Acta Dermatovener (Stockholm) 58: 413–419, 1978

LOCAL ABSORPTION OF ZINC FROM WOUNDS TREATED WITH DIFFERENT CONCENTRATIONS OF ZINC SULPHATE

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Abstract. In the present study it was shown in rats that zinc is absorbed from excisional wounds treated with zinc sulphate. Systemic toxic effects were observed in the group treated with 20% zinc sulphate. Local toxic effects were seen in wounds treated with 0.2%, 2% and 20% zinc sulphate. An inhibitory effect of zinc on the migration of granulocytes was suggested on the basis of microscopic observation. In the operated groups which were not treated with zinc and the group treated with 0.02% zinc sulphate a decline was observed in the concentration of zinc in serum. The zinc concentration in serum increased in proportion to the zinc sulphate concentration (0.2%, 2% and 20%) applied to the wounds, while the copper concentration decreased in the groups treated with 2% and 20% zinc sulphate. In all operated groups an increase in zinc and copper concentrations was observed in liver. This was most pronounced in groups treated with higher concentrations of zinc sulphate (0.2%, 2% and 20%). The groups treated with higher concentrations of zinc sulphate also had higher pancreas zinc concentrations than the remaining groups.

Local absorption of zinc has previously been demonstrated from excisional wounds in rats (8) and from burn wounds in humans (9) treated with zinc tape which contains zinc oxide. Local absorption of zinc has also been shown to take place from excisional wounds in rats treated with other zinc compounds (10). In the latter study the most pronounced absorption of zinc was recorded from wounds treated with zinc peroxide. A substantial absorption was also observed from wounds treated with zinc oxide, while the absorption of zinc from wounds treated with 0.2% zinc sulphate solution was very small. It was suggested that zinc is absorbed in ionic form from zinc sulphate and that the absorption is proportional to the concentration of zinc sulphate in the solution applied. It was therefore decided to investigate whether or not this assumption was true.

In rats, zinc absorption from excisional wounds treated with different concentrations of zinc sulphate was investigated. Zinc and copper concentrations were determined not only for serum but also for pancreas and liver in order to discover how the early process of wound healing and local zinc absorption affects the metabolism of these minerals and to what extent locally absorbed zinc is distributed to these organs. Biopsies taken from the wounds and the surrounding skin were investigated by microscope in order to study the effects, in particular some toxic and anti-inflammatory ones, of different concentrations of zinc sulphate applied locally to the tissue.

MATERIAL AND METHODS

Animals

Fifty-two male albino rats of the Sprague-Dawley strain weighing 170–300 g were used. The rats were housed in groups of seven and fed a standard laboratory diet containing 100 μg zinc/g and tap water *ad libitum*. Forty-two animals were subjected to operations and the remaining 10 served as unoperated controls.

Operations

Two circular wounds were made on the dorsolateral aspect of the back. The hair covering the wound sites was removed with electric clippers and the outlines of the intended wounds marked with the end of an inked metal tube, 4 cm in diameter. The excision was made through the panniculus carnosus to the fascia using curved scissors. Treatment of the wounds was initiated immediately post-operatively. Twenty-four hours later all 52 animals were killed by decapitation under ether anaesthesia.

Wound treatment

Gauze pads soaked in 2 ml of zinc sulphate solution (0.02, 0.2, 2 or 20%) were applied to the wounds. Each concentration was used on 7 animals. Two methods of treatment not containing zinc were used for comparison. A dry, sterile gauze pad was placed on the wound surface in 7 animals and in 7 animals a sterile gauze pad soaked in 2 ml of de-ionized water was used.

Zinc and copper determinations

Samples of serum were diluted 10 times in 0.1 M HCl. The zinc and copper concentrations were determined by

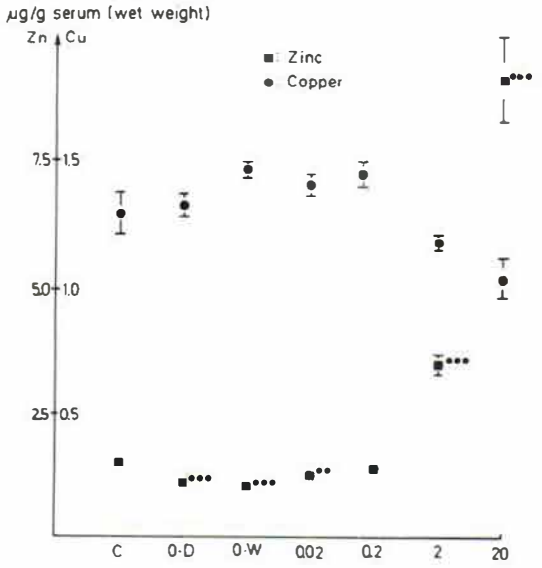

Fig. 1. Zinc and copper concentrations in serum (µg/g wet weight). Explanation of symbols: C: unoperated controls (all other groups surgically operated); 0-D: dry gauze pads; 0-W: gauze pads soaked in de-ionized water; 0.02, 0.2, 2, 20: gauze pads soaked in different concentrations of zinc sulphate, expressed as percentages. The results are given as means±S.E.M. The *t*-test results related to controls are indicated in the figures. The decline in copper concentration seen in animals treated with 2 and 20% zinc sulphate is significant ($p<0.01$) compared with the other two zinc-treated groups.

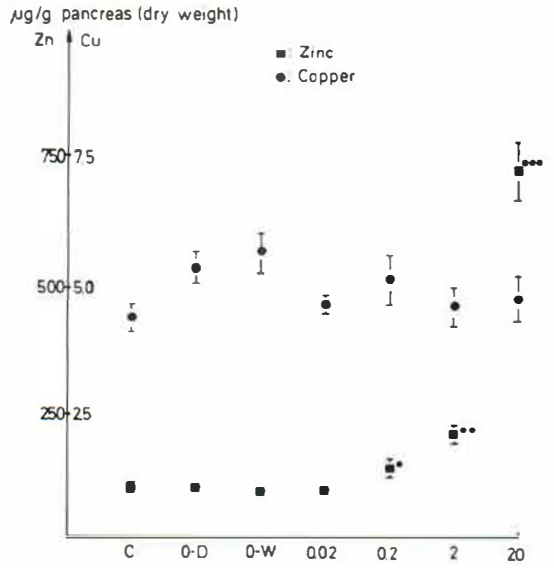

Fig. 2. Zinc and copper concentrations in pancreas (µg/g dry weight). For explanation of symbols, see Fig. 1. The results are given as means±S.E.M. The *t*-test results related to controls are indicated in the figure. No significant differences among the groups were observed in the copper concentration.

atomic absorption spectrophotometry (Varian AA-6DB) with reference samples of zinc and copper in 0.1 M HCl. Samples of pancreas and liver were taken as described earlier by Hallmans (7). The samples were placed in HCl-washed Pyrex tubes, weighed, dried and ignited as described by Bergman et al. (3) and the zinc and copper concentrations determined by atomic absorption sepctrophotometry at 213.9 nm and 324.7 nm respectively.

Table 1. *The correlation (r) of the zinc concentrations in serum (wet weight), pancreas and liver (dry weight) to the concentration of zinc sulphate applied to the wound surface (0–20 %)*

n = number of observations,
p = levels of significance

	r	*n*	*p*
Serum	0.96	33	<0.001
Pancreas	0.96	33	<0.001
Liver	0.85	33	<0.001

Histology

Samples from the wounds and surrounding skin were fixed in 10% neutral formalin, embedded in paraffin, sectioned and stained with hematoxylin-eosin or Ladewig's stain.

Statistics

The differences between group means for various variables were tested using Student's *t*-test for unpaired observations. The test was modified if the variances were of significant magnitude ($p<0.01$; *F*-test). Product moment correlation coefficients (r) were calculated for selected paired variables and tested using Student's *t*-test.

RESULTS

Zinc concentrations

A decline in the *serum* concentration of zinc was seen in the two groups of operated animals not treated with zinc as well as in the group treated with 0.02% zinc sulphate (Fig. 1). In the group treated with 0.2% zinc sulphate there was no difference in serum zinc concentration, compared with controls. In the animals treated with 2% and 20% zinc sulphate, high levels of serum zinc were found. The increase in the latter group was pronounced (6 times that of controls). The increase in serum

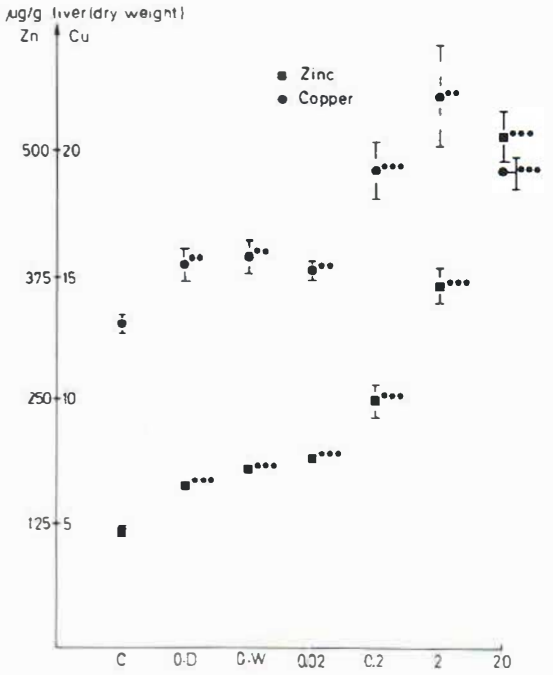

Fig. 3. Zinc and copper concentrations in liver (µg/g dry weight). For explanation of symbols, see Fig. 1. The results are given as means±S.E.M. The *t*-test results related to controls are indicated in the figure. The increases in copper concentration seen in animals treated with 0.2, 2 and 20% zinc sulphate are nearly significant ($p<0.05$) compared with the group treated with 0.02% zinc sulphate.

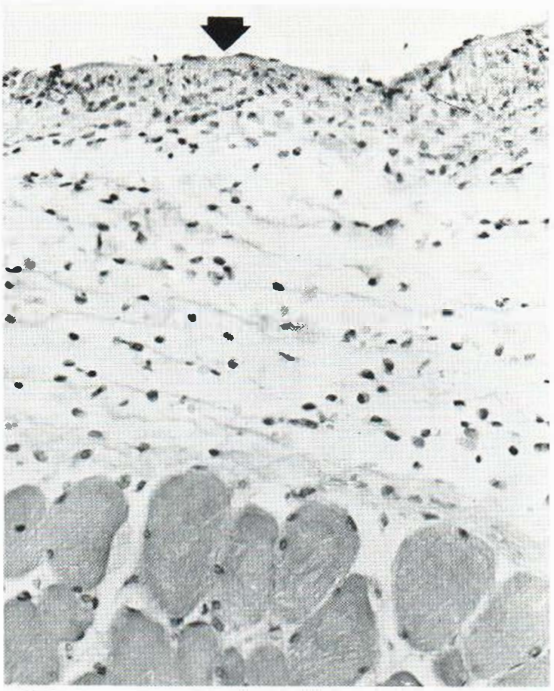

Fig. 4. Microscopic appearance of the tissue adjacent to the surface of a wound treated with 0.02% zinc sulphate. The surface is covered with a thin layer of fibrin (*arrow*). A few inflammatory cells, mostly mononuclear cells, and some erythrocytes are seen in the oedematous tissue beneath the surface. The underlying muscle has a normal appearance. Hematoxylin-eosin, ×215.

zinc was directly proportional to the concentration of zinc sulphate applied on the wound surface (Table I).

In *pancreas* no differences in the concentration of zinc were seen among the control group, the two groups of operated animals not treated with zinc, or in the group treated with 0.02% zinc sulphate (Fig. 2). In the three remaining zinc-treated groups an increase was observed which was most pronounced in the group treated with 20% zinc sulphate (7 times that of controls). The increase in concentration of zinc in pancreas was directly proportional to the concentration of zinc sulphate applied to the wound surface (Table I) as well as to serum zinc ($r=0.97$; $p<0.001$).

In *liver* an increase in the zinc concentration was observed in all operated groups, again most pronounced in the 20% zinc sulphate group (4 times that of controls) (Fig. 3). The increase was directly proportional to the concentration of zinc sulphate applied to the wound surface (Table I) as well as to the serum zinc concentration ($r=0.90$; $p<0.001$).

Copper concentrations

In *serum* a decline in the copper concentration was seen in the groups treated with 2% and 20% zinc sulphate (Fig. 1). In the remaining operated animals a slight but not statistically significant increase was observed.

In *pancreas* no differences were seen among the groups (Fig. 2).

In *liver* an increase was seen in all operated groups. The increase was most pronounced in the 0.2, 2 and 20% zinc sulphate groups (Fig. 3). One animal in the dry gauze pad treated group had a very high value (33.6 µg/g) and was therefore eliminated.

Morphological observations

After 24 hours of treatment the surface of the wounds was bright red in animals treated with dry

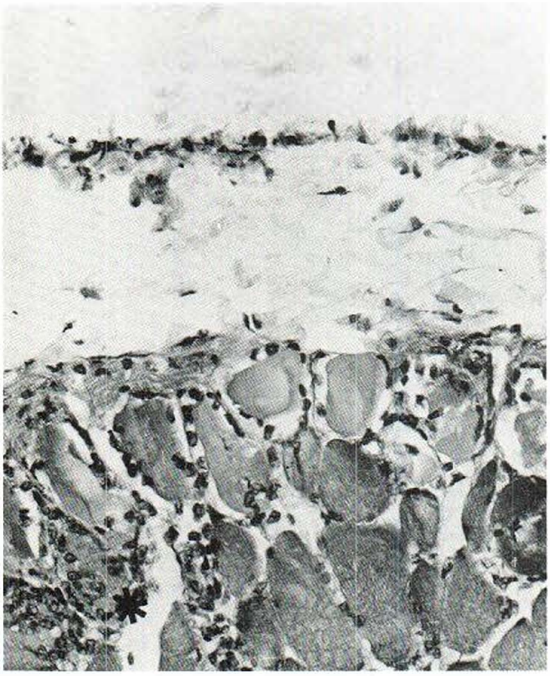

Fig. 5. Microscopic appearance of the tissue adjacent to the surface of a wound treated with 0.2% zinc sulphate. No inflammatory cells are seen in the oedematous tissue beneath the surface. The fibres of the underlying muscle show signs of degeneration and necrosis, with reduction of the fibre size and accumulation of cells (star). Some inflammatory cells, mostly macrophages, are seen within the muscle fibres. Hematoxylin-eosin, ×210.

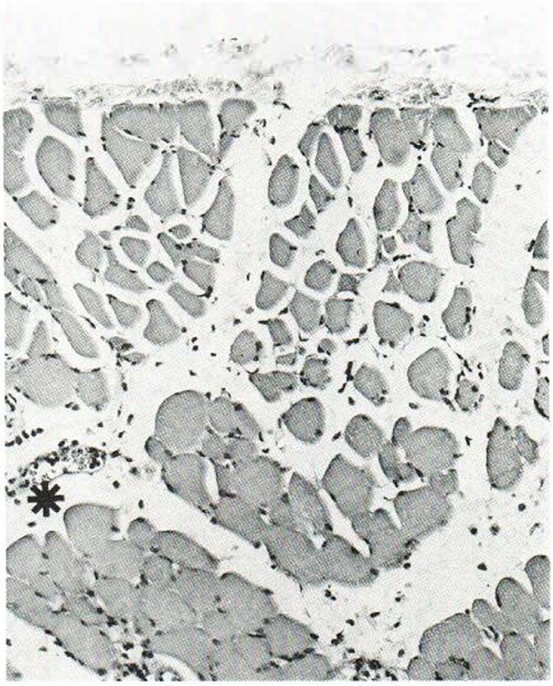

Fig. 6. Microscopic appearance of the tissue adjacent to the surface of a wound treated with 2% zinc sulphate. There are no signs of inflammatory cells near the surface of the wound seen in the upper part of the figure. The fibres of the surface layer of the underlying muscle are shrunken and separated by oedema. The nuclei of the muscle cells are pyknotic. The muscle cells in the lower part of the figure appear to be normal. Some inflammatory cells are seen within and around the vessels (star). Hematoxylin-eosin, ×145.

gauze pads as well as in wounds treated with gauze pads soaked in de-ionized water, or with 0.02 or 0.2% zinc sulphate. The wounds treated with 2% zinc sulphate had a pale, reddish appearance, whereas the wounds treated with 20% zinc sulphate were dark grey. The animals of the last group were generally debilitated and appeared intoxicated. One animal in this group died during treatment.

In wounds treated with dry gauze pads or gauze pads soaked in de-ionized water or 0.02% zinc sulphate there was a diffuse accumulation interstitially in the oedematous wound tissue of inflammatory cells, mostly mononuclear cells (Fig. 4).

In wounds treated with gauze pads soaked in 0.2% zinc sulphate, few inflammatory cells, mononuclear cells or granulocytes, were seen near the wound surface, whereas more such cells were present some distance away, near and within the underlying muscle. Some of the surface muscle fibres were necrotic, with accumulation of macrophages within the fibres. The tissue surrounding the wound was oedematous (Fig. 5).

In most wounds treated with 2% zinc sulphate, no inflammatory cells were seen near the wound surface. The fibres of the surface layer of the underlying muscle were shrunken and separated by oedema. The nuclei of these muscle cells were pyknotic. Granulocytes were present between and beneath the degenerated muscle fibres. A pronounced leukocytic diapedesis was observed from some vessels within the muscle (Fig. 6).

In principle a similar microscopic picture was observed in wounds treated with 20% zinc sulphate. No inflammatory cells were seen near the wound surface and the entire muscle layer adjacent to the wound was necrotic. In the vessels

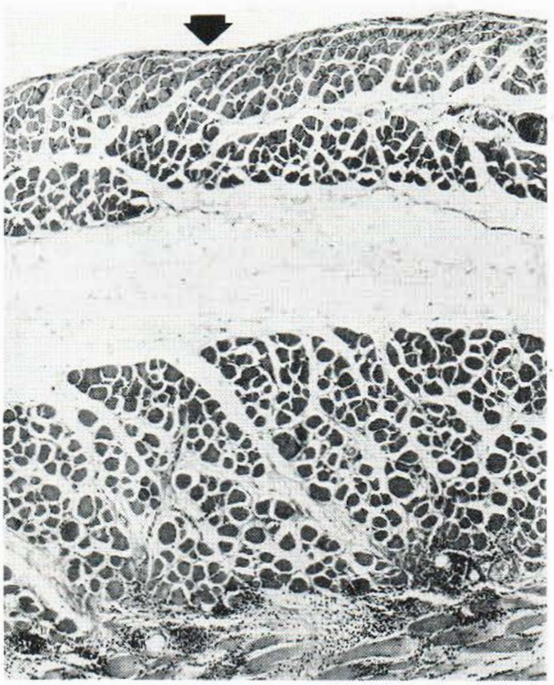

Fig. 7 a. Microscopic appearance of the tissue beneath the surface of a wound treated with 20% zinc sulphate. The wound surface is seen at the top of the figure (arrow). No inflammatory cells are seen either adjacent to the wound surface or in the upper layers of the muscle, where the fibres are shrunken and separated by oedema. In the bottom part of the figure an obvious border line is seen in the muscle, demarcated by inflammatory cells. Hematoxylin-eosin, ×55.

within and beneath the necrotic muscle, diapedesis of granulocytes with or without signs of vasculitis was observed. Most granulocytes were localized in or near the vessels and few granulocytes were seen only a short distance from the vessels (Fig. 7 *a* and *b*).

DISCUSSION

The present results demonstrate that zinc is readily absorbed in ionic form from zinc sulphate treated excisional wounds and that the absorption is dependent upon the concentration of zinc sulphate in the gauze pads.

Comparison with previous results (10) reveals that more zinc is absorbed from 20% zinc sulphate within 24 hours than from different zinc oxide preparations applied in excess on the wound surface as well as from a zinc peroxide suspension. Zinc may also be absorbed to serum from suspensions of zinc oxides and zinc peroxide in an ionized form, as in both cases zinc ions are liberated—even if ever so slowly in the case of zinc oxide.

In addition to the local, toxic effects of high concentrations of ionized zinc from the wound tissue, the risk of systemic, toxic effects of the absorbed zinc must always be kept in mind. This problem is clearly demonstrated in the present study in the 2% and 20% zinc sulphate treatment groups in

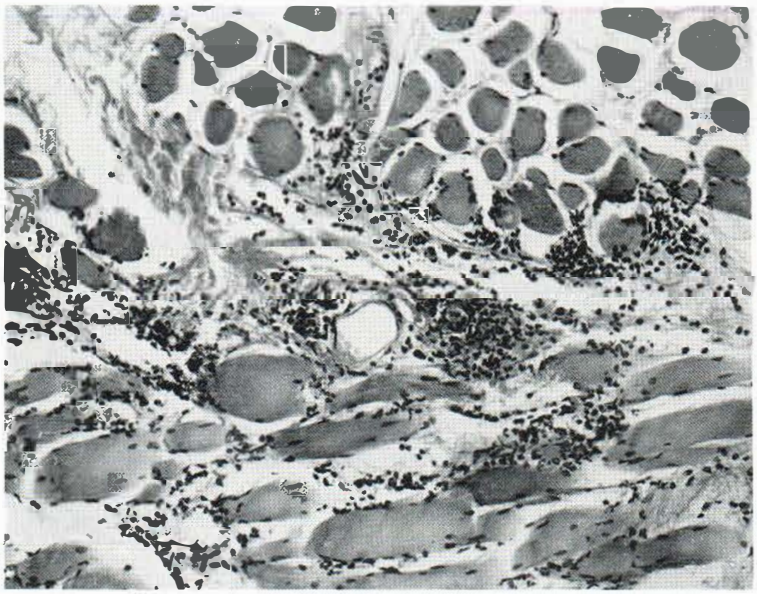

Fig. 7 b. Higher magnification of the bottom left side of Fig. 7 *a*. The inflammatory cells, almost exclusively granulocytes, are seen to be accumulated, especially around vessels, sometimes creating a picture similar to vasculitis. In the upper part of the figure, where the muscle cells are necrotic, very few inflammatory cells are seen. In the bottom part of the figure many granulocytes are seen infiltrating between and sometimes within the muscle fibres. Hematoxylin-eosin, ×165.

which morphologically obvious toxic effects were seen in the wounds concomitant with high concentrations of zinc in serum, pancreas and liver. In the case of the 20% zinc sulphate group the toxic limit of zinc in the body must in all probability have been exceeded.

In all zinc-treated groups except the 0.02% group a more or less obvious line of demarcation between normal and degenerated muscle was seen. Around this line, inflammatory cells, mostly granulocytes, were always present and the depth of the line was dependent upon the concentration of zinc sulphate used. It is likely that higher concentrations of zinc had an inhibitory effect on the migration of granulocytes, at least in the case of treatment with 20% zinc sulphate. These cells preferred to migrate in the direction away from the wound rather than towards the wound where the concentration of zinc was presumably higher.

The observation that the serum zinc concentration is reduced in groups of operated animals which were not treated with higher concentrations of zinc sulphate is in agreement with previous findings (10). The increase seen in the liver concentration of zinc in these groups is most likely a response to the acute inflammatory condition in the wounds (2). The increase was more pronounced in the animals treated with 0.02% zinc sulphate, although no differences were seen in the serum and pancreas concentration of zinc between these animals and the operated animals which were not treated with zinc. This indicates that the increase in the liver zinc in these animals is caused by the acute inflammatory condition rather than an uptake of locally absorbed zinc. In the animals treated with 0.2% and 2% zinc sulphate a more pronounced increase in the concentration of zinc was seen in the liver than in serum or pancreas, compared with controls. The increases in zinc concentrations in serum and in pancreas paralleled each other in the animals treated with 0.2%, 2.0% and 20.0%, compared with the other three operated groups. In pancreas the surgical trauma and the acute inflammatory conditions in the wounds did not seem to affect the concentration of either zinc or copper.

A slight but not statistically significant increase was seen in the serum concentration of copper in all operated animals except those treated with 2% and 20% zinc sulphate. An increase in serum copper and ceruloplasmin concentration in rats has previously been demonstrated to occur more slowly than the decrease in zinc concentration after injection of a leukocytic endogenous mediator (LEM) (12). The fall in the level of serum copper in the animals treated with 2% and 20% zinc sulphate was probably caused by zinc intoxication in these animals. Similar results have previously been observed after wound treatment with zinc peroxide (10, 11) and after feeding rats a diet containing high levels of zinc (6). An increase in the copper concentration in the liver occurred in all surgically treated animals. In those animals with a slight increase in the serum concentration of copper this probably indicates an increased synthesis in the liver of ceruloplasmin, an acute phase protein (12). The increased liver copper levels in the animals treated with 2% and 20% zinc sulphate and to some extent even in animals treated with 0.2% zinc sulphate may denote an accumulation of copper for excretion, as the major part of copper excretion occurs via the bile (1).

The changes in the concentrations of zinc and copper in the liver in some of the operated groups are probably a response to the inflammatory reaction in and around the wound, including accumulation of activated, phagocytic cells. These cells release a low molecule weight protein, LEM, which passes into the blood. LEM initiates a variety of effects in the liver, including influx of zinc, iron and amino acids as well as an increased production of proteins including several acute phase reactive proteins (2). The present results also indicate that there is an influx of copper. The reduction or absence of inflammatory cells near the surface of wounds treated with 0.2, 2 and 20% zinc sulphate indicates a toxic effect and/or an anti-inflammatory effect. Probably both explanations are true, at least in the case of wounds treated with 2% and 20% zinc sulphate. Inhibitory effects of zinc on macrophages and also granulocytes have previously been reported (4, 5, 13).

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Professor S. Lidén, Department of Dermatology, Umeå, for valuable advice and to Ann-Marie Nilsson for skilful technical assistance.

This work has been supported by grants from the Swedish Medical Research Council (Proj. No. B76-17X-04518-03) and "Förenade Liv" Mutual Group Life Insurance Company, Stockholm, Sweden.

REFERENCES

1. Beisel, W. R. & Pekarek, R. S.: Acute stress and trace element metabolism. *In* International Review of Neurobiology (ed. C. C. Pfeiffer), Suppl. 1, p. 53. Academic Press, New York and London, 1972.
2. Beisel, W. R., Pekarek, R. S. & Wannemacher, R. W., Jr: Homeostatic mechanisms affecting plasma zinc levels in acute stress. *In* Trace Elements in Human Health and Disease (ed. A. S. Prasad), vol. 1, p. 87. Academic Press, New York, San Francisco and London, 1976.
3. Bergman, B., Sjöström, R. & Wing, K. R.: The variation with age of tissue zinc concentrations in albino rats determined by atomic absorption spectrophotometry. Acta Physiol Scand *92:* 440, 1974.
4. Chvapil, M., Zukoski, C. F., Hattler, B. G., Stankova, L., Montgomery, D., Carlson, E. C. & Ludwig, J. C.: Zinc and cells. *In* Trace Elements in Human Health and Disease (ed. A. S. Prasad), vol. 1, p. 269. Academic Press, New York, San Francisco and London, 1976.
5. Chvapil, M., Stankova, L., Żukoski IV, C. & Zukoski III, C.: Inhibition of some functions of polymorphonuclear leukocytes by in vitro zinc. J Lab Clin Med *89:* 135, 1977.
6. Grant-Frost, D. R. & Underwood, E. J.: Zinc toxicity in the rat and its interrelation with copper. Austral J Exp Biol *36:* 339, 1958.
7. Hallmans, G.: Wound healing with adhesive zinc tape. An animal experimental study. Scand J Plast Reconstr Surg *10:* 177, 1976.
8. Hallmans, G.: Zinc resorption from zinc-tape during wound healing. An experimental study in rats. Scand J Plast Reconstr Surg *11:* 27, 1977.
9. Hallmans, G.: Treatment of burns with zinc-tape. A study of local absorption of zinc in humans. Scand J Plast Reconstr Surg *11:* 155, 1977.
10. Hallmans, G.: Local absorption of zinc from wounds treated with various zinc-compounds. Acta Dermatovener (Stockholm). *58:* 251, 1978.
11. Hallmans, G. & Stigbrand, T.: Unpublished results 1978.
12. Pekarek, R. S., Powanda, M. C. & Wannemacher, R. W., Jr: The effect of leukocytic endogenous mediator (LEM) on serum copper and ceruloplasmin concentrations in the rat. Proc Soc Exp Biol Med *141:* 1029, 1972.
13. Stankova, L., Drach, G. W., Hicks, T., Zukoski, C. F. & Chvapil, M.: Regulation of some functions of granulocytes by zinc of the prostatic fluid and prostate tissue. J Lab Clin Med *88:* 640, 1976.

Received March 21, 1978

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