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# Studies on Zinc in Wound Healing

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*To Margareta and Axel*

This thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Strömberg H-E, Ågren MS. Topical zinc oxide treatment improves arterial and venous leg ulcers. Br J Dermatol 1984; 111: 461-468.

II. Ågren MS, Strömberg H-E, Rindby A, Hallmans G. Selenium, zinc, iron and copper levels in serum of patients with arterial and venous leg ulcers. Acta Derm Venereol (Stockh) 1986; 66: 237-240.

III. Ågren MS, Chvapil M, Franzén L. Enhancement of re-epithelialization with zinc oxide in porcine partial-thickness wounds. J Surg Res 1991 (in press).

IV. Ågren MS, Söderberg T, Reuterving C-O, Tengrup I, Hallmans G. The effect of topical zinc oxide on bacterial growth and inflammation in full-thickness skin wounds on normal and diabetic rats. Submitted to Acta Chir Scand.

V. Ågren MS, Franzén L. Influence of zinc deficiency on breaking strength of 3-week- old skin incisions in the rat. Acta Chir Scand 1990 (in press).

VI. Ågren MS, Krusell M, Franzén L. Release and absorption of zinc from zinc oxide and zinc sulfate in open wounds. Submitted to Acta Derm Venereol (Stockh).

VII. Ågren MS. Percutaneous absorption of zinc from zinc oxide applied topically to intact skin in man. Dermatologica 1990; 180: 36-39.

# Abstract

Topical zinc is widely used in wound treatment although the beneficial effect of zinc has only been documented in zinc-deficient patients who were given zinc orally. The main purpose of this study was to investigate the effect of topically applied zinc on leg ulcer healing and examine its effect on some mechanisms in wound healing using standardized animal models. Additionally, absorption of zinc into wounds and intact skin treated topically with zinc was studied.

In a double-blind trial involving 37 leg ulcer patients with low serum zinc levels, topical zinc oxide promoted cleansing and re-epithelialization. Infections and deteriorations of ulcers were less common in zinc oxide treated patients.

Re-epithelialization, an important mechanism in the closure of leg ulcers, was enhanced with zinc oxide applied topically on partial-thickness wounds in pigs with normal zinc status. Zinc sulfate at three different concentrations did not, however, result in this beneficial effect on the resurfacing of wounds. The inflammatory reaction was diminished in zinc treated wounds except when a high zinc sulfate concentration was applied.

Bacterial growth and concomitant diseases such as diabetes can complicate wound healing. In normal rats, bacterial growth in full-thickness wounds was reduced with topical zinc oxide but not in hyperglycemic diabetic rats. The anti-bacterial mechanism of zinc oxide seemed to be more indirect and to be mediated via local defense systems rather than being directly toxic to the bacteria.

Healing of 21-day-old skin incisions was impaired in zinc deficiency, as measured by a significantly decreased wound breaking strength in zinc-deficient rats compared with that of pair-fed controls. The decreased breaking strength did not seem to be due to differences in collagen concentration of the wounds.

Zinc oxide was slowly but continuously solubilized when applied on open wounds in rats. On the other hand, with zinc sulfate, the zinc concentrations, either locally or systemically, did not maintain a constant level for the 48-hour post-operative treatment period as they did with zinc oxide.

Zinc absorption in and through normal human forearm skin was demonstrated after treatment with a zinc oxide medicated occlusive dressing by increased zinc levels in epidermis, interstitial fluid and dermis compared with the non-zinc control dressing.

In conclusion, topical zinc may stimulate leg ulcer healing by enhancing re-epithelialization, decreasing inflammation and bacterial growth. When zinc is applied on wounds it not only corrects a local zinc deficit but also acts pharmacologically. In wound treatment topical administration, with slow delivery over an extended period of time as with zinc oxide seems to be superior to rapid delivery of zinc ions as with zinc sulfate. No toxic or adverse effects were found with zinc oxide in wounds. Zinc also appears to play a role during the remodeling of wounds.

*Key words:* tissue repair, topical therapy, zinc oxide, zinc sulfate, leg ulcers, re-epithelialization, inflammation, bacterial growth, wound breaking strength, hydroxyproline, wound absorption, percutaneous absorption.

# Contents

# Introduction

That zinc is essential for living organisms was first reported for microorganisms in 1869, for plants in 1926, for rats in 1934, for pigs in 1955 and for man in 1961.[107]

When ionized, zinc exists only in the oxidation state of +2. Zinc is essential for the activity of several metalloenzymes, i.e. enzymes in which zinc is tightly bound to the active site and participates in the catalytic process.[134] Zinc can also render structural stability to enzymes.[134] To date there are at least 200 known zinc metalloenzymes involved in different biological processes, such as protein and nucleic acid synthesis or degradation, and carbohydrate and lipid metabolism.

Although the biological activity of zinc is commonly attributed to its role in enzymes, zinc is also important for the structure and function of biomembranes.[13]

## Zinc and wound healing

*Wound healing process*

Wound repair involves complex biological events which have been arbitrarily divided into three overlapping healing phases, viz. inflammatory (lasting 4-6 days after wound infliction), fibroplasia (from 3-5 to 14-21 days) and remodeling phase (from 14-21 days).[28,77,102]

Platelet aggregation, blood coagulation, and production of vasoactive and chemotactic substances take place first. Platelets are not only important for hemostasis but also release mitogenic substances. Mast cells secrete histamine which primarily changes vascular permeability. Polymorphonuclear leukocytes (PMNs) next migrate into the wounded area reaching a maximum number 1 to 2 days after wounding. The main function of PMNs is to eliminate contaminating bacteria from wounds. Macrophages, derived from blood monocytes, arrive to the wound simultaneously as PMNs but their number culminates later. Apart from phagocytosing bacteria, macrophages also aid PMNs in debridement and play a key role in the transition from the inflammatory into the fibroplasia phase. The role of lymphocytes in wound healing is still uncertain although they do produce lymphokines which can stimulate fibroblast proliferation and collagen synthesis.

During the fibroplasia phase granulation tissue is formed, i.e. a loose matrix containing macrophages, ingrowing blood vessels and fibroblasts and composed of fibrin, fibronectin, glucosaminoglycanes and collagen. The rate of collagen synthesis reaches its height between day 5 and 7.

Re-epithelialization starts within hours after the infliction of cutaneous wounds. The process begins with epidermal cell migration during the first two post-operative days and is followed by a burst in cell proliferation after 2 to 3 days. As there is no basement membrane the migrating epithelial cells are guided by a tempory matrix of fibrin and fibronectin. When the advancing epithelial cells meet at the wound center their mitosis ceases and they resume their pre-wound phenotype, a phenomenon known as contact inhibition.

Contraction is another important mechanism by which wounds are closed. This is believed to be accomplised by altered fibroblasts – the myofibroblasts.

During remodeling, the matrix tissue deposited is gradually changed. Although no net increase in collagen quantities occurs, the collagen deposited undergoes morphological and chemical changes, such as increased caliber, reorientation, transition from type III to type I collagen and formation of cross-links. These changes result in progressively increased wound strength.

*Zinc and cellular activities*

Since this study deals mainly with epithelial healing, inflammation and bacterial growth, this section will focus on the effects of zinc on epithelial cells, inflammatory cells and bacteria.

*Epithelial cells.* Zinc is important for the structure and function of chromatin,[46] and for enzymes linked with deoxyribonucleic acid synthesis.[106] It has been found that zinc is proliferative for epidermal cells in zinc-deficient rats.[123] There are, however, very few reports on the effect of supplementary zinc on epithelial cells.[14,82,83] Even though a slightly mitogenic effect of zinc (10% above control) was found at 5 μg/mL, zinc arrested the *proliferation* of epithelial cells above this level.[14,82] However, the anti-proliferative effect of zinc was less pronounced in the presence of serum and at higher pH of the medium.[83] The *migration* of epidermal cells from porcine 300 μm skin explants was enhanced by zinc oxide, but only in a suboptimal medium.[79]

*Inflammatory cells.* The acute inflammatory reaction after tissue injury is crucial to the reparative processes. Extensive studies mainly carried out by Chvapil et al[21,25-27] have shown that zinc is one factor that modulates the activity of inflammatory cells.

The aggregation of platelets and release of serotonin was inhibited by extra zinc.[27] Mast cells exposed to zinc *in vitro* (0.6-2.0 μg/mL) released less histamine the higher the zinc concentration.[88] Zinc was found to be a competitive antagonist of the $Ca^{2+}$ induced histamine release.[88] Zinc up to 5.5 μg/mL added to peripheral blood PMNs *in vitro* inhibited the oxygen consumption, phagocytosis and bacterial killing in relation to the zinc content of the cells.[26] However, these inhibitory effects of zinc were reversible, less pronounced in the presence of plasma in the incubation medium and seen only in activated cells.[21] Similar effects of zinc have been documented for macrophages.[25] For example, macrophages exposed to zinc chloride in concentrations up to 50 μg/mL did not show any ultrastructural changes when returned to the normal medium, indicating a non-toxic action of zinc. After the injection of mineral oil the mobilization of PMNs and macrophages to the peritoneal cavity was reduced in rats and guinea pigs with a two-fold increase in serum zinc level (≈2 μg/mL). In agreement with these findings, zinc supplementation inhibited the penetration of PMNs in a dose-dependent manner into a pleural inflammatory exudate in rats.[139]

The following mechanisms for the action of zinc on the membrane level (1-3) and intracellularly (4-6) have been proposed:[21] (1) formation of mercaptides with thiol groups; (2) inhibition of enzymes, e.g. ATPase; (3) masking of receptors; (4) inhibition of oxidation of NADPH; (5) interference with the contractile elements; and (6) inhibition of superoxide dismutase and glutathione peroxidase.

*Bacteria.* The acute inflammatory reaction will persist as long as bacteria are present in wounds.[28] Although zinc is necessary for normal growth of procaryotic and for eucaryotic cells, concentrations of zinc exceeding physiological zinc levels inhibit growth of most bacteria *in vitro*.[71,92,124,126,127]

Gram-positive bacteria seem to be more sensitive to $Zn^{2+}$ than are gram-negative bacteria.[92,126,127] For example, minimum inhibitory concentrations (MICs) of $Zn^{2+}$ on aerobic

bacteria isolated from human wound infections, or the urine from patients with urinary tract infections were determined.[127] Four susceptibility grades emerged from the study: 1 (MICs< 32-130 µg/mL): *Streptococcus* groups A, C, G; 2 (MICs< 130-260 µg/mL): *Staphylococcus aureus*, *Streptococcus* group B; 3 (MICs< 260-520 µg/mL): *Escherichia coli*, *Klebsiella* sp., *Enterobacter* sp.; and 4 (MICs< 520-2080 µg/mL): *Proteus* sp., *Pseudomonas aeruginosa*, *Enterococcus* sp. The same sensitivity pattern was found in strains isolated from full-thickness rat wounds.[127] Several possible mechanisms for the anti-bacterial action of zinc have been suggested, such as inactivation of enzyme systems and promotion of bacterial aggregation.[124]

Although the antiseptic property of zinc oxide is mentioned in most Pharmacopeia, its anti-bacterial effects *in vivo* have only been reported during the last decade.[65,127] However, the effect of zinc oxide *per se* was not ascertained in these *in vivo* investigations.

## Zinc metabolism in man

Zinc deficiency is assessed by clinical signs and by using different diagnostic methods.[107] The most common method is plasma or serum zinc determination. In serum, 30-40% of the zinc is tightly bound to alpha-2-macroglobulin and 50-60% is lightly bound to albumin;[53] the remainder is bound to amino acids and transferrin. However, serum or plasma zinc are influenced by stress (e.g. surgical trauma), infection, malignancies and major liver diseases and thus may not reflect the nutritional zinc status.[107]

*Surgical patients*

The decreased serum zinc level found after surgical trauma seems to be maximal 6 hours post-operatively returning to normal after 3-4 days.[131] The decline is larger the more extensive the surgery.[131] Although hypozincemia is accompanied by zincuria, zinc is mainly redistributed to the liver after operations.[62,111] Goldblum et al [54] have suggested interleukin-1 as partly responsible for the accumulation of zinc in the liver most likely due to the induction of metallothionein synthesis. Metallothioneins are cystein-rich proteins which play an important role in maintaining homeostasis and in binding excess free zinc.[110]

Surgical patients with impaired healing capacity had lower zinc concentrations in granulation tissue than patients with adequate healing capacity.[72] Furthermore, zinc concentrations are higher in the wound tissue than in adjacent unwounded skin.[72]

*Leg ulcer patients*

Most leg ulcers are caused by impaired circulation which prevents adequate supply of nutrients and oxygen. Therefore, leg ulcers heal slowly and when healed they often recur. Ulcers caused by venous insufficiency should primarily be treated by compression of the affected limb, whilst ulcers of arterial origin should, if possible, be surgically intervened.[67] In 1980 about 0.3% of the population of Göteborg, Sweden had ulceration of the lower leg.[67] Forty per cent were due to venous insufficiency, 21% were of arterial origin and the rest were caused by a combination of the two etiological factors.[67] These epidemiological figures are in the same range as those in Great Britain and the USA.[113]

Decreased retention and shorter biological half-life was seen in leg ulcer patiens given an oral $^{65}Zn$ dose.[70] Theoretically this impaired zinc metabolism would eventually lead to depleted zinc stores. It has also been found that leg ulcer patients often have reduced

serum zinc concentrations.[30,31,40,63,101,118] However, uninvolved skin or cells have not been found to be depleted of zinc[30,31,40,101] although the zinc concentration in ulcerated tissue has not been reported.

## Zinc treatment

*Oral*

The positive results of zinc supplementation on the healing of excised rat wounds reported by Strain et al[105] as early as 1953 have subsequently been re-evaluated. Except for the result of one study,[80] oral or parenteral zinc proved effective only when the animals were zinc-deficient.[42,81] Clinically, the fall in serum zinc after trauma can be reduced with general zinc supplementation.[61] The benefit of oral zinc supplementation on wound healing was first reported in connection with excision of pilonidal sinuses.[104] More recent studies though, have mainly concerned healing of venous leg ulcers and not surgically inflicted wounds.

Several double-blind studies have been reported on the effect on leg ulcer healing of zinc sulfate given as tablets or capsules in a dose of 220 mg three times a day in combination with meals.[29,49,56,59,63,74,103,120,142] However, some results were difficult to interpret due to lack of serum zinc measurements, ulcer area differences and inadequate healing-rate measurements.[60] Zinc was found to be effective in the two earliest controlled studies.[74,120] Hallböök and Lanner[63] subdivided their 27 out-patients into those with low serum zinc levels and those with normal levels, and by this stratification found that oral zinc supplementation was beneficial only in the patient group with low initial serum zinc levels. Haeger and Lanner[58] confirmed these results in a controlled, albeit neither prospective nor double-blind, trial on patients with arterial leg ulcers. Results were, however, negative for Ølholm Larsen et al,[142] Phillips et al[103] and Floersheim[49] despite the fact that they followed the same stratification procedure as Hallböök and Lanner.[63] However, Ølholm Larsen et al[142] maintained their zinc therapy for only 6 weeks which is thought to be too a short period for the oral zinc supplementation to be effective.[1,63]

Nevertheless, the results from the controlled trials seem to favor the conclusion that oral zinc sulfate is a valuble adjunct in the treatment of leg ulcers, but only for patients with subnormal serum zinc levels.

*Topical*

Zinc sulfate may be used topically in lotions (1.5% w/v or 3.4 mg Zinc/mL) to promote granulation of indolent ulcers, and in aqueous solutions (0.25% or 0.57 mg Zinc/mL) to relieve chronic inflammation in conjunctivitis.[17] Topical zinc sulfate restored the healing of open wounds in malnourished children with low serum zinc levels.[55]

Zinc oxide is used mostly in ointments, pastes and lotions, for various skin disorders because of its protective, astringent and antiseptic properties.[8,17,32] In the treatment of ulceration of the lower leg, zinc oxide alone or with other substances is usually incorporated into paste bandages[17,43,68,113] but can also be used in adhesive tapes.[16,52] However, the clinical trials performed with these zinc oxide dressings were not designed to investigate the effect of zinc oxide specifically but rather to study the dressings in ordinary clinical practice.[16,43,113]

On the other hand, the effect of topical zinc on wound healing has been performed in a controlled and standardized manner in rats and guinea pigs. As seen in Table I below,

only one study demonstrated a beneficial effect of topical zinc oxide on wound healing and that was a transient effect.[66] The only experimental study on the effects of zinc sulfate reported a promotion of early granulation tissue formation.[97] Thus, the use of zinc oxide and zinc sulfate seems to be based on emperical rather than on scientific experience.

Table I. *Vehicle-controlled experimental studies on the effect of topical zinc oxide (ZnO) and zinc sulfate ($ZnSO_4 \cdot 7H_2O$) on wound healing*

| Investigation | Species | Zinc application | Results |
|---|---|---|---|
| Murray and Rosenthal[94] | Wistar male rats (average body weight 243 g) | ZnO (30 mg) applied as a single dose onto a musculofascial sutured wound | No difference in wound breaking strength on the 7th post-operative day between experimentals and controls |
| Norman et al[98] | Lister rats (250-300 g) and English guinea pigs (400-500 g) | ZnO (1% in petrolatum) applied every second day on one 1 $cm^2$ square full-thickness skin excision on each animal | Time to 10, 50 and 90% healing of the wounds did not differ between zinc and control treated animals irrespective of species |
| Williams et al[138] | Sprague-Dawley male rats (350 g) | ZnO (15 mg powder) applied twice daily to a caudad or a cephalad circular 4 $cm^2$ full-thickness skin excision | Healing rate of the wounds did not differ between experimentals and controls over a 21-day treatment period |
| Hallmans and Lasek[66] | Sprague-Dawley male rats (initial body weight 96 g) given zinc-deficient or zinc-sufficient diets | ZnO (1% in a polyacrylic-based adhesive) applied every second day on circlar 4 cm full-thickness skin excisions | Wound area reduction over a 12-day treatment period was enhanced by ZnO in both zinc-deficient and zinc-sufficient animals |
| Niedner et al[97] | Pirbright guinea pigs | $ZnSO_4 \cdot 7H_2O$ (0.5% in polyacrylamide-agar-gel) applied daily to full-thickness skin excisions | Amount of granulation tissue formed was doubled in the zinc group compared with controls after 7 days of treatment |

## Chemical properties of zinc oxide and zinc sulfate

Zinc oxide (ZnO, molecular weight 81.37) exists as colorless hexagonal crystals or as a white, odorless powder.[95] Zinc oxide is almost insoluble in pure water, about 4-6 µg/mL at 25°C.[35] It is amphoteric and the solubility product for zinc oxide in 1 M KOH and 1 M NaOH was recently determined to $2.2 \times 10^{-17}$ at 25°C.[36] Zinc oxide in water gives rise to an alkaline pH. Due to the presence of zinc binding ligands the solubility is increased in human plasma buffered with Tris(hydroxymethyl)-aminomethane (pH 7.4) to about 80 µg/mL at 37°C.[50]

Zinc sulfate ($ZnSO_4 \cdot 7H_2O$, molecular weight 287.56) is colorless, odorless, efflorescent and highly soluble in water (965 mg/mL). Aqueous solutions of zinc sulfate have an acidic pH.

## Zinc absorption

It has been estimated that about 98% of all the zinc in the body (2 g in the human adult) is found intracellularly.[119] The zinc uptake processes have mostly been studied in enterocytes and relatively little information is available on the uptake mechanisms in epithelial and connective tissue cells. However, a recent paper focused on the uptake of zinc by human skin fibroblasts in a medium containing 10% serum.[2] The uptake was rapid during the initial 10 min period which corresponded to the binding of zinc on the cell surface and was followed by a slower linear phase which possibly reflected internalization. The same biphasic uptake pattern has previously been reported for PMNs.[26,75] Futhermore, the uptake was reported to be carrier-mediated and saturable.[2]

*Wound*

Hallmans[64] made a thourough study of the absorption of zinc through open rat skin wounds treated topically with different zinc compounds. Absorption was demonstrated into blood and body from zinc oxide (alone, in petrolatum (40%) and incorporated into an adhesive mass), from zinc sulfate aqueous solutions and from activated zinc peroxide powder in glycerin.

It was also found that with different concentrations of a zinc ion solution the absorption was dose-dependent.

Furthermore, the distribution of the absorbed zinc in the body from zinc oxide did not differ from that of zinc administered parenterally in ionized form.

*Intact skin*

When $^{65}$Zn-ZnO was applied on intact rat skin, the $^{65}$Zn activity in blood was 1/100 of that registered when $^{65}$Zn-ZnO was applied on the same surface area of excised wounds indicating a slower absorption rate of zinc through intact skin.[64] Skin penetration of zinc in hairless mice has also been shown from zinc oxide in petrolatum (20%) applied twice daily, measured quantitatively but indirectly by induction of metallothionein in the skin.[51] The penetration was, however, less than that of topically applied synthetic corticosteriods.[51]

The permeability of human skin is generally lower than that of rodents. Percutaneous absorption of zinc in man has previously been studied by measuring the serum zinc level before and after topical treatment with zinc oxide of extensive skin areas. For example, zinc oxide (40%) in petrolatum applied on more than 50% of the total body surface area of normal skin did not result in an increase of the serum zinc level after 3 hours of treatment.[33] Using the same technique Morgan et al[93] also failed to show a percutaneous zinc absorption from zinc oxide applied on extensive areas of psoriatic skin. Thus, there is a lack of data to support that zinc from topically applied zinc oxide can penetrate human skin.

# Aims of the present study

The overall objective of the study was to see whether topically applied zinc improves healing of chronic wounds in humans and to examine its mode of action on some important mechanisms in the healing by using established animal wound models. Zinc absorption in wounds and intact skin were also investigated after topical zinc application. Thus, the following effects were studied:

- topically applied zinc oxide on the healing of leg ulcers and ulceration of the lower leg on the serum zinc level (I, II),
- topically applied zinc in two chemical forms, zinc oxide and zinc sulfate, on re-epithelialization of partial-thickness wounds and on inflammatory response in underlying dermis in normal pigs (III),
- topical zinc oxide on bacterial growth and inflammatory response in excised skin wounds on normal and diabetic rats (IV),
- zinc deficiency on wound breaking strength of 3-week-old skin incisions in rats (V), and
- topical zinc on zinc absorption in excised rat wounds and in intact human skin (VI, VII).

# Materials and methods

## Zinc dressings

Dressings A-E were prepared by impregnating a gauze web (Ph Eur) with zinc oxide (Ph Eur, Rånäs Bruk, Rimbo, Sweden) suspensions or zinc sulfate heptahydrate (Ph Eur, Merck) solutions.[7] The gauze web was fed continuously through a press nip of a pair of rolls, to which the propeller-stirred suspensions or solutions were supplied. As the web passed through the nip, the zinc oxide particles were pressed into the structure of the gauze. The suspensions and solutions also contained polyvinyl pyrrolidone (PVP, USP, Kollidon® 90, BASF), which was added in order to bind the zinc oxide particles to the fibres. After being dried in an oven, the web was converted into dressings (Fig. 1). The PVP content of the dressings was 5-40 mg/g.

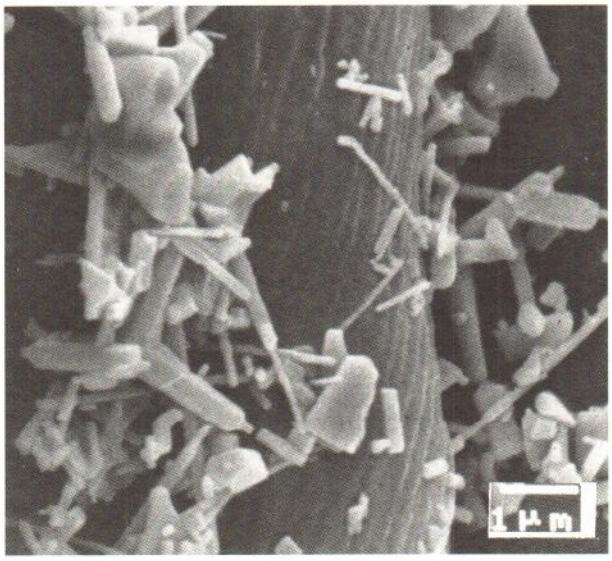


*Fig. 1.* Scanning electron micrograph of ZnO particles adhering to a gauze fiber (marked with a star) in dressing A. The particles were identified as zinc with energy dispersive X-ray fluorescence. Particles were not seen in gauze impregnated only with PVP (not shown). Note the differences in morphology of the ZnO particles. X10,000; Bar= 1 µm.

For dressings F and G a bovine derived collagen cross-linked sponge (C. Freudenberg, FRG) was impregnated manually with a zinc oxide suspension containing PVP, and with a zinc sulfate solution containing PVP.

Portuguese gum rosin (Ph Nord, SOCER, Lisbon, Portugal), natural rubber (Ph Nord) and white mineral oil (Ph Eur) were dissolved in n-heptane and mixed with zinc oxide. The adhesive was laminated to PVC-coated cotton fabric to make the adhesive, occlusive dressing H.

Dressings were ethylene dioxide sterilized and composed according to Table II below.

Table II. *Composition of the dressings used in the different studies. Each dressing will henceforth be referred to by a capital letter*

| Dressing | Zinc | Zinc content | | Vehicle | Study |
|---|---|---|---|---|---|
| | | $\mu g/cm^2$ | mg/g | | |
| A | ZnO | 250 | 14 | Gauze+PVP | I, III, IV, VI |
| B | ZnO | 1000 | 56 | Gauze+PVP | IV |
| C | $ZnSO_4$ | 6.6 | 0.3 | Gauze+PVP | III |
| D | $ZnSO_4$ | 65 | 3.0 | Gauze+PVP | III, VI |
| E | $ZnSO_4$ | 620 | 27 | Gauze+PVP | III |
| F | ZnO | 510 | 26 | Collagen sponge+PVP | III |
| G | $ZnSO_4$ | 130 | 6.0 | Collagen sponge+PVP | III |
| H | ZnO | 2700 | 250 | Gum rosin, rubber, mineral oil | VII |

Dressings A-E and H were manufactured by Mölnlycke Health Care AB, Mölnlycke, Sweden.

## Zinc analyses

These were performed with flame atomic absorption spectrophotometry (AAS) with background correction (Perkin-Elmer). The sample, which must be in a liquid form, is atomized in an acetylene/air flame. A photomultiplicator is used to register the absorption of a light beam of a wavelength specific for the element (213.9 nm for zinc) in the flame through which it passes. The concentration is proportional to the absorption.

For the analysis the different samples in the study were prepared as follows: *serum and wound fluid* were diluted at least 10 times with deionized water or with 1% nitric acid (v/v) and then aspirated directly into the flame; *skin (whole, epidermis and dermis), wound tissue and liver* were dry ashed at 450°C for 16 hours and the ash subsequently dissolved in 0.6 M nitric acid; and *dressings and diets* were wet ashed in 6 M nitric acid at 130° C for 30 min.

All glassware were acid washed.

Reference material (Bovine liver 1577a, National Bureau of Standards, Washington, DC, USA) with certified zinc content was run in parallel with, and identically to the tissue samples to establish the accuracy of the analyses.

The variability of the measurements was estimated from time to time to less than 2% and of duplicate samples to 1.4%.

## Wound healing (I-VI)

These studies were performed on patients with ulcerations of the lower leg (I, II), on pigs with partial-thickness skin wounds (III), on normal (IV, VI) and diabetic (IV) rats with full-thickness skin excisional wounds, and on zinc-deficient rats with full-thickness skin incisions (V).

*Healing of leg ulcers (I, II)*
The effect of zinc oxide (A) applied topically was compared with compresses containing only the binder, PVP in a double-blind trial.

Thirty-seven patients (median age 78 years) were randomized to zinc oxide or placebo treatment from the order in which they came to the clinic. Diagnosis was made from clinical signs and using the Doppler technique. Nineteen of the patients (14 women) had arterial and 18 (14 women) venous leg ulcers. The ulcer area was determined planimetrically from tracings of the ulcer outline on plastic foil. This method is more accurate than area determinations from photographs.[133] Ulcers were limited to areas measuring between 0.5 and 100 $cm^2$.

After a treatment period of eight weeks, two physicians working independently assessed the effect of the treatment basing their judgement on the appearance of the ulcers and on the percentage ulcer area change. For the result to be recorded as "successful" (criteria determined *before* the trial start): (1) there had to be visible granulations in the ulcer after treatment, or visible granulations had to be present before treatment, the initial ulcer area had to be reduced by 25% and by 50% for arterial and venous ulcers, respectively; and (2) the ulcer had to be free of slough. The result was judged "unsuccessful" if the initial ulcer area increased by 50% or more, if antibiotics were needed or if criteria (1) and (2) were not fulfilled. It was also noted whether the ulcers were completely healed or not after 12 weeks for the patients who completed the assigned treatment.

The plasma zinc levels were measured at entrance at the hospital laboratory. However, at the end of the trial it was revealed that those values were unreliable. A comparison of parallel venous blood samples (n=34) with AAS at the hospital laboratory and at another laboratory[64] showed that the plasma zinc levels were significantly ($p<0.001$, paired $t$-test) higher (1.00 ± 0.14 µg/ml, mean ± SD) than the serum zinc values (0.66 ± 0.12 µg/ml). We compared these serum zinc values with those of a control group made up of 40 patients suffering from dementia (median age 80 years) but without leg ulcer history. The serum albumin concentration was also determined for all patients.[53]

*Animal investigations (III, IV, V, VI)*
*Re-epithelialization (III)* was assessed morphometrically using a pig-wound model.[22] For example, the effects of epidermal growth factor and shock waves on re-epithelialization have been documented with this model.[23,69] Pigs were chosen mainly because of the structural and functional similarities of pig skin to that of humans.

The female Yorkshire piglets were given a zinc-adequate diet (55 mg zinc/kg) to ascertain normal nutritional zinc status of the animals. Sixteen or 24 wounds (2.2 cm x 2.2 cm) were inflicted on the backs with an electrokeratome set to a cutting depth of 400 µm. This procedure removes the entire epidermis with hair follicles, sebaceous and sweat glands

remaining in the wound bed as an additional source of epithelial cells apart from the wound edges.[22] The area of the wounds corresponded to about 1.5% of the total body surface area.

Zinc oxide (A) and zinc sulfate (C-E) in gauze as well as in the collagen sponge (F, G) were applied on the wounds on each pig in single doses for 48 and 64 hours. The dose-dependency between topical zinc and re-epithelialization was examined by using three concentrations of zinc sulfate in gauze (C-E). Dressings were moistened with saline (0.9% NaCl w/v) and covered with a semipermeable adhesive polyurethane film (Tegaderm®, 3M). This film is atoxic towards keratinocytes in culture.[112]

The percentage coverage of at least one cell-layer thick epithelium was evaluated in a blinded fashion for 8 histological sections from each wound at a total magnification of 63 times.

*Inflammatory response (III, IV, VI)* was estimated after topical zinc application (A, C-G) in dermis 48 and 64 hours after the infliction of the porcine wounds (III), and in granulation tissue after the skin excisions in rats 24, 48 (A, D) and 96 (A, B) hours post-operatively (IV, VI). It was assessed as the infiltration of inflammatory cells by light microscopy on a semi-quantitative (III, VI) or strict quantitative basis (IV),[109] or indirectly by measuring alkaline phosphatase,[137] a marker enzyme for PMNs (IV).

*Bacterial growth (IV)* was studied in early granulation tissue of normal rats and hyperglycemic diabetic Wistar female rats. Diabetes was induced by a single intravenous injection of alloxan monohydrate.[130] The rats were not given insulin and were operated on 20 days after the induction of diabetes. Two concentrations of zinc oxide (A, B) were applied on the wounds on the backs of rats during the 4-day-post-operative period with an intermediate dressing change 2 days post-operatively. Dressings were moistened with saline after dressing applications.

Biopsies from the wounds were weighed and homogenized in broth. The homogenate was plated on agar and the number of colony-forming units counted.[127]

*Breaking strength (V)* was measured of 3-week-old skin incisions in male Sprague-Dawley rats given a zinc-deficient diet (1.4 mg/kg) or in rats given a zinc-adequate diet (33 mg/kg) of the same amount as that consumed by the zinc-deficient rats 2 weeks pre-operatively. The maximal strength of a 0.5-cm wide wound strip cut perpendicular to the wound alignment was measured using a materials testing machine (Instron®). The hydroxyproline (as a measure of collagen) and zinc concentration of the wound tissue were determined.

## Zinc absorption (VI, VII)

*Wound (VI)*

Rats were chosen as the experimental animals for these studies because relatively large wounds can be inflicted on these animals without severely affecting their general health and thus enabling recording of zinc absorption through the wounds without the need of radiolabelled zinc. Furthermore, most of the information available about zinc absorption from wounds has been derived with the rat.[64]

Two circular wounds with a total area of 22 $cm^2$ were made on the back of male Sprague-Dawley rats. According to Diack´s formula[34] this wound area corresponds to about 8% of the total body surface area. The absorption from zinc oxide (A) or zinc sulfate (D) in gauze moistened with saline and covered with Tegaderm® was compared. The control group was treated with the non-zinc containing gauze vehicle.

Wounds were treated for 4, 24 and 48 hours, and then the zinc concentration was determined in wound fluid, wound tissue, serum and liver. The protein concentration of wound fluid was also determined.

*Intact skin (VII)*

Percutaneous absorption was investigated in normal human forearm skin treated with zinc oxide in an occlusive, adhesive dressing (H) and with a control dressing without added zinc oxide. The use of this vehicle for zinc oxide was selected for the following reasons: (1) the drug is restricted to a defined and delimited area; (2) the drug is protected from external disturbances; (3) a controlled skin humidity is ascertained; and (4) the occlusion (70% reduction of the normal transepidermal water loss) provided probably enhances the absorption.

The local zinc concentrations were determined after 48 hours of treatment in 5 healthy volunteers (median age 33 years, 4 men). For this purpose, suction blisters were raised by applying a negative pressure of 250 mm Hg for about 2 hours on the treated skin area which enabled zinc analysis of epidermis, blister fluid (interstitial fluid) and dermis. Histological examination of the blistering technique confirms that epidermis separates from dermis at the dermoepidermal junction (Fig. 2).

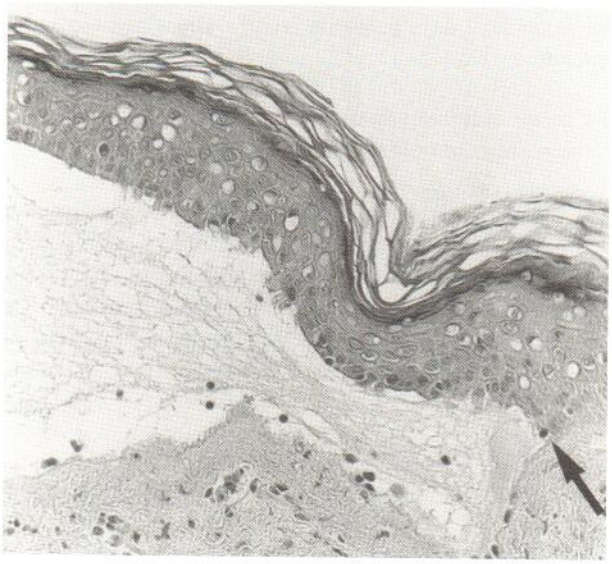

*Fig. 2.* The edge of a blister formed on skin treated with a zinc oxide occlusive dressing (H). The site of separation between dermis and epidermis is marked with an arrow. The blister fluid is rich in fibrin but essentially free of inflammatory cells. Hematoxylin-eosin .X400.

In another study, the permeability for the arm skin to four different molecules was found to be lower than that for abdominal, postauricular and forehead skin.[85]

## Statistical tests

The following statistical tests were used: Sequential analysis (I), Student´s *t*-test (II, III, V, VII), Duncan´s multiple range statistics (III) and Wilcoxon rank sum test (IV).

# Results

## Clinical investigations (I, II)

In this investigation the effect of topical zinc oxide on leg ulcer healing was assessed. By the predefined assessment criteria the success-rate was significantly higher ($p<0.05$) after 8 weeks for the zinc treated group (83%) than for the placebo treated group (42%). In addition, 11 of the 18 ulcers treated with zinc oxide had healed completely after 12 weeks compared with only 4 of the 19 placebo treated ulcers.

Due to ulcer infections the treatment was discontinued in six of the placebo treated patients but in only one of the zinc treated patients.

It was found that the leg ulcer patients had a significantly ($p<0.001$) reduced mean serum zinc level (0.66 µg/mL), about 35% lower than an age-matched control group without leg ulcers. The reduced serum zinc level was not attributed to a decreased albumin concentration since no difference was found between the two groups concerning serum albumin concentration. Although no correlation between serum zinc and healing was found, the two patients with the lowest serum zinc levels (0.52 and 0.38 µg/mL, respectively) seemed to respond better to topical zinc oxide than the other zinc oxide treated patients (87% and 100% ulcer area decrease after 8 weeks of treatment, respectively). Both patients had had their arterial ulcers for more than two years and had not responded to local treatments previously.

## Animal investigations (III-VI)

*Re-epithelialization (III)*
Re-epithelialization was enhanced by including zinc oxide both in the gauze (A) and in the collagen sponge (F) vehicles by more than 30% compared with corresponding non-zinc containing vehicles. However, zinc in the form of zinc sulfate produced no measurably beneficial effects for either concentration or vehicle used. In contrast, re-epithelialization was slightly and profoundly retarded with the lowest (C) and highest zinc sulfate concentration (E), respectively (Fig. 3).

Re-epithelialization was also significantly ($p<0.05$) enhanced by incorporating zinc oxide in an adhesive of an occlusive dressing (H) compared with gauze (98% versus 68% at 64 hours).

*Inflammatory response (III, IV, VI)*
Inflammatory response was less pronounced with zinc in either chemical form or vehicle used in the dermis underlying the partial-thickness wounds in the pigs. However, a more pronounced inflammatory reaction was seen when the highest concentration of zinc sulfate was used.

In granulation tissue the infiltration of inflammatory cells was slightly more pronounced in full-thickness rat wounds treated with zinc (A, B, D) than in non-zinc treated wounds. Biochemically, zinc oxide treatement (B) reduced the alkaline phosphatase activity, in-

dicating reduced activity of PMNs.

No foreign body reactions to zinc oxide were observed in the wounds (III, IV, VI).

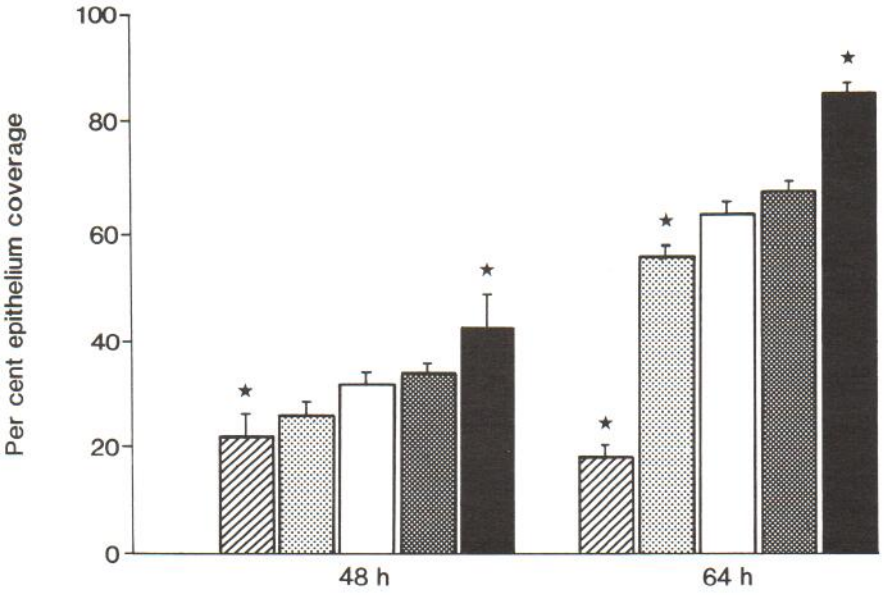


*Fig. 3.* Effect of two forms of zinc on re-epithelialization of partial-thickness wounds in pigs after 48 and 64 hours (h) of treatment.
$ZnSO_4$: E ( ▨ ); $ZnSO_4$: C ( ▩ ); Control ( □ ); $ZnSO_4$: D ( ■ ); ZnO: A ( ■ ).
Mean ± SEM. *$p<0.05$ compared with control at each point in time.

*Bacterial growth (IV)*
It was found that bacterial growth in full-thickness skin excisions was inhibited with zinc oxide (A, B) in normal rats. The bacterial count was lowered by 1 log unit with the lowest zinc oxide concentration (A) and by 2 log units with the highest zinc oxide concentration (B) compared with that of non-zinc treated wounds. A dose-response relation was also found *in vitro* when the two concentrations of zinc oxide were tested against *S. aureus*. However, in the diabetic rats no significant difference regarding colony-forming units/g granulation tissue was found between zinc oxide and control-treated animals.

*S. aureus* was cultured from 62% (18/29) of control-treated wounds compared with 30% (9/30) for the zinc oxide treated wounds. *Enterococcus* sp. was most common in zinc oxide treated wounds, occurring in 83% (25/30) of these wounds. The corresponding figure for the control-treated wounds was 24% (7/29).

*Healing of incisional wounds (V)*
Healing was impaired in zinc-deficient rats compared with pair-fed controls, as was evident from the significantly ($p<0.01$) decreased 3-week-post-operative wound breaking strength, 554 ± 148 for the zinc-deficient and 720 ± 204 g/0.5 cm for the pair-fed control group. The zinc concentration in wound tissue was significantly lower in the zinc-deficient group, whereas no difference was found between the two groups regarding the hydroxyproline concentration of the wounds.

## Zinc absorption (VI, VII)

*Wound (VI)*

The zinc delivery over time after a single application of zinc oxide (A) and zinc sulfate (D) was compared in freshly excised wounds in rats. With the zinc sulfate dressing the zinc concentration in the wound fluid decreased during the 48 hour experimental period with the sharpest decline occurring within the first 4 post-operative hours. In the zinc oxide treated group the wound fluid zinc remained fairly constant, although it increased to about 55 µg/mL in the 48th post-operative hour, due probably to increasing protein concentration in the wound fluid.

Wound tissue zinc concentration was 4-5 times higher than in controls after a single zinc oxide application (≈40 µg/g wet tissue) over the 2 day post-operative period. With a single zinc sulfate application, on the other hand, the zinc concentration in the wound tissue increased 30 times on the first post-operative day but it was only about 7 times higher than in control-treated wounds on day 2.

The changes of the zinc concentration of the serum followed essentially the same kinetic pattern over time as that of the wound fluid zinc levels.

*Percutaneous (VII)*

Zinc concentrations increased in blister dome (epidermis) and blister fluid (interstitial fluid) after 48 hours of treatment of normal human skin with zinc oxide in an occlusive dressing (H) . However, during the formation of the blisters the zinc penetration increased which indicates that the barrier function was less effective. Therefore, the procedure was modified to include 10 pre-blistering tape strippings to remove superficial solubilized and unsolubilized zinc oxide. After the tape strippings the zinc levels were still higher after zinc oxide treatment than after control treatment in epidermis (130 versus 50 µg/g) and in blister fluid (0.55 versus 0.30 µg/mL), and also in 3 mm deep dermal biopsies (24 versus 15 µg/g) taken in the bottom of the blisters.

After these modifications, the use of suction blisters for the study of percutaneous absorption satisfies most requirements of a valid model.[117]

# Discussion

Zinc oxide has been considered to be biologically inert, due probably to its limited water-solubility. However, zinc oxide is widely used in formulations intended for external use on skin and wounds and there is recent evidence indicating that zinc oxide plays a pharmacologically role when applied on wounds.[64,66,125,127]

For many years the standard local wound treatment has been gauze dressings but synthetic and occlusive dressings have become more interesting of late.[45] It has been shown in animals and man that occlusive dressings increase re-epithelialization of excised partial-thickness wounds as compared with gauze dressings possibly by preventing dehydration of wounds.[5,87,109] The main disadvantage of occlusive dressings is the risk of enhancing bacterial growth,[10,127] which might lead to an infection that delays wound healing.

Although these newer dressings are claimed to be advantageous also in the treatment of leg ulcers, unambiguous clinical evidence of their efficacy is lacking.[45] For example, Rubin et al[113] demonstrated higher healing rate (95%) with a zinc oxide medicated bandage (Unna´s boot) than with a polyurethane foam dressing (41%), using uniform compressive treatment in the two treatment groups. Although the efficacy was evaluated blindly the effect of the zinc oxide itself was not ascertained since Unna´s boot also contains glycerin and a small amount of ferric oxide.[113]

In order to enable a double-blind evaluation of a topical agent, the active compound must be masked in the vehicle. Gauze was found to fulfill this criterium since compresses impregnated with zinc oxide could not visually be distinguished from placebo compresses impregnated only with the binder and thus it was used in study I.

It was shown that topical zinc oxide improved healing compared with placebo in the treatment of arterial and venous leg ulcers as judged from loss of slough, granulation and re-epithelialization (I). It is thought that a prerequisite for the effectiveness of zinc supplementation, at least per os, is that the patients have low serum-zinc levels.[60,63] Eighty-five per cent of the patients in study I had serum zinc levels below the normal range (<0.77 µg/mL) and an average serum zinc level which was significantly lower than an age-matched control group without leg ulcers (I, II). The reduced serum zinc was not attributed to a concomitant hypoalbuminemia as was earlier suggested as being the cause for low serum zinc levels in geriatric patients.[136]

No correlation between the serum zinc level and ulcer healing was found, but possibly due to the advanced age of the patients their overall healing ability was impaired.[41] Based on these findings it cannot be concluded whether zinc oxide applied topically is also beneficial for patients with normal serum zinc levels. In an attempt to elucidate the action of zinc in wound healing re-epithelialization, inflammation, bacterial growth and remodeling were studied using standardized animal wound-healing models. As zinc is necessary for many enzymes and present in cell membranes it is involved in many different biochemical reactions. Thus, its mode of action in the wound healing process is probably multifactorial.

In study III, re-epithelialization of porcine partial-thickness wounds was assessed morphometrically.[22] These wounds resemble the human donor site wounds. Zinc oxide (A, F) applied topically in single doses on the wounds enhanced re-epithelialization. In contrast,

none of the three zinc sulfate concentrations were effective, and in fact re-epithelialization was retarded with the lowest and with the highest zinc sulfate concentrations. These latter findings could be explained by the general dose-response behavior of metals.[134] Concentrations higher than those required for normal function, but lower or higher than pharmacological concentrations, hamper normal activity.[134] Thus to achieve the desired effect the dosing of zinc is critical.

It is possible that the rapid cell division in wounds is connected to an increased need for zinc and that this need of extra zinc is satisfied by zinc administered on the wound site. Due to the fact that zinc is necessary for cellular proliferation,[19,106] it is tempting to speculate that zinc stimulates the proliferation of epidermal cells in the wounds only when delivered slowly over an extended period of time as was the case with zinc oxide.

In another study the increased re-epithelialization of partial-thickness wounds with a permeable dressing, as compared with a semipermeable dressing, was attributed to the presence of a fibrin clot layer on the wounds.[76] Although *in vitro* studies indicate that zinc ions can promote the formation of fibrin clots[89] and inactivate plasmin,[78] it is unknown whether these effects of zinc are of significance for the migration of epithelial cells in wounds.

Due to the membrane-stabilizing/protective effect of zinc it is also possible that cell turnover was reduced by an inhibited release and effect of toxic metabolites in the wounds.[11,13,21,48,126]

Although zinc sulfate in water yields an acidic pH, in study III the pH of zinc sulfate (C-E) and zinc oxide (A) dressings moistened with saline *increased* slightly compared with the control dressing without zinc. Although theoretically, application of the zinc dressings would then, at least initially, increase the pH of the wounds compared with the control dressing, the pH would most likely return to normal shortly after application. Moreover, re-epithelialization increased when the pH of wounds was reduced below 7.4.[122] However, the effect of the sulfate anion itself is unknown, although the effects of zinc in cell culture systems were independent of the counter-ion (acetate, chloride, sulfate) used.[48,114]

The discrepancy between our topical zinc oxide results and those of other investigators who found that topical zinc oxide was ineffective on tensile strength, wound contraction and time to complete healing of non-zinc-deficient skin wounds might be ascribed to factors such as experimental models, dosage and differences in healing characteristics between species (Table I). Norman et al[98] used relatively small full-thickness excisional wounds (1 $cm^2$) which heal quickly and are difficult to measure accurately. In the study by Williams et al,[138] one wound was treated with zinc oxide and the other on the same animal remained untreated. Söderberg and Hallmans[125] showed that systemically absorbed zinc from a zinc oxide treated wound increased the healing rate of the contralateral control wound. Williams et al[138] applied zinc oxide twice daily at a higher dosage than in study III. In addition, the above mentioned investigations involved full-thickness rodent wounds which heal primarily by contraction and to a lesser extent by re-epithelialization. For example, the zinc oxide dressing (A) used here did not enhance the closure rate of 5 $cm^2$ full-thickness wounds on rats.[128]

Zinc influences many of the factors that cause tissue inflammation.[99] *In vivo*, zinc administered subcutaneously near an established skin inflammation was found to diminish the infiltration of leukocytes in rabbits.[57] Zinc topically administered to partial-thickness

wounds on pigs was associated with a less pronounced inflammatory reaction in the underlying dermis (III). Thus these findings could be ascribed to the inhibitory effects of zinc on the migratory abilities of PMNs and macrophages.[18,21,139] However, unlike nonsteroidal anti-inflammatory agents e.g. indomethacin zinc does not seem to inhibit prostaglandin synthesis.[9,20] When an established model for inflammation is used, both zinc and indomethacin administration resulted in less edema of the paw than controls.[121] However, with a high zinc ion concentration, initially about 6000 µg/mL, a more pronounced inflammatory reaction was seen in the porcine wounds, possibly caused by toxicity.[64]

Although the dermal component affects re-epithelialization[6] the anti-inflammatory effect of zinc in dermis was probably not the primary cause of the enhanced healing seen with zinc oxide, as these effects were found with zinc sulfate as well. For example, indomethacin reduced inflammation without affecting the rate of re-epithelialization of partial-thickness porcine wounds.[4] On the other hand, most corticosteroids retard wound healing presumably due to their anti-inflammatory action.[4,37]

Topical zinc oxide inhibited the bacterial growth in 4-day-old granulation tissue in normal rats (IV). Although the dose-dependent inhibition of zinc oxide on bacterial growth *in vitro*[126,127] was indicated also *in vivo* in study IV, the anti-bacterial effect of zinc oxide was probably not solely due to a direct toxic effect of zinc on the bacteria in the wounds for two reasons: (1) zinc concentration in zinc oxide treated granulation tissue is lower than MICs for most bacteria cultured from the wounds (VI); and (2) the lack of any anti-bacterial effect in hyperglycemic diabetic rats. Another conclusion that can be drawn from study IV is that the *inhibitory* effects of zinc on the phagocytosis by leukocytes observed after general zinc supplementation[18,26] do not appear to be operative in wounds after topical zinc oxide treatment. As opposed to some common anti-bacterial agents used in wound treatment e.g. chlorhexidine and povidone-iodine,[91,96] no toxic or detrimental effects of zinc oxide on wound healing were found. Actually a *stimulatory* action of zinc oxide on the phagocytosis by PMNs was indicated, as evidenced by reduced activity of alkaline phosphatase in zinc oxide treated wounds.[86]

The influence of bacteria on the healing of leg ulcers has not yet been fully established[44] although regimens that reduce bacterial counts to less than $10^5$ colony-forming units/g tissue may affect healing favorably.[84] Chronic venous leg ulcers with no bacterial growth healed more rapidly than those colonized by bacteria.[3]

In study I, a cleansing effect was observed in zinc oxide (A) treated ulcers which placebo did not possess. The cleansing effect of zinc oxide was also indicated when compared with a streptokinase-streptodornase solution (Varidase®) in the treatment of pressure ulcers.[141]

That zinc plays a role during fibroplasia has been shown indirectly by the reduced strength of 14-day-old incisional wounds in zinc-deficient rats.[100,108,116] The impaired healing has been attributed to a general depression of the protein synthesis, also evident in zinc-deficient rats rather than to a specific inhibition of collagen synthesis.[47] Recently, Hicks and Wallwork[73] showed that the primary defect in protein synthesis in zinc deficiency occurred at the translational rather than at the transcriptional level. However, in the case of parenteral zinc supplementation to rats which were not zinc deficient, no effect of zinc was found on the synthesis of collagen in granulation tissue.[129] When zinc as

zinc oxide was applied topically on full-thickness rat skin excisions, no effect on the collagen synthesis was seen either.[137] In addition, supplementary zinc did not influence the rate of collagen synthesis by fibroblasts in culture.[135] Therefore zinc does not seem to affect collagen synthesis in nutritionally balanced subjects.

It is not known whether zinc affects healing beyond the fibroplasia phase, i.e. during the remodeling of the scar. Although net collagen quantities do not increase during this healing phase, the wound strength increases, primarily due to the increased cross-linking of collagen.[28,77,102] The reduced wound breaking strength in zinc-deficient rats, three weeks post-operatively, i.e. during the early remodeling phase, seemed to be unrelated to differences in the concentration of collagen, since the hydroxyproline concentration did not differ between zinc-deficient and pair-fed control rats (V). However, our wound tissue samples for the hydroxyproline assays probably also contained adjacent skin collagen which could have masked a true intergroup difference. On the other hand, the hydroxyproline values were only slightly higher than those found in polyvinyl sponges implanted near skin incisions.[115] A function of zinc during the formation of cross-links in collagen has been suggested based on biochemical analyses of unwounded skin of zinc-deficient rats.[90] It has also been shown that the activity of lysyl oxidase, which catalyzes the formation of cross-links, is depressed in zinc-deficient rats.[24]

Although topically applied zinc is intended to act locally, most of the available information on zinc absorption pertains to the uptake of zinc from wounds into more distant tissues, i.e. blood, liver, pancreas, kidney and bone.[64] In the pharmacokinetic study (VI), zinc oxide (A) was compared to the highly water soluble zinc sulfate (D) on the release of zinc to wounds and its absorption into and through the wounds. In contrast to zinc sulfate, zinc oxide delivered zinc ions over an extended period of time, i.e. a sustained release pattern was achieved with zinc oxide. The presence of zinc-binding proteins in the wound fluid was probably an important contributing factor to the solubilization of zinc oxide. Also, wound tissue zinc concentrations were constant with zinc oxide, whereas they decreased after a single zinc sulfate application over the 2-day post-operative period (VI).

The effect of systemically versus locally administered zinc was not compared directly in this investigation. However, it is conceivable that increased zinc concentrations at the target site are more easily accomplished via the local than via the general route. Tennican et al[132] found that topical but not systemically administered zinc increased tissue zinc concentrations and was effective in eradicating herpes genitalis. They also noted that parenteral but not topical zinc caused systemic effects.[132] The lack of difference between serum zinc levels in zinc and placebo treated patients in study I indicates that no appreciable systemic zinc absorption occurred probably as a result of the proportionally small ulcer areas. To correct a zinc-deficient state a general zinc supplementation is required.[59]

Leg ulcer patients often have sensitized skin.[67,68] Zinc oxide applied on the skin does not seem to elicit contact allergy.[68,126]

Zinc absorption through human skin has not yet been shown although topical zinc is often used in the treatment of inflammatory conditions of the skin.[33,93] In study VII, percutaneous zinc absorption through normal human forearm skin was demonstrated by

the increased zinc levels in epidermis, blister fluid and dermis seen beneath zinc oxide in a vehicle containing gum rosin (H) compared with the plain vehicle. Dreno et al[38] could demonstrate an improvement of inflammatory acne with oral zinc supplementation. They have also shown increased zinc levels in epidermis and blister fluid after oral zinc supplementation although the zinc levels[39] were lower than those found in study VII after topical administration of zinc oxide.

In a subsequent study to study VII, the zinc oxide dressing (H) was also compared with zinc oxide incorporated in a rubber-based hydrophilic vehicle in terms of percutaneous absorption.[140] No absorption through intact human skin was found with this vehicle, indicating that the vehicle for zinc oxide is an important determining factor for percutaneous zinc absorption. Zinc oxide forms zinc soaps with gum rosin – the zinc resinates – which are almost insoluble in water but more soluble in non-polar (organic) solvents, e.g. octanol.[15] A generally accepted theory is that a drug´s ability to penetrate skin is governed, not only by its molecular weight and water solubility, but also by its octanol/ water partition coefficient.[12] Theoretically the inorganic zinc oxide can be converted into organic zinc resinates in dressing H and these are then able to penetrate the skin. In addition, the dissociation of zinc oxide in buffered saline at pH 7.4 was increased in the presence of gum rosin (unpublished observations). Rosin may also increase the permeability of the skin *per se* thereby enabling zinc transportation through the skin.

# Summary and conclusions

Zinc applied topically, mostly as zinc oxide but also as zinc sulfate, is widely used in wound treatment. Most research on zinc in wound healing has been performed with zinc administered parenterally or orally. The conclusions of the previous studies are that zinc supplementation is effective only if the patients or animals are zinc deficient. The results of the present study indicate that when zinc is administered topically as zinc oxide it can favorably influence wound healing in non-zinc-deficient subjects as well. The discrepancy between the effect on healing with systemic and with local zinc administration may be explained by the fact that pharmacological zinc concentrations can be reached when zinc is administered locally.

Topical zinc oxide improved healing (83% success-rate) compared with placebo (42% success-rate) in a double-blind trial on humans. Since the patients had subnormal serum zinc levels, indicating that they were zinc deficient, it could not be concluded that topical zinc oxide is effective in normal subjects as well. In pigs with normal zinc status local zinc oxide treatment promoted healing (re-epithelialization) by more than 30% compared with control-treated wounds. Since re-epithelialization is an important mechanism in the closure of leg ulcers these results taken together imply that topically applied zinc might increase the healing rate not only in patients with low serum zinc levels but in patients with normal zinc status as well.

Zinc sulfate at three different concentrations had, however, no beneficial effect on the healing rate of the porcine wounds. These findings indicate that not only the administration route but also the type of zinc compound is important in achieving the positive effect. It was shown that the delivery of zinc from zinc oxide resulted in fairly constant zinc concentrations over time in wound fluid (about 55 μg/mL) and wound tissue (40 μg/g) after a single application on open wounds, whereas with zinc sulfate, the zinc concentrations in wound fluid declined from 500 to 30 μg/mL and from 200 to 80 μg/g in wound tissue at the end of the 2-day treatment period. Furthermore, the solubilization of zinc oxide seemed to depend on the protein concentration of the wound fluid.

The effect of topical zinc on the acute inflammatory response in partial-thickness porcine wounds was also assessed. Both zinc oxide and zinc sulfate reduced the infiltration of inflammatory cells into the underlying dermis of the porcine wounds. Furthermore, zinc oxide reduced the bacterial growth in the granulation tissue of rats. The anti-bacterial effect of zinc oxide seemed more indirect, acting via local defense systems rather than being directly toxic to the bacteria. However, it is unclear to what extent the anti-inflammatory and anti-bacterial effects contributed to the observed enhanced healing effect of zinc oxide.

In conclusion, zinc as a topical wound-treatment modality seems to promote healing also in subjects which are not zinc deficient. This indicates that apart from being an essential nutrient, zinc exerts a pharmacological action on wound healing. However, when zinc is applied as zinc ion solutions the therapeutic range is achieved with difficulty. At zinc concentrations above physiological, but below or above pharmacological levels, zinc may inhibit essential functions (Fig. 3). When zinc is administered in the form of zinc oxide, on the other hand, it provides a depot of zinc which releases zinc ions at a proper rate. These zinc levels are below toxic levels and may have stimulatory effects on some biological systems during wound healing. Although topical zinc oxide can promote re-epithelialization,

diminish inflammation and reduce bacterial growth in wounds, the exact mechanisms by which zinc exerts these effects are still not clear. The mechanisms are probably complex due to the interaction of zinc with many enzyme systems and with biomembranes.

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