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Pharmacokinetics of sulphadoxine and trimethoprim in horses. Half-life and volume of distribution of sulphadoxine and trimethoprim and cumulative excretion of [^{14}C]-trimethoprim

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A proprietary combination product containing sulphadoxine and trimethoprim was administered to horses by intravenous injection. Protein-binding of sulphadoxine was dependent on the concentration in plasma and decreased from 72% at 50 μg/ml to 14% at 450 μg/ml. Sulphadoxine is eliminated from plasma in accordance with a three compartment open model. The elimination half-life was on average 14 h while the volume of distribution was found to be 0.39 l/kg. Trimethoprim was eliminated from plasma in accordance with a two compartment open model. The elimination half-life was on an average 3 h. Experiments in which trimethoprim was administered alone showed that the elimination half-life was not dependent on the simultaneous administration of sulphadoxine. About 50% of trimethoprim was bound to plasma proteins, but in contrast to sulphadoxine there was no dependence between plasma concentration and protein binding. The protein binding of trimethoprim was independent of the presence of sulphadoxine and *vice versa*. Experiments with ^{14}C-labelled trimethoprim showed that it was excreted in almost equal amounts in urine and faeces. 97% of the administered dose was recovered in urine and faeces during the course of the first 4 days after administration.

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INTRODUCTION

Sulphadoxine and trimethoprim (TMP) are eliminated from plasma partly by metabolism and partly by excretion of the unchanged compounds in urine or faeces. Differences in the extent of any of these processes may lead to large variations in pharmacokinetic parameters between species such as have been reported for both sulphadoxine and TMP.

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The information on the pharmacokinetics of sulphadoxine and TMP in horses is very scarce. Therefore, these parameters have been further studied in this species using a proprietary combination of sulphadoxine and TMP (Duoprim vet., Wellcome) and ^{14}C-labelled TMP.

MATERIALS AND METHODS

Eleven experiments were performed on six clinically healthy horses (two stallions and four mares), aged from 0.7 to 6 years of body weights 174–352 kg.

The sulphadoxine/TMP combination was administered intravenously in the left jugular vein. In six experiments, 40 mg sulphadoxine and 8 mg TMP per kg were injected and in two experiments, a dose of 120 mg sulphadoxine and 24 mg TMP per kg were used. In the last three experiments, ^{14}C-TMP (specific activity 0.2 μCi/mg) was administered alone in a dose of 8 mg/kg.

Blood samples were collected through a plastic cannula (Braunüle 2R) placed in the right jugular vein just before and 5, 10, 15, 20, 30, 40 and 50 min, and 1, 1.5, 2, 3, 4, 5, 6, 7, 9, 13, 24, 30 and 48 h after injection.

The general clinical appearance of the horses (behaviour, defecation, pulse and respiration) was observed in order to consider any unfavourable reaction due to the administration of the drug.

In three experiments where ^{14}C-TMP was given alone, urine was sampled for 32 h by means of a balloon catheter (Rüsch No. 28, 75 ml) placed in the bladder for the whole sampling period. Faeces were collected quantitatively up to 96 h after administration.

Analytical methods

The sulphadoxine concentration in plasma was determined by the method of Bratton & Marshall (1939). TMP was measured spectrofluorometrically according to Schwartz, Koechlin & Weinfeld (1969).

The concentration of ^{14}C-TMP in plasma and urine samples (TMP + metabolites) was determined by liquid scintillation counting. The composition of the scintillation liquid was: 8 g PPO, 200 mg dimethyl-POPOP, 100 ml BBS-3 (Beckman) and 1900 ml toluene. The concentration of ^{14}C-TMP in faeces was estimated after wet combustion according to Packard (1974).

Protein-binding

Buffered aqueous solutions (pH = 7.4) of different concentrations of sulphadoxine and of TMP were added to plasma samples from two horses using a constant volume ratio, 1 : 19, and concentrations of 50, 150 and 450 μg sulphadoxine per ml or 1, 5 and 25 μg TMP per ml (Rieder, 1963). In addition, a buffered solution of sulphadoxine and TMP was added to plasma samples using the same volume ratio in order to obtain a concentration of 450 μg sulphadoxine plus 25 μg TMP per ml. Ultrafiltration of the samples was performed by the method of Poulsen (1956).

Pharmacokinetic analysis

The experimental data was analysed by a non-linear iterative curve-fitting program AUTOAN (Sedman & Wagner, 1976).

The volume of the central compartment (V_c) and the apparent volume of distribution ($V_{d(B)}$) was calulated using the following formulae (*vide* Baggot, 1977):

$$V_c = \frac{D}{A + B} \qquad \text{(two-compartment model)}$$

$$V_c = \frac{D}{P + A + B} \qquad \text{(three-compartment model)}$$

$$V_{d(B)} = \frac{D}{B} \qquad \text{(two- and three-compartment model)}$$

where D = dose (mg/kg b.wt) and P, A and B are zero-time plasma drug concentration intercepts. A and B are based on the distribution and elimination phases. P is based on the rapid initial distribution phase. The statistical calculations were performed by standard methods.

RESULTS

Protein-binding

The results from the *in vitro* determination

of sulphadoxine protein-binding are shown in Fig. 1. It is seen that the percentage of protein-binding decreases with increasing concentration of sulphadoxine in plasma. The protein-binding of TMP was on an average 51% and it was not dependent on the plasma concentration within the concentration range investigated. There was no interference in the protein-binding between sulphadoxine and TMP.

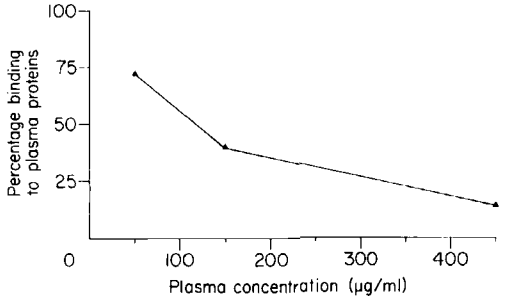

FIG. 1. The relationship between plasma concentration and binding of sulphadoxine to plasma proteins *in vitro*.

Pharmacokinetic parameters

The results from determination of the plasma concentration of sulphadoxine and TMP in a single experiment are shown in Fig. 2.

The figure shows that sulphadoxine is eliminated from plasma in accordance with a three-compartment open model which may be described by the formula:

$$C_t = P\,e^{-\pi t} + A\,e^{-\alpha t} + B\,e^{-\beta t}$$

where C_t = plasma concentration at time t.

The use of the non-linear regression analysis (AUTOAN) showed that the experimental data from all the experiments with 40 mg as well as 120 mg sulphadoxine per kg fit this model. The data obtained from the analyses of the experiments are listed in Table I.

The distribution may be described as the sum of two exponential functions ($e^{-\pi t}$ + $e^{-\alpha t}$) reflecting differences in the rate of distribution. The half-lives calculated from these two functions were on average $t_{½(\pi)}$ = 5 ± 1 min and $t_{½(\alpha)}$ = 80 ± 15 min. The elimination half-life $t_{½(\beta)}$ was on an average 851 ± 95 min varying from 410 to 1081 min.

The volume of the central compartment (V_c) was on average 0.18 ± 0.01 l/kg and the apparent volume of distribution ($V_{d(B)}$) was 0.39 ± 0.02 l/kg.

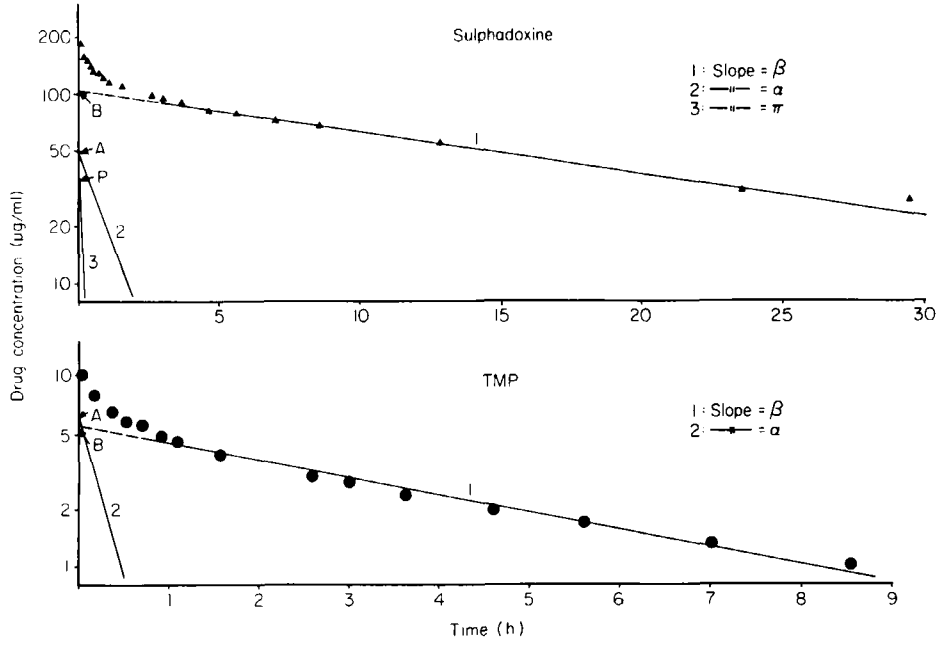

FIG. 2. Plasma concentration of sulphadoxine (▲) (40 mg/kg) and TMP (●) (8 mg/kg) after intravenous injection. The solid lines are based on non-linear regression analysis.

TABLE I. The pharmacokinetic parameters of sulphadoxine calculated by means of the curve-fitting computer program after intravenous infusion of 40 mg/kg to horses

Horse No.	Sex	Age (years)	Body weight (kg)	P (μg/ml)	A (μg/ml)	B (μg/ml)	π (min^{-1})	α (min^{-1})	β (min^{-1})	$t_{½}(\pi)$ (min)	$t_{½}(\alpha)$ (min)	$t_{½}(\beta)$ (min)	V_c (l/kg)	$V_d(B)$ (l/kg)
H1	F	3	282	28.3	40.1	99.2	0.09	0.010	0.00073	7	70	947	0.24	0.40
H2	M	1	200	65.6	71.8	93.1	0.16	0.008	0.00075	4	83	925	0.17	0.43
H3	M	0.7	174	55.9	46.4	98.9	0.13	0.014	0.00074	5	51	939	0.20	0.40
H4	F	6	352	113.6	69.6	94.0	0.17	0.005	0.00064	4	148	1081	0.14	0.43
H5	F	4.5	330	66.4	41.3	135.2	0.18	0.009	0.00169	4	77	410	0.17	0.30
H6	F	2.5	275	68.6	49.5	104.1	0.15	0.014	0.00086	5	49	803	0.18	0.38
	Average									5	80	851	0.18	0.39
	± SEM									± 1	±15	±95	± 0.01	± 0.02
H2*	M	1.0	185	220.3	125.0	96.2	0.07	0.009	0.00073	9	79	947	0.27	0.54
H3*	M	0.7	176	218.4	336.9	150.4	0.20	0.010	0.00068	4	73	1018	0.17	0.55

*Dose: 120 mg/kg

For TMP, Fig. 2 shows an elimination from plasma corresponding to a two-compartment open model which is described by the formula:

$$C_t = A\ e^{-\alpha t} + B\ e^{-\beta t}$$

The non-linear regression analysis fits the experimental data from all the experiments (8 mg and 24 mg TMP per kg) to this model. The data obtained from the analyses of the experiments are listed in Table II.

The distribution half-life ($t_{½(\alpha)}$) was on average 10 ± 2 min. The elimination half-life ($t_{½(\beta)}$) was on average 189 ± 17 min varying from 110 to 214 min.

The volume of the central compartment (V_C) was on average 0.76 ± 0.07 l/kg while the apparent volume of distribution ($V_{d(B)}$) was 1.51 ± 0.06 l/kg.

Dividing the concentration of a drug in plasma (mg/l) by the dose (mg/kg), and relating this ratio to time will result in superimposing curves for drugs eliminated by first order kinetics. A superimposition plot for sulphadoxine based on the two dose-levels of the combination formulation of sulphadoxine/trimethoprim in a horse (no. H2) is shown in Fig. 3a. The plot for the high dose-level of sulphadoxine shows constantly lower values than the plot for the low dose-level. A superimposition plot for TMP is given in Fig. 3b which shows almost identical values for the two dose levels. Comparable results were obtained for both sulphadoxine and TMP in horse no. H3.

The plasma concentration of TMP obtained by spectrofluorimetry and by liquid scintillation counting after intravenous infusion of ^{14}C-TMP were analysed by non-linear regression analysis and the calculated parameters are given in Table III. The average results obtained by the two methods were compared by Student's t test. This analysis did not show any significant difference between $t_{½(\alpha)}$, $t_{½(\beta)}$, V_C and $V_{d(B)}$ ($P > 0.05$) obtained by the specific spectrofluorometric analysis and by the non-specific determination by liquid scintillation counting which includes metabolites of TMP.

The average pharmacokinetic parameters based on spectrofluorometric analysis after intravenous infusion of TMP alone (Table III) were compared with those obtained after administration of TMP in combination with sulphadoxine (Table II) using Student's t test. The pharmacokinetic parameters obtained after administration of TMP alone did not differ significantly from those obtained after administration of TMP in combination with sulphadoxine ($P > 0.05$).

Cumulative excretion

The cumulative excretion of ^{14}C-TMP (TMP + metabolites) in urine and faeces and the total excretion are shown in Fig. 4. About 30% of the dose was excreted in urine in 6 h. In 32 h, a total of 46% was recovered in urine. Faecal excretion comprised 51% of the administered dose after 96 h. This means that the total excretion during 96 h was 97% of the dose administered.

DISCUSSION

The percentage of sulphadoxine protein-bound in equine plasma is dependent on the concentration of sulphadoxine present, and declines from 72% at 50 μg/ml to 40% at 150 μg/ml and 14% at 450 μg/ml. These findings agree with the results obtained in cows (Nielsen & Rasmussen, 1977).

The protein-binding of TMP in plasma was not dependent on the concentration at the plasma levels of TMP examined. This is a rather common finding for bases (Baggot, 1977) and in accordance with the findings for TMP in cows (Davitiyananda & Rasmussen, 1974).

The pharmacokinetic behaviour of sulphadoxine in horses is best described by a three compartment open model (Fig. 1). Similar observations in horses have been made for oxytetracycline (Baggot, 1977), while the pharmacokinetics of sulphadoxine are described by a two-compartment open model in cows (Davitiyananda & Rasmussen, 1974), swine (Nielsen & Rasmussen, 1975d) and goats (Nielsen & Rasmussen, 1976).

The distribution phase of sulphadoxine in horses may be described as a sum of two first order reactions which expresses differences in the rate of distribution. This is possibly caused by differences in the rate by which the drug can penetrate into the tissues. Equilibration between plasma and tissue concentrations of

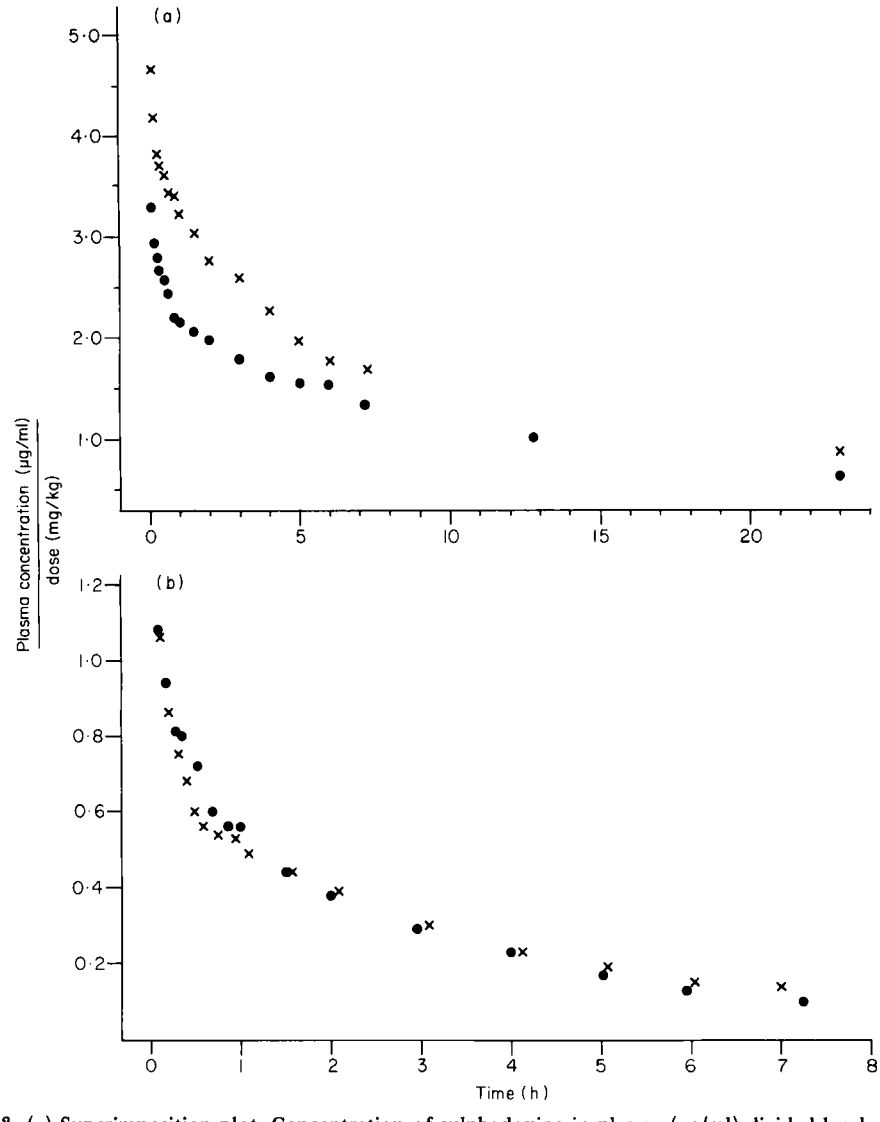

FIG. 3. (a) Superimposition plot. Concentration of sulphadoxine in plasma (μg/ml) divided by dose, 40 mg (×) and 120 mg (•) sulphadoxine/kg, versus time following intravenous administration. (b) Superimposition plot. Concentration of TMP in plasma (μg/ml) divided by dose, 8 mg (×) and 24 mg (•) TMP/kg, versus time following intravenous administration.

sulphadoxine would occur more rapidly in tissues into which sulphadoxine penetrated rapidly, rather than those into which it penetrated slowly. This would be reflected in different rates in decline of plasma concentration in the distribution phase and may be postulated as a contribution to the bi-exponential distribution phase.

Another factor of importance is the degree of protein-binding. The fact that protein-binding of sulphadoxine is dependent on the plasma concentration means that the fraction of non-protein-bound sulphadoxine is high just after intravenous administration of the drug and decreases as the plasma concentration declines. Since it is only the non-protein-bound fraction of drugs which can cross biological membranes, the proportion available for diffusion into tissue compartment is highest initially and gradually decreases with de-

TABLE II. The pharmacokinetic parameters of trimethoprim calculated by means of the curve-fitting computer program after intravenous infusion of 8 mg/kg to horses

Horse No.	Sex	Age (years)	Body weight (kg)	A (μg/ml)	B (μg/ml)	α (min^{-1})	β (min^{-1})	$t_{½(\alpha)}$ (min)	$t_{½(\beta)}$ (min)	V_c (l/kg)	$V_{d(B)}$ (l/kg)
H1	F	3	282	1.8	5.5	0.049	0.0033	14	212	1.10	1.45
H2	M	1	200	5.0	6.5	0.094	0.0038	7	181	0.70	1.61
H3	M	0.7	174	6.9	6.3	0.113	0.0063	6	110	0.61	1.28
H4	F	6	352	6.3	4.6	0.073	0.0032	14	214	0.73	1.74
H5	F	4.5	330	6.0	5.4	0.095	0.0034	7	203	0.71	1.49
H6	F	2.5	275	6.2	5.4	0.056	0.0032	12	214	0.69	1.50
Average ± SEM								10 ±2	189 ±17	0.76 ± 0.07	1.51 ± 0.06
H2*	M	1.0	185	14.4	16.2	0.060	0.0044	12	156	0.78	1.48
H3*	M	0.7	176	24.0	18.1	0.085	0.0033	8	211	0.57	1.33

*Dose: 24 mg/kg

TABLE III. The pharmacokinetic parameters of ^{14}C-labelled TMP (8 mg/kg 0.2 μCi/mg) calculated by means of the curve-fitting computer program

Horse No.	Sex	Age (years)	Body weight (kg)	A (μg/ml)	B (μg/ml)	α (min^{-1})	β (min^{-1})	$t_{½}(\alpha)$ (min)	$t_{½}(\beta)$ (min)	V_c (l/kg)	$V_d(B)$ (l/kg)
H1*	F	3	327	3.0	3.1	0.079	0.0041	9	169	1.31	2.58
H5*	F	4.5	320	6.7	4.0	0.084	0.0037	8	190	0.75	2.03
H6*	F	2.5	320	6.4	6.5	0.090	0.0052	8	134	0.62	1.23
Average								8.3	164	0.89	1.95
± SEM								± 0.3	±16	± 0.21	± 0.39
H1†	F	3	327	5.3	5.5	0.127	0.0039	5	180	0.75	1.46
H5†	F	4.5	320	6.0	6.0	0.099	0.0025	7	214	0.62	1.33
H6†	F	2.5	320	5.6	6.6	0.099	0.0041	7	168	0.66	1.21
Average								6	187	0.68	1.33
± SEM								± 1	±14	± 0.04	± 0.07

*Spectrofluorometric method.
†Liquid scintillation counting.

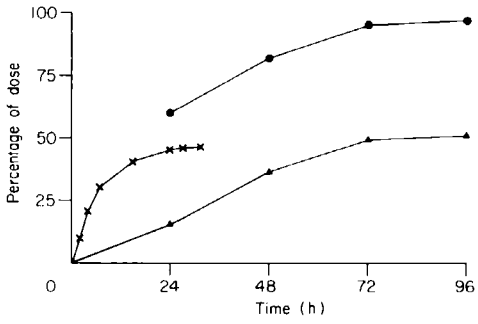

FIG. 4. Cumulative excretion of TMP and its metabolites (▲ faecal excretion, x urinary excretion and ● total excretion) after intravenous administration of ^{14}C-TMP (8 mg/kg, 0.2 μCi/mg). The results are the average of three experiments.

creasing plasma concentration. Consequently the division of the distribution phase into two separate processes may be caused partly by the concentration-dependent changes of the protein-binding of sulphadoxine and partly by differences in tissue penetration rate.

The elimination half-life of sulphadoxine averaged 851 min (14 h) at both dose levels in horses (Table I). This value is a little greater than that observed in cows (11 h) (Davitiyananda & Rasmussen, 1974) and goats (12 h) (Nielsen & Rasmussen, 1976), and considerably longer than that observed in swine (8 h) (Nielsen & Rasmussen, 1975a). Compared to humans (123–205 h) (Portwich & Büttner, 1964; Madsen & Iversen, 1964; Bünger, 1967; Struller, 1968; Böhni, Fust, Rieder, Schaerer & Havas, 1969), the half-life of sulphadoxine in horses is very short.

The volume of the central compartment (V_c) of sulphadoxine in horses was on an average 0.18 l/kg and corresponds well to the volume of the extracellular body water. The apparent volume of distribution ($V_{d(B)}$) was 0.39 l/kg (dose: 40 mg/kg) and in accordance with observations in cows (0.37 l/kg; Davitiyananda & Rasmussen, 1974), swine (0.35 l/kg; Nielsen & Rasmussen, 1975d) and goats (0.27 l/kg; Nielsen & Rasmussen, 1976). Administration of 120 mg/kg sulphadoxine to two horses resulted in an increase of the apparent volume of distribution to 0.55 l/kg. This increase may be explained by the decrease in protein-binding caused by the higher plasma concentration. This will result in a greater fraction of non-protein-bound sulphadoxine available for diffusion into tissue compartments with a consequent increase in the apparent volume of distribution. This is further confirmed by the superimposition plot (Fig. 3).

In horses the elimination of TMP from plasma follows a two-compartment open model (Fig. 2). Similar observations have been reported previously in horses (Alexander & Collett, 1975) and rabbits (Ladefoged, 1977). The distribution half-life was on average 10 min. The elimination half-life was on average 189 min (3 h) at both dose-levels in the horses (Table II). Alexander & Collett (1974; 1975) found variations in elimination half-life from 3 to 6 h. The elimination half-life in horses is considerably shorter than in humans (9–15 h) (Bushby & Hitchings, 1968; Schwartz & Ziegler, 1969; Schwartz & Rieder, 1970; Bergan & Brodwall, 1972; Craig & Kunin, 1973; Fowle, 1973; Nolte & Büttner, 1973; Andreasen, Elsborg, Husted & Thomsen, 1978), and about the same as in dogs (3 h) (Kaplan, Weinfeld, Cotler, Abruzzo & Alexander, 1970) and in swine (2.5 h) (Nielsen & Rasmussen, 1975b). The apparent volume of distribution was 1.51 l/kg, thus resembling the values obtained in humans (1.4 l/kg; Andreasen *et al.*, 1978), cows (1.1 l/kg; Davitiyananda & Rasmussen, 1974), swine (1.4 l/kg; Nielsen & Rasmussen, 1975b) and goats (1.2 l/kg; Nielsen & Rasmussen, 1972). A value greater than 1 indicates that the concentration of TMP in tissue is higher than in plasma. This has been confirmed by the estimation of concentrations of TMP in tissues from mice (Bushby & Hitchings, 1968); rats (Schulz, 1972); humans (Lykkegård Nielsen & Hansen, 1972); pigs (Nielsen & Rasmussen, 1975b) and goats and cows (Nielsen & Rasmussen, 1975c).

There was no difference between the pharmacokinetic parameters for TMP based on the spectrofluorometric analysis and those based on liquid scintillation of ^{14}C-TMP. This indicates that the concentration of metabolites in plasma was very low and thus in accordance with findings in swine (Nielsen & Rasmussen, 1975d) but in contrast to those in goats where there appears to be a relatively high fraction of TMP-metabolites in plasma (Nielsen & Rasmussen, 1976). These findings in goats are in accordance with the rapid rate of metabolism of TMP in this species which results in very short half-life and the excretion of only 2%

unchanged TMP in urine (Nielsen & Rasmussen, 1975b). The pharmacokinetic parameters obtained for TMP in the experiments where it was administered alone were not different from those obtained after administration together with sulphadoxine. This means that the distribution and elimination of TMP and sulphadoxine in horses take place independently of each other. Similar results have been obtained with TMP and sulphamethoxazole in humans (Schwartz, Vetter & Englert, 1970).

The urinary and faecal excretion of ^{14}C-TMP were almost equal to each other in horses. The same has been reported in goats and cows (Nielsen & Rasmussen, 1975c) while humans (Schwartz, *et al.*, 1970) and pigs (Nielsen & Rasmussen, 1975d) excrete a much higher fraction in urine than in faeces.

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