined colorimetrically by phenylhydrazone method, as described by Buzzard et al. (1956). For determining the concentration of nitrofurantoin in the urine, the experimental urine samples were diluted to 1:25 ml, if the dilutions did not give colour reaction, urine dilutions of 1:10 or even undiluted urine was used.

Principle : - The method of estimation of nitrofurans as described by Buzzard et al. (1956) is based on the formation and colorimetric estimation of 5-Nitro-2-furfural-dehyde

phenylhydrazone, which is the condensation product, containing the trivalent-N-N=C- group, resulting from the action of phenyl-hydrazine HCl (Ph. NH. N : CHR) on the aldehyde or Ketone group of nitrofuran.

Plasma :

Standard solutions of different concentrations of nitrofurantoin in plasma were prepared as follows :

Finely powdered 100 mg of the compound was dissolved in 100 ml of N, N-dimethyl formamide in a measuring cylinder. Then 2.5 ml of this solution was diluted to 25 ml with plasma, to obtain a concentration of 100 ug/ml. From this plasma drug solution, 2.5 ml was taken and diluted to 25 ml with the plasma, to obtain a concentration of 10 ug per ml of plasma. Then 0.5, 1, 2 and 3 ml of 10 ug per ml of solution was pipetted out and placed in separate pyrex test tubes and each of these were made to 3 ml volume by adding 2.5, 2 and 1 ml of plasma respectively. The standard concentrations thus obtained were 1.67, 3.33, 6.667 and 10 ug/ml respectively.

Urine :

Standard solution of urine was also prepared by the above procedure excepting that urine in the dilution of 1 in 25 was used in place of plasma.

Three ml of each of the experimental sample of plasma and urine collected at different intervals were taken in separate sets of pyrex tubes. Three ml of control and standard solution were also taken in separate pyrex tubes. Then 1 ml of 1.5 per cent phenyl-hydrazine and 1 ml of 5N-HCl was added to each of the tubes. The tubes were thoroughly stirred with the help of glass rods for complete mixing the reagents and were heated at 70°C for 25 minutes. The tubes were then kept for 5 minutes under running tap water and subsequently 5 ml toluene was added to each of these tubes. The tubes were vigorously shaken 30 times. The content of each tube were transferred to centrifuge tubes and was centrifuged for 15 minutes to get a clear toluene layer. Whenever, it was found that toluene layer was not clear re-centrifugation was done. The clear toluene layer at the top of the tubes was gently taken out and kept in clean separate tubes, which were plugged with cotton. The drug concentration were read in the Klett-Summersen Colorimeter by using blue filter at 430 mu against a toluene blank.

Standard solutions and unknown plasma and urine samples of a particular buffalo calf were always carried out by the same procedure at the same time. Standard curve for each compound was prepared by plotting the absorbance of each drug obtained at different concentrations.

The reading of the control samples were substracted from the unknown samples and the final Klett-reading of the

unknown samples were obtained. Final readings were now compared with the standard curve and the concentrations in the original plasma and urine samples were obtained. By dividing the concentrations, by three (the volume of plasma and urine samples used), the concentration per ml of biological fluid was obtained.

The final concentration of urine was derived by multiplying with diluting factor, wherever required.

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R E S U L T S

R E S U L T S

DISTRIBUTION IN PLASMA AND URINE.

Sulfamoxole.

Plasma :

The plasma concentration of free and conjugated sulfamoxole in buffalo calves No . 1, 2, 3, 4 and 5 after a single oral dose of 200 mg per kg body weight have been shown in Table I. The mean concentration of free and conjugated drug in plasma have been presented in Table II and Fig. I.

The free drug concentration in plasma of buffalo calf Nos. 1, 2, 3, 4 and 5 at 1 hour was 3.75, 2.75, 2.50, 2.50 and 4.25 mg per cent respectively.

The peak plasma concentration of free drug was observed to be 12, 12.50, 10.50, 11.25 and 12.50 mg per cent in buffalo calf Nos. 1, 2, 3, 4 and 5 respectively between 9 - 12 hours.

The 96 hours samples revealed a free drug concentration of 1.75, 2.50, 1.75, 0.75 and 1 mg per cent in buffalo calf Nos. 1, 2,3 , 4 and 5 respectively. The mean free sulfamoxole concentration in buffalo plasma at 1, 3, 6, 9, 12, 18, 24, 48, 72 and 96 hours were 3.15, 5.35, 7.77, 11.60, 10.75,

9.83, 7.60, 4.61, 2.82, 1.55 mg per cent.

The minimum therapeutic concentration of drug was maintained in plasma from 3 to 24 hours, post oral administration of drug.

TABLE - I.

Individual plasma concentration of sulfamoxole after single oral dose administration at the rate of 200 mg per kg body weight.

All concentrations are expressed in mg per cent of plasma

Hour	Buffalo calf No. I.			Buffalo calf No. 2		
	F	T	C	F	T	C
1	3.75	3.75	0.00	2.75	3.12	11.85
3	4.75	5.25	9.52	6.00	6.75	11.11
6	9.00	9.75	7.69	8.12	9.50	14.52
9	12.00	13.75	12.72	12.50	15.25	18.03
12	11.25	12.25	8.16	9.50	11.75	19.14
18	10.75	12.00	10.41	-	11.00	-
24	7.00	8.25	15.15	9.00	10.50	14.28
48	3.80	4.00	5.00	4.75	5.00	5.00
72	2.50	2.50	0.00	3.25	3.25	0.00
96	1.75	1.75	0.00	2.50	2.50	0.00

TABLE - I (Cont'd)

Hours	Buffalo calf No. 3			Buffalo calf No. 4		
	F	T	C	F	T	C
1	2.50	2.50	0.00	2.50	2.75	9.09
3	6.50	7.00	7.14	3.75	4.00	6.25
6	8.50	9.00	5.55	5.00	6.00	16.66
9	10.50	13.25	20.75	10.50	11.75	10.63
12	9.75	10.50	7.14	11.25	12.50	10.00
18	-	-	-	7.00	7.75	9.67
24	6.25	6.75	7.40	7.00	7.75	9.67
48	6.25	6.50	3.83	3.50	3.75	6.66
72	2.75	2.75	0.00	2.25	2.25	0.00
96	1.75	1.75	0.00	0.75	0.75	0.00

Hour	Buffalo calf No. 5		
	F	T	C
1	4.25	4.25	0.00
3	5.75	6.25	8.00
6	8.25	9.25	10.81
9	12.50	14.25	12.27
12	12.00	13.25	9.43
18	11.75	13.00	9.61
24	8.75	10.00	12.50
48	4.75	5.00	5.00
72	3.37	3.50	3.71
96	1.00	1.00	0.00

F = Free. T = Total.

C = Conjugated - expressed as per cent of total.

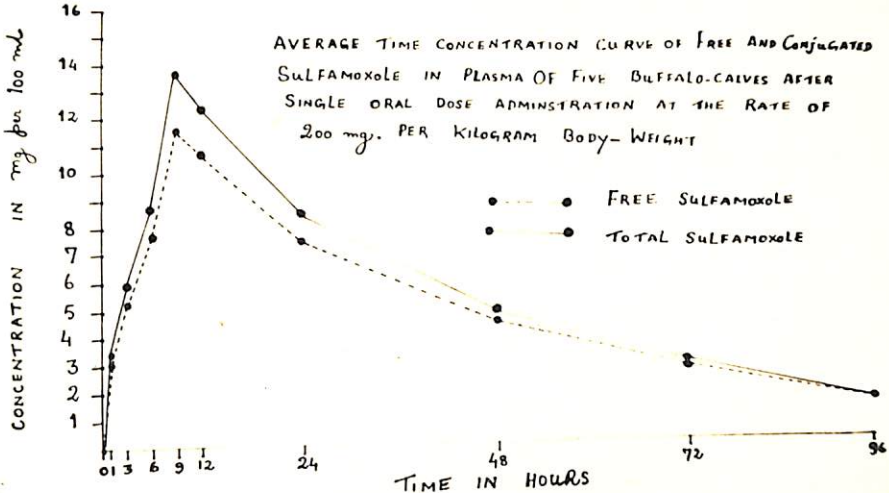

AVERAGE TIME CONCENTRATION CURVE OF FREE AND CONJUGATED SULFAMOXOLE IN PLASMA OF FIVE BUFFALO-CALVES AFTER SINGLE ORAL DOSE ADMINSTRATION AT THE RATE OF 200 mg. PER KILOGRAM BODY-WEIGHT
FREE SULFAMOXOLE
TOTAL SULFAMOXOLE
CONCENTRATION IN mg per 100 ml
16
14
12
10
8
7
6
5
4
3
2
1
0 1 3 6 9 12 24 48 72 96
TIME IN HOURS

TABLE - II.

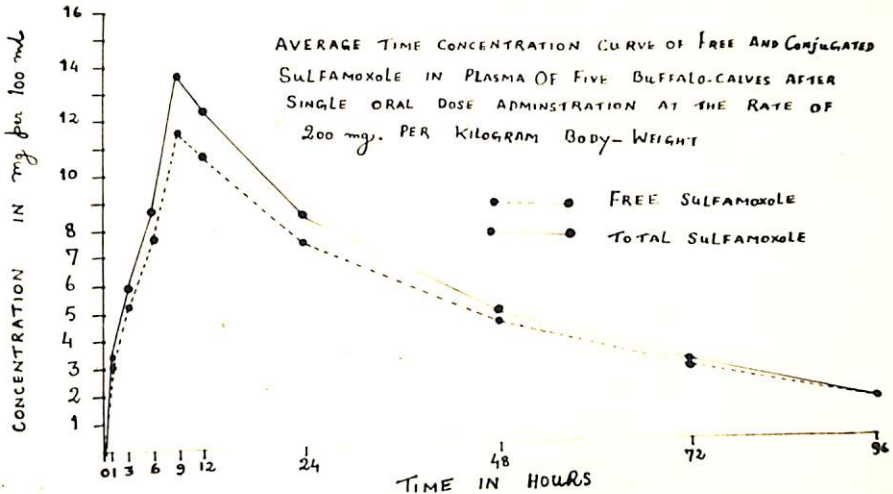
Mean plasma concentration of free and total sulfamoxole and its conjugated percentage for five buffalo calves

All concentrations are expressed in mg per cent of plasma.

Hour	F	T	C
1	3.15 (0.35)	3.27 (0.34)	3.98 (0.87)
3	5.35 (0.49)	5.85 (0.55)	8.40 (0.57)
6	7.77 (0.69)	8.70 (0.68)	11.04 (2.11)
9	11.60 (0.45)	13.65 (0.57)	14.88 (1.88)
12	10.75 (0.48)	12.05 (0.68)	10.77 (2.13)
18	9.83 (1.44)	10.93 (0.96)	9.89 (0.26)
24	7.60 (0.53)	8.65 (0.70)	11.80 (1.17)
48	4.61 (0.45)	4.85 (0.51)	5.09 (0.55)
72	2.82 (0.21)	2.85 (0.23)	0.74 (0.28)
96	1.55 (0.31)	1.55 (0.31)	0.00

F = Free. T = Total. C = Conjugated - expressed as per cent of total.

() = Values in the parentheses denote standard error.


AVERAGE TIME CONCENTRATION CURVE OF FREE AND CONJUGATED SULFAMOXOLE IN PLASMA OF FIVE BUFFALO-CALVES AFTER SINGLE ORAL DOSE ADMINSTRATION AT THE RATE OF 200 mg. PER KILOGRAM BODY-WEIGHT
FREE SULFAMOXOLE
TOTAL SULFAMOXOLE
CONCENTRATION IN mg per 100 ml
16
14
12
10
8
7
6
5
4
3
2
1
0 1 3 6 9 12 24 48 72 96
TIME IN HOURS

Urine :

The excretion of sulfamoxole in the urine of buffalo calves following a single oral dose of 200 mg per kg body weight have been depicted in Table III. The mean urinary concentration of the drug has been shown in Table IV.

The 0-1 hour concentration of free drug in the urine of buffalo calf Nos. 1, 2, 3, 4 and 5 were 65.00,62.75, 72.00, 35 and 45 mg per cent respectively. The peak urine concentrations of free drug were 307.50, 275, 287 and 475 mg per cent in buffalo calf Nos. 1, 2, 4 and 5 respectively, during at 12-24 hours. In buffalo calf No. 3 however the peak level of 475 mg per cent was attained between 6-9 hours post oral drug administration. The free drug was detected in urine samples collected between 72-96 hours in the concentration of 37.50, 14.25, 12.75, 15.00 and 15.00 mg per cent in buffalo calf Nos. 1, 2, 3, 4 and 5 respectively.

The mean concentration of free drug in the urine at 0-1, 1-3, 3-6, 6-9, 9-12, 12-24, 24-48, 48-72 and 72-96 hours were 55.95, 141.33, 332.83, 291.44, 266.75, 320.25, 202.95, 80.35 and 18.90 mg per cent respectively.

The urine samples at some hours could not be collected as the animals did not micturate between those time-intervals.

TABLE - III.

Individual urinary concentration of sulfamoxole of five buffalo calves after a single oral dose administration at the rate of 200 mg/kg body weight.

(All concentrations are expressed as mg per cent).

Hour	Buffalo calf No. 1			Buffalo calf No. 2		
	F	T	C	F	T	C
0-1	65.00	71.75	9.40	62.75	68.75	8.72
1-3	-	-	-	-	-	-
3-6	168.50	218.50	22.88	-	-	-
6-9	218.75	268.75	18.60	256.25	293.75	12.76
9-12	237.50	287.50	17.38	231.25	275.00	15.90
12-24	307.50	393.75	21.90	275.00	312.50	12.00
24-48	262.25	337.50	22.29	206.25	275.00	25.00
48-72	75.25	84.25	10.68	55.50	62.50	11.20
72-96	37.50	37.50	0.00	14.25	15.00	5.00

Hour	Buffalo calf No. 3			Buffalo calf No. 4		
	F	T	C	F	T	C
0-1	72.00	80.00	10.00	35.00	37.50	6.66
1-3	162.50	206.25	21.21	135.50	148.50	8.75
3-6	455.00	555.00	18.02	-	-	-
6-9	475.00	560.00	15.18	215.75	251.75	14.29
9-12	-	-	-	248.25	303.25	18.13
12-24	256.25	306.25	16.32	287.50	400.00	28.12
24-48	240.00	280.00	14.28	156.25	193.75	19.30
48-72	58.25	66.25	12.07	112.50	125.00	10.38
72-96	12.75	13.25	3.77	15.00	15.00	0.00

TABLE - III (Cont'd)

Hour	Buffalo calf No. 5		
	F	T	C
0-1	45.00	51.25	12.19
1-3	126.00	162.50	22.46
3-6	375.00	425.00	11.76
6-9	-	-	-
9-12	350.00	400.00	12.50
12-24	475.00	525.00	9.52
24-48	150.00	168.75	11.11
48-72	100.25	118.25	15.22
72-96	15.00	16.25	7.69

F = Free

T = Total

C = Conjugated - expressed as mg per cent.

TABLE - IV.

Mean urinary concentration of free and total sulfamoxole and its conjugated percentages for five buffalo calves.

(All concentration are expressed in mg per cent)

Hour	F	T	C
0-1	55.95	61.85	9.36
1-3	141.33	172.41	17.47
3-6	332.83	399.50	17.55
6-9	291.44	343.56	15.20
9-12	266.75	316.43	15.97
12-24	320.25	387.50	17.41
24-48	202.95	251.00	18.39
48-72	80.35	91.25	11.91
72-96	18.90	19.40	3.29

F = Free.

T = Total.

C = Conjugated - expressed as per cent of total.

(Not taken for statistical analysis).

Cumulative excretion in urine.

The average and individual cumulative excretion of sulfamoxole has been shown in Table V and Fig. II.

It is evident from the results that a major quantity of drug was recoverable in urine between 12-24 hours. The mean cumulative per cent excretion between 0-12, 12-24, 24-48, 48-72, 72-96 hours were 8.08, 19.94, 29.14, 32.82, 33.56 per cent. Total mean cumulative excretion of sulfamoxole in this species was found to be 33.56 per cent (at 0-96 hours post oral administration of drug).

Conjugation (Plasma and urine).

Plasma :

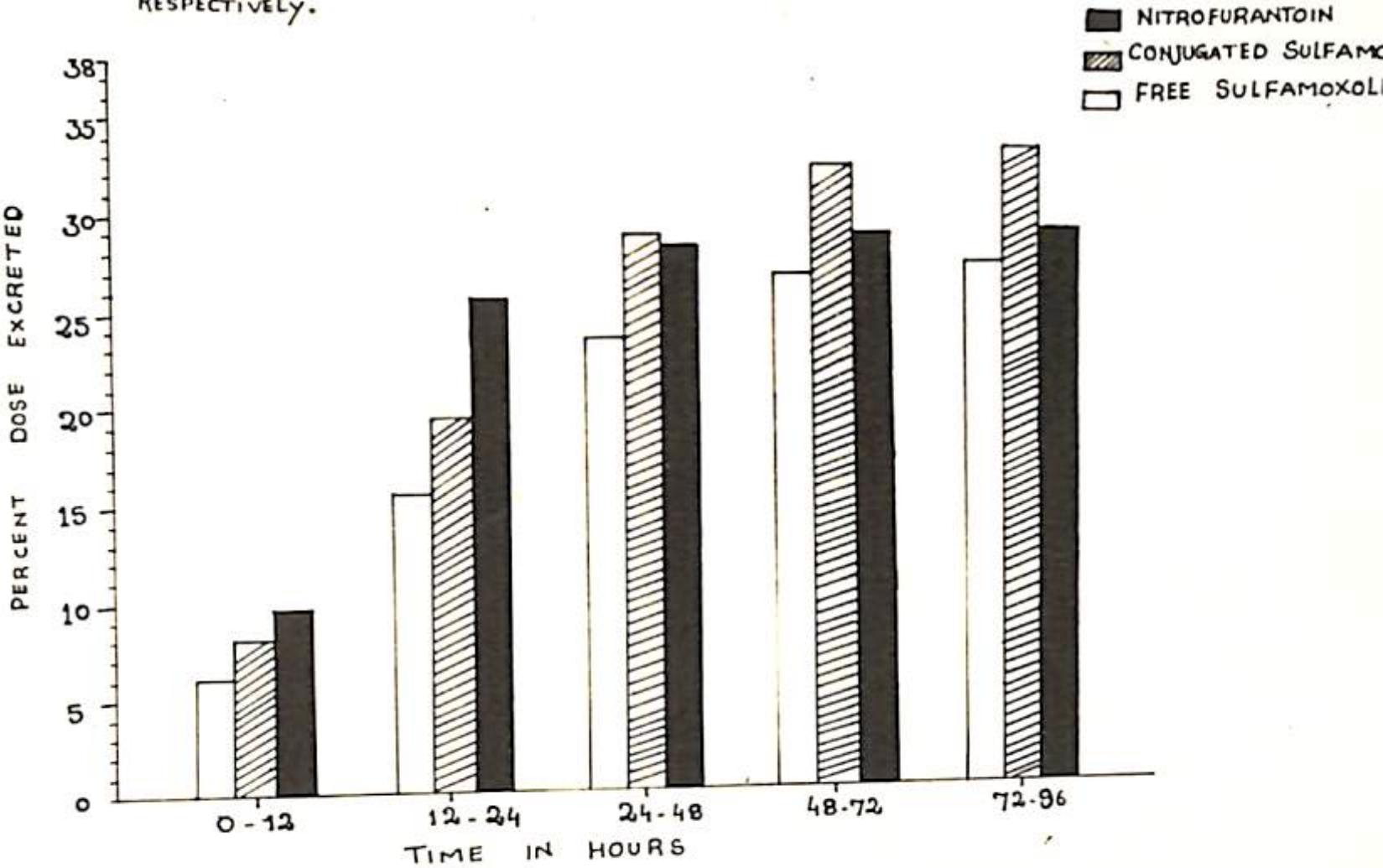
The result incorporated in Table I and Table II indicates that conjugated derivative of the drug appeared in buffalo plasma after 1 hour of oral administration. The mean per cent conjugations for five buffalo calves were found to be 3.98, 8.40, 11.04, 14.88, 10.77, 9.89, 11.80, 5.09 and 0.74 at 1, 3, 6, 9, 12, 18, 24, 48, 72 and 96 hours respectively. The overall mean per cent conjugation in plasma was noted to be 7.65.

TABLE - V.

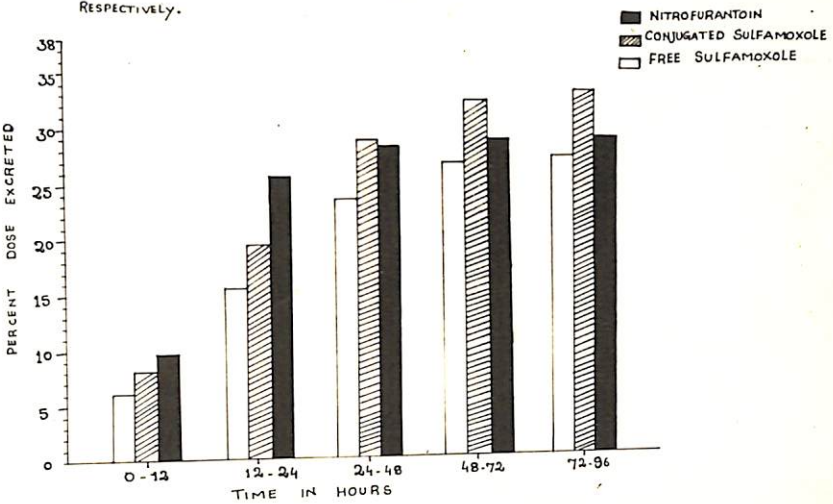
Average and individual cumulative excretion of sulfamoxole in buffalo calves urine after a single oral dose administration at the rate of 200 mg per kg body weight (0-96 hours).

Hour	Per cent of dose excreted					
	Buffalo calf No.1	Buffalo calf No.2	Buffalo calf No.3	Buffalo calf No.4	Buffalo calf No.5	Average ± S.E.
0-12	5.5	9.2	9.3	9.6	6.8	8.08 ±0.83
12-24	13.2	19.7	22.7	25.6	18.5	19.94 ±2.05
24-48	25.2	28.0	28.7	35.0	28.8	29.14 ±1.60
48-72	28.8	29.5	30.1	39.3	36.4	32.82 ±2.11
72-96	30.0	30.0	30.6	39.9	37.3	33.56 ±2.10
Total cumulative excretion upto 96 hours.	30.0	30.0	30.6	39.9	37.3	33.56 ±2.10

AVERAGE CUMULATIVE URINARY EXCRETION OF NITROFURANTOIN, FREE AND CONJUGATED SULFAMOXOLE IN BUFFALO CALVES AFTER A SINGLE ORAL DOSE AT THE RATE OF 10mg AND 200mg PER KILOGRAM BODY-WEIGHT - RESPECTIVELY.



AVERAGE CUMULATIVE URINARY EXCRETION OF NITROFURANTOIN, FREE AND CONJUGATED SULFAMOXOLE IN BUFFALO CALVES AFTER A SINGLE ORAL DOSE AT THE RATE OF 10mg AND 200mg PER KILOGRAM BODY WEIGHT - RESPECTIVELY.



Urine :

The conjugation was evident in urine of all the experimental buffalo calves during 1 to 96 hours post oral administration of sulfamoxole (Table III and IV). The mean conjugation per cent in urine were found to be 9.36, 17.47, 17.55, 15.20, 15.97, 17.41, 18.39, 11.91 and 3.29 in samples collected at 0-1, 1-3, 3-6, 6-9, 9-12, 12-24, 24-48, 48-72 and 72-96 hours respectively. The overall per cent conjugation was 14.06 per cent in buffalo urine.

Distribution of sulfamoxole in the plasma after intravenous administration.

The determination of plasma concentration of sulfamoxole was carried out in five buffalo calves after a single intravenous dose at the rate of 50 mg/kg body weight. The result obtained has been presented in Table VI, VII and Fig. III.

The peak concentrations of the free sulfamoxole in the plasma were 13.25, 14.00, 15.25, 11.50, 11.00 mg per cent at ½ hour in buffalo calf Nos. 1, 2, 3, 4 and 5 respectively. The concentration declined to a level of 2.75, 4.00, 3.87, 2.50, 3.50 mg per cent at 8 hours.

No; significant unchanged drug concentration was evident at 48 hours sample. The mean ½ hour plasma concentration

TABLE - VI.

Individual plasma concentration of sulfamoxole after intravenous administration of a siggle dose at the rate of 50 mg per kg body weight.

(All concentrations are expressed in mg per cent of plasma)

Hour	Buffalo calf No. 1			Buffalo calf No. 2		
	F	T	C	F	T	C
½	13.25	16.00	17.18	14.00	16.75	16.41
1	12.50	14.50	13.79	13.25	15.50	14.51
2	10.00	11.00	9.09	11.25	12.00	6.25
4	7.00	7.50	6.66	8.25	8.75	5.71
8	2.75	3.00	8.33	4.00	4.00	0.00
24	0.25	0.25	0.00	1.00	1.00	0.00
48	0.00	0.00	0.00	0.50	0.50	0.00

Hour	Buffalo calf No. 3			Buffalo calf No. 4		
	F	T	C	F	T	C
½	15.25	17.00	10.29	11.50	13.00	11.53
1	13.00	14.38	9.59	9.75	10.75	9.30
2	9.25	9.75	5.12	6.25	6.75	7.40
4	7.00	7.25	3.44	4.00	4.25	5.88
8	3.87	3.87	0.00	2.50	2.50	0.00
24	1.75	1.75	0.00	1.50	1.50	0.00
48	0.50	0.50	0.00	0.00	0.00	0.00

TABLE - VI (Cont'd)

Hour	Buffalo calf No. 5		
	F	T	C
½	11.00	12.50	12.00
1	8.25	9.25	10.81
2	7.25	8.00	9.37
4	4.75	5.00	5.00
8	3.00	3.00	0.00
24	2.00	2.00	0.00
48	1.00	1.00	0.00

F = Free

T = Total

C = Conjugation - expressed as per cent of total.

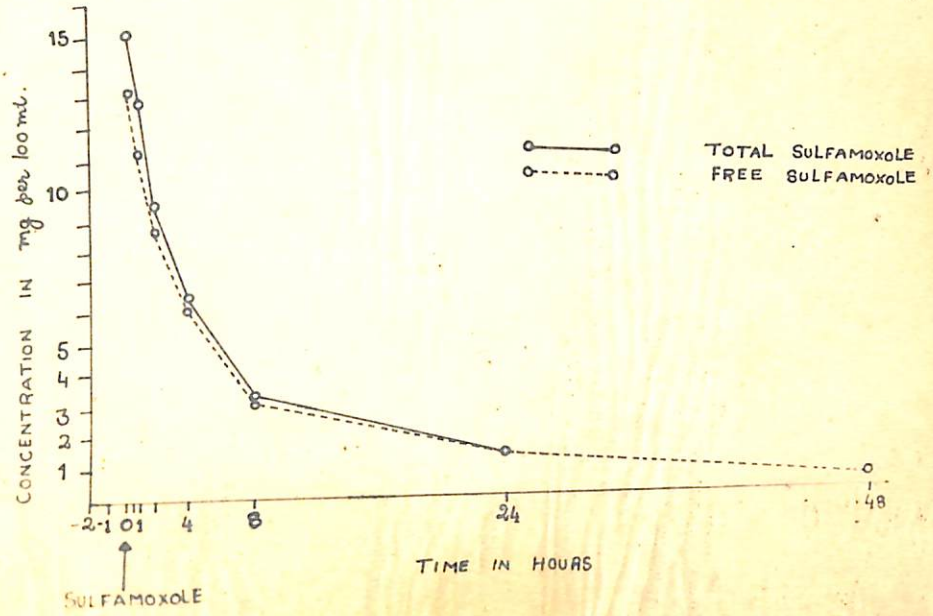
TABLE - VII.

Mean plasma concentration of free and conjugated sulfamoxole for five buffalo calves after intravenous administration.

Hour	F	T	C
¼	13.00 (0.78)	15.05 (0.82)	13.48 (1.38)
1	11.35 (1.09)	12.88 (1.21)	11.60 (1.42)
2	8.80 (0.90)	9.50 (0.95)	7.44 (0.81)
4	6.20 (0.78)	6.55 (0.83)	5.33 (0.44)
8	3.22 (0.29)	3.27 (0.28)	1.66 (0.02)
24	1.30 (0.30)	1.30 (0.30)	0.00
48	0.40 (0.01)	0.40 (0.01)	0.00

F = Free.
T = Total.
C = Conjugated - expressed as per cent of total.
() = Value in parenthesis denote standard error.

AVERAGE PLASMA CONCENTRATION OF FREE AND CONJUGATED SULFAMOXOLE OF FIVE BUFFALO-CALVES AFTER INTRAVENOUS ADMINSTRATION OF A SINGLE DOSE AT THE RATE OF 50 mg PER KILOGRAM BODY-WEIGHT



of free drug was found to be 13.00 mg per cent which is also the mean peak of unchanged drug. The mean free plasma concentration of drug at 2, 4, 8, 12 and 24 hours were 8.80, 6.20, 3.22, 1.30 mg per cent respectively.

The mean zero time concentration was found to be 16 mg per cent. The zero time concentration was derived by plotting the plasma concentration against time in semilogarithmic scale and extrapolating it to zero time. The mean biological half-life of the drug was calculated to be 2 hours 10 minutes.

The mean per cent apparent volume distribution of sulfamoxole was 31.25 per cent.

Conjugation :

It would be evident from Table VII that conjugation occurred between ½ to 8 hours only after intravenous administration of drug. The mean peak conjugation was 13.48 per cent at ½ hour in buffalo plasma.

Nitrofurantoin.

Plasma :

The individual and mean plasma concentration of nitrofurantoin following single oral dose of 10 mg/kg body weight in six buffalo calves have been depicted in Table VIII

and Fig. IV.

The drug concentration in plasma at 1 hour were 0.3, 0.7, 0.86, 0.36, 0.76 and 0.50 µg/ml in buffalo calf nos. 1, 2, 3, 4, 5 and 6 respectively. The peak drug concentration were 1.60, 2.60, 2.30, 2.80, 2.80, 2.10 µg/ml in buffalo calf Nos. 1, 2, 3, 4, 5 and 6 respectively between 12-24 hours.

No drug moiety was evident at 72 and 96 hours samples in all the six experimental buffalo calves.

The mean concentration in buffalo plasma were found to be 0.58, 1.10, 1.30, 1.94, 2.28, 1.88, 0.25, at 1, 3, 6, 9, 12, 24 and 48 hours samples respectively.

Urine :

Mean and individual urinary concentration of drug have been incorporated in Table IX, following a single oral administration of drug at the rate of 10 mg/kg body weight in six buffalo calves.

The samples collected at 0-1 hour revealed a concentration of 8.75, 7.00, 8.60, 8.60, 8.60, 5.50 ug/ml in buffalo calf Nos. 1, 2, 3, 4, 5 and 6 respectively. The peak concentration attained between 6-12 hours were 47.75, 71.50, 75.00, 82.00, 87.50, 75.00, [illegible] µg/ml respectively.

A concentration of 10.30, 35.00, 13.00, 11.00, 9.00, and 10.00 µg/ml were noted at 24-48 hours in buffalo calves Nos. 1, 2, 3, 4, 5 and 6 respectively.

Insignificant concentration of drug moiety was found in the urine samples of buffalo calves collected after 48 hours of administration.

The mean concentration of drug were 7.84, 24.00, 43.29, 51.30, 72.45, 49.00, 14.71, 2.08 and 0.36 µg/ml at 0-1, 1-3, 3-6, 6-9, 9-12, 12-24, 24-48, 48-72 and 72-96 hours respectively.

TABLE - VIII.

Mean and individual plasma concentration of nitrofurantoin after an single oral dose administration of drug at the rate of 10 mg per kg body weight.

(All concentration are expressed in µg/ml).

Hour	Buffalo calf No.1	Buffalo calf No.2	Buffalo calf No.3	Buffalo calf No. 4	Buffalo calf No.5	Buffalo calf No.6	Mean ± S.E.
1	0.30	0.70	0.86	0.36	0.76	0.50	0.58 ± 0.09
3	1.20	1.30	1.30	0.86	0.91	1.03	1.10 ± 0.07
6	1.23	1.60	1.50	1.20	1.10	1.20	1.30 ± 0.08
9	1.50	1.80	2.10	2.50	2.10	1.63	1.94 ± 0.14
12	1.60	2.10	2.30	2.80	2.80	2.10	2.28 ± 0.20
24	-	2.60	1.80	2.10	1.33	1.56	1.88 ± 0.22
48	-	-	0.20	0.30	0.25	-	0.25 ± 0.06
72	0.00	0.00	0.00	0.00	0.00	0.00	0.00
96	0.00	0.00	0.00	0.00	0.00	0,00	0,00

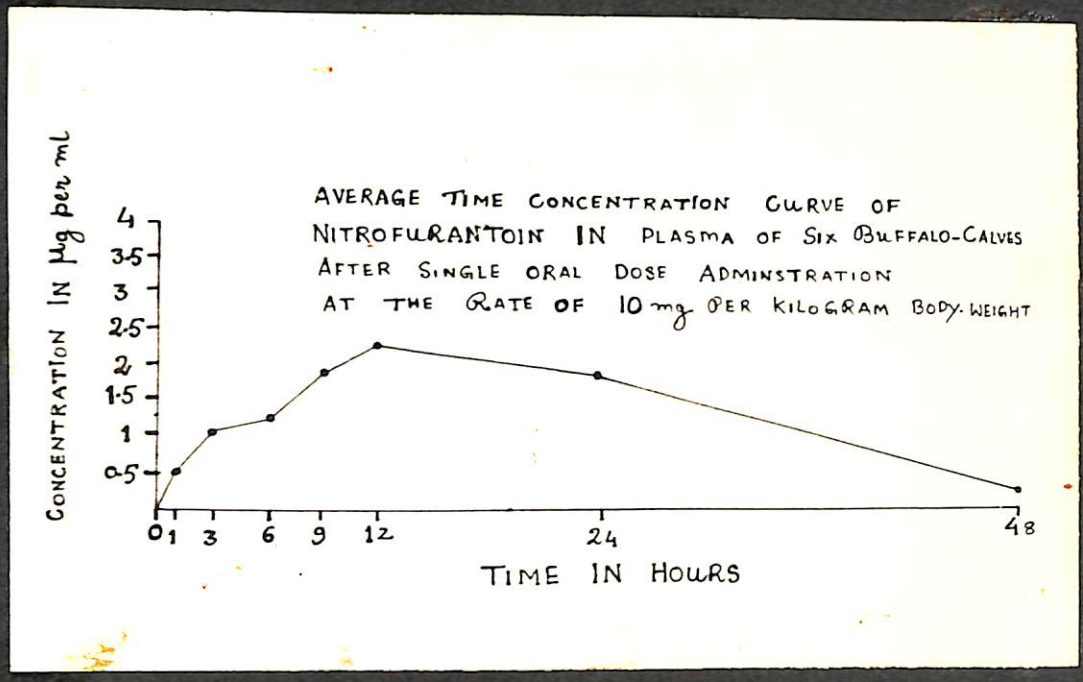

AVERAGE TIME CONCENTRATION CURVE OF
NITROFURANTOIN IN PLASMA OF SIX BUFFALO-CALVES
AFTER SINGLE ORAL DOSE ADMINSTRATION
AT THE RATE OF 10 mg PER KILOGRAM BODY WEIGHT
CONCENTRATION IN µg per ml
4
3.5
3
2.5
2
1.5
1
0.5
0 1 3 6 9 12 24 48
TIME IN HOURS

FIGURE - IV.

TABLE - IX.

Average and individual urinary concentration of nitrofurantoin after single oral administration of drug at the rate of 10 mg/kg body weight.

(All concentrations are expressed in µg/ml).

Hour	Buffalo calf No.1	Buffalo calf No. 2	Buffalo calf No. 3	Buffalo calf No. 4	Buffalo calf No. 5	Buffalo calf No. 5	Average ± S.E.
0-1	8.75	7.00	8.60	8.60	8.60	5.50	7.84 ± 0.54
1-3	19.50	17.50	30.00	27.00	20.00	30.00	24.00 ± 2.28
3-6	43.75	50.75	50.75	37.00	27.50	50.00	43.29 ± 3.85
6-9	47.75	56.25	65.00	33.75	-	53.75	51.30 ± 5.19
9-12	43.75	71.50	75.00	82.00	87.50	75.00	72.45 ± 6.40
12-24	40.00	54.00	55.00	40.00	55.00	50.00	49.00 ± 2.94
24-48	10.30	35.00	13.00	11.00	9.00	10.00	14.71 ± 4.13
48-72	3.50	4.50	0.00	4.50	0.00	0.00	2.08 ± 0.94
72-96	1.08	1.1	0.00	0.00	0.00	0.00	0.36 ± 0.22

Cumulative urinary excretion.

The cumulative urinary excretion of nitrofurantoin have been shown in Table X and Fig. II.

Total cumulative urinary excretion of drug were found to be 26.9, 27.00, 31.2, 30.2, 31.1 and 28.1 per cent of the total dose administered in buffalo calves Nos. 1, 2, 3, 4, 5 and 6 respectively upto 72 hours post oral administration of drug at the rate of 10 mg per kg body weight.

The mean cumulative excretion of drug were 9.6, 25.8, 28.6, 29.06 and 29.1 per cent of total dose administered at 0-12, 12-24, 24-48 and 48-72 hours samples.

It would be evident from the results that majority of drug was excreted in the urine of buffalo calf between 12-24 hours post oral administration.

TABLE - X.

Average and individual cumulative excretion of nitrofurantoin in buffalo calves urine after a single oral dose administration at the rate of 10 mg per kg body weight.

Hour	Per cent of dose excreted						
	Buffalo calf No. 1	Buffalo calf No. 2	Buffalo calf No. 3	Buffalo calf No. 4	Buffalo calf No. 5	Buffalo calf No. 6	Average ± S.E.
0-12	12.9	7.9	14.4	7.7	6.7	8.0	9.6 ± 1.30
12-24	24.7	19.8	29.1	28.1	27.5	25.8	25.8 ± 1.36
24-48	26.1	25.8	31.2	29.7	31.1	28.1	28.6 ± 0.97
48-72	26.9	26.8	31.2	30.2	31.1	28.1	29.06± 0.82
72-96	26.9	27.0	31.2	30.2	31.1	28.1	29.1 ± 0.81
Total cumulative excretion.	26.9	27.0	31.2	30.2	31.1	28.1	29.1 ± 0.81

D I S C U S S I O N

D I S C U S S I O N

SULFAMOXOLE.

Plasma :

It is evident from the results (Table I and II) that sulfamoxole appeared in plasma of buffalo calves after one hour of oral administration of drug. This provides an evidence that absorption of drug takes place from the rumen of buffalo calves.

Austin and Stowe (1962) confirmed that absorption of various sulfonamides from calf rumen depends on pH-pka relationship. Sulfamoxole is a newly introduced sulfonamide and its pka value is not known. However, it would be expected that at buffalo calf ruminal pH value (between 6.3 to 7) the drug remained mostly in unionized state. The unionized molecules due to lipid soluble characteristics diffused through the ruminal mucosa and appeared in the plasma at concentrations ranging between 3.75 to 4.25 mg per cent (Table 1).

It would be further seen from Table I that at 1 hour, the plasma drug concentration of buffalo calf No. 5 was slightly nigher than other experimental buffalo calves which may have been either due to favourable ruminal pH variation or lower ruminal dilution facilitating a better absorption rate of the drug from the rumen of this buffalo calf.

The mean peak plasma level of free drug (11.60 mg per cent) was attained between 9-12 hours in this species and the free drug could be detected till 96 hours (Table II).

It would be evident from Table II that free drug plasma concentration above 5 mg per cent was maintained till 24 hours post single dose oral administration. Thereafter, the concentration declined slowly reaching 4.61 ± 0.45 mg per cent at 48 hours. At 96 hour, the concentration was significantly low. These observations indicate that between 24 hours and 48 hours, the disappearance rate of the drug from the plasma was slow. This relatively slower rate of disappearance may have been due to renal reabsorption processes or saturated drug metabolising capabilities.

A sulfonamide blood level of 5 mg per cent and more has been reported to be essential for antimicrobial activity against succesptible organisms (Maclay and Slavin, 1947; Stowe et al., 1956 and Brander and Pugh, 1971). In the present investigation it was found that a mean concentration of 5.35 to 7.60 mg per cent of free drug was maintained between 3 to 24 hour, after a single oral dose administration of sulfamoxole at the rate of 200 mg per kg body weight. Thus sulfamoxole may prove to be an useful antimicrobial for systemic use in buffaloes and will be required to be repeated every 24 hours. The necessity to repeat administration at 24 hours interval provides a distinct clinical advantage, thus obliviating the necessity of short span frequent administration. The persistance

of minimum therapeutic concentration for 24 hours may indicate that sulfamoxole is a long acting sulfonamide in buffaloes. The observations further indicate that there is a species variation in the distribution of sulfamoxole since Banerjee et al. (1977) have reported that therapeutic concentration in the poultry could only be achieved till 6 hours.

Urinary excretion :

The results incorporated in Table III and IV showed that a therapeutic mean concentration of 18.90 to 332.83 mg per cent in the urine was maintained till 96 hours following oral administration of sulfamoxole at the rate of 200 mg per kg body weight.

The presence of sulfamoxole for a longer period in the urine may indicate that at a urine pH of 7.5 (Gans, 1970), the ratio between ionized to unionized molicules was such that would facilitate higher non-ionic tubular reabsorption in the kidney thus prolonging its duration in the plasma which is turn increased its urinary excretion time. Baggot (1968,1970) observed that renal tubular reabsorption of sulfonamides in dogs, calves and pigs was dependent on pH-pka relationship.

The prolonged therapeutic concentration of sulfamoxole maintained in the urine of buffalo calves may be of advantage in urinary tract infection where usually a long term therapy is required.

In the present investigation the higher concentration (18.90 to 332.83 mg per cent) and persistance of sulfomoxole for a long period found in the urine of buffalo calves is well in agreement with the observations of Weinstein (1970) that sulfonamides and nitrofurans may be considered as best among the antimicrobials for their use in urinary tract infection without abstructive uropathy.

It would be seen from Table III and IV that minor variations were evident in the time concentration of the drug in the urine among the five experimental buffalo calves. These variations in the urine concentrations may be due to the variation in the volume output of urine and time and quantity of fluid intake by individual animals.

In the present study peak sulfamoxole level of unchanged drug was 332.83 mg per cent which was significantly higher than the concentration obtained with sulfanilamide, sulfapyridine, sulfadiazine, sulfadimidine and sulfaphenazole. On the basis of these findings it is inferred that sulfamoxole was superior to sulfanilamide, sulfapyridine, sulfadiazine, sulfadimidine and sulfaphenazole for its therapeutic use in urinary tract infection in ruminants specially in buffaloes.

As shown in Table V, 33.56 per cent of free and conjugated drug was excreted through urine till 96 hours post single oral administration of sulfamoxole at the rate of 200 mg/kg body weight. The rest 66.44 remained unaccounted for till 96 hours. It is possible that a part of the drug was

metablised and the other fraction excreted through urine and other routes subsequently. However, in ruminants, some drug molecules are destroyed in the rumen during macerative process. Furthermore, drugs may be so mixed with indigestible fiber as to pass through the digestive tract largely unabsorbed. In the present investigation it was found that majority of the drug (59 per cent of total excreted drug) was excreted through urine between 12-24 hours, presumably showing a saturated reabsorption mechanism which augmented faster renal excretion during the above said period.

The urinary concentration obtained with sulfamoxole when given orally at the rate of 200 mg/kg body weight being much higher than the desired therapeutic level, its dose rate could be reduced specially in cases of specific urinary tract infections, when higher blood level was not required. This reduction of dose simultaneously will minimize the chances of renal toxicity and at the same time reducing the cost of treatment.

BIOTRANSFORMATION OF SULFAMOXOLE.

Plasma :

The rate and extent of biotransformation plays an important role in the determination of duration of action of a drug. The metabolic degradation of sulfonamides in general,

takes place through conjugation reaction in which acetylation plays the most important role (Stowe, 1966). It would be evident from Table II that conjugated derivatives were present in the plasma between 1 to 48 hours post administration. The mean plasma conjugation per cent was only 3.98 per cent of the total drug at one hour at the corresponding plasma free drug concentration of 3.15 mg per cent.

The per cent conjugation increased with the increase of plasma free drug concentration. At 9 hours the maximum conjugation of 14.88 per cent of the total drug was recorded, at the corresponding plasma peak free drug concentration of 11.60 mg per cent. After 9 hours, per cent conjugation declined and became insignificant at 72 hours. These findings advanced evidence that conjugation quantitatively increased with the increase in plasma drug concentration and thereafter declined with the decrease in plasma drug concentration.

It may be possible that enzymatic reaction, increases with the increase in the concentration of drug and with time because whatever sulfonamide is present in the body has a greater chance to pass through the liver and become metabolised. The more the plasma concentration of the drug more molecules of drug will be metabolised due to collision frequency although corresponding per cent conjugation may fall as would be evident from Table II. The overall mean per cent conjugation was found to 7.65 per cent in buffalo plasma.

Singh (1974) observed overall mean per cent acetylation

of 8.34, 9.64, 9.37 and 8.37 for sulfamethazine, sulfaphenazole, sulfapyridine and sulfadiazine respectively in buffalo calves. Thus it would be evident that extent of acetylation with sulfamoxole (7.65 per cent) was very similar to the per cent acetylation of sulfamethazine, sulphaphenazole, sulfapyridine and sulfadiazine in buffalo plasma.

Urine :

Table III and IV indicated that conjugation product appeared in the urine till 96 hours. The percentage conjugation range of sulfamoxole in the urine of buffalo calves was 3.29 to 18.39 between 1 to 96 hours. The overall mean per cent conjugation was 14.06 per cent of the total drug excreted. However, the presence of comparatively lower per cent of acetylated drug in the urine of buffalo calves as compared to sulfanilamide, 72.7 per cent; sulfathiazole, 43.1 per cent; sulfamerazine, 37.3 per cent as observed by Stowe et al.(1956) in bovine urine, gives a distinct clinical advantage for its use since the chances of nephro-toxicity becomes meagre. The alkaline pH of buffalo urine further minimizes precipitation of acetylated derivative in the renal tubules.

DISTRIBUTION OF SULFAMOXOLE IN THE PLASMA AFTER SINGLE INTRAVENOUS ADMINISTRATION.

Plasma concentration of sulfamoxole was studied

after a single intravenous dose of 50 mg per kg body weight and the results have been presented in Table VI and VII. The therapeutic concentration (5 mg per cent and above) was present till 4 hours post administration of drug. The peak concentration of free drug in buffalo plasma was 13 mg per cent at ½ hour. Inspite of the fact it maintained a good therapeutic concentration the administered dose of 50 mg per kg body weight seems to be low as the therapeutic level (5 mg per cent) was maintained till only 4 hours. To maintain a therapeutic level for longer period and to avoid frequent repeatation, the present dose level needs to be raised.

This experiment was conducted chiefly for the purpose of obtaining values so as to determine the biological half-life and apparent volume distribution of the drug as such higher dose levels were not tried.

Apparent volume distribution indicates the degree of localization of a drug in extracellular as well as in intracellular compartments of the body. The concept of volume distribution of drug can be help ful in understanding body disposition of drugs and in devising optimal methods of using drugs in practical therapeutics. The mean apparent volume distribution (per cent) of sulfamoxole in buffalo was calculated to be 31.25 per cent. In ruminants the extracellular space ranges between 15 to 20 per cent. As such, it may be concluded on the basis of the present findings that sulfamoxole will be distributed mainly to the extracellular space and may

also diffuse into intracellular compartments to some extent.

It is worthwhile to mention here that degree of localization of drug outside the plasma, would be associated with the slow disappearance of drug from the body as there is definite relationship between volume distribution to the Kinetics of elimination of drugs. Thus marginal diffusion of sulfamoxole into the intracellular space might have contributed to some extent for the slow elimination of sulfamoxole from the buffalo urine (Table II and III). However, the role of tubular reabsorption (as discussed previously) can not be ignored for explaining the persistance and longer duration of presence of this drug in buffalo urine.

The mean biological half-life of sulfamoxole in buffalo calves was calculated to be 2 hours 10 minutes.

NITROFURANTOIN.

Plasma :

The drug appeared within one hour post oral administration in the plasma (Table VIII). This suggested absorption of nitrofurantoin from rumen of buffalo calves as food takes 3-5 hours to reach small intestine. The absorption of drug seems to be pH-pka dependent. Nitrofurantoin being a weak organic acid with pka of 7.2 (Paul et al., 1960), at a buffalo ruminal pH of 6.3, about 90 per cent of the drug would be expected to remain in unionized form. The higher unionization would have expectedly facilitated faster absorption of the drug across the ruminal wall. The absorption of drug across simple stomach mucosa as well as ruminal mucosa dependent on pH-pka relationship has been reported by several workers (Schanker et al., 1957; Stowe et al., 1956; Banerjee et al., 1977).

The peak plasma concentration of nitrofurantoin (2.28 µg/ml) was observed at 12 hours post single dose oral administration of the drug at the rate of 10 mg per kg body weight. However, the peak plasma concentration in buffalo calf No. 2 was observed at 24 hours, in this case the delayed absorption may have been occurred due to ruminal stasis which raised ruminal pH as well as which may have delayed the passage of drug to the small intestine the site of absorption.

The level of nitrofurantoin in plasma has been studied by many workers in different species of animals and man. Paul et al. (1959, 1960) observed nitrofurantoin concentration in plasma of rat and found a concentration of 1.2 mg/l and 2.6 mg/l at the dose level of 25 and 100 mg per kg body weight. Schirmeister et al. (1965) reported 1 to 4 mg per litre of nitrofurantoin concentration in human plasma at therapeutic dose level. Pandey (1974) reported a concentration of 1.71 µg/ml of nitrofurantoin in goat plasma at 12 hours post oral administration of drug at the rate of 10 mg/kg body weight. In this experiment however the peak plasma concentration was higher (2.28 ug/ml) at 12 hours as compared to that in goat as reported by Pandey (1974).

On the basis of findings recorded by the above workers it is confirmed that nitrofurantoin did not achieve an appreciable therapeutic concentration in plasma of different species of animals. Low concentration recorded in plasma inspite of good absorption of the drug may be due to the rapid plasma protein binding. Paul et al. (1960) reported that nitrofurantoin was bound to the extent of 50-90 per cent in rat plasma. The author also reported the low blood level of this drug following clinical use in dog and rat.

Ronald and Turk (1967) observed in a vitro study that the concentration of 16 µg/ml could inhibit the growth of about 100 per cent of the sensitive strains of E. coli.

It would be evident then that nitrofurantoin in buffaloes gave therapeutic plasma concentration between 12 to 24 hours if 2-8 µg/ml is taken as minimal inhibitory concentration (Vomel, 1963). Thus, this compound failed to give optimal antimicrobial concentration in the plasma between 0-12 hours and after 24 hours besides giving a very marginal therapeutic concentration between 12 to 24 hours.

These results thus indicate that nitrofurantoin may not be of clinical usefulness in cases of systemic infection. However, it may be appreciated that a low dose level of only 10 mg/kg was used in this experiment, irrespective of the fact that in buffaloes the ruminal dilution was apt to play its role of slow absorption. Actually in ruminants, the dosage level of drug is generally higher than in case of animals having simple stomach. In this experiment however, established dosage level was used to find out the plasma levels at that dosage level. As such higher dosage were not used.

Urinary excretion :

The data depicted in Table IX indicates that 1 hour of urine sample showed a mean concentration of 7.84 µg/ml. The mean peak concentration was 72.45 µg/ml at 12 hours in the buffalo calves. However, in buffalo calf No. 1 the peak urinary drug concentration was observed at 6-9 hours.

Several investigations (Paul et al. 1959; Conklin

and Hollifield (1965) advanced evidences that a species variation occurred in the concentration, persistance and excretion of nitrofurantoin. In the present investigation, in buffaloes, the peak concentration was observed at 12 hours which persisted till 24 hours whereas in simple stomached animals like dog, and rat the majority of drug was found at 0-8 hours (Paul et al., 1959). This deviation might be due to slower rate of absorption in buffalo. In ruminants usually food takes 3-5 hours to reach small intestine whereas in dog and human being digestion is complete in 3-5 hours. These situations may lead to somewhat more variable responses to drug therapy in ruminants than is noticed in simple stomached animals.

In the present study only 29 per cent of the orally administered drug was detectable in buffalo calves till 96 hours. It may be quoted here that 30-50 per cent of the orally administered drug was recoverable in urine in animals and human beings (Paul et al., 1959; Miura and Rockendorf, 1967; Buzzard et al., 1961). The rest 71 per cent remained unaccounted for upto that period. It is possible that this amount of the drug was metabolised and a portion excreted through other routes and through urine subsequently. The present study revealed that the persistance of drug in the buffalo plasma was comparatively longer than in the dog, rats and human being. This persistances enhance the chances of metabolism to a greater extent than in other species of animal in which the drug is eliminated through the body more rapidly.

Nitrofurantoin a weak organic acid with pka of 7.2 at the alkaline urine pH remains mostly in ionized state (Paul and Paul, 1964; 1966), in tubular urine the ionized drugs are least absorbed across tubular membrane so it was excreted in urine in high concentration.

It would be evident from the findings of present investigation that effective therapeutic concentration was maintained in the buffalo urine from 3 to 48 hours post oral administration of drug. The high concentration of drug excreted through the kidney is advantageous for its use in urinary tract infection. Weinstein (1970) has preferred the use of nitrofurans and sulfonamides in urinary tract infection without obstructive uropathy.

It has been reported that optimum therapeutic effect of nitrofurantoin can only be achieved in acidic urine (Paul et al., 1960; Miura and Rockendorf, 1967; Schirmeister et al., 1965). So, in view of the above findings alkaline urine of buffaloes could be adjusted to some extent by the administration of acidifying agents like ammonium chloride to get optimum antimicrobial effect of this compound. Future, pharmacokinetic investigation on the above line may throw light on the extent of efficacy of this drug in ruminants in urinary tract infections.

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S U M M A R Y

S U M M A R Y

1. The present study revealed that in buffaloes after an oral dose of 200 mg per kilogram body weight of sulfamoxole therapeutic blood level was maintained till 24 hours. Thus sulfamoxole can be considered as a longacting sulfonamide in buffaloe.

2. The longer duration of sulfamoxole in the buffalo blood indicates its possible effective use in systemic infections succeptible to sulfonamide therapy.

3. After a single intravenous administration of sulfamoxole at the rate of 50 mg per kg body weight, therapeutic blood level was observed between 30 minutes to 4 hours indicating that the drug may be useful in acute infections. However, the present dose (50 mg/kg body weight) seems to be less as the drug will be required to be repeated at every 4 hours as such to minimize the frequency of administration the dose rate could be increased.

4. Contrary to the sulfamoxole, the nitrofurantoin failed to achieve an appreciable therapeutic concentration in plasma of buffalo calves (0.58 to 2.28 µg/ml) after an single oral dose of 10 mg per kg body weight. Thus nitrofurantoin may be ineffective as an antimicrobial in systemic

infections, particularly at the recommended dose level of 10 mg per kg body weight.

5. A therapeutically effective sulfamoxole urinary concentration in buffaloes was observed from 1 to 96 hours after a single oral dose administration at the rate of 200 mg per kg body weight. The higher and sustaining rate of excretion of sulfamoxole in the buffaloe urine makes it a potential good choice for urinary tract infection where usually a long term therapy is required. Further, the present dose level (200 mg per kg body weight) could be comfortably slashed down thus decreasing the cost of medication.

6. A therapeutically effective urinary concentration of nitrofurantoin was observed in the buffaloe between 3 to 48 hours after a single dose oral administration of the drug at the rate of 10 mg/kg body weight. Thus in buffaloes, nitrofurantoin may be fruitfully used in urinary tract infections along with a urine acidifier.

7. In buffaloes also conjugation was found to be the chief route of sulfamoxole biotransformation. In buffalo plasma and urine the overall conjugation was 7.65 mg per cent and 14.06 mg per cent respectively. Thus the comparatively lower rate of acetylation involved with this drug gives it an added advantage in regard to its possible minimal nephrotoxic side effects.

8. The apparent volume distribution of sulfamoxole was found to be 31.25 per cent indicating that the drug would diffuse into the extracellar space and to a small extent into intracellular space.

9. The mean biological half-life of sulfamoxole was determined.

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