labor is shown in Fig. 1C. The specificity of this method was also confirmed by mass spectral studies. The HPLC eluate corresponding to ritodrine was found to exhibit MS characteristics identical to those of pure ritodrine. The spectrum shows a base peak at m/z 121 and a strong fragment ion at m/z 164. No molecular ion is detectable for ritodrine in the electron-ionization spectrum.

The calibration curve for ritodrine using the internal standard bears a linear relationship ($r^2 = 0.9910$) over the range (2–80 ng/mL) studied. Table I illustrates the reproducibility of the method. To demonstrate the applicability of this method for the therapeutic monitoring of ritodrine, a serum sample from a pregnant woman receiving ritodrine for premature labor was analyzed. The patient received an intravenous infusion of ritodrine at 100 μg/min for 3.5 h. The serum level of ritodrine 20 min after the end of the infusion was 12.8 ng/mL. The concentration compares favorably with the results obtained by the RIA method (7). The degree of sensitivity achieved by the HPLC–EC method is suitable for drug monitoring and pharmacokinetic studies of ritodrine, since serum concentrations of the drug during clinical use usually range from several to ~100 ng/mL. The linearity of the calibration curve indicates that the assay methodology is appropriate for these measurements.

The usefulness of electrochemical detection has been demonstrated in the HPLC analysis of catecholamines (8–10), uric acid (11), ascorbic acid (11), and acetaminophen (12) in biological fluids and pharmaceutical preparations. Ritodrine, a compound structurally related to catecholamines, evokes a strong electrochemical response due to the two phenolic moieties in the molecule. The method described herein permits a rapid analysis of ritodrine at the nanogram level.

The sensitivity of the electrochemical detector is quite comparable with that of the RIA technique, and the assay speed of the two methods is also very comparable. The RIA method appears to be more convenient because no extraction is required. However, it takes ~40 min to incubate and equilibrate a sample with the antiserum plus additional time for scintillation counting, not to mention the time for preparing the antiserum. On the other hand, the analysis time by HPLC requires ~15 min according to the protocol. Most importantly the HPLC–EC method may be superior to the RIA in terms of specificity. Previous work in the assay of ritodrine by RIA showed negligible cross-reactivity with the drug conjugates (7). However, the possibility of cross-reactivity with endogenous catecholamines has not been evaluated. The fact that abnormal variability was observed in the data during the RIA quantitation of ritodrine (7) points to the potential problem of cross-reactivity. It is known that serum catecholamine levels are elevated during labor (13). This may cause erroneous determination of ritodrine by RIA under such conditions. On the other hand, the accuracy of the HPLC–EC method is not affected by these endogenous substances.

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Remarks on the Structure–Activity Relationship of Silver Sulfanilamides

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Abstract □ The biological activity of a series of 10 silver sulfanilamides is studied in relation to the physical parameters pK_a, log K, and the aqueous solubility. None of the parameters demonstrate a simple relationship with the activity. A discussion of the significance of log K and the solubility in relation to the activity is given.

Keyphrases □ Silver sulfanilamides—structure-activity relationships, correlation between activity and pK_a, log K, and solubility □ Structure–activity relationships—silver sulfanilamides, correlation between activity and pK_a, log K, and solubility

An increasing interest in the silver sulfanilamide complexes was stimulated by the successful use of silver sulfadiazine (I) as an efficacious topical antibacterial agent in burn treatment (1). A number of efforts were made to account for the good *in vivo* activity of I as compared with other silver sulfanilamides (2–4). In this study the relevance of some physical parameters in relation to the *in vivo* antimicrobial activity are discussed.

BACKGROUND

The antimicrobial activity of I is thought to result from alteration of the mesosomal function of the microbial cell by the silver moiety of the molecule (5). The sulfadiazine moiety does not enter the cell and does not contribute appreciably to the antimicrobial action; therefore, I is not antagonized by *p*-aminobenzoic acid (3). A possible role of sulfadiazine is to localize the action of silver to the cell. The undissociated molecule I seems to interact with the microbial cell and is dissociated next at the cell surface into silver and sulfadiazine (6, 7).

The role of the sulfadiazine anion and other anions is rather unclear. Wysor assumed as a prerequisite for an active silver sulfanilamide that, like I, these compounds need to be stable in a chloride-containing solution

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Table I—Biological and Physical Data of the Silver Sulfanilamides

	Compound	mortality, %[a]	pK_a[b]	log K[c]	log K'[d]	Silver Ion Concentration, mg/100 mL[e]
I	Silver sulfadiazine	25.6	6.46 ± 0.02	3.62 ± 0.05	3.57	0.05 ± 0.001
II	Silver sulfathiazole	40	7.19 ± 0.01	4.18 ± 0.22	3.97	0.01 ± 0.003
III	Silver sulfisomidine	60	7.47 ± 0.01	3.69 ± 0.13	3.35	0.11 ± 0.003
IV	Silver sulfamethizole	65	5.63 ± 0.01	3.55 ± 0.18	3.54	0.10 ± 0.004
V	Silver sulfanilamide	77	10.57 ± 0.10	4.13 ± 0.11	0.96	0.70 ± 0.003
VI	Silver sulfamerazine	90	6.88 ± 0.11	3.91 ± 0.04	3.80	0.06 ± 0.003
VII	Silver sulfapyridine	75	8.57 ± 0.01	4.02 ± 0.04	2.82	0.11 ± 0.003
VIII	Silver 5-methylsulfadiazine	80	6.68 ± 0.05	3.66 ± 0.08	3.58	0.19 ± 0.002
IX	Silver 5-methoxysulfadiazine	80	6.67 ± 0.04	3.48 ± 0.08	3.41	0.06 ± 0.002
X	Silver benzenesulfonamidopyrimidine	100	6.12 ± 0.08	3.42 ± 0.04	3.40	0.05 ± 0.005

[a] Mortality for untreated mice: 88%; mortality for mice treated with ointment base: 65% (8). [b] Mean ± *SD*; n = 4–8. [c] Mean ± *SD*; n = 9–14. [d] Conditional stability constant (log K') = log K − log $\alpha_{L(H)}$ at pH 7.4. [e] Mean concentration of silver ion in a saturated aqueous solution ± *SD*; n = 5.

(no formation of silver chloride) (2). Only sulfanilamides with 2-pyrimidino as the N-1 substituent meet this criterion. Use of this information did not lead to the successful design of active compounds. Fox and Modak partly explained the large differences in *in vivo* antimicrobial activity of the silver sulfanilamides by differences in dissociation constants (pK_a) of the acidic N(1)—H of the parent sulfanilamides (3). Nesbitt and Sandmann reported a nonlinear relationship between the *in vivo* activity and pK_a + log K_s (solubility product) (4).

EXPERIMENTAL

A series of 10 silver sulfanilamides was selected for this study (Table I). The *in vivo* activity data (percentage of mortality of mice with burns over 30% of their bodies which were infected with *Pseudomonas aeruginosa*) were derived from the literature (8) or obtained from C. L. Fox, Jr., Columbia University, New York, N.Y. The acid dissociation constants (pK_a) of the sulfanilamides and the stability constants (log K) of their silver complexes were measured according to the method of Boelema *et al.* (9), using a microcomputer-controlled titration method in which the silver ion[1] and hydrogen ion[2] concentrations were determined in a combined measurement. The conditional stability constants (log K') were calculated from the pK_a and log K values using log K' = log K − log $\alpha_{L(H)}$, where $\alpha_{L(H)} = 1 + [H_3O^+]\cdot K_a^{-1}$ is the side reaction coefficient of the sulfanilamide with the hydrogen ion. The sulfanilamides (I–IX) used in this study were obtained commercially and recrystallized from acetone–water; X was synthesized as described previously (10).

The solubility measurements of the silver sulfanilamides were performed by equilibration in double-distilled water at 25 ± 0.1°C for 1 week in 100-mL vials wrapped with aluminum foil with covered[3] rubber closures. The vials were rotated during equilibration. The saturated solutions were filtered through a 25-mm diameter (1.2-μm average pore size) filter[4]; the first 25 mL of filtrate was discarded. The pH[5] of the filtrate was measured, and the filtrates were analyzed for sulfanilamide content by UV[6] and silver by atomic absorption spectroscopy[7]. The silver compounds used in this study are described elsewhere and were micronized[8] (10, 11).

RESULTS AND DISCUSSION

In Table I the biological and physical data of the 10 silver sulfanilamides are summarized. Efforts to measure the distribution coefficient (log P) for octanol–water failed; the silver compounds decomposed with the formation of metallic silver. Calculation of the correlation coefficients between percent mortality and pK_a, log K, log K', and the silver ion concentration reveals that a simple linear relationship between these sets of data does not exist; the coefficients are r = 0.07, 0.21, 0.21, and 0.19, respectively. Nesbitt and Sandmann (4) previously reported a nonlinear relationship between pK_a + log K_s and percent mortality. From Fig. 1 it is clear that this relationship is confirmed by our extended series; the points are spread randomly.

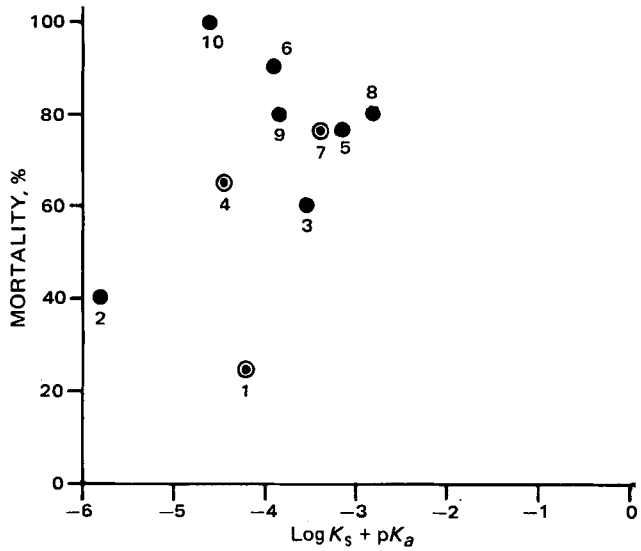

Figure 1—*Relationship between percent mortality and log* K_s + pK_a. *The points of Nesbitt and Sandmann (4) are marked by encircled dots. The solubility product* K_s *is calculated from* $[Ag]^2 = ([H_3O^+]\cdot K_s/K_a) + K_s$ *(4).*

The data for log K' (pH 7.4), the conditional stability constant, can explain in a qualitative way some of the observations made in earlier studies. Wysor (2) found that the silver sulfanilamides with 2-pyrimidino as the N-1 substituent (I, VI, VIII, IX, and X) were stable in a chloride-containing solution; V and VII, with lower log K' values, were unstable. Another example is the reaction of the silver compounds with human serum (3). Silver nitrate and V reacted completely within 2 h with the components of the serum, I reacted at a moderate rate (80% in 40 h), and II and VI reacted very slowly (~30% in 40 h). The log K' values of these compounds increase in the same order.

One unexpected result is that treatments with ointment bases containing the silver sulfanilamides V–X give rise to higher mortality rates than the ointment base alone. This is especially hard to understand for VI and VIII, which are structurally very similar to I. Also hard to understand in relation to the mortality data are the *in vitro* activities of the silver compounds. The minimum inhibitory concentration (MIC) of all the silver compounds in Table I are approximately the same for one type of microorganism. The MIC values for 10 different organisms vary between 6.3 and 200 μg·mL^{-1} (12). Thus, the differences in *in vivo* activity between the silver compounds are in no way reflected by their MIC values.

[1] Ion-selective silver sulfide electrode, Orion research 941600.
[2] Glass electrode, Radiometer G 2040.
[3] Using parafilm *M*.
[4] Selectron-filter, type ST 69; Schleicher-Schüll, Dassel, West Germany.
[5] pH-Meter, type PHM 22 r; Radiometer, Copenhagen, Denmark.
[6] Perkin-Elmer 124.
[7] Perkin-Elmer, type 303, acetylene-air flame, lamp: 3 UAX/Ag-Cathodeon Ltd, 328.1 nm.
[8] Trost Gem T Mill; Helme Chemicals, Inc., Helmetta, N.J.

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COMMUNICATIONS

Enhancement of Fluoride Concentration in Saliva after Topical Application of Fluoride Sustained-Release Dosage Form on Orthodontic Plates

Keyphrases □ Fluoride, sustained release—topical application, concentration in whole saliva

To the Editor: The beneficial role of fluoride topical applications for caries prevention is now well established. The effectiveness of a topical treatment resides in the amount of fluoride incorporated in apatitic form (1) and in the continuous supply and renewal of the fluoride content of tooth enamel (2–3). None of the topical dosage forms of fluoride marketed today (dentifrices, mouth rinses, and gels) are able to supply fluoride for a long period of time. A sustained-release dosage form of fluoride for topical application should provide conditions that will result in the major product being a stable fluoroapatite. The research effort expended to date on the development of a sustained-release delivery system for fluoride is minor compared with efforts expended in the areas of other drugs; however, significant progress has been made.

Three general approaches are being taken now to develop a sustained-release delivery system of fluoride: (*a*) matrix tables (4), (*b*) aerosol containing microcapsules for direct application on the tooth surface (5), and (*c*) intraoral system (6).

In our previous *in vitro* work (7, 8) it was shown that fluoride release could be sustained by embedding NaF or CaF_2 in ethylcellulose polymers. The aim of this work was to establish the fluoride level of saliva in children using orthodontic plates coated with a long-acting fluoride application.

A group of nine children aged 7–12 undergoing orthodontic treatment participated in the study. The subjects resided in the Jerusalem area, which had ~0.4 ppm fluoride in the water supply. None of the children had brushed their teeth with a fluoride dentifrice or received a fluoride supplement (including tea) 2 d before or during the experiment. The palatinale part of the upper orthodontic plates was coated by immersing the plates in a 10% NaF suspension in ethanol solution of ethylcellulose and allowing them to dry at room temperature (8).

The amount of the coating layer on the orthodontic plates was determined by weighing the difference between the uncoated and coated materials. The fluoride (F) concentration was calculated from the weight ratio of fluoride and polymer used and was in the range of 4.8–5.1 mg/plate. Whole saliva samples were collected by chewing paraffin wax, and the F concentration was determined in the centrifuged sample with a fluoride ion electrode (9). Statistical significance was determined by the paired t test.

F release resulted in a significant ($p < 0.005$) increase in the amount of fluoride in the saliva during the first 4 days of the study but returned to its initial level on the 5th day (Table I). By the use of fluoride dentifrices or ingestion of 1-mg fluoride tablets, similar increases in the F content in the saliva were reported (10). However, the increase was for a short period only, with the F concentration returning to the initial levels after 90 min.

A relationship between the natural fluoride content in water and the prevalence of dental caries is well established today.

In communities with a fluoride content of ≥1 ppm, the prevalence of dental caries was less than in those communities whose water supplies contained fluoride at a concentration ≤0.6 ppm.

It has been shown by Yao and Grøn (9) that the fluoride content in the saliva of children who consumed drinking water containing 1 ppm of fluoride was 0.01–0.05 ppm.

These values are similar to the fluoride concentration obtained in the saliva of the children using sustained release application of fluoride in this study.

In our previous work (7) it was shown that thickness of the coating applied on the orthodontic plate did not affect

Table I—F Content in Mixed Saliva of Nine Children after Fluoride Sustained Release Application [a]

	Day after Application				
	1	2	3	4	5
F, mean ± *SD*	0.015 ± 0.003	0.02 ± 0.003	0.016 ± 0.003	0.01 ± 0.002	0.007 ± 0.0002
P	<0.005	<0.005	<0.005	<0.005	NS[b]

[a] Control (F concentration in saliva before the experiment) 0.008 ± 0.002 ppm. [b] NS = not significant.

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