Effect of Supplements of Zinc Salts on the Healing of Incised Wounds in the Rat and Guinea Pig

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ABSTRACT Zinc deficiency affects healing as shown by measurement of two parameters of wound healing in the rat, namely the rate of healing of an excised, granulating wound and the development of tensile strength in an incised wound. The rate of healing of granulating wounds in two species of animals, the rat and the guinea pig, is not affected by the addition of supplements of zinc salts to the normal diet (J. Nutr. 105, 815-821). In this study, the development of tensile strength in incised wounds in rats and guinea pigs was measured at 7 and 14 days after wounding in animals given supplements of zinc salts by either the oral or parenteral route. No difference in tensile strength was observed at these times in the wounds of either rats or guinea pigs given zinc supplements. J. Nutr. 105: 822-826, 1975.

INDEXING KEY WORDS tensile strength of wounds • dietary zinc supplements • guinea pigs

The claim made by Pories et al. (1, 2) that the addition of dietary supplements of zinc salts to the normal diet of normal men will accelerate wound healing, is of such fundamental importance to our understanding of the forces that control and influence tissue growth, it is important to investigate this claim fully. We have already investigated the influence of adding zinc supplements to the normal diet of two species of animals and have been unable to detect any difference between the rate of healing of excised granulating wounds in test and control animals, whether the zinc supplements were administered orally, parenterally, or topically (3). It has been pointed out by Murray and Rosenthal (4), however, that a substance that affects healing in a granulating wound may have no effect on healing in an incised wound. Thus, it seemed worthwhile to continue our investigation to determine whether the addition of supplementary zinc salts to the normal diet of rats and guinea pigs would influence the development of tensile strength in standard incised wounds in these animals.

MATERIALS AND METHODS
Rats of the hooded Lister strain weighing between 250 and 300 g were used throughout as were English guinea pigs weighing between 300 and 400 g. These were fed standard laboratory diet (3), and water was freely available throughout. The animals were divided into groups of 10 for study, and the control and test groups were matched for sex and weight. Zinc sulfate 2 was administered in a daily dose of 1 mg dissolved in 1 ml of water to all test animals either orally or parenterally by the techniques previously described (3). The control animals were given 1 ml of water by the same route at the same time.

Wounding in both species was carried out in an identical manner using the same preparations and precautions as previously described for excised wounds (3). In each animal, the site of incision and suture was measured and marked before two long-
Tudinal incised wounds were made in the midline of the back, each 2 cm long and through the full thickness of skin and subcutaneous tissues. Each wound was sutured by three simple sutures using 0000 silk.

Measurement of tensile strength. The tensile strength of both test and control wounds was measured either for 7 or 14 days after wounding. The animals were killed, and three 1-cm strips were cut across the wounds with a double-bladed scalpel. Two strips were used for the measurement of tensile strength, and one was used for histological examination.

The breaking strength of these strips was measured in a specially designed tensiometer, in which strain gauges recorded the distortion of a beam, which resulted from the forces applied in distracting the tissue under test. Distraction was produced by the action of a uniform motor drive, and the output of the strain gauge system was amplified and then recorded on a two-channel, ultraviolet recorder, which was calibrated before each measurement was made.

The cross-sectional area of the wound was also measured before the wound was disrupted by means of a micrometer screw gauge. The measurement was repeated five times, and the mean of five consecutive measurements was taken as the cross-sectional area of the wound. This measurement was used to express the breaking strength of the wound in terms of load applied per unit of cross-sectional area, which is the true tensile strength of the wound.

Experiment 1. This experiment consists of two separate but almost identical studies that were carried out on two batches of 40 rats each. In both batches, the animals were divided into 4 groups of 10 rats each, and incised wounds were made in all animals. In the first batch the animals were killed, and the tensile strength of the wounds was measured 1 week after wounding, while in the second batch the animals were killed, and the wounds were tested at the end of the second week. In both batches the four groups of rats were:

Group 1. This group was the control. Before and after wounding, they were given standard laboratory diet and received no zinc supplement.

Group 2. This group was given 1 mg/day zinc sulfate orally for 1 week before wounding; the zinc sulfate administration was continued until the end of the experiment.

Group 3. This group was given standard laboratory diet for 1 week before wounding; zinc sulfate was given orally only after wounding, and zinc supplements were continued until the end of the experiment.

Group 4. This group was given 1 mg/day zinc sulfate 7 days before wounding. The zinc administration was stopped on the day of wounding.

Results. The body weight was recorded every second day throughout the experiments. In the series killed at 7 days after wounding, the body weight of group 1 was 283.5 ± 10.0 g at the beginning of the experiment and 294.1 ± 8.5 g (mean ± SEM) at the end, while the weight of the rats in group 2 was 320.0 ± 17.1 g at the beginning of the experiment and 317.4 ± 19.3 g at the end. In group 3 the weight increased from 306.4 ± 15.0 to 324.1 ± 21.9 g during the experiment, and in group 4 the weight increased from 291.7 ± 14.7 to 308.3 ± 14.1 g. In the series killed at 14 days after wounding, the body weight of the controls (group 1) increased from 246.0 ± 7.3 g at the beginning of the experiment to 255.2 ± 7.5 g at the end, while the weight of the rats of group 2 increased from 260.6 ± 13.7 to 274.3 ± 12.7 g. The weight of group 3 rats increased from 274.2 ± 11.7 to 281.5 ± 8.1 g during the experiment, and the weight of group 4 rats increased from 275.8 ± 11.5 to 271.3 ± 10.1 g. The relationship of body weight between the four groups of each batch did not vary significantly throughout the experiments.

The measurements of tensile strength in a wound from each rat in the first study, killed 7 days after wounding, may be seen in figure 1, and the measurements made in the rats of the second study, made 14 days after wounding, may be seen in figure 2. Each point represents the mean value of the measurements made from each of the two wounds on the back of each rat. The mean value and standard error found in each group is also shown. It can be seen that there is no difference between the tensile strength measurements made in each batch of four groups.
Fig. 1 Measurement of tensile strength (open circles are means ± SEM) made 7 days after wounding in 4 groups of 10 rats given zinc supplements by the oral route. Group 1 was the control, while group 2 was given zinc supplements from 7 days before wounding until the end of the experiment. Group 3 was given zinc supplements from the time of wounding and group 4 for 7 days prior to wounding only.

Since it is known that absorption of zinc salts from the alimentary tract is incomplete and variable (5, 6) and since no difference in tensile strength was found between the wounds of test animals receiving zinc supplements orally and their controls, it seemed important to examine the wounds of a series of animals given zinc supplements by a parenteral route.

Experiment 2. Two batches of 20 rats each were studied and each batch consisted of a group of 10 test rats and a group of 10 controls. In the test group, zinc sulfate was administered intraperitoneally in a dose of 1 mg/day from the day of wounding until the day of killing. The same volume of deionized water was injected into the control animals each day. The tensile strength of wounds from the first batch of rats was measured 7 days after wounding, and measurements were made on wounds of the second batch of rats 14 days after wounding. The body weight of each rat was measured every second day during the experiment.

Results. In the experiment where the tensile strength of the wounds was measured 7 days after wounding, the weight of the control animals was $277 ± 5.6$ g (mean ± SEM) at the beginning of the experiment and $276 ± 5.0$ g at the end, while the mean weight of the test animals was $278 ± 3.5$ g at the beginning and $264 ± 3.7$ g at the end. The weight loss in the test group was highly significant ($P < 0.0005$), and the pattern of weight changes between test and control groups was significantly different ($P < 0.0025$). In the experiment where the tensile strength of the wounds was measured 14 days after wounding, the mean body weight of the control animals was $286 ± 4.9$ g at the beginning of the experiment and $294 ± 6.3$ g at the end, while again the mean weight of the test animals was $297 ± 4.2$ to $285 ± 8.4$ g. The weight gain in the control group and the weight loss in the test group did not reach statistical significance, but the pattern of weight changes between test and control groups was significantly different ($P < 0.05$).

This suggests mild zinc toxicity that is probably due to the increased absorption of zinc from the peritoneal cavity, compared with that of the gut. The rats showed no other signs of zinc toxicity. The tensile strength measurements were, however, identical between test and control groups as may be seen in figure 3.

Before concluding that the administration of zinc supplements does not influence the development of tensile strength in incised wounds, it seemed reasonable to examine another species of animal, the guinea pig.

Experiment 3. In this experiment, 20 male and 20 female guinea pigs weighing between 300 and 400 g were used. Stan-
standard incised wounds were made in all these animals, and supplements of 1 mg of zinc sulfate were administered by intraperitoneal injection each day to each of the animals in the test groups from the day of wounding until the end of the experiment, while the same volume of water was administered by the same route to each of the animals of the control groups.

Seven days after wounding, the first 20 animals were killed and the tensile strength of the wounds was measured; 14 days after wounding, the second 20 animals were killed and the same measurements were made.

Results. In the experiments where the tensile strength of the wounds was measured 7 days after wounding, the mean body weight of the control animals was 420 ± 7.9 g (mean ± SEM) at the beginning of the experiment and 475.0 ± 10.3 g at the end, while the mean weight of the test animals was 406.0 ± 10.1 g at the beginning and 388.8 ± 12.4 g at the end. The weight gain in the control group was significant (P < 0.0005) as was the weight loss in the test group (P < 0.05), and the pattern of weight changes between the groups was significantly different (P < 0.0005). In the experiments where the tensile strength of the wounds was measured 14 days after wounding, the mean weight of the control animals at the beginning of the experiment was 395.5 ± 12.7 g and at the end it was 492.8 ± 13.2 g, but the mean weight of the test animals once again decreased from 408.9 ± 12.3 g at the beginning to 406.4 ± 20.8 g at the end. The weight gain in the control animals was highly significant (P < 0.0005), but there was no significant change in the weight of the test group during the experiment. Once again the pattern of weight changes between the test and control groups was significantly different (P < 0.0005).

The lack of weight gain in the test group was interpreted as evidence of mild zinc toxicity. On the other hand, there was no difference in the tensile strength of the wounds between test and control animals measured at 7 and 14 days (fig. 4).

DISCUSSION

This study has shown that the administration of supplements of zinc salts to the normal diet of normal rats and guinea pigs has no measurable effect on the tensile strength developed in incised wounds at 7 or 14 days after wounding. The zinc salts were administered both by the oral and parenteral routes to overcome the difficulties reported of zinc absorption from the alimentary tract. The dose range was sufficient to cause mild toxic effects in the groups of both rats and guinea pigs given zinc supplements by intraperitoneal injec-
tion. In certain other groups, the zinc salts were administered for 7 days before wounding on account of the claim by Pories et al. (1, 2) that zinc supplements caused the maximum effect in accelerating wound healing 14 days after the administration of the salts was begun, but once again there was no significant difference in the rate of healing in these groups and their controls.

Tensile strength measurements have not been previously reported in animals receiving zinc supplements at 14 days after wounding, but in general these results are in agreement with the findings of other workers (4, 7-10).

We have thus been unable to detect any effect of zinc salts on the healing rate of either incised or excised granulating wounds (3) in two species of normally nourished animals. On the other hand, there is little doubt that zinc salts are involved in the processes of wound healing (11-13), and there seems little doubt that zinc deficiency, which has been described in malnourished man (14, 15), interferes with normal wound healing (16, 17). Although the subjects in whom zinc deficiency was discovered were grossly malnourished people from very underdeveloped areas of the world, it is possible that seriously ill people could develop zinc deficiency after several weeks maintenance with purified intravenous infusions. This type of patient is known to have healing problems. In normally nourished subjects, on the other hand, it is also possible that indolent, granulating wounds could develop a local state of zinc deficiency if they were invaded by organisms that required zinc for the manufacture of their metalloenzyme systems. It seems worthwhile studying these two areas when techniques are currently available to make measurements of zinc levels in tissue and fluids. Apart from these situations, however, there seems little evidence that zinc supplements will in any way influence the rate of healing of wounds.

LITERATURE CITED