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MAMMARY AND RENAL EXCRETION OF SULPHADOXINE AND TRIMETHOPRIM IN COWS*

By

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DAVITIYANANDA, DANIS and FOLKE RASMUSSEN: *Mammary and renal excretion of sulphadoxine and trimethoprim in cows.* Acta vet. scand. 1974, *15*, 340—355. — In 21 experiments on 5 healthy, non-pregnant cows are sulphadoxine and trimethoprim infused intravenously for maintenance of constant levels of the drugs through the experimental periods. The experiments show that both sulphadoxine and trimethoprim are bound to the proteins in blood plasma and milk. Further it is demonstrated that sulphadoxine (an acid) is excreted into milk in concentrations lower than in blood plasma while trimethoprim (a base) is excreted into milk in concentrations higher than in blood plasma. Both results are consistent with the theory that drugs are excreted through the mammary gland by passive diffusion.

Glomerular filtration and back-diffusion are both involved in the renal handling of sulphadoxine and trimethoprim. For trimethoprim active tubular secretion is also demonstrated. Both the mammary and renal handling of sulphadoxine as well as trimethoprim are influenced by the pH of milk and urine, respectively. The experiments underline that it is the unionized, non-protein-bound fraction of the drugs which diffuses through biological membranes.

sulphadoxine; trimethoprim; mammary excretion; renal excretion; cow.

In earlier publications it has been shown that only the non-protein-bound and unionized fraction of weak electrolytes diffuses through the mammary gland epithelium. This means that acids are excreted into milk in concentrations equal to or lower than those in plasma, while bases are excreted into milk in con-

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centrations equal to or higher than those in plasma (*Rasmussen* 1958, 1959, 1966, 1971, 1973, *Sisodia & Stowe* 1964, *Stowe & Plaa* 1968, *Ziv & Risenberg-Tirer* 1969, *Ziv & Sulman* 1973 a, b). Further, it has been shown that the unionized fraction of chemotherapeutics in renal tubular fluid is responsible for the back-diffusion from tubular lumen to blood circulation (*Beyer et al.* 1944, *Dalgaard-Mikkelsen* 1951, *Dalgaard-Mikkelsen & Poulsen* 1956, *Sharpstone* 1969, *Rasmussen* 1970, *Jørgensen & Rasmussen* 1972). The ionization of the acid sulphadoxine and the base trimethoprim is changed in opposite directions when the pH in the medium is changed. These drugs are normally used in fixed combinations and it seemed, therefore, to be of interest to investigate the mammary and renal handling of simultaneously infused sulphadoxine and trimethoprim.

EXPERIMENTAL

Twenty-one experiments were conducted on 5 healthy, non-pregnant cows with individual milk yield varying from 3 to 8 kg per day. The kidneys and udders were clinically normal. In each experiment an initial intravenous infusion of sulphadoxine* (4-sulphanilamido-5,6-dimethoxy-pyrimidine) and/or trimethoprim** (2,4-diamino-5-(3,4,5-trimethoxybenzyl)-pyrimidine) was given, and subsequently, the concentration in blood plasma was kept constant by continuous intravenous infusion of sulphadoxine through a plastic catheter (Intracath®) placed in the left jugular vein and by infusion of trimethoprim through another plastic catheter in the same vein. The lowest and highest priming doses of sulphadoxine used were 7 and 55 mg/kg b.wt. followed by maintenance doses of 2 and 15 mg/kg b.wt. per hr., respectively. For trimethoprim the priming doses were 3 and 6 mg/kg b.wt. followed by maintenance doses of 0.7 and 1.6 mg/kg b.wt. per hr. The priming dose of sulphadoxine was dissolved in 75 ml distilled water by adding 5N-NaOH (0.7—1 ml 5N-NaOH per g sulphadoxine) and infused in 10 min. The solutions of sulphadoxine used for the continuous intravenous infusion were prepared in the same way and diluted with 0.9 % sodium chloride solution. The priming dose of trimethoprim was dissolved in

* Sulphadoxine was generously supplied from the Wellcome Research Laboratories, Beckenham, Kent, England, and

** Trimethoprim from Grindstedværket, Aarhus, Denmark.

50 ml distilled water added lactic acid (1 ml lactic acid per g trimethoprim). The solution of trimethoprim used for the continuous intravenous infusion was diluted with distilled water.

Blood samples were taken at intervals of 30 min. through a plastic cannula (Braunüle 2 R) placed in the right jugular vein. The bladder was emptied at 30-min. intervals via a balloon catheter (Rüsch no. 28, 75 ml). Before the start of the infusion the mammary glands were milked out completely. During the experiment the glands were milked out at intervals of 30 min. In each experiment 5 or 6 blood, urine and milk samples were taken for determination of sulphadoxine, trimethoprim and creatinine. The sampling was started 30 min. after establishment of the continuous intravenous infusion.

Sulphadoxine in blood plasma, urine and milk was measured ad modum *Bratton & Marshall* (1939) modified for milk by *Rasmussen* (1958). Trimethoprim was estimated according to the method of *Schwartz et al.* (1969).

The protein binding of sulphadoxine and trimethoprim in blood plasma and milk was determined by ultrafiltration by the method of *Poulsen* (1956). The cellophane membrane used was "Kalle" dialysing tube, diameter 32 mm, pore size 20—80 Å, which should permit molecules with a molecular weight up to 5,000 to pass through.

The endogenous creatinine clearance was used to express the glomerular filtration rate. Creatinine was estimated by the method described by *Bonsnes & Taussky* (1945).

The pH of the blood, milk and urine samples was measured immediately after each experiment by means of a potentiometer with glass electrode (Radiometer, Copenhagen) at 37°C.

The pK_a value of sulphadoxine is 6.1 (*Struller* 1968) and of trimethoprim 7.6 (*Rasmussen* 1970).

Calculations of the theoretical distribution (R) of non-protein-bound drug between milk (M. Ultr.) and plasma (P. Ultr.) were made on the basis of the pK_a value of the drug and the pH in blood (pH_b) and milk (pH_m), by means of the formula (*Rasmussen* 1958, 1966):

$$R = \frac{\text{M.Ultr.}}{\text{P.Ultr.}} = \frac{1 + 10^{(pH_m - pK_a)}}{1 + 10^{(pH_b - pK_a)}} \text{ for acids and}$$

$$R = \frac{\text{M.Ultr.}}{\text{P.Ultr.}} = \frac{1 + 10^{(pK_a - pH_m)}}{1 + 10^{(pK_a - pH_b)}} \text{ for bases.}$$

The statistical calculations were done in accordance to standard methods (*Kemp* 1955) and the results are given as average ± s.e.m. The relationships between the renal clearance of each drug, and the concentration of drug, urine pH, urine flow-rate

were examined by means of correlation matrices and multiple regression analyses (*Dixon* 1967).

RESULTS

Protein-binding

In Tables 1 and 2 are listed the concentrations of sulphadoxine and trimethoprim in plasma and milk and in ultrafiltrates of plasma and milk. The protein-binding of sulphadoxine was highest (66 ± 2 %, n = 6) at concentrations of sulphadoxine in plasma lower than 100 µg/ml and lowest (48 ± 1 %, n = 9) at concentrations higher than 150 µg/ml, this difference is significant ($P < 0.05$). The binding of sulphadoxine to the plasma proteins was not influenced by the presence of trimethoprim. The binding of trimethoprim to plasma proteins was in average 57 ± 2 % independent of the concentration of trimethoprim, and the binding of trimethoprim was not influenced by sulphadoxine in plasma. Both sulphadoxine and trimethoprim were bound to the proteins in milk 4 ± 2 % and 41 ± 2 %, respectively.

Table 1. The concentration of sulphadoxine in plasma and milk and in ultrafiltrate of plasma and milk.

Cow no.	Experiment no.	Plasma µg/ml	Milk µg/ml	Ratio M/P	Ultrafiltrates of		Experimental ratio M. Ultr. / P. Ultr.	Blood pH	Milk pH	Theoretical ratio M. Ultr. / P. Ultr.
					plasma µg/ml	milk µg/ml				
3	1	29	2	0.1	—	—	—	7.50	6.80	0.2
2	2	34	6	0.2	—	—	—	7.55	7.15	0.4
2	3	34	5	0.2	12	5	0.5	7.50	7.10	0.4
2	4	37	6	0.2	12	6	0.5	7.50	7.15	0.5
5	5	43	4	0.1	12	4	0.4	7.45	6.95	0.3
4	6	53	7	0.1	16	7	0.5	7.60	7.10	0.3
4	7	56	8	0.2	13	8	0.6	7.50	7.10	0.4
1	8	82	9	0.1	28	9	0.3	7.45	7.00	0.3
2	9	84	19	0.2	39	14	0.4	7.65	7.30	0.5
1	10	88	15	0.2	32	15	0.5	7.50	7.05	0.4
3	11	96	14	0.2	35	12	0.4	7.50	7.20	0.5
2	12	157	28	0.2	87	28	0.3	7.55	7.05	0.3
3	13	218	64	0.3	108	63	0.6	7.45	7.20	0.6
5	14	232	74	0.3	126	72	0.6	7.45	7.25	0.6
5	15	248	39	0.2	132	35	0.3	7.45	6.90	0.3
4	16	254	68	0.3	129	65	0.5	7.45	7.15	0.5
4	17	263	63	0.2	130	62	0.5	7.45	7.15	0.5

— too low to calculate the ratio.

Table 2. The concentration of trimethoprim in plasma and milk and in ultrafiltrates of plasma and milk.

Cow no.	Experiment no.	Plasma µg/ml	Milk µg/ml	Ratio M/P	Ultrafiltrates of plasma µg/ml	Ultrafiltrates of milk µg/ml	Experimental ratio M.Ultr./P.Ultr.	Blood pH	Milk pH	Theoretical ratio M.Ultr./P.Ultr.
3	1	0.6	1.7	3.2	0.2	1.0	5.0	7.50	6.80	3.2
2	18	0.6	0.9	1.4	0.3	0.6	1.9	7.55	7.20	1.7
2	3	0.9	1.4	1.6	0.3	0.9	2.5	7.50	7.10	1.4
5	5	0.9	1.9	2.0	0.3	1.0	3.0	7.45	6.95	2.3
2	2	1.2	1.3	1.1	0.6	0.9	1.5	7.55	7.15	1.8
3	19	1.2	2.2	1.8	0.6	1.0	1.7	7.50	7.00	2.2
2	9	1.5	1.7	1.1	0.7	0.9	1.3	7.65	7.30	1.6
3	11	1.6	2.8	1.8	0.7	1.5	2.1	7.50	7.20	1.6
1	8	1.7	4.0	2.4	0.7	2.6	3.6	7.45	7.00	2.1
4	16	1.8	2.3	1.3	0.8	1.7	2.2	7.45	7.15	1.6
1	10	1.9	3.7	1.9	1.0	2.5	2.6	7.50	7.05	2.0
4	6	2.0	4.3	2.1	0.8	2.1	2.5	7.60	7.10	1.6
2	12	2.1	2.6	1.2	0.6	1.4	2.5	7.55	7.05	2.1
5	20	2.5	3.6	1.4	1.1	2.1	1.9	7.40	7.20	1.4
5	21	3.7	4.6	1.2	1.6	2.8	1.8	7.40	7.00	1.9
5	14	7.8	9.3	1.2	3.7	5.1	1.4	7.45	7.25	1.3

Mammary excretion

The mammary excretion of sulphadoxine and trimethoprim was examined in 17 and 16 experiments, respectively. The results in Table 1 show that the concentrations of sulphadoxine were much lower in milk than in plasma (ratio M/P and M.Ultr./P.Ultr.). The experimentally found ratios between the concentration of non-protein-bound sulphadoxine in milk and plasma (ratio M.Ultr./P.Ultr.) are given in column 8 of Table 1. Furthermore, the pH of the blood and milk, together with the theoretically calculated ratios between the concentration of non-protein-bound sulphadoxine in ultrafiltrates of milk and plasma, can be seen from the last 3 columns.

The concentrations of trimethoprim in milk were 1.1—3.2 times higher than in plasma (Table 2, ratio M/P). The experimentally found ratios between the concentration of non-protein-bound trimethoprim in milk and plasma (M.Ultr./P.Ultr.) are shown in Table 2. They varied from 1.3 to 5.0. The pH of blood and milk and the theoretically calculated ratio between the con-

centration of trimethoprim in ultrafiltrates of milk and plasma, can be seen from the last 3 columns.

From Tables 1 and 2 it is seen that an increase in the pH of milk means an increase in the ratio M.Ultr./P.Ultr. for sulphadoxine and a decrease in the ratio M.Ultr./P.Ultr. for trimethoprim. This is also illustrated in Fig. 1 demonstrating the results obtained in an experiment with nearly constant levels in ultrafiltrate of plasma of sulphadoxine (14—17 μg/ml) and trimethoprim (0.7—1.0 μg/ml) and with rather big variation in pH of the milk samples during the experimental period. The concentrations of drug in the milk samples varied appreciably, but the concentrations of sulphadoxine were in all cases lower than in plasma, while the concentrations of trimethoprim in milk in all cases were higher than in plasma.

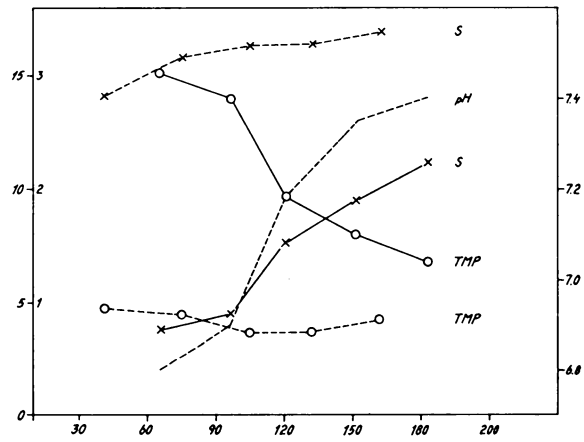

Figure 1. Concentrations of non-protein-bound sulphadoxine and non-protein-bound trimethoprim in plasma and milk at different pH values in milk.

Ordinate on the left: Concentration of sulphadoxine (0—15 μg/ml) and of trimethoprim (0—3 μg/ml).

Ordinate on the right: pH of the milk.

Abscissa: Time in minutes.

x------x: Sulphadoxine in ultrafiltrate of plasma.

x——x: Sulphadoxine in ultrafiltrate of milk.

o------o: Trimethoprim in ultrafiltrate of plasma.

o——o: Trimethoprim in ultrafiltrate of milk.

---------: pH of the milk.

Renal clearance

Clearance of endogenous creatinine, sulphadoxine and trimethoprim was determined in 21, 17 and 16 experiments, respectively, and the average results of each experiment are shown in Table 3.

Sulphadoxine

From Table 3 it is seen that the plasma clearances of sulphadoxine and even non-protein-bound sulphadoxine ($Clear_{S.ultr.}$) were much lower than the clearance of endogenous creatinine (ratio columns 9 and 10).

By means of multiple regression analysis 2 regression equations have been calculated. Both equations give the influence of the concentration of non-protein-bound sulphadoxine ($C_{S.ultr.}$), the urine pH and the urine flow rate (V) on the renal clearance of sulphadoxine in comparison to the clearance of creatinine. The regression equations are

$$\text{Clearance ratio} = \frac{\text{Clear}_{S.ultr.}}{\text{Clear}_{Cr.}} =$$

$$-1.74 - 0.00012\ C_{S.ultr.} + 0.25\ pH + 0.023\ V \quad (1)$$

and

$$\text{log. clearance ratio} = \log. \frac{\text{Clear}_{S.ultr.}}{\text{Clear}_{Cr.}} =$$

$$-6.29 - 0.00062\ C_{S.ultr.} + 0.70\ pH + 0.044\ V \quad (2)$$

Correlation analysis showed significant correlation between clearance ratio and both the concentration of non-protein-bound sulphadoxine ($C_{S.ultr.}$) and the pH of the urine, while clearance ratio and the urine flow rate were not significantly correlated (Table 4). The regression equations show that the pH of the urine is by far the most important of the above mentioned factors which influence the renal clearance of sulphadoxine.

The first regression equation (1) explains about 55 % of the variations in the clearance ratio while the second one (2) explains about 73 % of this variation.

The found relation between the renal excretion of non-protein-bound sulphadoxine and the pH of the urine is further demonstrated in Fig. 2 giving the results of 5 experiments on 2

Table 3. Renal clearance of endogenous creatinine, sulphadoxine and trimethoprim.

Experiment no.	Kg b.wt.	Urine pH (min.-max.)	Plasma clearance/100 kg b.wt.			Clearance of non-protein-bound drug ($Clear_{ultr.}$)/100 kg b.wt.		Ratio $\frac{Clear_{S.}}{Clear_{Cr.}}$	Ratio $\frac{Clear_{S.ultr.}}{Clear_{Cr.}}$	Ratio $\frac{Clear_{TMP}}{Clear_{Cr.}}$	Ratio $\frac{Clear_{TMP\ ultr.}}{Clear_{Cr.}}$
			creatinine ml/min.	sulphadoxine ml/min.	TMP ml/min.	sulphadoxine ml/min.	TMP ml/min.				
1	450	8.00 (8.00—8.10)	179	16	96	53	276	0.09	0.3	0.5	1.5
2	347	7.25 (7.10—7.50)	208	6	141	29	262	0.03	0.1	0.7	1.3
3	347	7.85 (7.80—7.90)	244	21	72	60	184	0.09	0.2	0.3	0.8
4	347	8.20 (8.20—8.22)	241	28		85		0.1	0.4		
5	390	8.20 (8.09—8.29)	134	13	34	45	102	0.09	0.3	0.3	0.8
6	400	8.15 (8.10—8.25)	130	8	47	28	110	0.07	0.2	0.4	0.8
7	400	7.60 (7.44—7.76)	174	4		19		0.03	0.1		
8	505	7.10 (6.50—7.50)	142	2	43	6	100	0.01	0.04	0.3	0.7
9	347	8.15 (8.10—8.20)	203	19	35	40	74	0.09	0.2	0.2	0.4
10	505	7.40 (7.16—7.91)	149	4	82	12	163	0.03	0.08	0.6	1.1
11	450	8.15 (8.12—8.22)	150	22	34	61	72	0.2	0.4	0.2	0.5
12	347	8.25 (8.21—8.29)	156	26	24	48	91	0.2	0.3	0.2	0.6
13	475	8.20 (8.18—8.20)	132	30		60		0.2	0.5		
14	390	8.15 (8.13—8.23)	125	17	5	32	10	0.1	0.3	0.04	0.08
15	390	8.15 (8.10—8.20)	152	24		45		0.2	0.3		
16	400	8.20 (8.17—8.22)	154	24	35	47	80	0.2	0.3	0.2	0.5
17	400	8.25 (8.22—8.26)	153	14		30		0.1	0.2		
18	350	7.90 (7.85—8.00)	196		83		169			0.4	0.9
19	475	8.15 (8.08—8.16)	144		59		120			0.4	0.8
20	400	8.04 (8.01—8.08)	118		27		62			0.2	0.5
21	400	8.15 (8.11—8.19)	112		19		44			0.2	0.4
Average + s.e.m.			164 + 8	16 + 2	54 + 9	41 + 5	125 + 19				

Table 4. Correlation coefficients (r) between variables i.e. clearance ratio and the concentration of non-protein-bound drug in blood plasma ($C_{S.ultr.}$ and $C_{TMP\ ultr.}$), the pH of the urine and the urine flow rate (V).

Variables		r	P
$\frac{Clear_{S.ultr.}}{Clear_{Cr.}}$	$C_{S.ultr.}$	0.26	$0.01 < P < 0.05$
	pH	0.71	< 0.001
	V	—0.02	n.s.
Log. $\frac{Clear_{S.ultr.}}{Clear_{Cr.}}$	$C_{S.ultr.}$	0.27	$0.01 < P < 0.05$
	pH	0.83	< 0.001
	V	—0.08	n.s.
$\frac{Clear_{TMP\ ultr.}}{Clear_{Cr.}}$	$C_{TMP\ ultr.}$	—0.55	< 0.001
	pH	—0.42	< 0.001
	V	0.42	< 0.001
Log. $\frac{Clear_{TMP\ ultr.}}{Clear_{Cr.}}$	$C_{TMP\ ultr.}$	—0.83	< 0.001
	pH	—0.38	< 0.001
	V	0.36	$0.001 < P < 0.01$

cows excreting urine with pH between 7.1 and 8.3. It will be seen that the excretion of sulphadoxine was highest in relation to the excretion of endogenous creatinine when the pH of the urine was high.

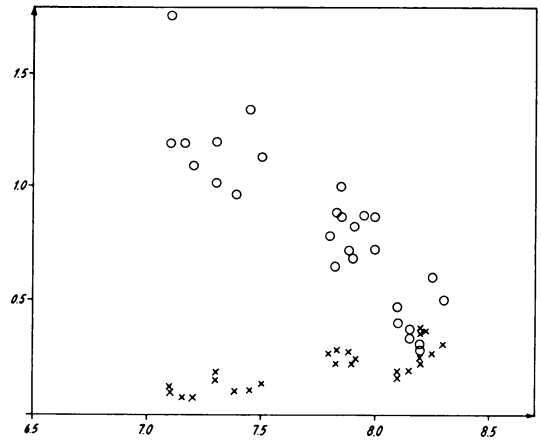

Figure 2. Ratio of the clearance of non-protein-bound drug to endogenous creatinine clearance in relation to urine pH.
Ordinate: Clear of non-protein-bound drug/Clear of creatinine.
Abscissa: Urine pH.
x Sulphadoxine.
o Trimethoprim.

As mentioned above the correlation analysis showed that the clearance ratio and the plasma concentration of non-protein-bound sulphadoxine ($C_{S.ultr.}$) were significantly correlated. The regression equations show, however, that the clearance ratio was only to a very small degree influenced by this factor in the concentration interval obtained in these experiments. This is further demonstrated in Fig. 3 giving the results from experiments with nearly constant pH in the urine (pH 8.0—8.3).

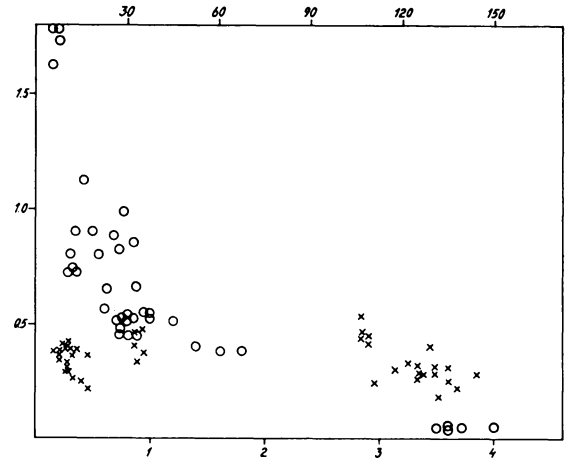

Figure 3. Ratio of the clearance of non-protein-bound drug to endogenous creatinine clearance in relation to the concentration of non-protein-bound sulphadoxine and non-protein-bound trimethoprim in plasma.

Ordinate: Clear of non-protein-bound drug/Clear of creatinine.
Abscissa above: Conc. of non-protein-bound sulphadoxine in plasma.
Abscissa below: Conc. of non-protein-bound trimethoprim in plasma.
x Sulphadoxine.
o Trimethoprim.

Trimethoprim

The clearance of trimethoprim from plasma was also lower than the clearance of creatinine, while the clearance of non-protein-bound trimethoprim varied from 8 to 150 % of creatinine clearance (last column in Table 3).

As in the case of sulphadoxine, 2 regression equations were calculated as follows

$$\text{Clearance ratio} = \frac{\text{Clear}_{\text{TMP ultr.}}}{\text{Clear}_{\text{Cr.}}} =$$

$$2.89 - 0.24\ C_{\text{TMP ultr.}} - 0.26\ \text{pH} + 0.102\ \text{V} \quad (3)$$

and

$$\text{log. clearance ratio} = \text{log.}\ \frac{\text{Clear}_{\text{TMP ultr.}}}{\text{Clear}_{\text{Cr.}}} =$$

$$1.32 - 0.29\ C_{\text{TMP ultr.}} - 0.17\ \text{pH} + 0.06\ \text{V} \quad (4)$$

Correlation analysis showed that the clearance ratio (Table 4) was significantly correlated with the concentration of non-protein-bound trimethoprim ($C_{\text{TMP ultr.}}$), the pH of urine and the urine flow rate (V).

The first equation (3) explains about 49 % while the second one (4) explains more than 81 % of the observed variations in the clearance ratio. Variations in the concentration of non-protein-bound trimethoprim influence the clearance ratio much more than urine pH, and the variations in the urine flow rate are of less importance. The excretion of trimethoprim in relation to clearance of creatinine diminishes when the concentration of non-protein-bound trimethoprim in plasma increases (Fig. 3). In the experiments included in this figure, the pH of the urine was constant (between 8.0 and 8.3) and has not influenced the renal excretion of trimethoprim. The influence of variations in urine pH on the renal excretion of trimethoprim is further demonstrated in Fig. 2, which reveals that the ratio ultrafiltrate clearance of trimethoprim/filtration clearance was lowest when the pH of the urine was high.

DISCUSSION

The binding of sulphadoxine to the plasma proteins in cows is 66 % at plasma levels below 100 µg/ml and 48 % at plasma levels above 150 µg/ml. A similar relationship between the binding of sulphadoxine to the proteins in plasma and the plasma level of sulphadoxine is seen in goats (*Jørgensen & Rasmussen* 1972). The binding of trimethoprim to the plasma proteins in cows is a little higher than in goats (*Rasmussen* 1970) and is of the same order as found by *Craig & Kunin* (1973) in humans. However, *Bushby & Hitchings* (1968) and *Schwartz & Ziegler*

(1969) found that only between 30 and 46 % of trimethoprim was bound to proteins in human plasma. The binding of sulphadoxine to plasma proteins is not influenced by trimethoprim and vice versa and this resembles *Schwartz & Ziegler*'s finding with trimethoprim and sulphamethoxazole in human plasma.

The binding of sulphadoxine to milk proteins in cows is lower (4 %) than in goats (16 %) (*Jørgensen* 1972), while the binding of trimethoprim to milk proteins in cows (42 %) is higher than in goats (12 %) (*Rasmussen* 1970).

According to the finding that only the non-protein-bound and unionized fraction of drugs can diffuse into milk and establish equilibrium across the mammary gland epithelium (*Rasmussen* 1958, 1966, 1971, 1973) sulphadoxine should give lower concentrations in milk than in plasma. In accordance to this the results in Table 1 show that the concentrations in milk were constantly lower than in plasma. During equilibrium conditions the experimentally found distribution (exp. M.Ultr./P.Ultr.) and the calculated distribution of sulphadoxine between the ultrafiltrates of milk and plasma show good agreement. Similar results were obtained with sulphadoxine in goats (*Jørgensen*).

The concentrations of the base trimethoprim in milk should be equal to or higher than in plasma (Table 2). The experimentally found distribution (exp. M.Ultr./P.Ultr.) and the calculated distribution of trimethoprim between ultrafiltrates of milk and plasma prove this hypothesis, which also is confirmed in experiments on goats (*Rasmussen* 1970). The fact that the unionized fraction of sulphadoxine and trimethoprim is important for concentrations of drug in milk compared with the concentration in plasma is further strengthened by the results shown in Fig. 1. A similar dependence between the pH of milk and the concentration of drug in milk is seen for other partly ionized drugs i. e. sulphadiazine (*Rasmussen* 1958, 1966), penicillin and erythromycin (*Rasmussen* 1959) and lincomycin (*Gyrd-Hansen & Rasmussen* 1967, *Ziv & Sulman* 1973 b).

The renal clearance of endogenous creatinine in cows express the glomerular filtration (*Poulsen* 1956). The renal clearance of endogenous creatinine in these experiments was found to be 164 ± 8 ml/min./100 kg b. wt. (Table 3) and resembles the results (168 ml/min./100 kg) obtained by *Poulsen*.

As the protein-bound fraction of drugs cannot filter through the glomeruli of the kidneys the excretory mechanism must be

determined on the basis of the comparison between glomerular filtration rate and the clearance of non-protein-bound sulphadoxine and trimethoprim. The clearance of non-protein-bound sulphadoxine was always much lower than the clearance of creatinine, which indicates in addition to filtration, a high degree of back-diffusion of sulphadoxine dependent on the pH of the urine (Fig. 2, Table 4). This back-diffusion is most pronounced in urine of low pH when sulphadoxine is more unionized (diffusible) than in urine of high pH. A similar influence of urine pH on the back-diffusion of sulphadoxine is seen in goats (*Jørgensen & Rasmussen*) and in humans (*Portwich & Büttner* 1964). The clearance of sulphadoxine is only to a small degree dependent on the variations in the concentrations of non-protein-bound sulphadoxine in plasma (equations 1 and 2). Therefore it is not possible from these experiments to decide whether an active tubular secretion is involved in the renal handling of sulphadoxine as seen in goats (*Jørgensen & Rasmussen*) and in humans (*Reber et al.* 1964, *Portwich & Büttner* 1964). However, the high degree of back-diffusion might shadow an active secretion.

The clearance of non-protein-bound trimethoprim varies from 8 to 150 % of the clearance of creatinine. This indicates that, in addition to filtration, both active tubular secretion and back-diffusion are involved in the renal handling of trimethoprim corresponding to observations in goats (*Rasmussen* 1970) and in humans (*Sharpstone* 1969, *Bergan & Brodwall* 1972).

The variations in the clearance of trimethoprim are related to the concentration of trimethoprim in plasma and the pH of the urine. High concentrations diminish (Fig. 3 and Table 4) drastically the renal excretion of trimethoprim, and these results indicate selfdepression and support the assumption that active tubular secretion is involved in the renal handling of trimethoprim.

The clearance of non-protein-bound trimethoprim is higher at low pH than at high pH in the urine (Fig. 2, Table 4). At high pH of the urine trimethoprim is more unionized (diffusible) than at low pH and back-diffusion is pronounced as also seen in goats (*Rasmussen* 1970) and in humans (*Sharpstone, Bergan & Brodwall, Craig & Kunin).*

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SAMMENDRAG

Mammær og renal ekskretion af sulfadoxin og trimethoprim hos køer.

I 21 forsøg på normale, ikke drægtige køer er der ved intravenøs infusion af sulfadoxin og trimethoprim opretholdt konstante koncentrationer af disse lægemidler. Forsøgene viser, at både sulfadoxin og

trimethoprim bindes til proteinerne i blodplasma og mælk. Det fremgår endvidere af forsøgene, at sulfadoxin, som er en syre, udskilles i mælken i koncentrationer, der er lavere end i blodplasma, mens trimethoprim, som er en base, udskilles i mælken i koncentrationer, der er højere end i blodplasma. De opnåede resultater er i overensstemmelse med teorien om, at lægemidler udskilles med mælken ved passiv diffusion.

Den renale udskillelse af sulfadoxin og trimethoprim involverer glomerulær filtration og tilbagediffusion. Hertil kommer, at trimethoprim tillige udskilles ved aktiv tubulær sekretion. Såvel den mammære som den renale ekskretion af sulfadoxin og trimethoprim er påvirkelig af pH i mælk og urin. Forsøgene har vist, at det er den ikke-ioniserede og ikke-proteinbundne fraktion af lægemidler, der diffunderer gennem biologiske membraner.

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