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In Vitro Study of Bacterial Growth Inhibition in Concentrated Sugar Solutions: Microbiological Basis for the Use of Sugar in Treating Infected Wounds

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The use of sugar for the treatment of infected wounds was investigated in in vitro experiments with bacteria pathogenic to humans, such as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Staphylococcus aureus*. Studies showed that solutions of appropriate sugar concentration incubated at pH 7.0 and 35°C were lethal to the bacterial species studied. On the basis of these results, it is proposed that an important function of sugar in the treatment of infected wounds is to create an environment of low water activity (a_w), which inhibits or stresses bacterial growth.

Various naturally occurring substances have been used in wound treatment throughout history. Among them, sugar (sucrose), honey (main constituents are glucose, fructose, and maltose), and molasses (main constituents are sucrose and glucose) are the most common. However, it is still uncertain how sugar acts on wounds, as recently reviewed by Forrest (9, 10).

In 1976, Herszage and Montenegro of Buenos Aires began treating wounds with ordinary sugar because of the complicated evolution (despite conventional therapy) and critical condition of two in-patients with postsurgical necrotic cellulitis. Sugar was used simply on the basis of existing folk therapy. In view of the success obtained, they began using sugar systematically for the treatment of infected wounds. Ordinary granulated sugar (purchased in the supermarket) was used since it was found not to contain any foreign substance with antibacterial properties; the sugar was not mixed with any antiseptic or any other substance with proven or supposed antibacterial action; and antibiotics were not used concurrently. The procedure consisted of (i) wide opening of the wound; (ii) drying of tissues with gauze; and (iii) filling the wound with as much sugar as possible, taking care to fill every cavity, and adding more sugar periodically. Herszage et al. (12) reported 120 cases with infected wounds and other superficial lesions which were treated with sugar with a cure rate of 99.2%. The time for cure varied between 9 days and 17 weeks; however, it was usual for odor and secretion to diminish within 24 h and to disappear after 72 to 96 h of treatment. This report, however, was only a short summary of their findings and did not include hundreds of photographs showing the evolution of cases treated. The patients varied between 3 months and 94 years in age and included 50 females and 70 males, of whom 6 were diabetics.

In most cases treated with sugar, it was observed that wounds were healed without debridement of necrotic tissues or any other surgical procedure except for a complete opening of the wound. After 5 or 7 days, it was possible to remove the necrotic tissue with forceps as if it were a piece of dressing; this process occurred even in wounds contaminated with fecal material. Diabetic patients showed the same response as others, even in the presence of hyperglycemia. Glossy protecting covering formed in the wounds, and the resulting cicatrices were unusually resistant. The pH values of all wounds treated with sugar ranged from 6.8 to 7.4. Photographs showing the evolution of wound healing in a representative case treated with sugar are shown in Fig. 1 and 2. For the sake of brevity, only one case is shown here. The work of Herszage et al. (12) was not a controlled study since the dramatic improvement resulting from this treatment initially prevented them, for ethical reasons, from undertaking a randomized controlled study. In spite of this, the accumulated evidence strongly suggests that sugar played a role in the treatment of infected wounds.

We are proposing that an important function of sugar in the treatment of infected wounds is to create an environment of low water activity (a_w) which inhibits or stresses bacterial growth. A low a_w also means high osmotic pressure (π) since both are thermodynamically related ac-

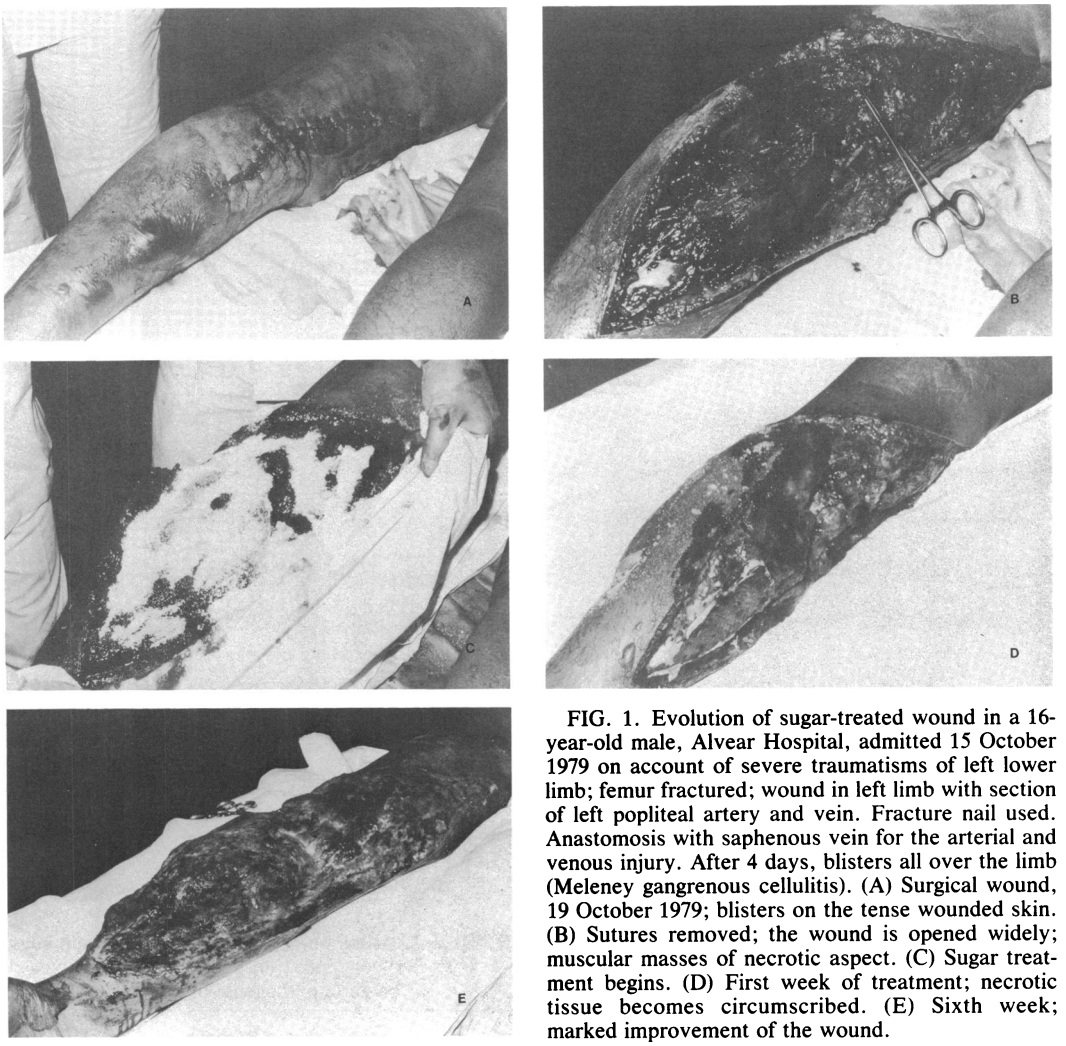

FIG. 1. Evolution of sugar-treated wound in a 16-year-old male, Alvear Hospital, admitted 15 October 1979 on account of severe traumatisms of left lower limb; femur fractured; wound in left limb with section of left popliteal artery and vein. Fracture nail used. Anastomosis with saphenous vein for the arterial and venous injury. After 4 days, blisters all over the limb (Meleney gangrenous cellulitis). (A) Surgical wound, 19 October 1979; blisters on the tense wounded skin. (B) Sutures removed; the wound is opened widely; muscular masses of necrotic aspect. (C) Sugar treatment begins. (D) First week of treatment; necrotic tissue becomes circumscribed. (E) Sixth week; marked improvement of the wound.

cording to the equation $\pi = (RT/\bar{V}\} \times \log (1/a_w)$, where $\bar{V}$ is the partial molal volume of water. In this way, a solution of low a_w has high osmotic pressure.

Bacteria, like all other forms of life, require water for growth, and these water requirements are best defined in terms of water activity (a_w) of the substrate (6, 18). When the aqueous solutions in the environment of the microorganism are concentrated by the addition of a solute such as sugar (sucrose), the consequences for microbial growth result mainly (although not only) from the change in a_w. At present, numerous data are available on the relationship between a_w and the ability of microorganisms to grow, and it has been reported that every microorganism has a limiting a_w below which it will not grow (5). Table 1 shows the limiting a_w values (and calculated equivalent sugar concentrations) for various bacteria pathogenic to humans, such as *Klebsiella* spp., *Salmonella* spp., *Pseudomonas* spp., *Escherichia coli, Clostridium perfringens*, and *Staphylococcus aureus*. Of the whole range of bacteria that infect human skin, subcutaneous tissues, and mucous membranes, the lowest a_w is tolerated by *S. aureus*, which can proliferate with an a_w as low as 0.86. We have recently reported some preliminary in vitro experiments on inhibiting *S. aureus* ATCC 6538P in sucrose solutions (3). *S. aureus* was chosen as a test organism since, as mentioned, it is the pathogen most resistant to low a_w.

This study has two objectives: (i) to extend the studies of bacterial growth inhibition in concentrated sucrose solutions to other pathogenic bacteria relevant to infected wounds and

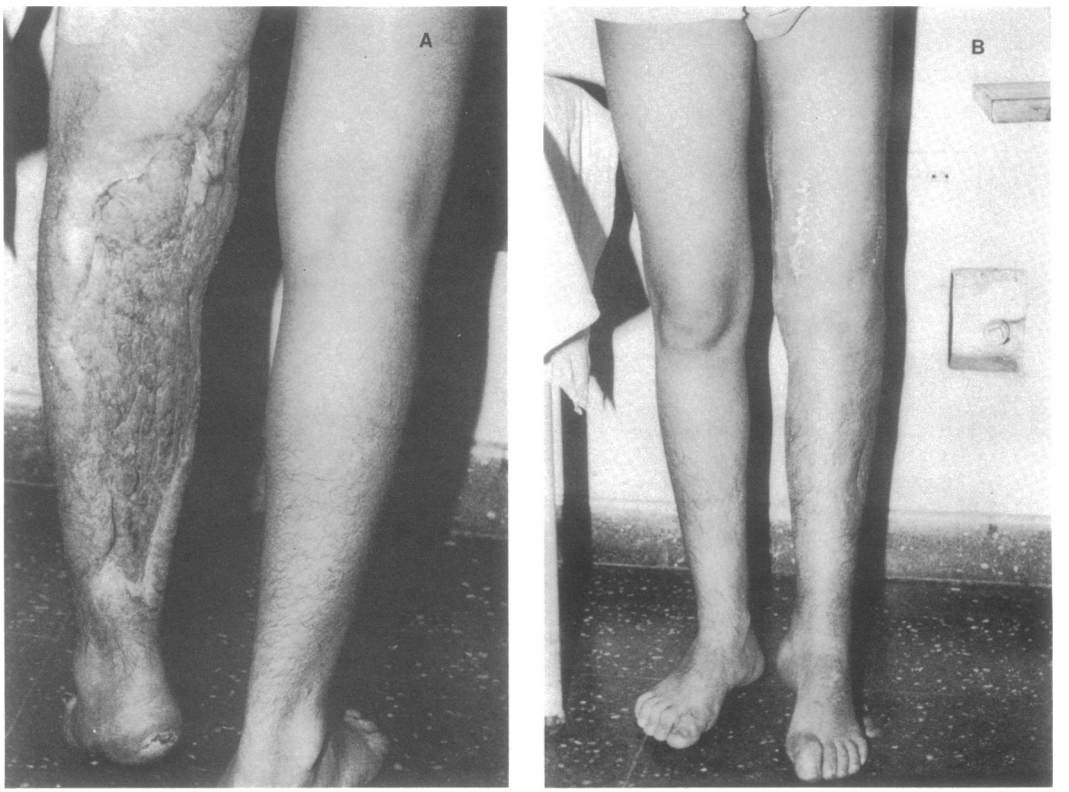

FIG. 2. Lower limb of same patient 6 months after the accident. (A) Posterior view. (B) Frontal view.

to other strains of *S. aureus*; and (ii) to present a theory of the mode of action of sugar in the treatment of infected wounds.

MATERIALS AND METHODS

Microorganisms. The strain numbers and sources of the bacteria used in this study are: *S. aureus* ATCC 6538P, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 10031, and *E. coli* ATCC 25922, American Type Culture Collection, Rockville, Md.; *S. aureus* NA and FM1, culture collection of the Instituto Nacional de Farmacología y Nutrición, Buenos Aires, Argentina; *S. aureus* 41/82 (a wild-type strain), Hospital Muñiz, Buenos Aires, Argentina; *P. aeruginosa* 15/4, *K. pneumoniae* 6440, and *E. coli* 11197 (wild-type strains), culture collection of the Instituto Nacional de Microbiología Dr. Carlos G. Malbrán, Buenos Aires, Argentina.

Media. For all bacterial strains, brain heart infusion (BHI) broth (Oxoid Ltd., London, England) was used for growth inhibition studies. Sugar (sucrose) was added to BHI before sterilization to adjust the a_w to the desired value. After the sucrose was dissolved, the pH was adjusted to 7.0 by the addition of 5 N NaOH. The different media were autoclaved with precautions to avoid the loss of water by evaporation, which could change the sugar concentration (and hence the a_w).

Sugar (sucrose). Refined granulated cane sugar was

TABLE 1. Minimum a_w values and equivalent sugar (sucrose) concentrations for growth of various bacteria pathogenic to humans

Bacterium	Minimum a_w	Equivalent sugar concn (g/100 g of water)
Klebsiella spp.	0.96[a]	66.7
	0.94[b]	92.3
Salmonella oranienburg	0.96–0.94[c]	66.7–92.3
Pseudomonas spp.	0.95[a]	78.6
	0.97[d]	51.5
Clostridium perfringens	0.97[e]	51.5
Escherichia coli	0.95[a]	78.6
Staphylococcus aureus	0.864[f]	185.7
	0.88[g]	167.4

[a] Leistner et al. (14).
[b] Measures (16); *Klebsiella aerogenes*.
[c] Christian (4); in sucrose solutions.
[d] Measures (16); *P. aeruginosa*.
[e] Christian (5); in sucrose solutions.
[f] Chirife et al. (3); in sucrose solutions.
[g] Scott (18); in sucrose solutions.

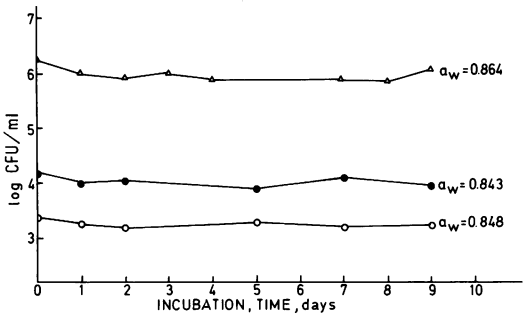

FIG. 3. Inhibition of growth of *S. aureus* ATCC 6538P (△), FM1 (●), and NA (○) in BHI with added sugar at pH 7.0, incubated at 35°C.

purchased in 1-kg packages from a local supermarket and used as it was.

Growth inhibition studies. Growth studies were made in 250-ml screw-top glass bottles containing about 18 g each of inoculated sugar-supplemented media, incubated at 35°C in a constant temperature cabinet.

Enumeration procedure. Counts were determined by the use of plate count agar (Difco Laboratories, Detroit, Mich.) for *S. aureus*, *K. pneumoniae*, and *E. coli*; CLED medium with Andrade indicator (Oxoid Ltd.) was used for *P. aeruginosa*. The samples were serially diluted with 0.1% peptone (Oxoid Ltd.) before plating. The plates were incubated at 35°C for 24 to 48 h, and the colonies were counted.

Determination of a_w. The a_w of sugar-supplemented media and the a_w in sugar-treated wound cavities were determined at 25.0 ± 0.1°C with an electronic hygrometer Humicap HMI 14 A manufactured by Vaisala, Helsinki, Finland, as described by Favetto et al. (G. Favetto, S. Resnik, J. Chirife, and C. Ferro Fontán, J. Food Sci., in press).

RESULTS

The relationship between a_w and sucrose concentration may be obtained from the equation $a_w = X_1 \exp(-6.47\ X_2^2)$, where X_1 and X_2 are molar fractions of water and sucrose, respectively, defined as X_1 = (moles of water)/(moles of water + moles of sucrose); $1 = X_1 + X_2$.

Figure 3 shows the behavior of *S. aureus* ATCC 6538P, NA, and FM1 in BHI media with the a_w adjusted with sugar to the range of 0.84 to 0.86, incubated at 35°C; initial inoculum levels were in the range of 10^3 to 10^6 CFU/ml. As expected, sugar produced complete growth inhibition of all three strains. Control experiments (BHI without sugar) were also performed (data not shown) and showed that the three *S. aureus* strains reached 10^9 (or above) CFU/ml after 24 to 36 h of incubation.

Figure 4 shows the inhibitory effect of a sugar-saturated broth (a_w, 0.828) on *S. aureus* at high initial inoculum levels (~10^8 CFU/ml). The number of CFU of strains ATCC 6538P and 41/82 per milliliter declined throughout the incubation period to the point of almost complete loss of viability by the end of the period studied (60 to 70 days).

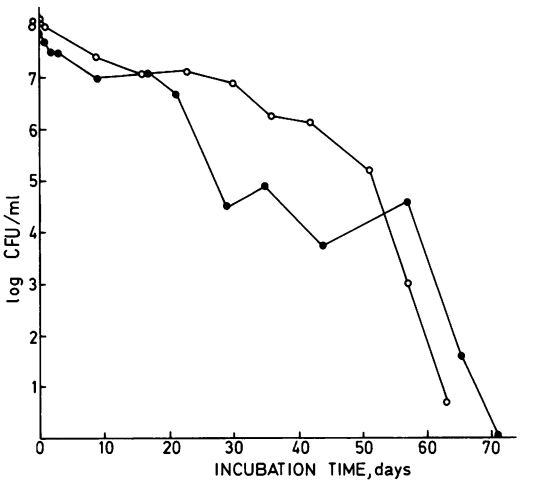

FIG. 4. Growth inhibition and survival of *S. aureus* 41/82 (●), and ATCC 6538P (○) in BHI saturated with sugar at pH 7.0, incubated at 35°C.

Figure 5 shows the results of experiments of growth inhibition and survival of *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 10031, and *S. aureus* ATCC 6538P in sugar-saturated broth (a_w, 0.828) incubated at 35°C, pH 7.0. Control experiments in broth without sugar were also performed (data not shown). It is noteworthy that viable cells of *P. aeruginosa*, *E. coli*, and *K. pneumoniae* declined rapidly, leading to almost total loss of viable population in 2 to 3 days of incubation. *S. aureus*, however, was far more resistant (although its viable population also declined continuously).

Figures 6 and 7 show similar growth inhibition

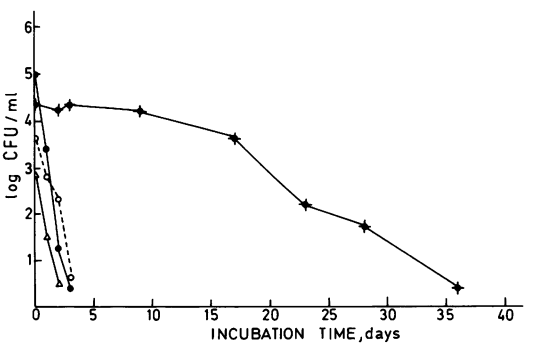

FIG. 5. Growth inhibition and survival of *E. coli* ATCC 25922 (●), *P. aeruginosa* ATCC 27853 (○), *K. pneumoniae* ATCC 10031 (△), and *S. aureus* ATCC 6538P (◆) in BHI saturated with sugar at pH 7.0, incubated at 35°C.

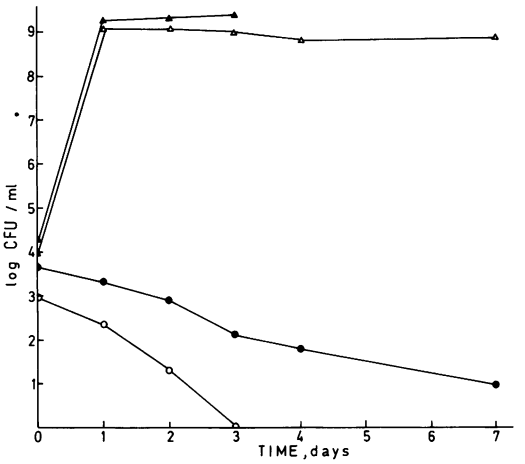

FIG. 6. Growth inhibition and survival of *K. pneumoniae* 6440 with sugar (●) and control (△) and *P. aeruginosa* 15/4 with sugar (○) and control (▲) in BHI at pH 7.0, incubated at 35°C.

and survival curves for other strains, *E. coli* 11197, *K. pneumoniae* 6440, and *P. aeruginosa* 15/4, in sugar-saturated broth (a_w, 0.828) incubated at 35°C, pH 7.0.

DISCUSSION

The explanation of the role of sugar in the treatment of infected wounds is complex and perhaps impossible to reduce to a single mechanism, such as its antibacterial action. Nevertheless, we are proposing here that an important function of sugar is to create an environment of low a_w which inhibits or stresses bacterial growth. Infected wounds in most normal persons heal by drainage and debridement; however, it has to be stressed that an infection is the result of the interrelationship between three factors: the medium, the bacteria, and the defenses of the host (15). Therefore, it would be reasonable to assume that in patients with lowered defense mechanisms, sugar may play a role in the control of infection by diminishing bacterial virulence.

Herszage et al. (12) followed the evolution of bacterial flora present in various infected wounds being treated with sugar. Bacteria initially present included, among others, *Steptococcus* spp., *Klebsiella* spp., *E. coli, C. perfringens*, and *S. aureus*, of which all but *S. aureus* disappeared during the first days of sugar treatment. These in vivo results have a striking similarity to our in vitro results (Fig. 5) and suggest that an a_w-based explanation of the sugar action is likely.

The a_w of a bacterial culture may also be reduced by exposing the medium to atmospheres of appropriate relative humidities; at equilibrium, the a_w of the medium is equal to the relative humidity divided by 100. Turner and Salmonsen (19) exposed very small volumes of cultures of three different serotypes of *Klebsiella* spp. to a relative humidity of 85% at 25°C and found that no bacteria survived after 24 h. This finding is in good agreement with our results in sucrose solution (Fig. 5) since in the experiments of Turner and Salmonsen (19), the a_w of the growth medium should have been close to 0.85, which resulted in the rapid death of *Klebsiella* spp.

In a recent preliminary report (3) on the effect of sucrose on the in vitro growth of *S. aureus*, we showed that the sucrose concentration needed to achieve complete growth inhibition of this microorganism was 183 g/100 g water. With this experiment, we tried to illustrate the convenience of maintaining a high sugar concentration, which can be obtained by initially filling the wound with as much sugar as possible (see Fig. 1C) and then adding periodically more sugar. We recommended this procedure bearing in mind the uptake of tissue water, which dilutes the sugar placed in the wound and which results in a low sugar concentration that may aid rather than inhibit bacterial growth. It is noteworthy, however, that the sucrose concentration needed to achieve growth inhibition of most human pathogens other than *S. aureus* is much lower than 183 g/100 g water (see Table 1). For instance, postoperative wounds are frequently contaminated with *E. coli* or *Pseudomonas* strains which may be inhibited by sugar concentrations as low as 52 and 79 g, respectively per 100 g of water.

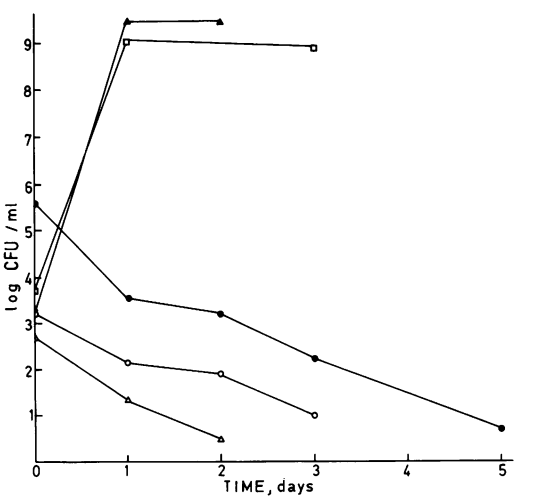

FIG. 7. Growth inhibition and survival of *E. coli* ATCC 25922 with sugar (△) and control (▲) and *E. coli* 11197 with sugar (●, ○) and control (□) in BHI at pH 7.0, incubated at 35°C.

The actual sugar concentration (or a_w) in a wound cavity is not constant but is a function of the frequency of the addition of fresh sugar and the amount of liquid released by the tissues, which is time dependent. At the beginning of every treatment, the sugar concentration should be very high, approaching saturation, which for body temperature is about 225 g/100 g water and corresponds to a_w about 0.83. Then, water activity is progressively raised owing to the uptake of water from the surrounding tissues until more sugar is again placed in the wound and the a_w drops to a very low value. In this way, bacteria in the wound cavity are subjected to a series of osmotic shocks owing to the continuous change of a_w of the medium; this implies that bacteria should be adapted to the differences in a_w inside and outside the protoplasm. It is known (1) that abrupt changes in the water activity of bacterial cultures cause injury and death of the cells, even without going beyond the conditions which are suitable for growth. Bayer (1) showed that 90% of logarithmically growing cells of *E. coli* survived exposure for 10 min to 50% sucrose followed by a slow dilution. However, when the cultures were subjected to an osmotic shock produced by a sudden reduction of the a_w of the medium, the viability of the cells decreased dramatically. Thus, although conditions in the sugar-treated wound are such that the limiting a_w for growth is temporarily surpassed, bacterial cells will be stressed, and it is reasonable to assume that under this condition it would be easier for the immune system to play its role.

We have shown that the limiting a_w with sucrose for in vitro growth of *S. aureus* at 35°C and pH 7.0 is around 0.864. At higher a_w values growth occurs, but the initiation of growth is delayed (lag period). Scott (18) reported that the lag period for *S. aureus* growth in various static liquid media at 30°C was 1 to 3 days at an a_w of 0.90. We have found that lag periods of *S. aureus* at 35°C in sucrose-supplemented liquid media were 1 to 2 days at an a_w of around 0.89 (3). These lag periods may be important in bacterial inhibition in sugar-treated wounds. The growth rate of bacteria is also very much affected by reduction in a_w. Scott (18) showed that the growth rate of various strains of *S. aureus* could be strongly diminished by decreasing the a_w from its optimal value for growth (0.993). At a_w 0.90 and 0.94 (both values well above the limiting a_w), the growth rates were only 12.3 and 51.5%, respectively, of the maximum. Christian (4) has shown that the limiting a_w for growth of *Salmonella oranienberg* at 30°C in sucrose-added medium was 0.96, which corresponded to about 67 g of sucrose per 100 g of water. However, a sucrose concentration of only 35 g/100 g of water was enough to reduce the growth rate to 59% of its maximum value.

It is well known that cells of *E. coli* and *Pseudomonas* spp. suspended in a hypertonic (low a_w) solution of a nonpenetrant solute (such as sucrose) plasmolyze rapidly as they adjust thermodynamically by losing water (13). Deplasmolysis is then essential for resumption of growth in a medium of lowered a_w (7, 8). Deplasmolysis consists of the rehydration of the cell, which occurs because of the entry of osmotically active solutes to balance intracellular and extracellular a_w. This process, however, is slow in the presence of sucrose. Kroll and Anagnostopoulos (13) have shown that deplasmolysis of *P. aeruginosa* in a sucrose solution having only 22.7 g of sucrose per 100 g water (a_w, 0.987) takes almost 3 h. The following experiment was performed on a 60-year-old male patient with a postsurgical abdominal wound who was receiving daily sugar treatment. The wound cavity was filled with about 265 g of ordinary granulated sugar and covered with gauze. After 2.5 h, the sugar was transformed into a syrup that still had undissolved sugar granules; a homogenized sample was taken, and the a_w was determined with an electronic hygrometer (as described above). The a_w was found to correspond to that of a saturated sucrose solution (a_w, 0.848 at 25°C). Additional samples were taken at 4 and 10 h (from the beginning of treatment), and the corresponding a_w values were 0.897 and 0.951, respectively. The last value is still low enough to inhibit or severely restrict growth of most human pathogens. The evolution of a_w in sugar-treated infected wounds is the subject of additional research.

All of the above facts may help to explain how sugar may reduce infection in a wound, even if there is a dilution owing to water uptake from the surroundings. It may be noted that this liquid flow contributes to cleaning the wound.

One may be concerned about the effect of the low water activity on the tissue cells with which the sugar is in contact. The internal water activity of the tissue cells ($a_{w\ cell}$) may be assumed to be >0.990 (11), whereas the water activity in the sugar side (or wound cavity) ($a_{w\ cav}$) is considerably lower; thus, a flux of water exists because of a water activity driving force. Thus, it may be argued that dehydration of the tissue surrounding the wound may eventually kill these cells. However, in living organisms it does not happen because a flux of water from the inner regions replaces water as fast as it is transferred from the surrounding cells to the wound cavity. In this way external tissue cells remain wet, and the healing process is not adversely affected by sugar (see Fig. 1 and 2). This does not occur, however, in a dead organism, in which sugar produces dehydration of the surrounding cells.

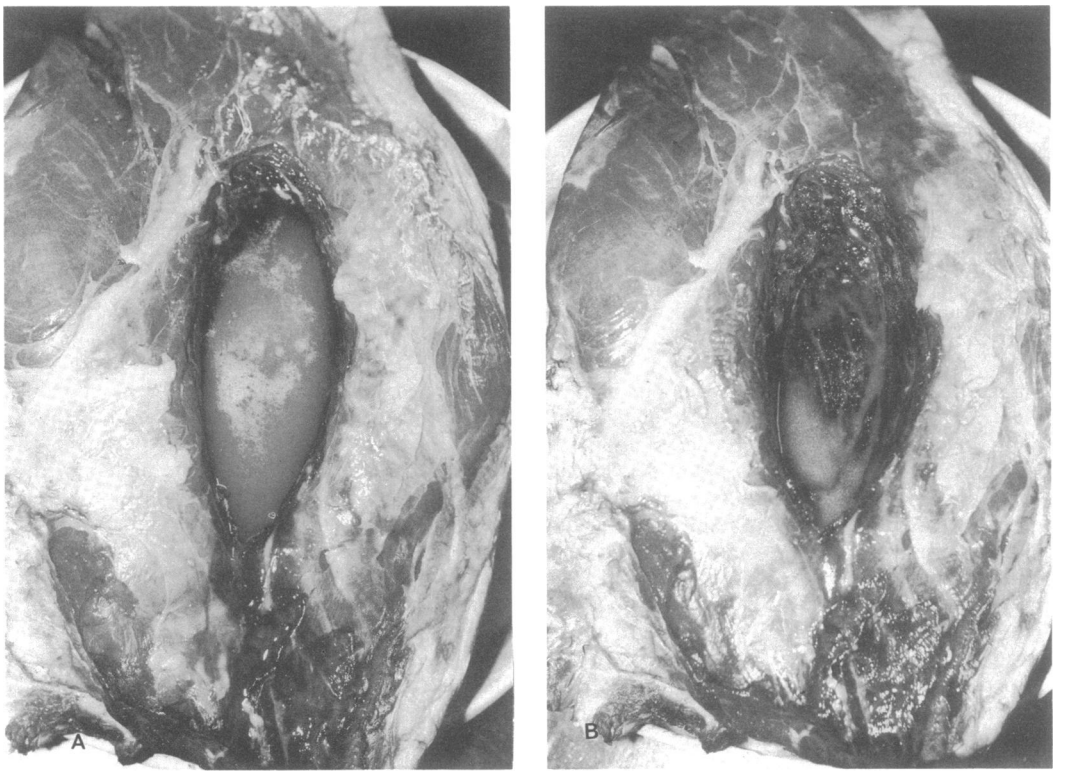

FIG. 8. Effect of saturated sugar solution on beef tissue after 24 h at room temperature. Dark areas caused by so-called osmotic dehydration may be observed in the tissue with which the sugar is in contact.

This was shown in the following experiment. A cavity was opened in the center of a large piece of beef (about 3 kg), filled with sugar, and allowed to remain for 24 h at room temperature. The sugar produced dehydration of the external tissue cells, as evidenced by the dark areas surrounding the cavity filled with sugar (Fig. 8).

Recently, Forrest (9, 10) reviewed the effective wound treatments that had been discovered by prehistoric and primitive peoples. He indicated that honey was among the agents most used by ancient civilizations for the treatment of wounds. Wounds were usually washed with water (or milk) and dressed with honey. Forrest (10) indicated that honey has antibacterial properties dependent on the production of hydrogen peroxide from glucose. However, it is also possible that honey may have acted on wounds by creating an environment of low water activity, which inhibits bacterial activity. Rüeg and Blanc (17) have measured the water activity of several commercially available natural liquid and crystallized honeys. They found that the mean a_w of liquid honeys was 0.562, and that of crystallized honeys was 0.589. These values are quite low and certainly would be inhibitory for bacterial growth.

We may conclude that it is likely that sugar reduces infection through lowering the a_w and that the experimental evidence indicates that sugar treatment does not negatively influence wound healing. There are, however, additional facts which must be studied, among them the effects of sugar on macrophages and the incidence on wound healing.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Bayer, M. E.** 1967. Response of cell walls of *Escherichia coli* to a sudden reduction of the environmental osmotic pressure. J. Bacteriol. **93**:1104–1112.
2. **Chirife, J., C. Ferro Fontán, and E. A. Benmergui.** 1980. The prediction of water activity in aqueous solutions in connection with intermediate moisture foods. IV. a_w Prediction in aqueous non-electrolyte solutions. J. Food Technol. **15**:59–70.
3. **Chirife, J., G. Scarmatto, and L. Herszage.** 1982. Scientific basis for the use of granulated sugar in the treatment of infected wounds. Lancet **i**:560.
4. **Christian, J. H. B.** 1955. The water relations of growth and respiration of *Salmonella oranienburg* at 30°C. Aust. J. Biol. Sci. **8**:490–497.
5. **Christian, J. H. B.** 1981. Specific solute effects on microbial water relations, p. 825–854. *In* L. B. Rockland and G. F. Stewart (ed.), Water activity: influences on food quality. Academic Press, Inc., New York.

6. **Christian, J. H. B., and J. A. Waltho.** 1962. The water relations of staphylococci and micrococci. J. Appl. Bact. **25**:369–377.
7. **Dhavises, G., and G. D. Anagnostopoulos.** 1979. Influence of amino acids and water activity on the growth of *Escherichia coli* B/r/1. Microbios Lett. **7**:105–115.
8. **Dhavises, G., and G. D. Anagnostopoulos.** 1979. Influence of amino acids and water activity on the growth of *Escherichia coli* B/r/1. Microbios Lett. **7**:149–159.
9. **Forrest, R. D.** 1982. Early history of wound treatment. J. R. Soc. Med. **75**:198–205.
10. **Forrest, R. D.** 1982. Development of wound therapy from the Dark Ages to the present. J. R. Soc. Med. **75**:268–273.
11. **Gimeno, A. L., and M. A. F. de Gimeno.** 1965. Nociones de fisiología celular. EUDEBA, Editorial Universitaria de Buenos Aires, Buenos Aires.
12. **Herszage, L., J. R. Montenegro, and A. L. Joseph.** 1980. Tratamiento de las heridas supuradas con azúcar granulado comercial. Bol. Trab. Soc. Argent. Cir. **41**:315–330.
13. **Kroll, R. G., and G. D. Anagnostopoulos.** 1981. Potassium fluxes on hyperosmotic shock and the effect of phenol and bronopol (2-bromo-2-nitropropan-1,3-diol) on deplasmolysis of *Pseudomonas aeruginosa*. J. Appl. Bacteriol. **51**:313–323.
14. **Leistner, L., W. Rodel, and K. Krispien.** 1981. Microbiology of meat and meat products in high and intermediate-moisture ranges, p. 855–916. *In* L. B. Rockland and G. F. Stewart (ed.), Water activity: influences on food quality. Academic Press, Inc., New York.
15. **Meakins, J. L.** 1976. Pathophysiologic determinants and prediction of sepsis. Surg. Clin. N. Am. **56**:847–857.
16. **Measures, J. C.** 1975. Role of amino acids in osmoregulation in non-halophilic bacteria. Nature (London) **257**:398–400.
17. **Rüeg, M., and B. Blanc.** 1981. The water activity of honey and related sugar solutions. Lebensm.-Wiss. Technol. **14**:1–6.
18. **Scott, W. J.** 1953. Water relations of *Staphylococcus aureus* at 30°C. Aust. J. Biol. Sci. **6**:549–564.
19. **Turner, A. G., and P. A. Salmonsen.** 1973. The effect of relative humidity on the survival of three serotypes of *Klebsiella*. J. Appl. Bacteriol. **36**:497–499.