

# In Vitro Studies of Water Activity and Bacterial Growth Inhibition of Sucrose–Polyethylene Glycol 400–Hydrogen Peroxide and Xylose–Polyethylene Glycol 400–Hydrogen Peroxide Pastes Used To Treat Infected Wounds

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**Water activity and bacterial growth inhibition have been studied in formulations comprising either sucrose or xylose along with polyethylene glycol 400 and hydrogen peroxide. The pastes are chemically stable for 6 months if stored at 2 to 8°C and have been shown to lower water activity to levels below those essential for bacterial growth and to be bactericidal even when diluted up to 50% with serum. Of the organisms tested, *Staphylococcus aureus* proved the least susceptible to the bactericidal effects of these pastes, and candida and gram-negative organisms proved the most susceptible. Pastes without hydrogen peroxide were less rapidly bactericidal than pastes with hydrogen peroxide, while polyethylene glycol 400 itself was found to have considerable antimicrobial activity. It is suggested that sucrose paste may be of benefit as a treatment for infected and malodorous wounds.**

In 1985 Trouillet et al. (8) reported on the use of sugar (sucrose) for the treatment of acute mediastinitis following cardiac surgery. They treated 19 patients and followed a regimen of packing the cavities every 3 to 4 h with ordinary commercially available granular sugar. Near complete debridement of wounds was followed by the rapid formation of granulation tissue and eradication of bacterial infection after an average of 7.6 days. A similar regime had been used previously by Chirife et al. (4).

Sugar was first used as a dressing at Northwick Park Hospital in 1982, when a paste formulation was developed (see Materials and Methods). Thick sugar paste has a consistency similar to that of modelling clay and can be packed into cavities with large openings, such as pressure sores. Thin sugar paste has a consistency similar to that of thin honey and is suitable for instillation into cavities with small openings. Polyethylene glycol (PEG) 400 was chosen as the lubricant because it does not interact with other components of the paste, is used in a wide variety of pharmaceutical preparations, and is relatively nontoxic (9). It exerts antibacterial activity because it has a low water activity (2). Hydrogen peroxide was added to the pastes as a preservative because they were packed into multidose ointment containers.

Sucrose pastes have been used clinically to treat infected and malodorous wounds. The pastes are safe to use in diabetic patients, as any sucrose that may be absorbed from a wound is excreted unchanged in the urine and does not affect diabetic control.

Microorganisms require water to grow and reproduce, and such water requirements are best defined in terms of water activity ( $a_w$ ) of the substrate rather than as water concentration (3). The water activity of a solution is expressed as  $a_w = p/p_0$ , where  $p$  is the water vapor pressure of the solution and  $p_0$  is the vapor pressure of pure water at the same temper-

ature. Addition of a solute (e.g., sucrose) to an aqueous solution in which a microorganism is growing will have the effect of lowering the  $a_w$  with a concomitant effect upon cell growth. Every microorganism has a limiting  $a_w$  below which it will not grow, e.g., for streptococci, *Klebsiella* spp., *Escherichia coli*, *Clostridium perfringens*, and *Pseudomonas* spp. the value is 0.95 (3). *Staphylococcus aureus* is most resistant and can proliferate with an  $a_w$  as low as 0.86.

Preliminary reports on the clinical value of sucrose paste have already been published (6, 7), while its effect on the formation of granulation tissue has been investigated in a novel model of moist wound healing in the domestic pig (1). In this study the antibacterial effects of thin sucrose and xylose pastes are described and compared with the water activity of pastes mixed with serum.

## MATERIALS AND METHODS

**Sugar pastes.** Sugar pastes were prepared in the hospital pharmacy according to the formulae in Table 1. Caster sugar and additive-free icing sugar were purchased from Tate & Lyle plc (London); xylose was purchased from Fluka Chemical Ltd. (Glossop, England); PEG 400, laboratory reagent grade, was purchased from British Drug Houses Ltd. (Poole, England) and hydrogen peroxide (30%) was purchased from Evans Medical (Greenford, England). The pastes were packed into white plastic screw-cap ointment pots and stored at 2 to 8°C. They were not sterilized.

**Quality control of sugar pastes.** All raw materials were identified before use. In addition the caster and icing sugars were assayed for added calcium and magnesium by the EDTA method. The hydrogen peroxide in the final product was assayed by a colorimetric method similar to that described by Garratt (5). In this study the  $A_{410}$  of the solution was measured.

**Microorganisms.** *S. aureus*, *Staphylococcus epidermidis*, *Streptococcus faecalis*, *E. coli*, group C beta-hemolytic streptococcus (*Streptococcus equisimilis*), *Proteus mirabi-*

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TABLE 1. Sugar pastes used in this study

Paste	Ingredient	Amt of ingredient in paste type	
		Thin	Thick
Sucrose	Caster sugar (fine granular sucrose)	1,200 g	1,200 g
	Icing sugar, additive free (powdered sucrose)	1,800 g	1,800 g
	PEG 400	1,416 ml (32% of total)	686 ml (18.5% of total)
	Hydrogen peroxide, 30% <sup>a</sup>	23 ml	19 ml
Xylose	Xylose	400 g	
	PEG 400	295 ml	
	Hydrogen peroxide, 30% <sup>a</sup>	4.75 ml	

<sup>a</sup> Final concentration of hydrogen peroxide, 0.15% (vol/wt). For studies requiring the use of paste without hydrogen peroxide, purified water replaced the hydrogen peroxide.

*lis*, and *Klebsiella* spp. were isolated from wounds of patients at Northwick Park Hospital and were maintained on Columbia agar slopes at 4°C.

**Media.** All media were purchased from Oxoid Ltd., London. All cultures were grown in nutrient broth, and 0.1% peptone water was used as the diluent for viable counts. Cysteine-lactose-electrolyte-deficient (CLED) agar was used for plating out *P. mirabilis*, and Columbia agar was used for all other microorganisms. Human serum was obtained from blood remaining after hematological tests. It was pooled and tested for hepatitis B antigen before use.

**Bactericidal effects of sucrose and xylose pastes.** Fresh cultures of the organisms under test, *S. aureus*, *S. faecalis*, *E. coli*, and *Candida albicans*, were suspended in 0.1% peptone water, and the concentration of cells was adjusted to approximately 10<sup>10</sup>/ml. One milliliter of the suspension was then added to approximately 100 g of the paste under test in a 150-ml sterile container. The cell suspension and pastes were mixed thoroughly, and 1-g samples were removed at 6, 24, and 48 h and at 7 and 28 days for serial dilution and subsequent counting. Peptone water was used for all dilutions, Tryptone soy agar was used for culturing bacteria, and Sabouraud dextrose agar was used for culturing candida.

Experiments were carried out at room temperature, and plates were incubated at 37°C for 3 days for bacteria and 5 days at room temperature for candida.

**Antibacterial activity of sucrose paste agars.** Peroxide-free thin sucrose paste was added to Columbia agar to give 10, 20, 30, 40, or 50% (vol/vol) sucrose agar. All the agar-sucrose paste mixtures were then autoclaved at 121°C for 15 min, and when cool, sterile horse blood was added to a final concentration of 7%. Approximately 20 ml of sucrose paste agar was then dispensed into each petri dish and allowed to set. Blood agar plates were used as controls.

Organisms under test were inoculated into nutrient broth and incubated for 5 h at 37°C. Cultures were then diluted 1 in 10,000 with peptone water, and 1 standard loopful of each was inoculated onto the sucrose paste-blood agar plates and control plates. A viable count was performed at the same time on the broth cultures. All plates were incubated for 24 h at 37°C.

**Water activity determinations.** A Humicap humidity meter

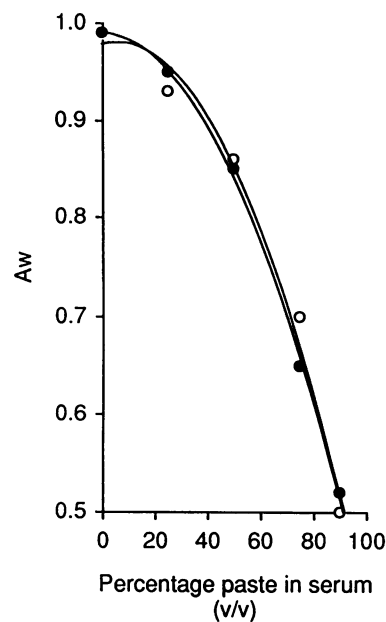


FIG. 1. Effects of  $a_w$  of adding hydrogen peroxide-free sucrose and xylose paste to serum. ●, xylose paste; ○, sucrose paste.

manufactured by Vaisala, Helsinki, Finland, was used for all determinations. Thin sucrose or xylose paste without peroxide was diluted with the appropriate amount of human serum to give a range of 0 to 100% paste in serum in stoppered conical flasks which were then wrapped to exclude light. Water activity was determined at  $25 \pm 0.1^\circ\text{C}$ .

**Viable count determinations.** Thin sucrose and xylose pastes, with and without peroxide, were used alone and also diluted with an appropriate amount of human serum to give 50 and 75% paste in serum. The final volume of paste and serum was 50 ml, and nutrient broth was used as the control. All were kept in stoppered conical flasks which were wrapped to exclude light. A 4-h broth culture of either *S. aureus* or *P. mirabilis* was diluted 10-fold with peptone water, and 0.5 ml of the resultant cell suspension was added to the paste-serum mixtures and control flasks. All flasks were incubated at 37°C for 130 min. Samples were taken at approximately 30-min intervals and serially diluted with peptone water for estimation of viable numbers. CLED agar was used for culturing *P. mirabilis*, and nutrient agar was used for culturing *S. aureus*. All plates were incubated at 37°C for 24 h, and the colonies were counted. A similar experiment was conducted with PEG 400 and *S. aureus* as a control without sugar and without hydrogen peroxide.

## RESULTS

The pastes are chemically stable for at least 6 months with little loss of hydrogen peroxide if stored in screw-cap containers at 2 to 8°C. Sucrose in the thin paste does settle out after a few weeks of storage, but the paste can be readily reconstituted by stirring with a spatula before use.

The effects on  $a_w$  of adding human serum to thin sucrose and xylose pastes are shown in Fig. 1. This plot indicates that an  $a_w$  of 0.86, the lowest  $a_w$  at which *S. aureus* can proliferate (3), is achieved with a mixture containing approximately equal parts of paste and serum.

Tables 2 and 3 give the results of challenging thin and thick

TABLE 2. Microbial challenge tests of thick and thin sucrose pastes with and without hydrogen peroxide

Sucrose pastes and organism	Log <sub>10</sub> bacteria/ml of sugar paste at following time of sampling (h):						
	Initial	0	6	24	48	168	672
<b>Thick</b>							
<b>Without peroxide</b>							
<i>S. aureus</i>	1 × 10 <sup>8</sup>	3 × 10 <sup>7</sup>	2 × 10 <sup>5</sup>	7 × 10 <sup>1</sup>	3 × 10 <sup>3</sup>	<10	<10
<i>S. faecalis</i>	1 × 10 <sup>9</sup>	2.3 × 10 <sup>7</sup>	7.3 × 10 <sup>4</sup>	7 × 10 <sup>3</sup>	8 × 10 <sup>1</sup>	2 × 10 <sup>1</sup>	<10
<i>E. coli</i>	1 × 10 <sup>8</sup>	7 × 10 <sup>4</sup>	<10	<10	<10	<10	<10
<i>C. albicans</i>	3 × 10 <sup>8</sup>	3.5 × 10 <sup>6</sup>	<10	4 × 10 <sup>1</sup>	<10	<10	<10
<b>With peroxide</b>							
<i>S. aureus</i>	1 × 10 <sup>8</sup>	>10 <sup>8</sup>	<10	<10	<10	<10	<10
<i>S. faecalis</i>	1 × 10 <sup>9</sup>	1.3 × 10 <sup>6</sup>	<10	<10	<10	<10	<10
<i>E. coli</i>	1 × 10 <sup>8</sup>	<10 <sup>3</sup>	<10	<10	<10	<10	<10
<i>C. albicans</i>	3 × 10 <sup>8</sup>	3 × 10 <sup>5</sup>	<10	<10	<10	<10	<10
<b>Thin</b>							
<b>Without peroxide</b>							
<i>S. aureus</i>	5.2 × 10 <sup>8</sup>	ND <sup>a</sup>	1.2 × 10 <sup>5</sup>	9.6 × 10 <sup>3</sup>	5.8 × 10 <sup>2</sup>	<10	<10
<i>S. faecalis</i>	4.4 × 10 <sup>10</sup>	ND	1.5 × 10 <sup>4</sup>	7.8 × 10 <sup>4</sup>	1.0 × 10 <sup>4</sup>	4.3 × 10 <sup>2</sup>	<10
<i>E. coli</i>	5.5 × 10 <sup>8</sup>	ND	<10	<10	<10	<10	<10
<i>C. albicans</i>	5.5 × 10 <sup>6</sup>	ND	1.3 × 10 <sup>3</sup>	<10	<10	<10	<10
<b>With peroxide</b>							
<i>S. aureus</i>	1 × 10 <sup>8</sup>	1 × 10 <sup>7</sup>	<10	<10	<10	<10	<10
<i>S. faecalis</i>	1 × 10 <sup>9</sup>	6.6 × 10 <sup>7</sup>	<10	<10	<10	<10	<10
<i>E. coli</i>	1 × 10 <sup>8</sup>	<10 <sup>3</sup>	<10	<10	<10	<10	<10
<i>C. albicans</i>	3 × 10 <sup>8</sup>	<10 <sup>3</sup>	<10	<10	<10	<10	<10

<sup>a</sup> ND, not determined.

sucrose and xylose pastes, with and without peroxide, with known numbers of microorganisms. In pastes with peroxide, the numbers of viable organisms declined rapidly, and none were detected 6 h after the initial inoculation. The pastes without peroxide exerted a less rapid bactericidal effect, and *S. faecalis* persisted for up to 168 h.

The effects of differing amounts of sucrose paste in blood agar on the growth of various microorganisms are shown in Table 4. These results demonstrate that *S. equisimilis* was most easily inhibited, whereas *S. aureus* was the most resistant.

Figures 2 and 3 show the effects of thin sucrose and xylose pastes, with and without hydrogen peroxide and various amounts of serum, on the viability of *S. aureus*. Viable

numbers declined most rapidly in the flasks containing paste with hydrogen peroxide, while serum alone was bacteriostatic. Figures 4 and 5 show the effects of thin sucrose and xylose pastes, with and without hydrogen peroxide, mixed with various amounts of serum, on the viability of *P. mirabilis*. The bactericidal effect of the pastes was more rapid than that observed against *S. aureus*. It appeared that the hydrogen peroxide made little contribution to the overall bactericidal effect.

Figure 6 shows the effects of PEG 400, without hydrogen peroxide, on the viability of *S. aureus*. It demonstrates its bactericidal property, which occurs because it has an  $a_w$  of 0. These results are similar to those of Chirife et al. (2) and are equivalent to those obtained with the sucrose and xylose pastes which contained only 32% PEG 400.

TABLE 3. Microbial challenge tests of thin xylose paste with and without peroxide

Xylose paste and organism	Log <sub>10</sub> bacteria/ml of sugar paste at following time of sampling <sup>a</sup> (h):		
	Initial	0	6
<b>Without peroxide</b>			
<i>S. aureus</i>	5.2 × 10 <sup>8</sup>	ND <sup>b</sup>	<10
<i>S. faecalis</i>	4.4 × 10 <sup>10</sup>	ND	1.5 × 10 <sup>4</sup>
<i>E. coli</i>	5.6 × 10 <sup>8</sup>	ND	<10
<i>C. albicans</i>	5.5 × 10 <sup>6</sup>	ND	9.0 × 10 <sup>2</sup>
<b>With peroxide</b>			
<i>S. aureus</i>	5.2 × 10 <sup>8</sup>	ND	<10
<i>S. faecalis</i>	4.4 × 10 <sup>10</sup>	ND	<10
<i>E. coli</i>	5.6 × 10 <sup>8</sup>	ND	<10
<i>C. albicans</i>	5.5 × 10 <sup>6</sup>	ND	<10

<sup>a</sup> Log<sub>10</sub> bacteria at 24, 48, 168, and 672 h in all cases was <10.

<sup>b</sup> ND, not determined.

## DISCUSSION

Chirife et al. (3) proposed that an important function of sucrose in a wound was to create an environment of low  $a_w$

TABLE 4. Effects of various amounts of hydrogen peroxide-free thin sucrose paste in blood agar on the growth of microorganisms

Organism	Bacterial growth on blood agar plates containing the following % of sucrose paste:				
	10	20	30	40	50
<i>S. aureus</i>	+	+	+	-	-
<i>S. faecalis</i>	+	+	-	-	-
<i>E. coli</i>	+	+	-	-	-
<i>S. equisimilis</i>	+	-	-	-	-
<i>S. epidermidis</i>	+	+	-	-	-
<i>Klebsiella</i> sp.	+	+	-	-	-

+, growth present; -, growth absent.

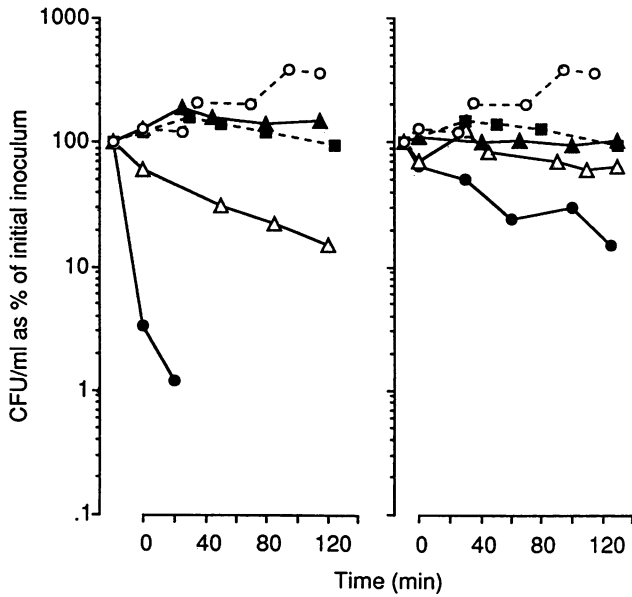


FIG. 2. Effects of sucrose pastes (with [left] and without [right] hydrogen peroxide) diluted with serum on the colony-forming ability of *S. aureus*. ○, broth control; ■, serum control; ▲, 50% paste with serum; △, 75% paste with serum; ●, 100% paste.

which inhibited and stressed bacterial growth. The formulation of sucrose paste and xylose paste had considerable effects in reducing  $a_w$ , which suggested that these pastes would have antibacterial activity. This premise was confirmed when it was shown that sucrose paste in blood agar had a good antibacterial effect. It was noted, however, that *S. aureus* was the least inhibited, a result in keeping with the findings of Chirife et al. (3), who demonstrated that a high concentration of sucrose (183 g/100 ml of water) was necessary to inhibit the growth of staphylococci.

The results of challenging the pastes with a variety of

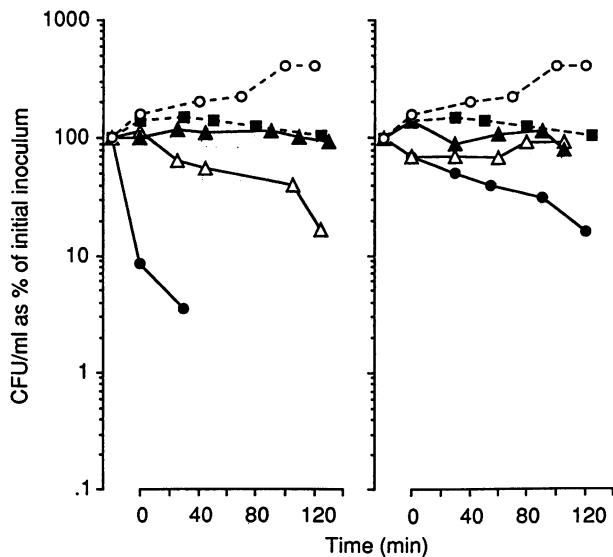


FIG. 3. Effects of xylose paste (with [left] and without [right] hydrogen peroxide) diluted with serum on the colony-forming ability of *S. aureus*. ○, broth control; ■, serum control; ▲, 50% paste with serum; △, 75% paste with serum; ●, 100% paste.

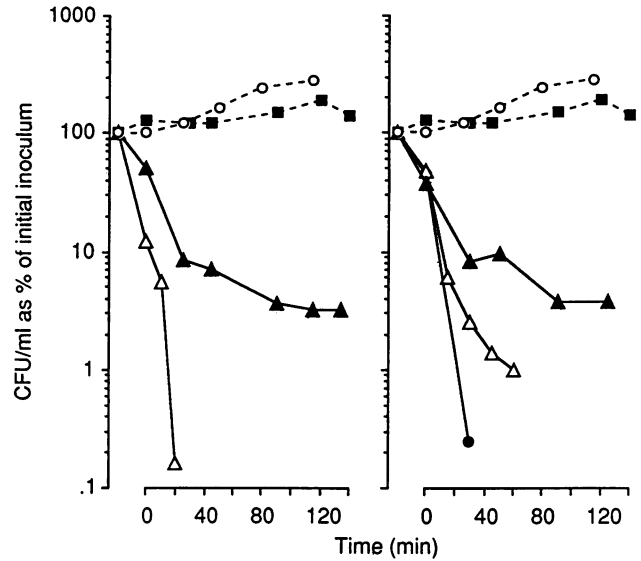


FIG. 4. Effects of sucrose paste (with [left] and without [right] hydrogen peroxide) diluted with serum on the colony-forming ability of *P. mirabilis*. ○, broth control; ■, serum control; ▲, 50% paste with serum; △, 75% paste with serum; ●, 100% paste.

microorganisms indicate that all the pastes have good antibacterial activity, which is enhanced by the addition of 0.15% (vol/wt) hydrogen peroxide.

The viability studies were performed on *S. aureus* and *P. mirabilis* because they are commonly found in infected wounds. In addition, *S. aureus* was demonstrated to grow in 30% sucrose in blood agar and thus presented the most severe challenge to the antimicrobial efficacy of the pastes.

The results indicated that *P. mirabilis* was very susceptible to the antibacterial activity of the pastes, which continued when they were diluted by 50%. The additional bacte-

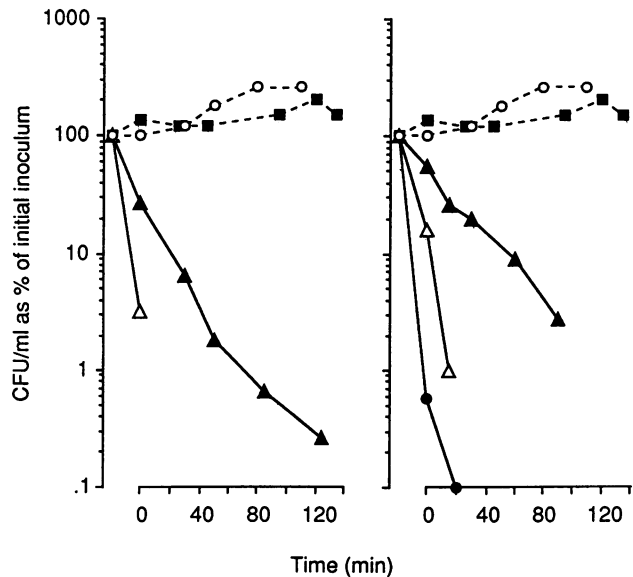


FIG. 5. Effects of xylose paste (with [left] and without [right] hydrogen peroxide) diluted with serum on the colony-forming ability of *P. mirabilis*. ○, broth control; ■, serum control; ▲, 50% paste with serum; △, 75% paste with serum; ●, 100% paste.

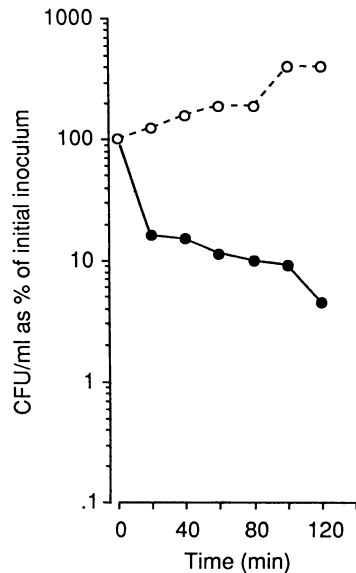


FIG. 6. Effects of PEG 400 on the colony-forming ability of *S. aureus*. ○, broth control; ●, PEG 400.

ricidal effect of peroxide may have been partly eliminated by the addition of serum, because of the well-described inactivation of hydrogen peroxide that occurs by organic matter.

These data also indicate that PEG 400 has good antimicrobial activity, but this compound could not be used alone as a wound treatment since it can be absorbed from a wound and may be nephrotoxic (9). Our thick sucrose paste (18.5% PEG 400) and thin sucrose paste (32% PEG 400) have been used often in many elderly and frail patients over the past 8 years with no detectable adverse side effects, but we do advocate caution in its use in patients with renal failure.

Clinically we usually use sucrose pastes, primarily because sucrose is less expensive than xylose. Treatment regimens need to reflect our laboratory data and require frequent application of paste. When sucrose paste is added to a wound, it invariably dissolves in fluid drawn into its cavity, creating an environment of low  $a_w$  which inhibits bacterial growth. As this dilution occurs within 3 to 4 h of addition of paste to the wound, repacking at least twice daily has been recommended. Although  $a_w$  may not be maintained at less than 0.86 for more than 3 h after packing, clinical experience with sucrose paste has shown that twice-daily dressing is adequate to remove infected (and necrotic) slough from dirty wounds within a few days and to deodorize malodorous wounds.

The findings of this study indicate that sucrose and xylose pastes have good antimicrobial activities. In a controlled trial in a pig model, full thickness wounds (9 mm deep) were packed with thick sucrose paste or gauze soaked in various antiseptic solutions (1). The wounds were then packed and kept moist by covering with a semipermeable self-adhesive plastic film dressing (Opsite). The clinical and histological results indicated that there was no difference between wounds left unpacked but covered with Opsite and those packed with sucrose paste and covered with Opsite, whereas all the wounds packed with gauze and an antiseptic showed evidence of delayed healing. This demonstrated that sugar paste neither inhibited or stimulated the normal cellular function of the wound healing process (1). Our data indicates that sucrose paste may be of benefit for infected and malodorous wounds which traditionally are treated with antiseptics that can be toxic to granulation tissue.

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