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Effect of Sucrose on Collagen Metabolism in Keloid, Hypertrophic Scar, and Granulation Tissue Fibroblast Cultures

Jyrki Kössi, M.D.,[1] Marko Vähä-Kreula, M.D.,[2] Juha Peltonen, M.D.,[2,3] Juha Risteli, M.D.,[4] Matti Laato, M.D.[1]

[1]Department of Surgery, University of Turku, Kiinamyllynkatu 4-8, FIN-20520 Turku, Finland
[2]Department of Medical Biochemistry, University of Turku, Kiinamyllynkatu 4-8, FIN-20520 Turku, Finland
[3]Department of Anatomy, University of Oulu, Kajaanintie 50-52, FIN-90220 Oulu, Finland
[4]Collagen Research Unit, Oulu Technology Center, Kajaanintie 50-52, FIN-90220 Oulu, Finland

Abstract. Sucrose has been used to treat wounds with excellent results and with minimal abnormal scarring. In this study the effects of sucrose on collagen metabolism in fibroblast culture was evaluated. Sucrose (5.5, 15, or 25 mM) was added to granulation tissue, hypertrophic scar, and keloid fibroblast cultures. mRNA levels and procollagen aminopropeptides for type I and III collagens in cell culture medium were studied. Sucrose decreased mRNA levels for proα1(I) and proα1(III) collagens in fibroblast cultures derived from hypertrophic scar and keloid. In normal granulation tissue fibroblast cultures, 5.5 mM sucrose increased mRNA levels for proα1(I) and proα1(III) collagen, and higher concentrations decreased them. The synthesis of type I collagen decreased dose-dependently in all cell strains, whereas the synthesis of type III collagen decreased only in granulation tissue fibroblasts. To conclude, in vitro high concentrations of sucrose down-regulate both collagen gene expression and synthesis in normal granulation tissue fibroblasts, whereas in fibroblasts derived from abnormal scar sucrose down-regulates only type I collagen gene expression and synthesis, changing the pattern of collagen metabolism toward normal.

Surgeons not infrequently encounter poorly healing wounds and ulcers, which are characterized by infection and tissue necrosis. Sugars such as sucrose [1–4] and honey [5, 6] have been used to treat these difficult wounds with excellent results. Clinical observations have shown that sucrose alone or supplemented with antiseptics rapidly sterilizes and débrides wounds and ulcers, initiating granulation tissue formation [1–4]. The débriding effect of sucrose has been attributed to its osmolar activity. The antimicrobial effect of sucrose has been confirmed experimentally [7, 8], but the suggested direct positive effect of sucrose on granulation tissue formation has not been satisfactorily studied with appropriate cell lines.

Hypertrophic scars and keloids are well known distinct forms of aberrant wound healing. Clinical studies have suggested that honey or sucrose may lower the incidence of hypertrophic scars and keloids [1, 9]. However, we could not find a single experimental study in the literature focusing on this matter. In this work the effects of sucrose on collagen metabolism in fibroblast cultures derived from human granulation tissue, hypertrophic scar, and keloid were studied.

Correspondence to: M. Laato, M.D., e-mail: matti.laato@tyks.fi

Materials and Methods

Cell Culture Studies

Fibroblast cultures were established from human granulation tissue, a keloid, and a postburn hypertrophic scar. The diagnosis of a fibrotic wound was confirmed clinically and histologically. Tissue samples taken from each wound were carefully dissected free from adjacent tissue, and small pieces were explanted on plastic tissue culture flasks (Nunc, Roskilde, Denmark). After a 2- to 3-week incubation, when fibroblasts originating from tissue pieces had reached visual confluence, cells were detached with trypsin and divided 1:3. Thereafter cells were maintained in Dulbecco's modification of Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) and further divided until passage three to seven was reached. Cells were then again divided into cell culture dishes (Nunc, Roskilde, Denmark) and grown in DMEM containing 5.5 mM D-glucose and supplemented with 10% FCS until they reached confluence. Cell cultures were then maintained in the same medium as above; but test cultures of granulation tissue fibroblasts, hypertrophic scar fibroblasts, and keloid fibroblasts were supplemented with 5.5, 15, or 25 mM sucrose (Sucros Oyj, Kantvik, Finland). The cultures were prepared in triplicate and incubated for 7 days in the presence of ascorbic acid 50 μg/ml (Merck, Darmstadt, Germany). During the experiments, the cell culture medium was changed daily. At day 7, when the experiments were terminated, the cell culture medium was collected from every dish, centrifuged and stored at −70°C for later analyses.

Isolation of RNA

Isolation of total cellular RNA from fibroblasts was performed as described by Chirgwin et al. [10]. The cells were lysed in 3 ml of 4 M guanidinium thiocyanate, 25 mM sodium acetate, and 0.14 M β-merceptoethanol. The lysates were layered onto a CsCl cushion (5.7 M CsCl, 25 mM sodium acetate) and centrifuged in a SW-55 rotor (Beckman) for 21 hours at 35,000 rpm at 20°C. Thereafter the aqueous guanidinium thiocyanate solution and CsCl were

removed, and the RNA pellets were washed with 200 μl 95% ethanol. The pellets were dissolved in 300 μl RNAse-free water, RNA was extracted with 300 μl of phenol/chloroform (1:1), and the aqueous phase precipitated with 1:10 (v/v) of 3 M sodium acetate and 750 μl of 95% ethanol at −20°C. The RNA pellet was dried and finally dissolved into RNAse-free water.

Hybridizations

Aliquots (7.5 μg) of total cellular RNA were fractionated electrophoretically on 0.75% agarose gel after denaturation with glyoxal and transferred to a Zeta Probe nylon membrane (Bio-Rad, Hercules, CA, USA). RNA was immobilized to membrane by baking at 80°C for 2 hours. The membrane was prehybridized for 2 hours in a solution containing 50% formamide, 0.25 M sodium biophosphate, 0.25 M sodium chloride, 7% sodium dodecyl sulfate (SDS), and 1 mM EDTA. Hybridization was performed overnight in an identical solution containing in addition a cDNA probe labeled by random priming with α-^{32}P-dCTP (Amersham Laboratories, Buckinghamshire, UK) to a specific activity of approximately 1×10^8 cpm/μg. The cDNAs used in hybridizations were probe 341-1 for 28S ribosomal RNA [11], pHCAL1U for human proα1(I) [12], and pHFS 3 for human proα1(III) [13]. After hybridizations the membrane was washed three times for 5 minutes in 2 × SSC/0.1% SDS at room temperature and two times for 15 minutes in 0.1 × SSC/0.1% SDS at 55° or 60°C. Kodak X-Omat films were exposed to membranes at −70°C, and the extent of hybridization was quantified densitometrically from the x-ray films.

Determination of Collagen Propeptides

The concentration of PINP (amino-terminal propeptide of type I procollagen) and PIIINP (amino-terminal propeptide of type III procollagen) were measured by competetive radioimmunoassay methods [14, 15] with commercially available reagents (Orion Diagnostica, Espoo, Finland). The assays are based on the use of human antigens and polyclonal rabbit antibodies. A 50 μl aliquot (diluted with buffer 1:10 or 1:40) of the cell culture medium was used for the assay of PINP and 200 μl of the dilution for the PIIINP analyses. The intra- and interassay coefficients of variation are around 5% for both assays for the concentrations obtained in this study.

Results

The effect of sucrose on steady-state mRNA levels for proα1(I) collagen and proα1(III) collagen are shown in Figures 1, 2, and 3. In granulation tissue fibroblast culture 5.5 mM sucrose caused a slight increase in both proα1(I) collagen and proα1(III) collagen mRNA, but the 15 and 25 mM concentrations caused a decrease. In fibroblast culture derived from hypertrophic scar, sucrose caused a dose-dependent decrease in both proα1(I) collagen and proα1(III) collagen mRNA levels. Sucrose also produced a clear decrease in proα1(I) collagen and a slight decrease in proα1(III) collagen mRNA levels in keloid fibroblast culture, but the greatest decrease was seen with the lowest concentration of sucrose.

The results of type I and type III collagen amino-terminal propeptide determinations are shown in Tables 1 and 2. Sucrose decreased dose-dependently the amounts of both PINP and PIIINP in granulation tissue fibroblast cultures; but in hypertrophic scar and keloid fibroblast cultures a decrease was seen only in PINP. The ratio of type I/III collagen decreased in hypertrophic scar and keloid fibroblast cultures toward the high sucrose concentration, whereas in granulation tissue fibroblast cultures the ratio remained close to the control level (Table 3).

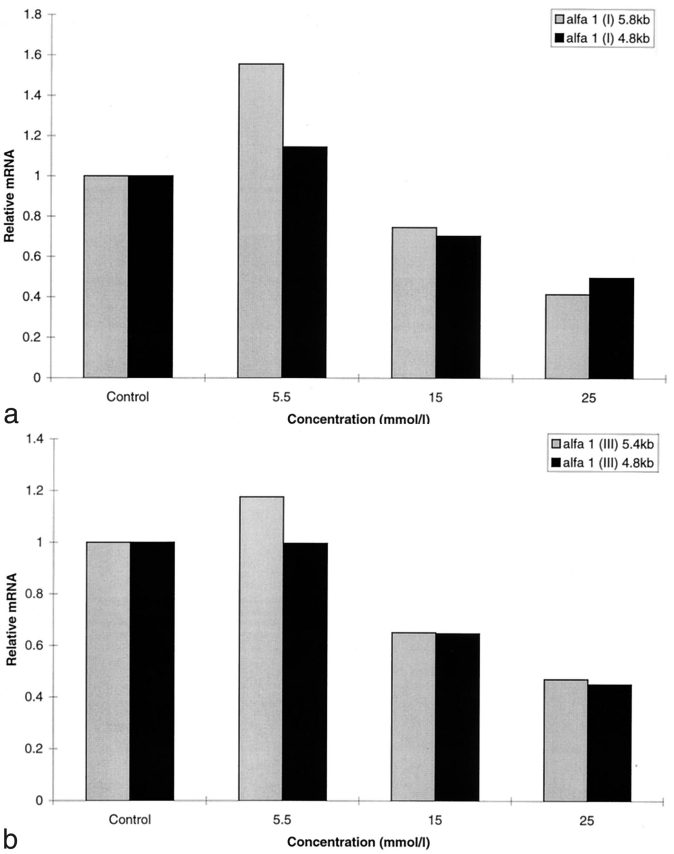

Fig. 1. a. Effect of sucrose on the steady-state level of proα1(I) collagen mRNA in granulation tissue fibroblasts. Values relative to the control group are indicated. The 5.8 kb transcript of proα1(I) collagen mRNA and the 4.8 kb transcript of proα1(I) collagen mRNA are shown. **b**. Effect of sucrose on the steady-state level of proα1(III) collagen mRNA in granulation tissue fibroblasts. Values relative to the control group are indicated. The 5.4 kb transcript of proα1(III) collagen mRNA and the 4.8 kb transcript of proα1(III) collagen mRNA are shown.

Discussion

The positive effect of sucrose on wound healing in necrotic and infected wounds, burns, or ulcers has been clearly shown in clinical studies [1–4]. Although uninfected wounds in clinical series healed normally, the effect of sucrose on clean wounds and on fibroblast behavior are not well established. In an experimental study Archer and coworkers [16] studied the effects of sugar paste on wound healing in a pig. They demonstrated that wound healing in sugar-treated wounds proceeded normally, but granulation tissue formation was not stimulated compared to that in the control (Op-site-treated; Smith & Nephew Medical, Hull, UK) wounds. Cell culture studies on the effect of sucrose on collagen metabolism have not been carried out to our knowledge, and studies

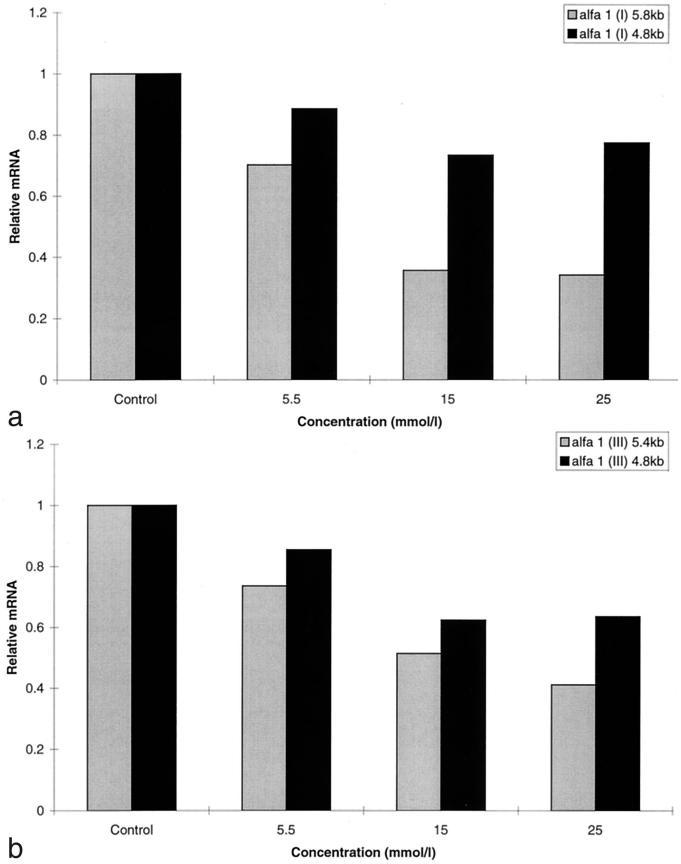

Fig. 2. a. Effect of sucrose on the steady-state level of proα1(I) collagen mRNA in hypertrophic scar fibroblasts. Values relative to the control group are indicated. The 5.8 kb transcript of proα1(I) collagen mRNA and the 4.8 kb transcript of proα1(I) collagen mRNA are shown. **b**. Effect of sucrose on the steady-state level of proα1(III) collagen mRNA in hypertrophic scar fibroblasts. Values relative to the control group are indicated. The 5.8 kb transcript of proα1(III) collagen mRNA and the 4.8 kb transcript of proα1(III) collagen mRNA are shown.

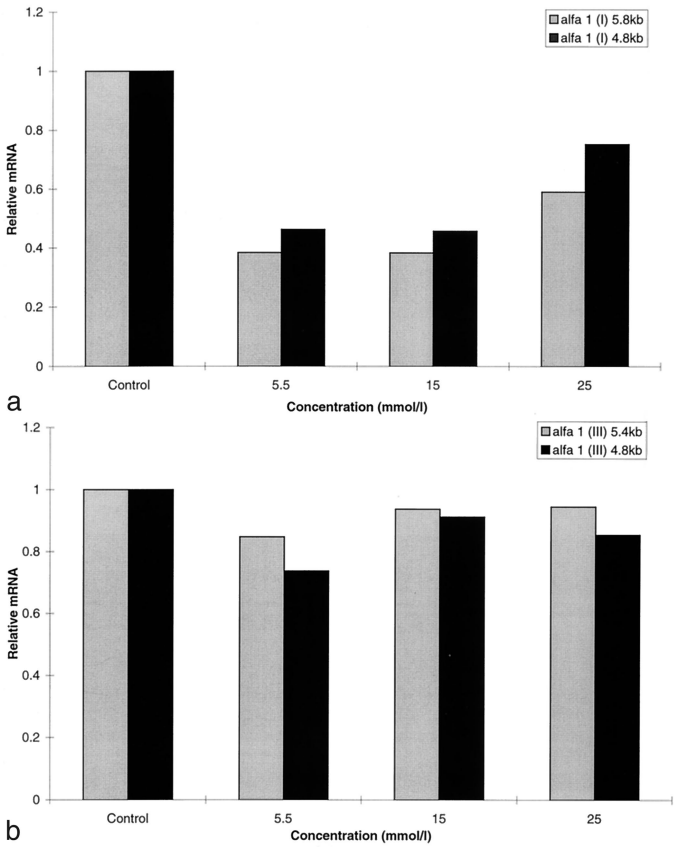

Fig. 3. a. Effect of sucrose on the steady-state level of proα1(I) collagen mRNA in keloid fibroblasts. Values relative to the control group are indicated. The 5.8 kb transcript of proα1(I) collagen mRNA and the 4.8 kb transcript of proα1(I) collagen mRNA are shown. **b**. Effect of sucrose on the steady-state level of proα1(III) collagen mRNA in granulation tissue fibroblasts. Values relative to the control group are indicated. The 5.4 kb transcript of proα1(III) collagen mRNA and the 4.8 kb transcript of proα1(III) collagen mRNA are shown.

focusing on the effects of sucrose on the behavior of fibroblasts are scarce. Sucrose has been used as an osmotic control in some fibroblast culture studies, and a slight increasing effect of sucrose on saturation density of dermal fibroblast correlating the sugar concentration has been observed previously [17]. On the other hand, 88 mM sucrose added to culture medium neither stimulated nor inhibited dermal fibroblast growth [18].

Although granulation tissue fibroblasts are closely related to skin fibroblasts, there are distinct differences between these cells. Cultured granulation tissue fibroblasts synthesize more type I and III collagens at early passage, grow more slowly, and respond differently to growth factors than do skin fibroblasts [19–21]. In the present study, granulation tissue fibroblasts showed synthesis of both type I and III collagen, and the ratio for the amount of type I/III collagen was 12.6 in the control cultures. The observed ratio is markedly higher than an earlier reported ratio in normal skin tissue (type I/III ratio 3.4) [22] and the ratio of steady-state synthesis of collagen by cultured normal skin fibroblasts or scar fibroblasts (ratios of 6.3 and 6.0, respectively) [23]. These findings provide more evidence that granulation tissue-derived fibroblasts differ from skin and scar fibroblasts.

Table 1. Effect of sucrose on type I procollagen synthesis in fibroblast cultures derived from granulation tissue, hypertrophic scar, and keloid.

	PINP in cell culture medium (μg/L)		
Sucrose concentration (mM)	Granulation tissue fibroblasts	Hypertrophic scar fibroblasts	Keloid fibroblasts
Control	1420	2729	1699
5.5	1141	2329	1596
15.0	851	1683	1426
25.0	576	1345	1250

PINP: amino-terminal propeptide of type I procollagen.

In contrast to our in vivo findings (unpublished observations), sucrose dose-dependently decreased the synthesis of both type I and III collagens in granulation tissue fibroblast culture. The concomitant down-regulation of proα1(I) and proα1(III) collagen mRNA at 15 and 25 mM sucrose concentrations suggest that sucrose might regulate, at least in part, collagen gene expression on the transcriptional level.

Table 2. Effect of sucrose on type III procollagen synthesis in fibroblast cultures derived from granulation tissue, hypertropic scar, and keloid.

	PIIINP in cell culture medium (μg/L)		
Sucrose concentration (mM)	Granulation tissue fibroblasts	Hypertrophic scar fibroblasts	Keloid fibroblasts
Control	135	98	62
5.5	99	100	59
15.0	67	93	57
25.0	59	92	55

PIIINP: amino-terminal propeptide of type III procollagen.

Table 3. Type I/type III collagen ratios in fibroblast cultures derived from granulation tissue, hypertrophic scar, and keloid.

Sucrose concentration (mM)	Granulation tissue fibroblasts	Hypertrophic scar fibroblasts	Keloid fibroblasts
Control	12.6	33.4	32.4
5.5	13.8	28.0	32.4
15.0	15.2	21.7	29.9
25.0	11.8	17.5	27.1

Hypertrophic scar fibroblasts showed, in control cultures, markedly increased synthesis of type I procollagen, whereas synthesis of type III procollagen was lower than that of granulation tissue fibroblasts, resulting in a markedly elevated type I/III collagen ratio. Additionally, the amount of synthesized type I procollagen was even higher than in keloid fibroblast culture. This is in contrast with previous cell culture studies [23] but may be explained by the fact that hypertrophic scars and keloids contain different subpopulations of fibroblasts [24–26]. The clear decrease in the collagen type I/III ratio toward high sucrose concentrations is an interesting finding. It suggests that sucrose might normalize the pattern of collagen synthesis in hypertrophic scar fibroblasts, and that type I and III collagen gene expression may be regulated separately. The parallel and proportionate decrease in the level of especially 5.8 kB transcript of proα1(I) collagen mRNA and synthesized type I collagen suggests that sucrose regulates type I collagen synthesis also in hypertrophic scar fibroblasts on the transcriptional level. However, other regulatory events are involved in type III collagen synthesis whereas there was a marked disproportion between the mRNA levels and amounts of synthesized type III collagen. The reason for this phenomenon remains to be elucidated.

In the present study, keloid fibroblasts in control cultures showed a slightly increased amount of synthesized type I procollagen, markedly decreased amount of synthesized type III procollagen, and a highly elevated type I/III ratio compared with granulation tissue fibroblasts. This is in line with previous cell culture studies [27], although the ratio in the present study is high. Sucrose decreased type I procollagen synthesis dose-dependently, whereas type III procollagen synthesis remained almost unaltered, resulting in lowering of the type I/III collagen ratio. This ratio, however, remained quite high. Surprisingly, mRNA hybridizations showed that the most profound decrease in the steady-state level of mRNA for type I and III collagens was caused by the lowest sucrose concentration. This is in marked contrast with the amounts of synthesized procollagens. The reason for this cannot be explained, but it may be the result of altered stability of mRNA or posttranscriptional regulation of collagen synthesis. Results of the present study suggest that sucrose may return the collagen synthesis of keloid fibroblasts toward normal.

Conclusions

Our data suggest that sucrose down-regulates type I and III collagen metabolism in granulation tissue fibroblast cultures, but it regulates differently type I and type III collagen metabolism in fibroblast cultures derived from fibrotic skin lesions, changing the collagen metabolism toward normal. The results of the present study suggest the need for further studies to determine the value of sucrose in the prevention and treatment of keloids and hypertrophic scars.

Résumé

Le sucrose a été utilisé pour traiter des plaies avec d'excellents résultats et un taux de cicatrisation anormale, minime. Dans cette étude, nous avons évalué les effets du sucrose sur le métabolisme du collagène des fibroblastes en culture. On a ajouté du sucrose 5,5 mMolar (mM), 15 mM ou 25 mM à des cultures de fibroblastes provenant du tissu de granulation, des cicatrices hypertrophiques et des chéloïdes. On a évalué le taux de mRNA et des aminopropeptides procollagènes pour les collagènes de type I et III en milieu de culture cellulaire. La sucrose a diminué les taux de mRNA pour les collagènes proα1(I) et proα1(III) en culture de fibroblastes dérivée des cicatrices hypertrophique et chéloïdes. Dans les cultures de fibroblastes issus du tissu de granulation normal, le sucrose 5.5 mM a augmenté les taux de mRNA-pour les collagènes proα1(I) et proα1(III) alors qu'à des concentrations plus élevées, ces taux ont diminué. La synthèse de collagènes de type I a diminué, étroitement en fonction de la dose, dans toutes les cultures cellulaires alors que la synthèse de collagènes de type III n'a diminué que dans les cultures de fibroblastes du tissu de granulation. En conclusion, les concentrations élevées de sucrose in vitro sous régule l'expression génétique du collagène et la synthèse des fibroblastes du tissu de granulation normal alors que dans les fibroblastes dérivés des cicatrices anormales, le sucrose sous-régule seulement l'expression génétique du collagènes de type I, tendant ainsi vers la synthèse normale de collagène.

Resumen

La sucrosa ha sido utilizada en el tratamiento de las heridas con excelentes resultados y mínima cicatrización anormal. En el presente estudio se evaluaron los efectos de la sucrosa sobre el metabolismo de colágeno en cultivos de fibroblastos de queloides. Se analizaron los niveles de mRNA y de aminopropéptidos procolágeno en los colágenos del tipo I y del tipo III en cultivos celulares. La sucrosa disminuyó los niveles de mRNA en los colágenos proα1(I) y proα1(III) en los cultivos de fibroblastos derivados de cicatriz hipertrófica y de queloide. En los cultivos de fibroblastos de tejido de granulación normal, la sucrosa 5.5 mM aumentó los niveles de mRNA para el colágeno proα1(I) y proα1(III), al tiempo que concentraciones mayores los disminuyó. La síntesis de colágeno tipo I decreció en forma dosis-dependiente en todas las cepas celulares, en tanto que la síntesis

del colágeno tipo III decreció solamente en los fibroblastos del tejido de granulación. En conclusión, las altas concentraciones de sucrosa *in vitro* disminuyen la expresión genética de colágeno y la síntesis de los fibroblastos de tejido de granulación normal, en tanto que en los fibroblastos derivados de cicatriz anormal la sucrosa disminuye solamente la expresión genética y la síntesis del colágeno tipo I, modificando metabolisomo del colágeno hacia el patrón normal.

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References

1. Knutson, R.A., Merbitz, L.A., Creekmore, M.A., Snipes, H.G.: Use of sugar and povidone-iodine to enhance wound healing: five years experience. South. Med. J. *74*:1329, 1981
2. Trouillet, J.L., Chastre, J., Fagon, J.Y., Pierre, J., Domart, Y., Gibert, C.: Use of granulated sugar in treatment of open mediastinitis after cardiac surgery. Lancet *2*:180, 1985
3. Middleton, K.R., Seal, D.: Sugar as an aid to wound healing. Pharm. J. *235*:757, 1985
4. Dawson, J.S.: Preiskel elective prize: the role of sugar in wound healing: a comparative trial of infected wounds using traditional gauze/antiseptic packing and granulated sugar, undertaken during elective period at Kagando Hospital, Uganda. Ann. R. Coll. Surg. Engl. *78*(Suppl. 2):82, 1996
5. Efem, S.E.E.: Clinical observations on the wound healing properties of honey. Br. J. Surg. *75*:679, 1988
6. Efem, S.E.E.: Recent advances in the management of Fourniers gangrene: preliminary observations. Surgery *113*:200, 1993
7. Chirife, J., Herszage, L., Joseph, A., Kohn, E.S.: In vitro study of bacterial growth inhibition in concentrated sugar solutions: microbiological basis for the use of sugar in treating infected wounds. Antimicrob. Agents Chemother. *23*:766, 1983
8. Ambrose, U., Middleton, K., Seal, W.: In vitro studies of water activity and bacterial growth inhibition of sucrose-polyethylene glycol 400-hydrogen peroxide and xylose-polyethylene glycol-hydrogen peroxide pastes used to treat infected wounds. Antimicrob. Agents Chemother. *35*:1799, 1991
9. Subrahmanyam, M.: Topical application of honey in treatment of burns. Br. J. Surg. *78*:497, 1991
10. Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J., Rutter, W.J.: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry *18*:5294, 1979
11. Irquela-Arispe, M.L., Hasselaar, P., Sage, H.: Differential expression of extracellular proteins is correlated with angiogenesis in vitro. Lab. Invest. *64*:174, 1991
12. Mäkelä, J.K., Raassina, M., Virta, A., Vuorio, E.: Human proα1(I) collagen: cDNA sequence for the C-propeptide domain. Nucleic Acids Res. *16*:349, 1988
13. Sandberg, M., Mäkelä, J.K., Multimäki, P., Vuorio, T., Vuorio, E.: Construction of a human proα1(I) collagen cDNA clone and localization of type III collagen gene expression in human fetal tissues. Matrix *9*:82, 1989
14. Risteli, J., Niemi, S., Trivedi, P., Mäentausta, O., Mowat, A.P., Risteli, L.: Rapid equilibrum radioimmunoassay for the amino-terminal propeptide of human type III procollagen. Clin. Chem. *34*:715, 1988
15. Melkko, J., Kauppila, S., Niemi, S., Risteli, L., Haukipuro, K., Jukkola, A., Risteli, J.: Immunoassay for intact amino-terminal propeptide of human type I procollagen. Clin. Chem. *42*:947, 1996
16. Archer, H.G., Barnett, S., Irving, S., Middleton, K.R., Seal, D.V.: A controlled model of moist wound healing: comparison between semipermeable film, antiseptics and sugar paste. J. Exp. Pathol. *71*:155, 1990
17. Sibbitt, W.L., Mills, R.G., Bigler, C.F., Eaton, R.P., Griffey, R.H., vander Jagt, D.L.: Glucose inhibition of human fibroblast proliferation and response to growth factors is prevented by inhibitors of aldose reductase. Mech. Ageing Dev. *47*:265, 1989
18. Kato, T., Okada, S., Yutaka, T., Yabuuch, H.: The effects of sucrose loading on lysosomal hydrolases. Mol. Cell. Biochem. *60*:83, 1984
19. Oda, D., Gown, A.M., Vande Berg, J.S., Stern, R.: Instability of the myofibroblast phenotype in culture. Exp. Mol. Pathol. *52*:221, 1990
20. Vande Berg, J.S., Rudolf, R., Poolman, W.L., Disharoon, D.R.: Comparative growth dynamics and actin concentration between cultured human myofibroblasts from granulating wounds and dermal fibroblasts from normal skin. Lab. Invest. *61*:532, 1989
21. Buckley-Sturrock, A., Woodward, S.C., Senior, R.M., Griffin, G.L., Glagsbrun, M., Davidson, J.M.: Differential stimulation of collagenase and chemotactic activity in fibroblasts derived from rat wound repair tissue and human skin by growth factors. J. Cell. Physiol. *138*:70, 1989
22. Abergel, R.P., Pizzurro, D., Meeker, C.A., Lask, G., Matsuoka, L.Y., Minor, R.R., Chu, M-L., Uitto, J.: Biochemical composition of the connective tissue in keloids and analysis of collagen metabolism in keloid fibroblast cultures. J. Invest. Dermatol. *84*:384, 1985
23. Friedman, D.W., Boyd, C.D., Mackenzie, J.W., Norton, P., Olson, R.M., Deak, S.B.: Regulation of collagen gene expression in keloids and hypertrphic scars. J. Surg. Res. *55*:214, 1993
24. Arakawa, M., Hatamochi, A., Mori, Y., Ueki, H., Moriguchi, T.: Reduced collagenase expression in fibroblasts from hypertrophic scar tissue. Br. J. Dermatol. *134*:863, 1996
25. Peltonen, J., Hsiao, L.L., Jaakkola, S., Sollberg, S., Aumailley, M., Timpl, R., Chu, M-L., Uitto, J.: Activation of collagen gene expression in keloids: co-localization of type I and VI collagen and transforming growth factor-β1 mRNA. J. Invest. Dermatol. *97*:240, 1991
26. Lee, K.S., Song, J.Y., Suh, M.H.: Collagen gene expression detected by in situ hybridization in keloid tissue. J. Dermatol. Sci. *2*:316, 1991
27. Friedman, D.W., Boyd, C.D., Mackenzie, J.W., Norton, P., Olson, R.M., Deak, S.B.: Regulation of collagen gene expression in keloids and hypertrophic scars. J. Surg. Res. *55*:214, 1993