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Release and antimicrobial activity of silver sulphadiazine from different creams

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Summary
The release and antimicrobial activity of silver sulphadiazine from five different creams were studied: unguentum emulsificans aquosum, unguentum hydrophylicum non ionogenicum, paraffin cream (15 per cent), a homemade preparation and a commercially available preparation (Flamazine). A diffusion cell was used to measure the release and the agar well diffusion technique to determine the antibacterial activity of the silver sulphadiazine released. The paraffin cream (15 per cent) preparation had the highest release rate, followed by the homemade cream and the commercially available cream. The antibacterial activity ran parallel with the release results. This study shows the silver sulphadiazine paraffin cream to be superior to the other four preparations, including the commercially available silver sulphadiazine cream, using release and antibacterial activity as criteria.

INTRODUCTION

Burn wound sepsis remains one of the major causes of death in burn victims. The main method of controlling burn wound sepsis is by using an appropriate topical antimicrobial agent. Silver sulphadiazine cream is commonly used in burn treatment to prevent bacterial colonization and infection of the wounds (Richards and Mahlangu, 1981; Pegg, 1982). The release of silver sulphadiazine from the cream base determines the bacteriostatic and bactericidal activity. The rate of release of the solid drug suspended in a cream base is determined by several factors, solubility being one of the most important (Higuchi, 1961). In the literature, no data were found concerning the rate of release of silver sulphadiazine. Therefore, the basic aim of this investigation was to determine the release of silver sulphadiazine from the commercially available cream Flamazine (Duphar, Weesp, The Netherlands), and to compare the release from this industrial cream with four homemade hydrophilic cream bases, mixed with 1 and 2 per cent silver sulphadiazine (Raunio et al., 1980; Lippold and Teubner, 1982).

Next, the antibacterial activity of the five creams, Flamazine and four homemade creams, was investigated. Finally, the practical applicability was measured using rheological parameters (Colnago et al., 1982).

MATERIALS AND METHODS

Materials

Cream bases

Five creams were tested: the commercially available silver sulphadiazine (Flamazine, 82C26) and four homemade hydrophilic creams, which were: unguentum emulsificans aquosum *(Deutsches Arzneibuch* (1978) **8**, 436), unguentum hydrophilicum non ionogenicum *(Pharmacopoeia Helvetica* (1981) vi, 685d), paraffin cream 15 per cent (Briedé, 1983) and preparation IV. The composition of these creams is shown in *Table I.* All the substances for the cream bases were of pharmacopoeial purity. Three cream bases were prepared as described in the quoted articles. For preparation IV (own formulation) the water

Table I. Composition of the four cream bases

*Unguentum emulsificans aquosum**	
Alcohol cetostearylatum	9%
Paraffin subliquidum	10·5%
Vaselinum album	10·5%
Aqua	70%
Unguentum hydrophylicum non ionogenicum†	
Cetanolum	10 g
Oleum arachidis hydrogenatum	20 g
Polysorbatum monostearylatum	5 g
Propylenglycolum	20 g
Aqua	ad 100 g
Paraffin cream 15%‡	
Carbomer watergel 1%	254 g
Polysorbate 80/sorbitan mono-oleate mixture āā	1 g
Paraffin subliquidum	45 g
Preparation IV§	
Alcohol cetostearylatum	5 g
Glycolum monostearylatum	9 g
Propylenglycolum	10 g
Paraffin subliquidum	10 g
Tween 60	2·5 g
Tween 80	2·5 g
Aqua	ad 100 g

* *Deutsches Arzneibuch* (1978) 8, 436.
† *Pharmacopoeia Helvetica* (1981) VI, 685d.
‡ *Pharmaceutisch Weekblad.*
§ Own preparation.

phase and fat phase were heated separately in a water-bath at 70°C. The water phase was added to the fat phase and the emulsion was stirred until the cream reached room temperature. The volume of water lost by evaporation was replaced. All these creams were prepared without preservatives. Silver sulphadiazine was suspended in the cream bases after fine grinding in a mortar and pestle. The concentrations in the four homemade preparations were 1 and 2 per cent silver sulphadiazine respectively.

Silver sulphadiazine

A commercial grade of silver sulphadiazine was used (ACF, Chemiefarma, Maarsen, The Netherlands; charge 780472/80E01). The particle size, given by the manufacturer, was: 100 per cent of the particles <50 μm, 99 per cent ≤20 μm and 90 per cent ≤10 μm.

Methods

Determination of the release

The in vitro release of silver sulphadiazine from different cream bases was studied by means of a diffusion cell (*Fig.* 1). The circular cell consisted of a ring (A) and a dish (B) of polymethacrylate.

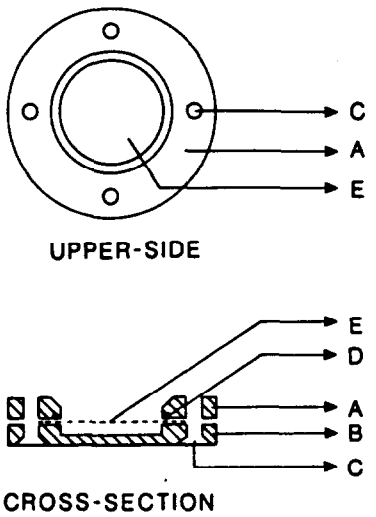

Fig. 1. Diffusion cell used for release experiments. A, a ring; B, a dish of polymethacrylate; C, screws; D, perspex O-ring; E, cellulose membrane.

The dish had a circular well (inner diameter 30 mm, depth 3 mm), which could be filled with cream. The excess cream was removed using the edge of a spatula to produce an even uniform surface of constant dimensions. A membrane (E) wetted with acceptor medium was placed over the surface of the dish containing the cream, in such a way as to avoid air bubbles in the cell. The semipermeable membrane consisted of cellulose with a molecular weight cut-off of 12 000–14 000 daltons (Spectra Medical Industries, Los Angeles, USA). The membrane was maintained in a flat position using a perspex O-ring (D) between the ring (A) and the dish (B). The ring of the diffusion cell was fixed with screws (C) onto the dish.

The diffusion cell was placed at the bottom of a 250 ml beaker, filled with 200 ml acceptor medium. An isotonic phosphate-buffered solution, pH=7·4 (*Dutch Pharmacopoeia* (1978) 8, 256), was used as acceptor medium. The beaker was kept in a constant-temperature water-bath maintained at 32°C. The acceptor medium was stirred with a four-bladed stirrer (l=50, h=10 mm), which was driven by a synchronous motor at 50 r/min. The distance between the diffusion cell and the stirrer was 40 mm. The diffusion cell was placed in the beaker at the beginning of the experiment. During a period of 6 h a 3 ml sample was taken every 30 min. Throughout the release run, the volume of the acceptor medium was kept constant by replacing the sample removed with an equal volume of acceptor

medium. The samples were assayed with a Beckmann ultraviolet spectrophotometer at 242 nm ($E^{1\%}_{1cm}$ of sulphadiazine = 600). Blank runs demonstrated the minimal presence of material in the external solution that might interfere with the measurements. All results were corrected for these deviations.

Data treatment

Each release experiment was performed at least four times and the averaged data were used to plot the amount of drug released (into 200 ml acceptor medium) per unit of area (cm^2) versus the time and the square root of time respectively. The correlation coefficient was calculated for the square root of time plots.

Microbiological evaluation

The agar-well diffusion technique described by Nathan et al. (1977) was used to evaluate the antimicrobial activity of identical concentrations of silver sulphadiazine in the five different creams. This diffusion technique was chosen since it incorporates the evaluation of the drug carriers which are critical factors for the antibacterial activity of topical agents (i.e., silver sulphadiazine). This in vitro method includes the following steps:

(1) Test plate preparation

A petri dish, 90 mm in diameter, was filled with 15 ml of agar containing standard brain–heart infusion (BHI) (BBL, Becton Dickinson and Company, Cockeysville, USA, Art. 11065). Five holes, 9 mm in diameter, were made in the agar by removing plugs cut with a cork-borer. The holes, evenly distributed on the plate, were spaced about 20 mm apart and 15 mm from the outer edge. All procedures were performed using sterile instruments to avoid contamination of the petri dish. Five separate syringes were filled with the five different silver sulphadiazine creams, and 18 gauge needles were attached to each syringe.

To evaluate the relation between the drug activity and the amount of drug, different amounts of silver sulphadiazine cream were tested: 10 mg, 20 mg, 30 mg, 40 mg, 50 mg and 60 mg of silver sulphadiazine cream per well. Each hole of a petri dish was filled with 10 mg of the five different creams; second, third, fourth, fifth and sixth petri dishes were filled with 20 mg, 30 mg, 40 mg, 50 mg and 60 mg, respectively, of each cream. The loaded syringes were weighed, an approximate amount of cream was extruded into the centre of each hole on the agar plate and each syringe was weighed again.

(2) Plate inoculation with a bacterial strain

Seven millilitres of melted BHI agar (at approximately 45°C) was mixed with 1 ml BHI broth containing 10^6 bacteria per ml. The bacterium used for inoculation was *Pseudomonas aeruginosa*. The test strain was maintained on agar slopes and transferred to the fluid BHI broth using a wire loop. The strain was incubated at 37°C for 18 h, leading to a pure culture suspension of 10^6 colony forming units (CFU) per ml of broth. The suspension of melted agar and bacteria was mixed on a mechanical agitator and poured onto the previously prepared five plates containing the five different creams to be tested. The holes were completely filled with the agar and the overlay was evenly distributed.

(3) Silver sulphadiazine concentrations

Two concentrations of silver sulphadiazine (1 and 2 per cent) were used in the four homemade creams. The commercially available cream contained 1 per cent silver sulphadiazine only.

(4) Incubation

The fluid agar overlay, containing the suspension of bacteria, solidified in about 1 min. The test plates were incubated at 37°C for 18 h.

(5) Reading the test plates

A clear area containing no colonies around a test well following the incubation period indicated that the drug was bactericidal against *Ps. aeruginosa*. The size of the cleared areas and the cream areas were measured with a marking gauge. The diameter (mm) of the clear area was determined by subtracting the cream diameter from that of the clear area.

Rheological evaluation

Rheograms were obtained using a rotational viscometer type Rotovisko RV 3, a cone and plate viscosity sensor system PK 1 in combination with a measuring head MK 500. A constant-temperature circulator (type FK) maintained the samples at a constant temperature of 32°C±0·02°C (Haake, D-Karlsruhe). In order to ensure that all the creams had been subject to the same 'shear history', the same preparation and loading procedures were employed in all experiments. Very small samples were transferred with a spatula onto the plate, taking care that no air bubbles were present. The flow curves were measured using a time linear r/min programme of 400 r/min.

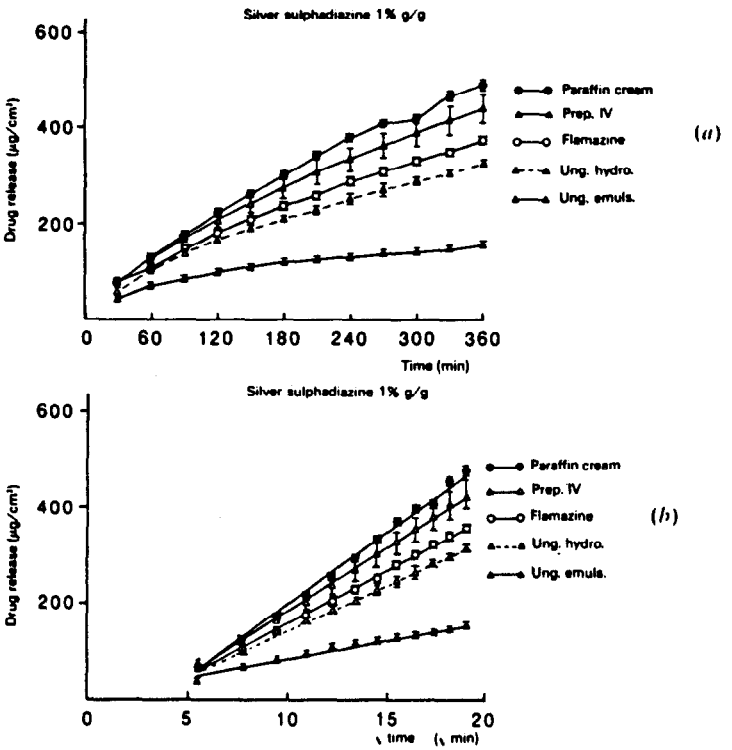

Fig. 2. Release (µg/cm^2) of silver sulphadiazine from different cream bases (1 per cent g/g) vs. time (min) (*a*) and vs. the square root of time (*b*).

Statistical evaluation of data

For each experiment, statistical differences were assessed using Student's *t* test and comparing experimental points corresponding to identical values on the axis. A significant difference between two bases was assumed to exist if all experimental points, except the first pair (1 h), were significantly different at the 5 per cent level (Armitage, 1974).

RESULTS

Release

Figs. 2 and 3 show the results of release from the different cream bases mixed with silver sulphadiazine at concentrations of 1 per cent and 2 per cent respectively. The amount of silver sulphadiazine released from the bases was plotted in *Figs.* 2(*a*) and 3(*b*) vs. the time and in *Figs.* 2(*b*) and 3(*b*) vs. the square root of time. The slopes between the different creams were all significantly different at a P value of less than 0·05 for both concentrations. The slope between Flamazine and paraffin cream was significantly different ($P<0·02$). A factor of about 1·4 was found between the amount of silver sulphadiazine released from the creams containing 1 per cent and 2 per cent of the drug.

Antimicrobial activity

The paraffin cream bases, containing 1 per cent and 2 per cent silver sulphadiazine respectively, both showed the best antimicrobial activity (*Fig.* 4). Thirty milligrams of silver sulphadiazine paraffin cream per well gave the maximal effect in the vitro test system. This amount was also found for the other bases, with the exception of ung. emulsificans and ung. hydrophylicum which showed a greater zone in relation to a higher amount of silver sulphadiazine cream. The slopes between paraffin cream, 1 per cent silver sulphadiazine and the other creams mixed with 1 per cent silver sulphadiazine were significantly different, including Flamazine ($P<0·05$).

Rheological evaluation

The dependence of shear stress on the rate of shear for the creams was measured, and some

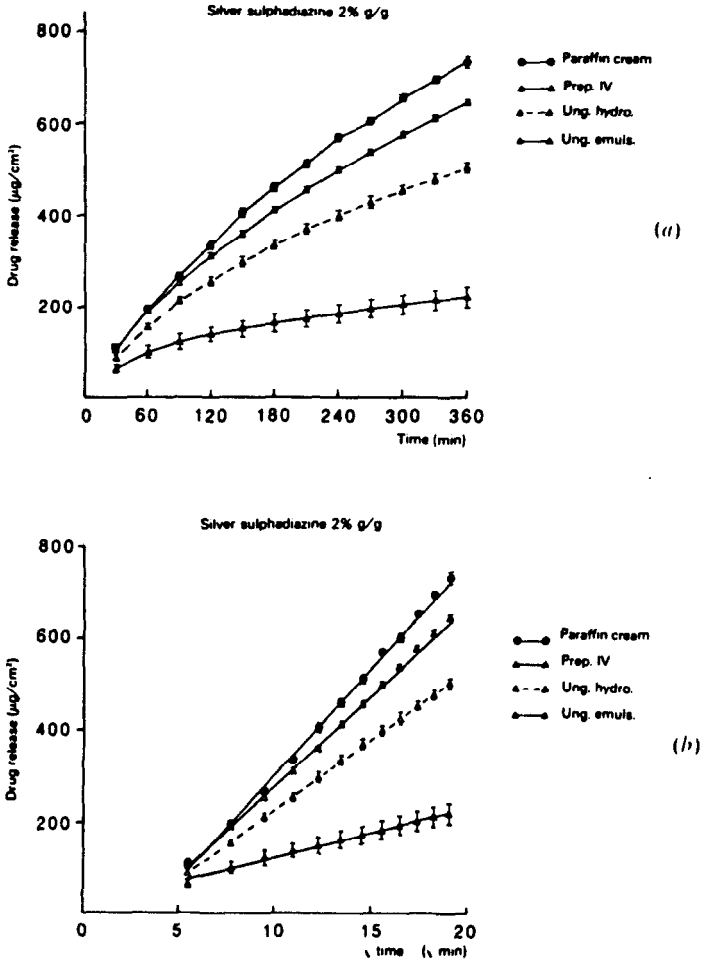

Fig. 3. Release (µg/cm²) of silver sulphadiazine from different cream bases (2 per cent, g/g) vs. time (min) (*a*) and vs. the square root of time (*b*).

characteristic rheograms are shown in *Fig.* 5. The 'up' and 'down' curves were not identical, showing a considerable thixotropic behaviour in some creams. The paraffin cream had a pseudoplastic character also.

DISCUSSION

The diffusion cell used in this study to measure the release of silver sulphadiazine was found to yield reproducible results. The relative standard deviation in the different determinations had an average value of 7 per cent. When the amounts of silver sulphadiazine released were plotted vs. the square root of time, straight lines were obtained with a good correlation coefficient *(Table II)*. The plots in *Figs.* 2(*b*) and 3(*b*) never passed through the origin. The presence of a lag time for the release of a drug across an artificial membrane has been reported earlier (Bottari et al., 1974). This phenomenon has been associated with the presence of the membrane, separating the bulk phase from the sink.

This study shows the paraffin base to be significantly superior to Flamazine cream from both the technological and microbiological point of view. The release from paraffin was found to be 1·5 times higher and the diameters of the cleared zones were about 4·8 mm compared to 3·6 mm for Flamazine. From the rheological point of view paraffin 'spreadability' is as good as that of Flamazine. Another factor which should be taken into

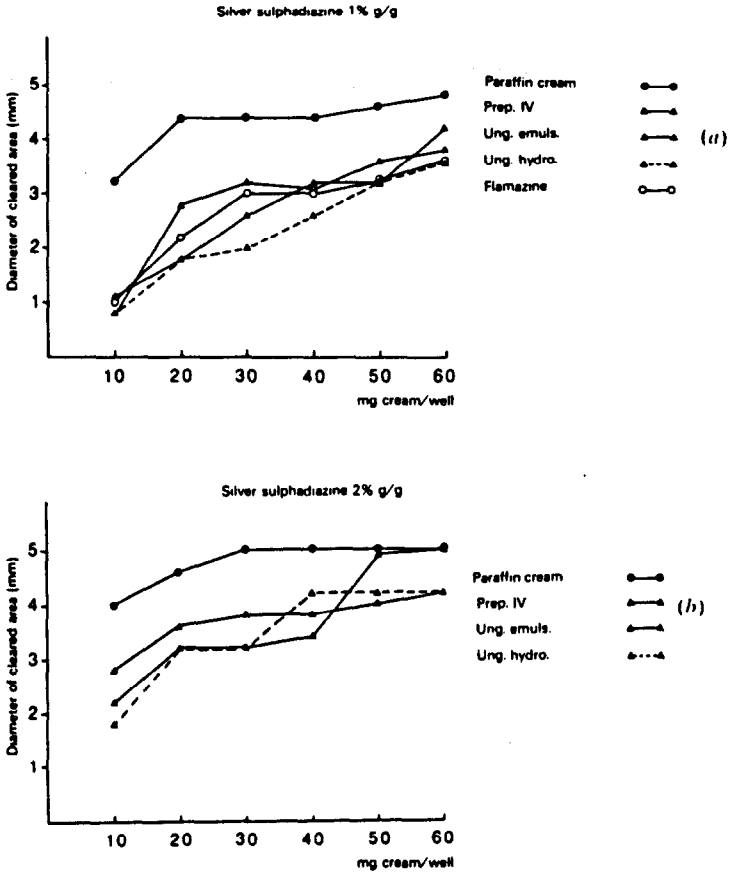

Fig. 4. The plot for the diameter of cleared area (mm) in the agar diffusion test with different cream bases containing 1 per cent silver sulphadiazine (g/g) (*a*) and 2 per cent silver sulphadiazine (g/g) (*b*) in the presence of *Pseudomonas aeruginosa*.

Table II. Correlation coefficients of silver sulphadiazine release from the different creams vs. time

Cream	*Silver sulphadiazine* 1 per cent g/g (*Fig.* 2(*b*))	2 per cent g/g (*Fig.* 3(*b*))
Paraffin cream	0·9966	0·9983
Prep. IV	0·9984	0·9990
Flamazine	0·9978	—
Ung. hydro.	0·9998	0·9998
Ung. emuls.	0·9912	0·9968

account is the toxicity of additives to creams: for example, emulsifying agents carboxypolymethylene and sorbitan derivatives. Toxicity has not been described for the low concentrations used in both the paraffin and Flamazine creams (*Martindale*, 1982).

The introduction of Flamazine by Fox (1968) has led to a remarkable improvement in the management of burn wounds. Different clinical studies show that Flamazine is a valuable topical cream for the prevention of burn wound infection (Fox, 1968; Stanford et al., 1969; Lowbury et al., 1971). However, careful microbiological studies have revealed that Flamazine does not prevent colonization by *Staphylococcus aureus* and *Ps. aeruginosa* (Gayle et al., 1978; van Saene and Nicolai, 1979). These observations can be explained by the relatively low concentrations of antibacterially active silver sulphadiazine, due to: (i) minimal inhibitory concentrations (MICs) of the agent, (ii) the small amount released from the cream base and (iii) inactivation of the agent by proteins. The MICs of silver sulphadiazine for most clinically important bacteria are relatively high: 50–100 mg/l (van Saene et al., 1983).

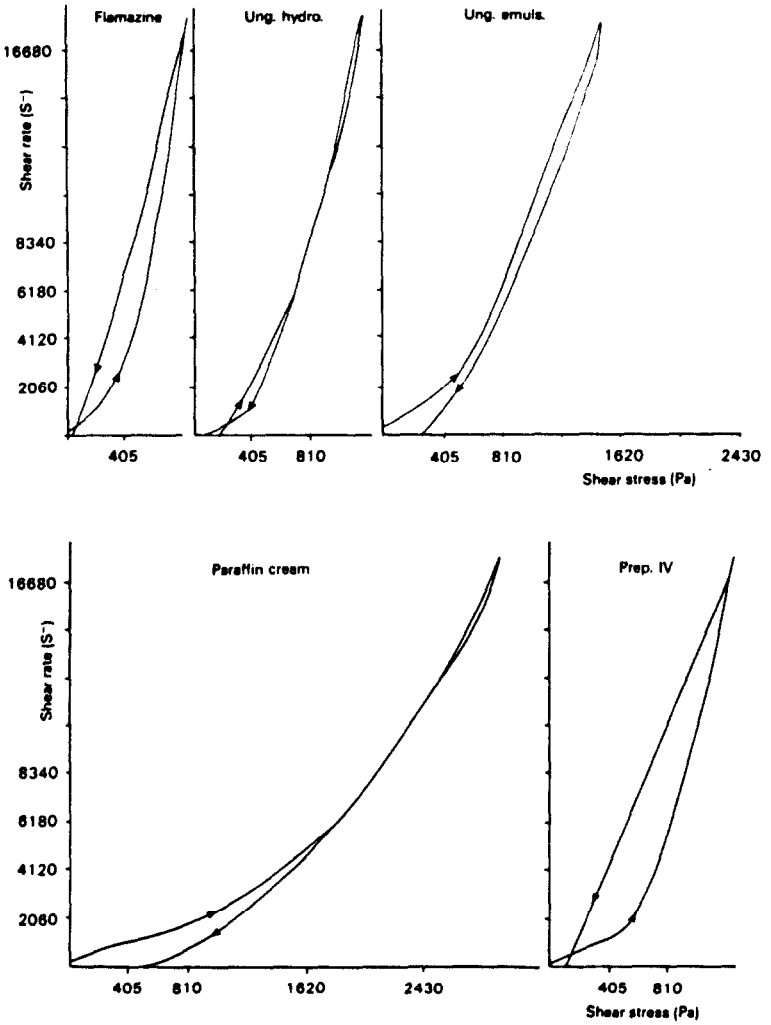

Fig. 5. Shear rate (S) dependent on stress of shear for different cream bases containing 1 per cent silver sulphadiazine.

Secondly, the release rate is found to be about 50 $\mu g/cm^2$/per h. Thirdly, silver sulphadiazine is known to be bound by proteins and necrotic tissue covering burn wounds (Bult, 1982). The theory of Fox and many others is that silver sulphadiazine is present in gross excess on the wound which alters the MIC, release rate and protein inactivation problems. High rates of bacterial colonization of wounds covered with Flamazine do not correlate with this theory. We believe that the satisfactory clinical results with Flamazine — which is also our daily experience — should be attributed primarily to the covering effect of the base (substituting damaged skin and preventing loss of fluids and proteins) and less to the antibacterial effect of silver sulphadiazine. An antibacterially more active disinfecting agent added to a paraffin cream base with identical rheological and technological properties as Flamazine, but characterized by a higher release rate, seems to us to be more promising in the management of burn wounds.

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