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The Influence of pH on Antimicrobial Substances in Canine Vaginal and Urethral Secretions

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Summary. Trimethoprim and rosamicin (a new basic macrolide antibiotic) were administered to normal and oophorectomised female dogs by constant intravenous infusion before and after oestrogen and androgen administration. Their concentrations in plasma and in urethral and vaginal secretions were determined by bioassay and correlated with the pH values of vaginal and urethral secretions. Both compounds were concentrated in the vaginal and urethral secretions in reverse correlation with the pH of these fluids. Trimethoprim and rosamicin have antimicrobial spectra well suited for the treatment of bacterial urethritis and vaginitis and require further clinical investigation.

Key words: Trimethoprim - Rosamicin - Vaginal and urethral secretions.

The mode of drug secretion by the urethral epithelium as well as by the female paraurethral glands, referred to as Skene's glands (6) or the female prostate (9), a terminology supported by embryological studies (3), seems to have many analogies with prostatic secretion in the male (2). Lipid solubility and a basic pKa are the necessary requirements for the secretion of antibacterial agents into the acid urethral and prostatic secretions in concentrations exceeding the simultaneous plasma concentrations (4). Protein binding appears to be of less importance.

The mechanism of diffusion and concentration of drugs in the acid vaginal secretion is similar and also occurs by nonionic diffusion across the vaginal epithelium. The difference between theoretical and true concentration gradients (vaginal secretion/plasma) may be explained by the lack of permanent trapping of vaginal secretion in the compartment since continuous seepage of vaginal secretion occurs (7). The influence of partial cervical excretion cannot be excluded (5).

In view of the nonionic diffusion of antibiotics with basic pKa into acid secretions, we were interested in investigating the influence of pH-changes of urethral and vaginal secretions on the antibacterial concentration ratios between these secretions and plasma. It was expected that with decreasing pH, the ratios would increase, resulting in higher antimicrobial substance concentrations in the vaginal and urethral mucosa.

In this study, we have investigated the distribution of trimethoprim (pKa 7.3)[1] and rosamicin (pKa 8.7)[2] (a new macrolide antibiotic with activity against gram-positive as well as gram-negative bacteria, chlamydia and mycoplasms) in plasma and urethral or vaginal secretions in dogs in relation to varying pH-values, induced either by oophorectomy of hormone application, or both.

[1] Trimethoprim, RO 5-6846, was supplied by Hoffman-LaRoche Inc., Nutley, NJ.

[2] Rosamicin was supplied as Rosamicin sodium dihydrogen phosphate by Schering Corp., Bloomfield, NJ.

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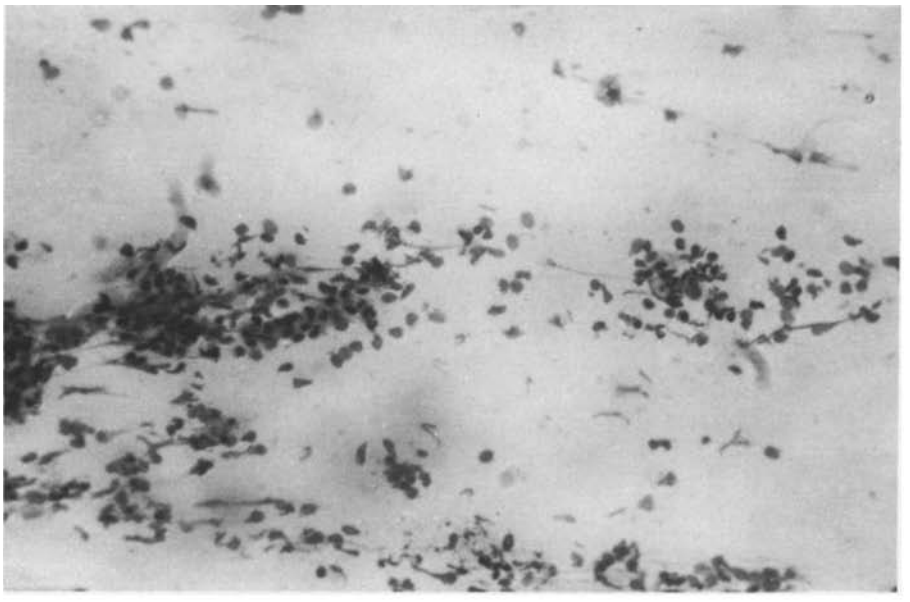

Fig. 1. Vaginal smear: Clusters of parabasal cells in autolysis; atrophic pattern

Fig. 2. Vaginal smear: Large intermediate and some superficial cells. Most of the cells are crowd ed, but flat. Good response to combined hormonal treatment

MATERIALS AND METHODS

Six adult female dogs weighing 17.6 ± 4.4 kg (mean ± 1 SD) were anaesthetised with sodium thiopental intravenously and a bilateral oophorectomy was performed. After a period of at least 42 days, studies were carried out as described below and the results compared to a control group of six other adult female dogs not being in oestrus and weighing 15.8 ± 2.6 kg. Three dogs from each group received either trimethoprim or rosamicin.

For the collection of specimens of urethral and vaginal secretions the dogs were anaesthetized with sodium thiopental intravenously, and the urethra was occluded at the bladder neck by a tourniquet to prevent urine entering the urethra. The bladder was drained by a suprapubic Foley catheter. The vaginal wall was then exposed with a speculum and cytological specimens were obtained, fixed, and stained according to the Papanicolaou technique for the purpose of evaluating the hormonal status of the vaginal mucosa. When sufficient fluid was available, the pH of urethral and vaginal mucosa was measured by a BMS 3 Mk 2 Blood Micro

Table 1. Vaginal and urethral pH (mean ± 1 SD) in dogs with and without oophorectomy before and after hormone application (n = 6 in each group).

		Before hormone application	After hormone application	pH Change
Oophorectomised dogs	Urethra	7.11 ± 0.30	6.98 ± 0.29	$p < 0.5$
	Vagina	7.16 ± 0.33	6.94 ± 0.18	$p < 0.2$
Control dogs	Urethra	7.04 ± 0.20	7.08 ± 0.25	$p < 0.8$
	Vagina	7.04 ± 0.25	7.04 ± 0.23	$p < 0.7$

System connected with a PHM 72 Mk 2 Digital Acid-Base Analyser (Radiometer, Copenhagen, Denmark). If only a minimal amount of fluid was present, the pH was estimated using pH paper. [3]

Blank paper disks, 6 mm in diameter, for baseline bioassay determinations were inserted with forceps into the urethra and vagina with a small thread attached making them easily retrievable. After 5 minutes in situ they were removed and the drug concentrations were determined by disk diffusion methods as follows:

Rosamicin disk content was determined by placing the saturated disk directly on a Streptomycin Assay Agar inoculated with Bacillus subtilis as test organism.

Trimethoprim disk content was determined by first washing out the content in 0.5 ml sterile ice cooled water and bioassay was carried out within 24 h on a Mueller-Hinton-Agar inoculated with Bacillus pumilus as test organism. At the same time blood samples were obtained for bioassay determinations of the respective drugs. Pilocarpine, 4 mg, was given intravenously immediately before the drug administration in order to stimulate the vaginal and urethral secretions.

In each experiment, an intravenous bolus of the antimicrobial agent was administered (Rosamicin 10 mg/kg body weight, Trimethoprim 4 mg/kg) followed by a continuous infusion (Rosamicin 3 mg/kg/h body weight, Trimethoprim 0.5 mg/kg/h) for 4 h.

Blood samples were obtained and disks applied immediately following the bolus injection and after 30, 60, 120, 180 and 240 min. The pH values were measured again after 90 min.

Following these studies, all dogs received an injection of Gynodian Depot intramuscularly (1 ml/65 kg body weight)[4], containing 200 mg 3 ß-Heptanoyloxy-androst-5-en-17-one and 4 mg Oestradiol-17-valerianate in 1 ml solution.

Five to seven days later, the experiments described above were repeated.

RESULTS

The vaginal smear in the six oophorectomised dogs confirmed the hormonal deficiency and the effect of oestrogen and androgen application (Figs. 1 and 2). The normal dogs, in all phases of their cycle, showed a clear tendency toward pyknotic, superficial and large intermediate cells in response to hormone application.

The pH values obtained by the "Radiometer" system showed that the values obtained by pH paper were satisfactorily accurate. The differences never exceeding 0.2 units in either direction. The means of all values in each dog are shown in Table 1. In the oophorectomised dogs, the pH in vagina and urethra decreased slightly but not significantly, following hormone application (paired t-test, vagina: $p < 0.2$, urethra: $p < 0.5$). The difference between both groups before hormone application was also not significant (student t-test, vagina: $p < 0.6$, urethra: $p < 0.2$). In all experiments, the pH became more basic following pilocarpine administration.

[3] Fisher Alkacid Tester, Range pH 6.0-8.5.

[4] Gynodian Depot, Schering AG, Berlin/Bergkamen, Germany.

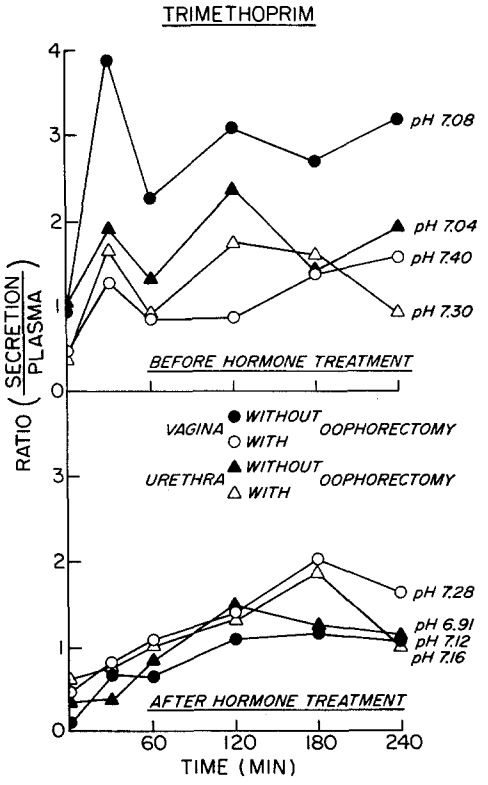

Fig. 3. Trimethoprim secretion/plasma ratios during constant intravenous infusion, before and after hormone treatment

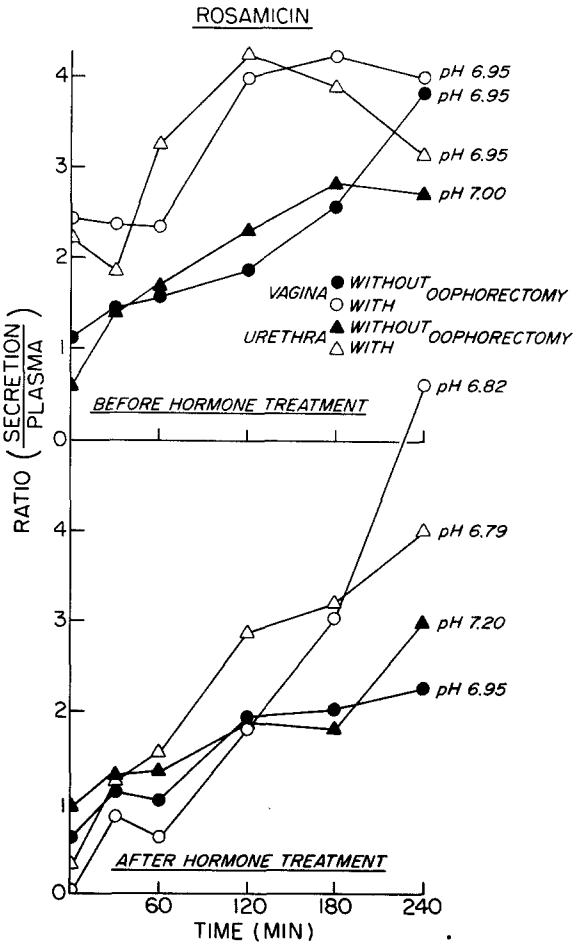

Fig. 4. Rosamicin secretion/plasma ratios during constant intravenous infusion, before and after hormone treatment

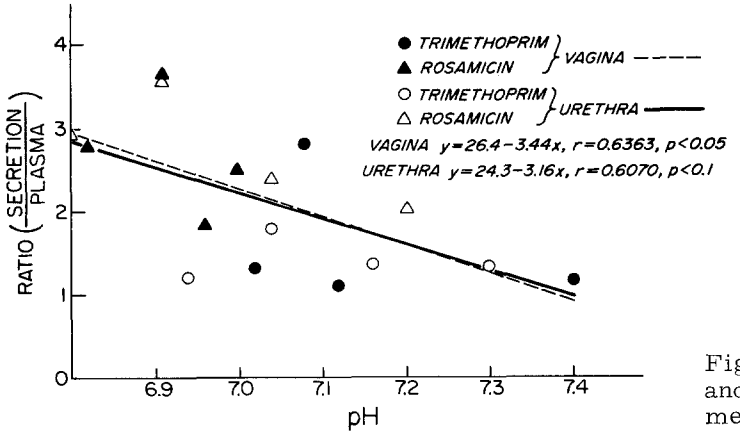

Fig. 5. Correlation between pH and vaginal and urethral secretions/plasma ratios for trimethoprim and rosamicin

The ratios between vaginal and urethral secretion respectively and plasma are high and fairly constant for both drugs, during the last 180 min in most of the series (Figs. 3 and 4), but without significant differences (student t-test) between the drugs or the groups, and also no significant differences between the hormone treated and non-treated animals as well as no differences between the ratios in vagina and urethra.

Table 2 and Figure 5 illustrate the relation between pH and antimicrobial agent ratios to plasma of vaginal and urethral secretion during the last 180 min of the experiments in all dogs.

Table 2. Concentrations of Rosamicin in plasma, (µg/ml), and plasma ratios of vaginal and urethral secretions (mean + 1 SE) during constant intravenous infusion (n = 3 in each group).

Dog group	Time (min)	Pre-infusion	0[a]	30	60	120	180	240
Oophorectomy	Plasma (PL), concentration	0	4.30±0.70	1.86±0.57	0.83±0.14	0.48±0.08	0.53±0.20	0.47±0.24
	Vaginal secretion (VS), ratio: $\frac{VS}{PL}$		2.44±1.09	2.38±0.78	2.35±0.19	4.00±0.43	4.27±0.90	4.01±1.04
	Urethral secretion (US), ratio: $\frac{US}{PL}$		2.23±0.83	1.86±0.53	3.27±0.99	4.26±0.67	3.90±0.73	3.14±0.90
Oophorectomy + hormones	Plasma (PL), concentration	0	7.90±3.34	1.66±0.13	0.95±0.09	0.76±0.13	0.73±0.09	0.54±0.11
	Vaginal secretion (VS), ratio: $\frac{VS}{PL}$		0.06±0.06	0.85±0.85	0.63±0.27	1.90±0.21	3.04±0.85	5.62±0.62
	Urethral secretion (US), ratio: $\frac{US}{PL}$		0.34±0.30	1.29±0.99	1.56±0.97	2.88±0.74	3.22±1.14	4.02±0.35
Control	Plasma (PL), concentration	0	4.23±0.56	1.76±0.08	1.13±0.08	0.61±0.09	0.64±0.15	0.51±0.09
	Vaginal secretion (VS), ratio: $\frac{VS}{PL}$		1.11±0.76	1.43±0.50	1.57±0.61	1.89±0.50	2.58±0.86	3.84±1.16
	Urethral secretion (US), ratio: $\frac{US}{PL}$		0.61±0.06	1.41±0.23	1.73±0.20	2.32±0.43	2.84±0.59	2.71±0.46
Control + hormones	Plasma, concentration	0	5.23±1.18	2.90±1.00	1.32±0.24	1.01±0.04	0.88±0.17	0.77±0.21
	Vaginal secretion (VS), ratio: $\frac{VS}{PL}$		0.61±0.19	1.12±0.01	1.03±0.12	1.90±0.38	2.04±0.45	2.27±0.56
	Urethral secretion (US), ratio: $\frac{US}{PL}$		0.97±0.31	1.30±0.05	1.35±0.08	1.89±0.47	1.82±0.24	3.00±0.72

[a]Immediately following bolus administration.

Table 3. Concentrations of Trimethoprim in plasma, (μg/ml) and plasma ratios of vaginal and urethral secretions (mean ± 1 SE) during constant intravenous infusion (n = 3 in each group).

Dog group	Time (min)	Pre-infusion	0[a]	30	60	120	180	240
Oophorectomy	Plasma (PL), concentration	0.04±0.03	6.73±1.66	2.33±0.09	2.53±0.59	2.63±0.39	2.26±0.09	2.53±0.24
	Vaginal secretion (VS), ratio: $\frac{VS}{PL}$		0.44±0.15	1.29±0.28	0.84±0.18	0.85±0.04	1.36±0.25	1.56±0.20
	Urethral secretion (US), ratio: $\frac{US}{PL}$		0.43±0.11	1.68±0.07	0.92±0.35	1.75±0.20	1.60±0.13	0.96±0.14
Oophorectomy + hormones	Plasma (PL), concentration	0	5.46±1.16	3.66±0.58	3.16±0.15	3.00±0.25	2.86±0.32	2.93±0.35
	Vaginal secretion (VS), ratio: $\frac{VS}{PL}$		0.48±0.43	0.81±0.09	1.09±0.05	1.40±0.30	2.02±0.72	1.63±0.28
	Urethral secretion (US), ratio: $\frac{US}{PL}$		0.63±0.46	0.78±0.24	1.04±0.13	1.32±0.32	1.88±0.97	1.07±0.20
Control	Plasma (PL), concentration	0	3.96±1.36	1.90±0.25	2.23±0.36	1.66±0.51	1.93±0.26	2.03±0.26
	Vaginal secretion (VS), ratio: $\frac{VS}{PL}$		0.95±0.47	3.87±0.68	2.26±0.31	3.07±0.93	2.69±0.72	3.17±0.36
	Urethral secretion (US), ratio: $\frac{US}{PL}$		1.10±0.41	1.94±0.20	1.34±0.31	2.37±1.27	1.42±0.33	1.94±0.25
Control + hormones	Plasma (PL), concentration	0.02±0.02	3.73±0.12	2.40±0.40	2.13±0.39	2.13±0.35	1.80±0.29	2.33±0.49
	Vaginal secretion (VS), ratio: $\frac{VS}{PL}$		0.12±0.12	0.68±0.12	0.65±0.43	1.09±0.13	1.15±0.12	1.08±0.40
	Urethral secretion (US), ratio: $\frac{US}{PL}$		0.33±0.33	0.37±0.21	0.84±0.21	1.44±0.27	1.26±0.27	1.13±0.19

[a]Immediately following bolus administration.

Table 4. Ratios between plasma and vaginal and urethral secretions (mean ± 1 SE) during last 180 minutes of constant intravenous infusion of Trimethoprim or Rosamicin in relation to pH of these secretions.

Antibiotic - dog group	Vagina		Urethra	
	pH	VS ratio: PL	pH	US ratio: PL
Trimethoprim				
Oophorectomy	7.40 ± 0.08	1.15 ± 0.25	7.30 ± 0.10	1.31 ± 0.29
Oophorectomy + hormones	7.02 ± 0.02	1.31 ± 0.41	7.16 ± 0.18	1.33 ± 0.49
Control	7.08 ± 0.17	2.80 ± 0.58	7.04 ± 0.15	1.77 ± 0.64
Control + hormones	7.12 ± 0.19	1.08 ± 0.23	6.94 ± 0.15	1.17 ± 0.24
Rosamicin				
Oophorectomy	6.91 ± 0.17	3.66 ± 0.77	6.91 ± 0.17	3.64 ± 0.76
Oophorectomy + hormones	6.82 ± 0.12	2.80 ± 1.24	6.79 ± 0.04	2.92 ± 0.76
Control	7.00 ± 0.15	2.47 ± 0.88	7.04 ± 0.11	2.40 ± 0.46
Control + hormones	6.96 ± 0.04	1.81 ± 0.45	7.20 ± 0.11	2.20 ± 0.50

Assuming a linear relationship and for both drugs, equal distribution in the narrow pH range investigated, we found significance for the vagina ($r = 0.6363$, $p < 0.05$).

DISCUSSION

Our experiments investigated the basic substances rosamicin and trimethoprim in the acid canine vaginal and urethral secretions in relation to the pH of these fluids. The mechanism concerning diffusion and concentration of drugs into the vagina to levels exceeding the simultaneous plasma values are described by Stamey (7) as occurring by nonionic diffusion across the vaginal epithelium. The behaviour of the urethral mucosa is very similar to the vaginal mucosa with regard to hormonal influence (1). In addition, the drug secretion may also occur in the prostate-like paraurethral glands.

Oophorectomy was effective in creating a different pH milieu on vaginal and urethral mucosa, although the influence of the adrenals could not be excluded. The differences between the rosamicin and trimethoprim groups following oophorectomy and also following hormone application could be explained by a longer interval (at least 14 days) between oophorectomy and experiment in the rosamicin group leading to better recovery by the animal and possibly hormone substitution by the adrenals. The vaginal smears, however, proved the efficacy of oophorectomy and hormone application in all dogs.

In these experiments, we were able to prove the dependence upon pH of the secretion of two basic drugs with slightly different pKa into more or less acid vaginal and urethral secretions. This held true although the plasma secretion ratio did not increase in all experiments following pH decrease after hormone application. This may have been due to exhaustion of the animal by the first experiment carried out 5-7 days earlier.

The ratios for trimethoprim between plasma and vaginal secretion are comparable to ratios found in humans (4). This occurs although the vaginal pH in dogs is considerably higher than in humans, even higher than in postmenopausal women, possibly due to the lack of Döederlein's flora in dogs. High concentrations of antimicrobial agents on the vaginal mucosa of women have been found capable of eradicating or preventing colonization of the vaginal vestibule with faecal bacteria, usually preceding urinary tract infections (7, 8). The corresponding ratios for the urethra were also in the same range. The ratios for rosamicin were slightly higher, resulting from the more basic pKa. Antimicrobial substances with a basic pKa, secreted by the paraurethral glands, may be effective in the treatment of bacterial urethritis, since they could reach the depth of the paraurethral glands and ducts in bacteriocidal concentrations.

Trimethoprim and rosamicin may be effective in the treatment of colonisation of the vaginal vestibule with faecal bacteria, usually preceding urinary tract infections as well as in the treatment of non-gonococcal chronic urethritis. Rosamicin also has the advantage of being effective against chlamydia and mycoplasms, and of having a higher secretion/plasma ratio than trimethoprim. A clinical study appears to be indicated following these animal studies.

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