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# The Microbial Flora in Venous Leg Ulcers without Clinical Signs of Infection

## *Repeated Culture Using a Validated Standardised Microbiological Technique*

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**The change of ulcer size in relation to the presence of species and quantities of microorganisms was analysed in 58 patients with venous leg ulcers, all without clinical signs of infection. Microbiological samples were taken on the day of inclusion and then repeated 4 times at monthly intervals or until the ulcer had healed or was too small to be cultured from. There was growth of microorganisms in all ulcers, and the numbers were below $10^4$ per $mm^2$ of ulcer surface in all cases. No correlation was found between ulcer size change and the species and amounts of microorganisms.**

**Sixty-nine species were isolated. *Staphylococcus aureus* was found in 88%, *Enterococcus faecalis* in 74%, *Enterobacter cloacae* and *Peptococcus magnus* in 29%, and fungi in 11% of the samples. One or more obligate anaerobe species was found in 41% of the samples and in half of the ulcers and constituted 62% of all bacterial species.**

**The colonising ulcer flora was markedly constant over time in the individual ulcers regardless of change in size. Resident bacterial species were found in 57 of the 58 ulcers.**

**If all samples were considered, the microorganisms were associated with not more than one fifth of the variability in healing rate, as shown by linear multiple regression analysis. The same species of microorganisms were found in ulcers that decreased (or healed) and in those that increased in size. Although an association between the microorganisms and ulcer healing could not be ruled out in this study, there seems to be no indication for routinely performed culture in the absence of clinical signs of infection in venous leg ulcers.** ***Key words: varicose ulcer; bacteria; fungi; wound healing.***

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Delayed ulcer healing has by some authors been linked to the influence of some special kind or to all kinds of microorganisms (1–3), while others have not found such a relationship (4–7). Certain bacteria have even been reported to stimulate healing (8–9). *Proteus mirabilis* and *Pseudomonas aeruginosa* have been associated with non-healing decubitus ulcers (10). A relationship between large quantities of microorganisms, $>10^5$ CFU/g tissue, and delayed healing of the ulcer has also been suggested (1), while others have reported no such effect even from heavy bacterial colonisation (5, 6, 11). The selection of the patients and the design of the published studies differ. Some authors report patients with clinical signs of infections together with patients without such signs; others include patients on treatment with systemic antibiotics or omit such information. Some reports concern experimental animal studies (11–13).

Microbial ulcer flora consistency over time has been studied in humans by some investigators (2, 5–7). They have all reported a noticeable persistence of the initial ulcer flora.

It can be concluded that several microbiological investigations of venous leg ulcers have revealed the growth of many different microbial species, but that the significance of microorganisms in relation to healing is still unclear.

Microbiological sampling from superficial ulcers has been described using several different non-quantitative as well as semiquantitative and quantitative methods. Culture of biopsy material is by some authors considered to be the most relevant sampling method (1, 14). Among non-invasive methods, cotton-tipped swabs are the most common, used without additives (15) or coated with serum (14) or impregnated with charcoal (4, 16, 17). Most authors consider swab methods to give only unsatisfactory semiquantitative estimates. Irrigation followed by aspiration has been found to be an acceptable non-invasive alternative to biopsy (18). Sampling with an absorbent pad followed by shaking in a machine was found to be superior to several other methods for quantitative determinations, especially when the bacterial density was low (14, 19, 20).

The aim of this study was to search for correlations between change of venous ulcer size and species and quantities of microorganisms present, as well as to observe the consistency of microbial findings during a period of 4 months.

## MATERIAL AND METHODS

### *Patients*

Consecutive out-patients treated at regular intervals at the Department of Dermatology, Sahlgrens' Hospital, Göteborg, with a clinical diagnosis of venous leg ulcer and without overt clinical signs of infection were included in the study. Patients with redness in the skin around the ulcers, and with warmth, oedema and pain were treated with systemic antibiotics and were excluded from the study. The initial ulcer width had to be at least 2 cm in the smallest direction. The largest ulcer was selected if there was more than one in the same patient. Significant arterial disease was excluded by means of Doppler examination. Three patients had mild diabetes (2 were treated orally and one with diet only). Sixty-four patients with an initial mean age of 75 years (41 females, mean age: 76 years; 23 males, mean age: 73 years) entered the study. Six patients were excluded for various reasons for the statistical analysis (ulcer infection which needed treatment with antibiotics, the ulcer healed and one person died), but 4 were included in the analysis concerning the initial ulcer size in relation to microbial species. The mean duration of the ulcers previous to the first sampling was 2 years and 7 months and the median duration was 6 months. Samples for quantitative culture were taken initially and then once each month to a maximum of 5 times or until the ulcers had healed to 1 cm in the shortest cross dimension. Fifty-eight patients remained in the study for 1 month, 44 for 2, 38 for 3 and 27 for 4 months. Twenty-one ulcers healed during the observation period.



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Table I. *The number of patients using different types of dressings and the mean initial ulcer size in cm² at the time of inclusion*

| Type of dressing | Number of patients | Mean initial ulcer size |
|---|---|---|
| Duoderm hydrocolloid dressing (Convatec, Squibb, USA) | 18 | 11 cm² |
| Zincaband N (Seton Products, Oldham, UK) | 16 | 30 cm² |
| Medicated stockings (Perstorp, Lund, Sweden) | 14 | 38 cm² |
| Unitulle petrolatum impregnated gauze (Roussel Lab Ltd., UK) | 7 | 42 cm² |
| Debrisan compresses (dextranomer beads) (Pharmacia, Uppsala, Sweden) | 5 | 27 cm² |
| Silastic foam (Dow Corning, Valbonne, France) | 1 | 17 cm² |
| Wet saline dressing | 1 | 36 cm² |

*Measurement of ulcer area*

On each sampling occasion, the area of the ulcer was determined twice, with the use of the following technique: the ulcer was covered with transparent plastic film and the ulcer periphery was outlined with a felt-tipped pen. The film was transferred to a piece of paper and photocopied onto a plastic overhead film of known weight per unit area. The ulcer pictures were cut out and weighed. The mean of the two weighings from each sampling occasion was used to calculate the ulcer area. This method had been validated by making five consecutive drawings from each of five randomly selected ulcers (SD = 0.02). Ulcers with an average decrease in size of at least 10% between sampling occasions were classified as decreasing and those with a 5% increase as increasing.

*Dressings*

The number of patients using different types of dressings and the mean initial ulcer size in cm² at the time of inclusion in the study are shown in Table I. The wet saline dressings were changed daily and the others 2–3 times a week.

An elastic compression bandage was always applied on top of the dressings. For Zincaband N and Medicated Stockings it was Coban Self-Adherent Wrap (3M Medical Products Division, USA), and for all the other types of ulcer dressings it was a compressive elastic bandage, Wero Normal (Wernli AG, Switzerland).

*Microbiological methods, evaluation of procedures*

Sterile charcoal-impregnated discs were made of 1 mm thick polyvinyl alcohol foam (PVA), (Mölnlycke Clinical Products AB, Mölnlycke, Sweden). The discs had a diameter of 10 mm and an absorption capacity of 0.29 ml. In survival tests, PVA discs had been compared with charcoal-impregnated cotton pellets. Approximately $5 \times 10^4$ bacterial cells from suspensions of representative aerobically and anaerobically growing bacterial strains were added to each of the two types of sampling vehicles. The vehicles were stored for various periods (1–3 days) in the modified transport medium VMGA III (21). (The original VMG-base was modified by anaerobic preparation and sterilisation and was dispensed in 5-ml portions in 6-ml screw-capped bottles, containing a layer of 3-mm glass beads and with oxygen-free nitrogen deposited over the medium surface). The bioburden of the discs was then determined by means of an appropriate technique. In these tests, the PVA discs gave as good or superior survival as the cotton pellets.

Sampling discs were put into the transport medium and transported within 2 h to the microbiological laboratory for culture. The bottles were heated to 36°C in order for the gelatin to melt in the medium and then shaken for about 20 s in a whirling mixer. From each bottle, a 0.1-ml portion was spread on one Brucella blood agar plate (BBL Cockeysville, MD, USA), with the addition of 5% defibrinated horse blood, 0.5% haemolysed blood and 5 mg/l menadione. It was incubated anaerobically (the hydrogen combustion method) (22) for 6–8 days. 0.1 ml was also spread on each of two blood agar plates, one of which was incubated aerobically for 2–3 days and one in air with the addition of 10% carbon dioxide for 3–4 days, all at 37°C. A further 0.2 ml was spread onto a Sabouraud glucose agar plate (Difco Laboratories, Detroit, MI, USA), which was incubated aerobically at 23°C for 5 days. Quantitative determinations were made according to the descriptions by Dahlén et al. (23).

In 10 ulcers the results from the following three sampling regimens were compared, all with the use of charcoal-impregnated PVA discs. Samples were taken immediately after removal of the dressing and after cleaning the ulcer bottom with a modified Ringer's solution (NaCl-content 0.5%) and gauze swabs. In both cases, a dry sampling disc was pressed lightly against the ulcer surface for 1 min or until saturated and thereafter immediately transferred to the transport medium. PVA discs were also enclosed under the dressing for 24 h and then taken out for culture. The comparison showed that about the same species and number of microbial cells were found regardless of method.

The microbial flora of the peripheral part and that of the central parts of the ulcers were compared. No clear differences were found between the counts obtained between the two sites.

As a result of the pilot tests, all samples in the present study were taken as "immediate" with charcoal-impregnated PVA discs after the bottom of the ulcer had been slightly moistened with modified Ringer's solution.

In 5 of the patients samples were also taken from healthy skin 10 cm from the border of the ulcer.

*Statistics*

A stepwise, multiple, linear regression analysis was used to study the extent to which the presence of different types of microorganisms in the ulcer influenced ulcer size change. The dependent variable was the difference between the logarithm of the ulcer area on one culturing occasion and the logarithm of the area 1 month earlier. The independent variables were the different types of microorganisms present in the ulcer and the age of the patient. Each difference between two consecutive samplings was regarded as one observation. A linear regression analysis of the effect of different bacterial strains on the initial ulcer size was also made.

The study was approved by the Ethical Committee of the Medical Faculty of the Göteborg University. The patients were informed about the study and consented to take part.

## RESULTS

In all, 69 species were isolated from the 58 ulcers, with a varying number of species per ulcer (Table II). The quantities of the microbial cells were all below $10^4$ per mm² of ulcer surface. *Staphylococcus aureus* was found in 88% and *Enterococcus faecalis* in 72% of the samples. More than one bacterial species was found in 50 (86%) of the ulcers. The predominant flora besides the above-mentioned were aerobes or facultative anaerobes such as *Staphylococcus epidermidis, Pseudomonas aeruginosa, P. fluorescens, P. maltophilia, Klebsiella oxytoca, K.mirabilis, K.morganii,* and *Escherichia coli.* One or more obligate anaerobe species, e.g. *Peptococcus prevotii, Gaffkya anaerobia, Propionibacterium acnes* and *Veillonella parvula,* was found in 50% of the ulcers, corresponding to 41% of the samples. Fungi (*Candida albicans, C. guilliermondii* and *Rhodotorula rubra*) were found in 11% of the samples.

A comparison of the microbial findings in consecutive samples from the same ulcer showed that one or more of the same species was re-isolated from all samples from 52 ulcers out of

Table II. *Microbial findings in 328 samples from 58 ulcers isolated in the initial or one of the following samples*

| Microorganisms | Occurrence in patients | | Per cent of positive samples. Distribution with regard to calculated cell density per mm² of ulcer area[a] | | | | Total |
|---|---|---|---|---|---|---|---|
| | Numbers | Per cent | $>0<10^1$ | $10^1$–$10^2$ | $>10^2$–$10^3$ | $10^3$–$10^4$ | Per cent |
| Staphylococcus aureus | 51 | 87.9 | 2.2 | 14.3 | 23.7 | 32.1 | 72.3 |
| capitis | 1 | 1.7 | – | – | 0.9 | – | 0.9 |
| epidermidis | 9 | 15.5 | 2.2 | 1.8 | 1.8 | 1.8 | 7.6 |
| hominis | 3 | 5.2 | 0.4 | – | 1.3 | – | 1.8 |
| simulans | 3 | 5.2 | 0.4 | 0.9 | 1.8 | 1.3 | 4.5 |
| warneri | 2 | 3.4 | – | 0.4 | – | 0.4 | 0.8 |
| hemolyticus | 2 | 3.4 | – | 0.4 | – | 0.4 | 0.8 |
| Aerococcus viridans | 2 | 3.4 | – | – | 0.4 | – | 0.4 |
| Micrococcus sp. | 1 | 1.7 | – | – | – | 0.4 | 0.4 |
| Streptococcus pyogenes | 1 | 1.7 | – | 0.4 | – | 0.4 | 0.4 |
| agalactiae | 5 | 8.6 | 0.4 | 0.9 | 2.7 | 0.4 | 4.5 |
| equisimilis | 6 | 8.6 | 0.4 | 0.9 | 2.7 | 0.4 | 4.5 |
| morbillorum | 2 | 3.4 | – | – | – | 0.4 | 0.4 |
| dysgalactiae | 2 | 3.4 | – | – | 1.8 | 1.8 | 3.6 |
| pneumoniae | 1 | 1.7 | – | – | 0.4 | – | 0.4 |
| mitis | 4 | 6.9 | – | 0.9 | 1.3 | 0.4 | 2.7 |
| milleri | 1 | 1.7 | – | – | 0.9 | 0.9 | – |
| sanguis | 3 | 5.2 | – | 0.9 | 0.9 | 1.8 | 3.6 |
| salivarius | 1 | 1.7 | – | 0.4 | 0.9 | – | 1.3 |
| Enterococcus faecalis | 42 | 72.4 | 5.3 | 12.5 | 14.3 | 9.8 | 41.5 |
| faecium 1 | 1 | 1.7 | – | 0.9 | 0.4 | – | 1.3 |
| avium | 1 | 1.7 | – | 0.9 | – | 0.9 | 1.8 |
| Corynebacterium equi | 1 | 1.7 | – | – | – | 0.4 | 0.4 |
| group 1 | 2 | 3.4 | 0.4 | – | 0.4 | 1.8 | 2.7 |
| group 2 | 1 | 1.7 | 0.4 | – | – | – | 0.4 |
| pseudodiphteriae | 1 | 1.7 | – | – | 0.4 | – | 0.4 |
| striatum | 5 | 8.6 | – | 1.3 | 1.3 | 0.9 | 3.6 |
| Arthrobacter sp | 3 | 5.2 | – | – | 0.9 | 0.9 | 1.8 |
| Pseudomonas aeruginosa | 11 | 19.0 | 0.4 | 2.7 | 5.3 | 5.3 | 13.8 |
| fluorescence | 6 | 10.3 | 0.4 | 1.3 | 0.4 | 0.9 | 3.1 |
| maltophilia | 7 | 12.1 | 0.4 | 1.3 | 1.3 | 6.2 | 9.3 |
| paucimobilis | 1 | 1.7 | 0.4 | – | 0.4 | 0.4 | 1.2 |
| stutzeri | 1 | 1.7 | – | – | – | 0.4 | 0.4 |
| sp | 4 | 6.9 | – | 0.9 | – | 0.4 | 1.3 |
| Klebsiella pneumoniae | 4 | 6.9 | – | 0.9 | – | 0.4 | 1.8 |
| oxytoca | 6 | 10.3 | – | 0.9 | 1.3 | 6.2 | 8.4 |
| Proteus mirabilis | 9 | 15.5 | 1.3 | 4.0 | 4.4 | 3.6 | 13.3 |
| morganii | 6 | 10.3 | 2.2 | 0.4 | 4.4 | 1.8 | 8.9 |
| vulgaris | 3 | 5.2 | – | – | 0.9 | 4.0 | 1.3 |
| Esherichia coli | 10 | 17.2 | 0.4 | 2.7 | 4.0 | 3.1 | 10.2 |
| Enterobacter cloacae | 17 | 29.3 | 0.4 | 2.7 | 9.3 | 11.1 | 23.6 |
| Acinetobacter calcoaceticus | 8 | 13.8 | 0.4 | 0.9 | 1.3 | 5.8 | 8.4 |
| Serratia liquefaciens | 1 | 1.7 | – | – | 0.4 | – | 0.4 |
| Citrobacter sp. | 2 | 3.4 | 0.9 | – | – | – | 0.9 |
| Alcaligeues faecalis/odorans | 1 | 1.7 | – | – | – | 0.4 | 0.4 |
| Peptococcus | | | | | | | |
| asaccharolyticus | 9 | 15.5 | 0.9 | 1.3 | 5.3 | 2.2 | 9.8 |
| constellatus | 1 | 1.7 | – | – | – | 0.4 | 0.4 |
| magnus | 17 | 29.3 | 2.2 | 3.1 | 6.7 | 7.6 | 19.6 |
| prevotii | 6 | 10.3 | 0.4 | 0.4 | 2.7 | 3.6 | 7.1 |
| saccharolyticus | 1 | 1.7 | – | – | – | 0.4 | 0.4 |
| Gaffkya anaerobia | 6 | 10.3 | – | – | 1.8 | 1.3 | 3.1 |
| Peptostreptococcus | | | | | | | |
| anaerobius | 2 | 3.4 | – | 0.4 | – | 0.4 | 0.8 |
| Propionibacterium acnes | 7 | 12.1 | 0.9 | 1.3 | 0.9 | 1.3 | 4.4 |
| avidum | 1 | 1.7 | – | – | – | 0.1 | 0.4 |
| lymphophilium | 1 | 1.7 | – | 0.9 | – | – | 0.9 |
| Arachnia propionica | 2 | 3.4 | – | – | 0.9 | 0.4 | 1.3 |
| Actinomyces odontolyticus | 2 | 3.4 | – | 0.4 | 0.9 | 2.7 | 4.0 |
| meyeri | 1 | 1.7 | – | 0.4 | – | – | 0.4 |
| Eubacterium lentum | 1 | 1.7 | – | – | – | 0.4 | 0.4 |
| Veillonella parvula | 6 | 10.3 | – | – | 1.3 | 3.6 | 4.9 |
| Bacteroides asaccharolyticus | 3 | 5.2 | – | 1.8 | 0.9 | – | 2.7 |
| corrodens | 1 | 1.7 | 0.4 | – | 0.4 | – | 0.8 |
| disiens | 1 | 1.7 | – | – | 1.3 | 0.4 | 1.7 |
| distasonis | 1 | 1.7 | – | – | – | 0.4 | 0.4 |
| multiacidus | 1 | 1.7 | – | – | 0.4 | – | 0.4 |
| thetaiotaomicron | 1 | 1.7 | – | – | – | 0.4 | 0.4 |
| Candida albicans | 3 | 5.2 | 1.8 | 0.9 | – | – | 2.7 |
| guilliermondii | 1 | 1.7 | – | 0.4 | – | – | 0.4 |
| Rodothorula rubra | 1 | 1.7 | 0.4 | – | – | – | 0.4 |

[a] estimated according to Dahlén et al. (23)

Table III. *Resident* bacterial flora in relation to change in ulcer size*

Number of cases in which the same bacterial species were re-isolated in the sampling series from 58 ulcers.

| Bacterial species | Ulcer development | | | | | |
|---|---|---|---|---|---|---|
| | Reduced** $n=41$ | | Varied $n=8$ | | Increased*** $n=9$ | |
| | Number of ulcers | % | Number of ulcers | % | Number of ulcers | % |
| Staphylococcus | | | | | | |
| aureus | 26 | 63.4 | 5 | 62.5 | 8 | 88.9 |
| epidermidis | 3 | 7.3 | – | – | – | – |
| simulans | 2 | 4.9 | 1 | 12.5 | – | – |
| Streptococcus | | | | | | |
| agalactiae | 3 | 7.3 | – | – | – | – |
| equisimilis | 1 | 2.4 | – | – | – | – |
| sanguis | 1 | 2.4 | – | – | – | – |
| dysgalactiae | – | – | 2 | 25.0 | – | – |
| Enterococcus | | | | | | |
| faecalis | 15 | 36.6 | 5 | 62.5 | 2 | 22.2 |
| avium | 1 | 2.4 | – | – | – | – |
| faecium | – | – | 1 | 12.5 | – | – |
| Corynebacterium | | | | | | |
| gr 1 | – | – | 1 | 12.5 | – | – |
| Pseudomonas | | | | | | |
| aeruginosa | 6 | 14.6 | 3 | 37.5 | – | – |
| fluorescens | 1 | 2.4 | 1 | 12.5 | – | – |
| maltophilia | 1 | 2.4 | – | – | 1 | 11.1 |
| paucimobilis | 1 | 2.4 | – | – | – | – |
| sp | 1 | 2.4 | – | – | – | – |
| Klebsiella oxytoca | 2 | 4.9 | – | – | 2 | 22.2 |
| Proteus | | | | | | |
| mirabilis | 4 | 9.8 | 1 | 12.5 | 2 | 22.2 |
| morganii | 5 | 12.2 | – | – | 1 | 11.1 |
| vulgaris | – | – | 2 | 25.0 | – | – |
| Escherichia | | | | | | |
| coli | 3 | 7.3 | – | – | – | – |
| Enterobacter | | | | | | |
| cloacae | 8 | 19.5 | 1 | 12.5 | 4 | 44.4 |
| Acinetobacter | | | | | | |
| calcoaceticus | 1 | 2.4 | 1 | 12.5 | – | – |
| Peptococcus | | | | | | |
| asaccharolyticus | 2 | 4.9 | 1 | 12.5 | – | – |
| magnus | 8 | 19.5 | 2 | 25.0 | 1 | 11.1 |
| prevotii | 2 | 4.9 | – | – | – | – |
| Propionibacterium | | | | | | |
| acnes | 1 | 2.4 | – | – | – | – |
| Actinomyces | | | | | | |
| odontolyticus | 1 | 2.4 | – | – | – | – |
| Veillonella | | | | | | |
| parvula | 1 | 2.4 | – | – | – | – |
| Bacteroides | | | | | | |
| asaccharolyticus | 1 | 2.4 | – | – | – | – |

* Bacterial species present in all but one sample (2–5) of a series. In series containing only 2 samples both contained the bacteria marked.
** Change between each sampling occasion 10% or more.
*** Change between each sampling occasion 5% or more.

the 58. In 5 ulcers out of the remaining 6, one or more of the same species was present in all but one of the samples. A resident flora, characterised as a bacterial species present in all or all but one sample of a series, is shown in relation to ulcer size change in Table III. Thus, according to this definition, resident bacteria were found in 57 of the 58 ulcers. Some of the most frequently occurring resident bacterial species were found both in ulcers which decreased and in those which increased in size. This was the case for *S. aureus, E. faecalis, Klebsiella pneumoniae, Proteus mirabilis, P. morganii, Enterobacter cloacae* and *Peptococcus magnus*. Some other resident bacterial species were isolated only from decreasing ulcers or ulcers which varied in size during the observation period, i.e. *P. aeruginosa, Proteus vulgaris, E. coli, Acinetobacter calcoaceticus*

Table IV. *Intra-individual comparison between the microflora of the ulcer and the skin in 5 patients*

| Patient No. | Microbial findings | |
|---|---|---|
| | Ulcer flora | Skin flora |
| 1. | Staphylococcus aureus<br>Enterococcus faecalis 1<br>Pseudomonas aeruginosa | Streptococcus morbillorum<br>Streptococcus salivarius<br>Propionibacterium acnes<br>Enterobacter cloaceae |
| 2. | Staphylococcus hominis<br>Enterococcus faecalis 1<br>Pseudomonas aeruginosa | Staphylococcus epidermidis<br>Enterococcus faecalis 2<br>Aerococcus viridans<br>Micrococcus sp<br>Kurthia sp<br>Acinetobacter sp |
| 3. | Proteus mirabilis<br>Escherichia coli<br>Candida albicans | Staphylococcus epidermidis<br>Staphylococcus hemolyticus<br>Micrococcus sp<br>Kurthia sp<br>Propionibacterium acnes<br>Enterobacter agglomerans<br>Arthrobacter sp |
| 4. | Enterococcus faecalis 1<br>Pseudomonas aeruginosa | Staphylococcus epidermidis<br>Staphylococcus warneri<br>Kurthia sp<br>Propionibacterium acnes<br>Arthrobacter sp |
| 5. | Enterococcus faecalis 2 | Staphylococcus epidermidis<br>Staphylococcus capitis<br>Propionibacterium acnes |

and *Peptococcus asaccharolyticus*. In 47 (84%) of the ulcers 2 or more (up to 6) resident bacterial species were found. Among these, *S. aureus*, isolated from 39 ulcers (83%), was by far the most common. This species was solitarily colonising 9 ulcers, and in the remaining 30 ulcers it was found together with one or more (up to 5) other ulcer-resident bacterial species. The other 2 single, resident bacterial species were *Staphylococcus simulans* and *E. faecalis*, which each occurred in one decreasing ulcer. Beside the resident bacterial flora, several transient microorganisms were found. Most of the obligate anaerobes belonged to this category. The total findings of transient bacteria may be seen by comparing the findings in Tables II and III.

The microbial flora of the 9 increasing ulcers is of special interest. In 8 of these *S. aureus* was constantly colonising; in 6 it was found together with 1–4 other species. The only remaining increasing ulcer was colonised by *E. faecalis* together with *P. mirabilis* and *P. morganii*.

The linear regression analysis of the different microbial strains found at the start of the study and the size of the ulcer showed that the presence of *P. aeruginosa* ($p < 0.01$) and *E. faecalis* ($p < 0.05$) was positively correlated to large size of the ulcer.

In the total number of observations, the stepwise, multiple, linear regression analysis did not show any single microbial strain that was significantly correlated to ulcer size change. The analysis revealed that the effect of the presence of different species could at most explain one fifth of the variability of the ulcer size change. The age of the patient was not seen to have any influence (n.s.).

Table IV shows the results of the intra-individual comparison in 5 patients between the ulcer flora and the surrounding skin flora. In none of the cases was the same bacterial flora found in the ulcer and on the skin of the same patient.

## DISCUSSION

Healing of venous leg ulcers probably depends on a combination of factors. The most important treatment for ambulant venous leg ulcer patients is compression therapy, which all patients had in this study.

Opinions still differ concerning the importance of microorganisms in the healing process in venous leg ulcers without clinical infection. This might partly be explained by different selections of patients, ulcers and treatments or by the use of different microbiological procedures. The techniques for sampling and microbiological analysis are of great importance for the quality of the observations. The sampling method used in the present investigation was a variant of the pad method proved by several authors (19, 20, 24) to be effective and reproducible. The PVA discs, which replaced the pads in this investigation, were uniform in size and have a known, constant and high absorption capacity. Therefore, they allowed reproducible sampling even from relatively small ulcers. Since the suitability of the PVA foam discs for survival of bacterial cells was previously unknown, they were compared with cotton pellets. Charcoal-impregnated discs were found to be as good as cotton pellets and were therefore used throughout this study.

It has been claimed that counts from homogenised biopsies show the status of microbial colonisation more truly. As biopsies cause damage to the vulnerable tissue, a method which could sample without trauma at least some of the interstitial liquid from the tissue would be advantageous. It was thought that PVA, due to its high absorption, could have this property. The results of culture reported here support this.

An ulcer may easily and accidentally become colonised by microorganisms. In venous leg ulcers, there is no microbial flora that can be regarded as normal. The questions are: which microorganisms are found in venous leg ulcers without clinical signs of infection and do they affect ulcer healing? Halbert et al. (2) investigated 100 leg ulcers in 82 patients and found that colonised ulcers had larger initial size, longer duration and longer healing time than uncolonised ones. In his study, up to 17% of the ulcers had no microorganisms. In our study all ulcers were colonised with microorganisms. This difference could either be caused by differences in the patients or in the sampling/culturing techniques.

Colonisation converting into overt clinical infection (erysipelas or cellulitis) in a venous ulcer may delay healing (24). Such manifestations are erythema, swelling, heat, a feeling of tenderness to touch in the tissue surrounding the ulcer and fever. The well-recognised pathogen *Streptococcus pyogenes* (2, 15) is most commonly reported to be the cause of these infections but several other bacteria such as *S. aureus, Hemophilus influenzae, P. aeruginosa, A. calcoaceticus, S. epidermidis* and *Bacteroides fragilis* have sometimes been implicated (26). Clinical infec-

tions, not only with bacteria but also with fungi, have been reported to affect ulcer healing (27).

The question remains if any particular species of microorganism could influence the change of ulcer size without overt clinical signs of infection. Scraibman (15) isolated *S. pyogenes* from several clinically infected ulcers which increased in size. This species was found in only one ulcer in the present study. The very low frequency of betahemolytic streptococci probably depends on the selection of patients with ulcers without clinical symptoms of infection and does not seem to be a consequence of the culture media or technique used.

*S. aureus* is also a well-defined pathogen, e.g. in infected operation wounds, and is a frequently reported finding in venous leg ulcers (7, 28). In the present study, it was isolated from 63% (26/41) of the ulcers with decreasing size, 63% (5/8) of the ulcers with varied size and 89% (8/9) of the ulcers with increasing size. This is not contradictory to the results of other investigators (28). It is interesting to note that such a potential pathogenic microorganism as *S. aureus* is the most frequent one in leg ulcers and at the same time, when occurring in relatively low numbers, was not shown to markedly influence the ulcer size change. This clearly strengthens the impression that bacteria in this type of ulcer are only of secondary importance. Unknown biochemical properties of the strain, rather than the species, may make the difference.

The more prevalent finding of *P. aeruginosa* and *E. faecalis* on the first culture occasion in large ulcers might be interpreted in at least two ways. *P. aeruginosa* and *E. faecalis* might make ulcers large, or larger ulcers might become colonised by these bacteria more readily. The findings shown in Table III or in the multiple regression analysis, where all sampling occasions are included, did not show any association between the presence of these species and ulcer size change. The high frequency of colonisation with these bacteria in large ulcers without visible influence on healing is in accordance with Halbert et al. (2).

The reported microbiological results ought not to have been influenced by any change in the conditions for the ulcer patients caused by the study. The type of treatment, the place where the treatment took place etc, were the same as before the patient was taken into the study.

No relationship was found between healing and the quantities of bacteria in this study, which might be explained by the low quantity of colonising bacteria throughout the studied population of ulcers.

The venous leg ulcers without clinical signs of infection in this study were colonised by a microbial flora that was largely constant over time, regardless of whether the ulcer was increasing or decreasing in size. This is in agreement with several previous investigations (5–7). The wide range of resident species found in this study does not indicate nosocomial transfer but rather accidental initial inoculation and/or ulcer specificity. A poor nutritional state in a slowly increasing ulcer may be more favourable for the colonisation of certain types of microorganisms than for others. Metabolic products, toxins, enzymes, the specific and non-specific defence factors of the host (including age) and interactions between microorganisms might also influence colonisation (25). This may partly explain why ulcers are colonised by different microorganisms.

It has been found that leg ulcers generally heal with a rate where the logarithm of the ulcer area changes linearly with time (29, 30). The stepwise, multiple, linear regression analysis gives a linear equation for the prediction of ulcer size in the presence of different variables, e.g. microorganisms and age, etc. Our analysis did not identify any single microbial strain significantly affecting the change in ulcer size in our selected ulcers, which were free from commonly accepted signs of infection. This is in contrast to the findings of Halbert et al. (2), but in concordance with several other investigations (4–7). There is naturally a variability in healing rate for leg ulcers. The effect of all microbial strains together could explain at most one fifth of the variability of the healing rate. In this material, the age of the patient could not be seen to have any influence (n.s.) either.

We have no definite explanation for most of the variability of the ulcer size change, but different degrees of circulatory insufficiency are probably the cause.

The conclusion we draw from this study is that several different aerobic and anaerobic microbial species can colonise a venous leg ulcer and that once they are established many of them continue to colonise. As shown by many authors, and also by the present investigation, the ulcer flora is different and more abundant and harbours notably more species than that of the surrounding normal skin. If no overt clinical signs of infection are seen, there is no reason to suspect that any particular microbial species colonising the ulcer could markedly influence the healing process.

Furthermore, this study did not demonstrate any significant association between microbial quantities and change in ulcer size. We therefore suggest that microbial analysis as a rule is not indicated in patients with venous ulcers without clinical signs of infection.

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