Effect of Zinc Sulfate on Wound Healing. An Experimental Study.

*Malti Gupta** *S.K. Khanna*** *T.C. Jain**** *P. Prasad***** *N.K. Jha******

Introduction

SINCE time immemorial man has tried to devise methods and invent drugs to increase the rate of healing of wounds. In fact the history of the healing of wound is the history of Surgery. Systemic and topical application of various substances to promote healing is reported in the literature (Carrel and Ebeling, 1921 ; Williams and Bissel, 1944; Nechel s et al, 1952; Prudden et al, 1957).

Use of Zinc to promote healing is not new. In ancient time the Egyptians used it topically in the form of calamine. Since then various salts of zinc have been used. Systemic use of zinc sulfate for acceleration of wound healing was first reported in 1953 (Strain etal) and only few reports are available on this subject. Thus a carefully controlled study was planned to evaluate the effect of oral Zinc Sulfate on the healing of wound in dogs.

Material & Method

Adult mongrel dogs weighing between 10-15 Kg. were used for this study. Intravenous Pentothal sodium (20-30 mg/kg. body weight) was used for anaesthesia.

The whole study was divided into two groups. One group was taken as control and the other as test group. In each group the same experiment was performed on ten dogs under standard conditions, but in the test series the animals were given 200 mgm. of Zn So_4, 7 H_20 (U.S. P) dissolved in milk, twice a day, from the first post-operative day till the healing was complete.

In each case a wound was produced with sterile precautions in the centre of the flank with the help of a standard measure which was pressed over the choosen site to produce a circular impression on the skin. The margin of the impression, so produced, was marked out with methylene blue. The skin and the subcutaneous tissue, of the size of the circular impression, were excised so as to leave a raw area, the floor of which was formed by the underlying deep fascia. After complete haemostasis the perimeter of the wound was measured. The wound was then dressed with normal saline. The dressing was covered with a square piece of wire mesh wrapped in many layers of dry gauze. This was stitched to the surrounding skin. A sterilised square cloth was then wrapped round the body, to cover the dressing, and a bandage was tied over it.

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*Tutor in Surgery. **Lecturer in Surgery. ***Reader in Surgery.
****Registrar in the Department of Surgery. *****Registrar in the Department of Surgery.
Department of Surgery, Jawahar Lal Nehru Medical College & Hospital, Ajmer (Rajasthan)

A plaster collar was given in the cervical region at the end of the operation. The dogs were fed on standard diet and no antibiotics were given in the postoperative period. Subsequent dressings were done at regular intervals. Following parameters were studied at the time of each dressing :

(1) Size and depth of the wound.
(2) Character of granulation tissue.
(3) Wound contamination.
(4) Character of exudate.
(5) Time taken for complete healing of the wound.
(6) Evidence of toxic effects of zinc sulfate.

Observations & Results :

The standard measure, having a perimeter of 8.5 cm. produced a circular impression but the wound tended to become oval in shape.

(1) Size & Depth of the Wound :

Inspite of all precautions there was some variation in the size of the fresh wounds in both the groups (Table 1)

Table 1

Perimeter of Fresh Wounds Centimeter

S. No.	Control	Test
1.	8.2	9.0
2.	9.6	8.5
3.	9.2	9.9
4.	9.9	9.8
5.	9.3	10.0
6.	9.4	8.9
7.	9.6	11.1
8.	8.7	9.2
9.	8.7	9.9
10.	9.5	9.7
Average	9.21	9.60

The perimeter of the wound immediately after production varied between 8.2 cms. to 9.5 cms. in the control group and 8.5 cms to 11.1 cms. in the test group.

Changes in the perimeter of the wound were noted at the time of each dressing (Table no. 2). On fourth day after operation it was observed that the size of the wound increasad in all the dogs in the control group and in 50% of the dogs in the test group. In the remaining 50% of the latter group there was diminution in size, (Fig. 1). The percentage increase in size varied between 2.12% to 28.17% and 5% to 16.66% in the control and the test groups respectively, while the diminution in size in 50% of the test group varied from 5.05% to 13.51%.

Table 2

Average perimeter in Cms. of the wounds on various days after operation

Postoperative days	Control	Test
Initial	9.21	9.60
4	10.37	9.76
8	9.28	7.55
12	6.58	5.65
15	5.17	3.71
19	3.71	1.77
Healing Complete	23 to 32 days	20 days

On eighth day there was no further retraction of the wound edges in either group except in one dog in the control group. (S. No. 9) and the healing was in progress as indicated by diminution in the size of the wound in comparison to the size on fourth day. However, in 60% dogs in control group the size was still bigger than

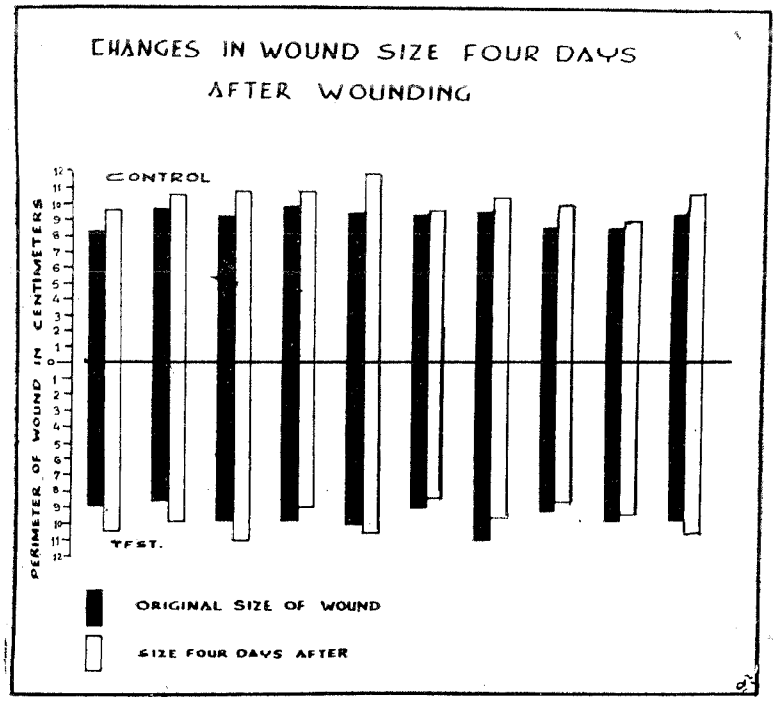

Fig. 1—Illustrates Changes in Wound Size four days after operation.

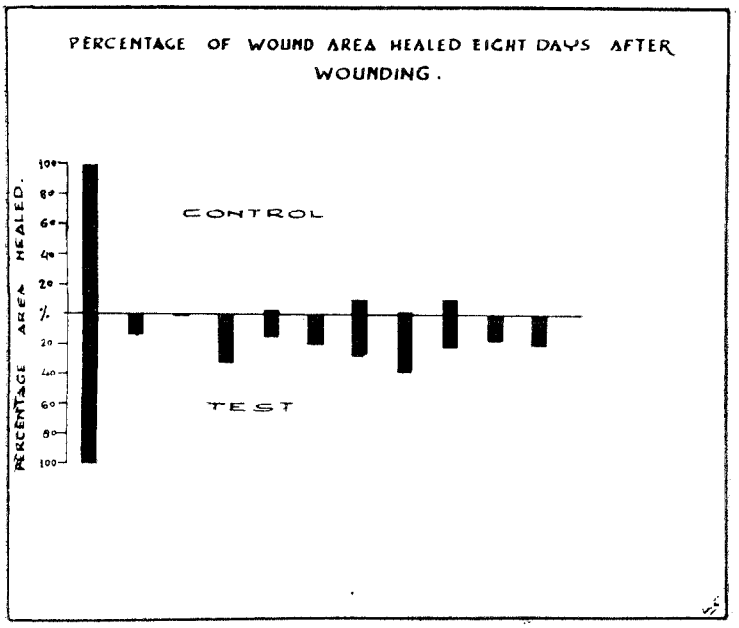

Fig. 2—Percentage of wound area Healed eight days after operation.

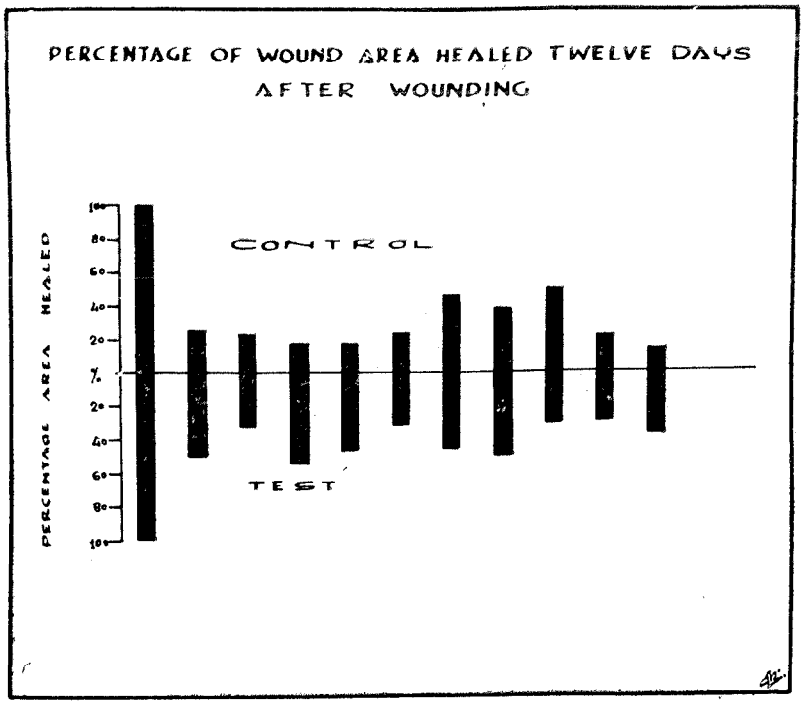

Fig. 3—Percentage of wound area healed twelve days after operation.

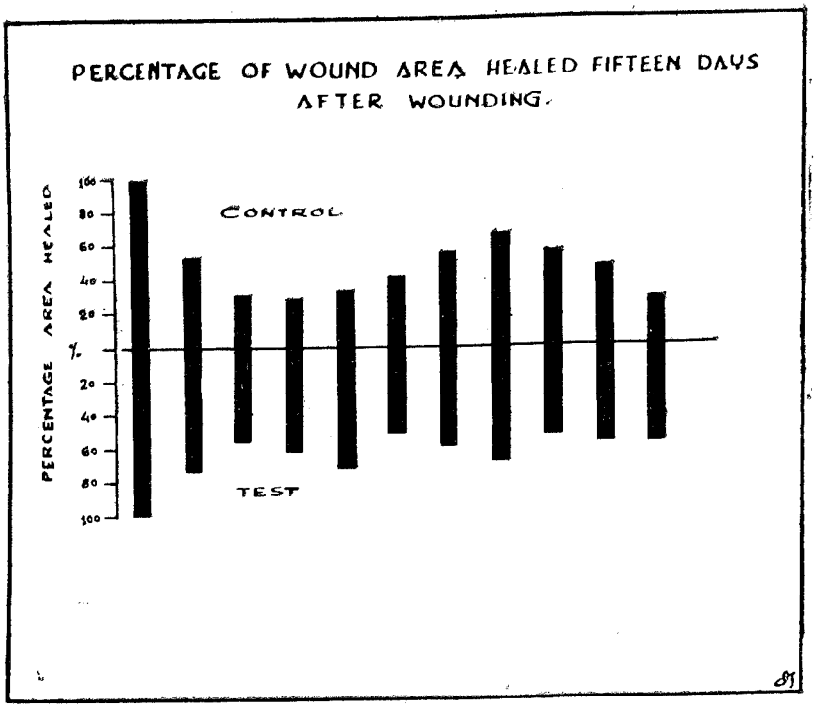

Fig. 4—Percentage of wound area healed fifteen days after operation.

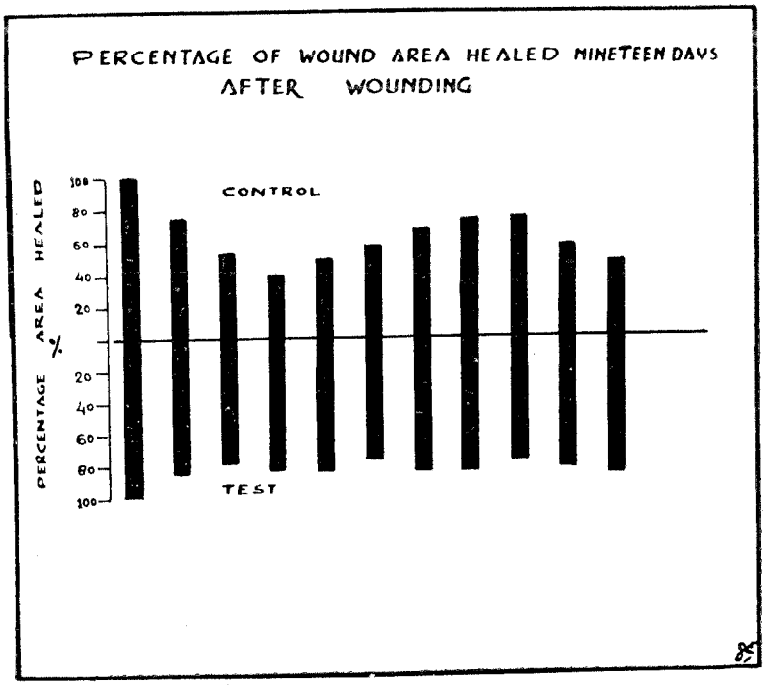

Fig. 5—Percentage of wound area healed ninteen days after operation

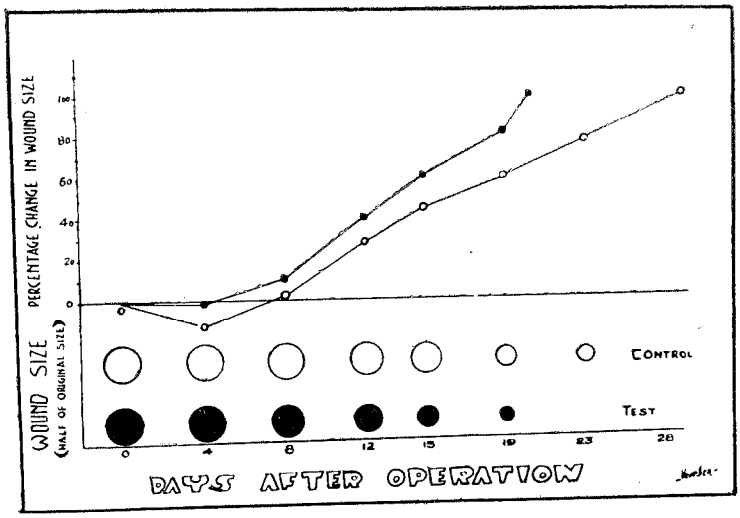

Fig. 6—The upper part of this figure illustrates the average percentage change in the wound area, while the Lower part illustrates the average change in the perimeter of the wound on various days after operation.

the original size while it was so only in one dog (S. No. 2) in the test group. In the latter group the diminution in size varied between 14.44% to 38.73% while it varied trom 1.01% to 11.49% in the control group, the average values being 20.63% and 2.13% respectively (Fig. 2 & 6).

By Twelfth day 13.68% to 49.42% of the wound had healed in the control group and 29.29% to 55.54% of the wound had healed in the test group. (Fig. 3) The average values were 28.364% and 40.895% respectively (Fig. 6).

By fifteenth day 28.42% to 66.66% of the area had healed in the control group and 51.00% to 74.44% of the wound area had healed in the test group (Fig. 4). The average values were 45.31% and 61.44% respectively (Fig. 6)

By Nineteenth day 40.21% to 75.86% of the wound area had healed in the control group and 76.00% to 85.86% of the wound area had healed in the test group (Fig. 5). The average values were 60.00% and 81.49% respectively (Fig. 6).

Thus the rate of healing process was 12.5% to 21.5% higher in the test group at the time of various dressings (Fig. 6).

(2) **Character of granulation tissue :**

It was observed that the granulation tissue was pink and healthy in all the dogs, except two, in the test group. One of these (S. No. 3) had pale granulation tissue and it remained pale till the last dressing. In the other dog (S. No. 9) there was pale hypergranulation tissue and it regained the normal character within 3 to 4 days. In the control group some degree of hypergranulation was present in all the dogs and this character persisted for 12 to 15 days. 60% of these dogs had unhealthy granulation tissue.

(3) **Wound Contamination :**

Moderate degree of infection was observed in the wound of three dogs (S.No. 8, 9 and 10) in the control group. The infection persisted for 3 days in two dogs (S. No. 8, 10) and for six days in one dog (S. No. 9). The latter dog had shown a further increase in the wound size on 8th day after operation. In all these dogs the infection cleared up without systemic or topical use of antibiotics. None of the dogs in the test group had infection in the wound.

(4) **Character of exudate :**

Seven out of ten dogs in the control group had unhealthy discharge in the dressing, three of these (S. No. 8, 9 & 10) had associated wound infection while the other four (S. No. 1, 4, 5 & 7) had no infection. The unhealthy discharge persisted for three to nine days only. Two of the ten dogs in (S. No. 3 & 9) the test group had healed unhealthy exudate which became normal within three days.

(5) **Time taken for complete healing of the wound.**

This varied between 23 days to 32 days in the control group & between 20 days to 21 days in the test group. On an average the wounds in the latter group about eight days earlier than in the control group (Fig. 6).

(6) **Toxic effects :**

None of the dogs receiving zinc sulfate manifested any evidence of anorexia, vomiting, diarrhoea, lethargy and significant loss of weight.

Discussion :

Usually the dogs have not been used for the experiments of wound healing. As these animals are cheap and easily available it was decided to use them for this study. It was realised that licking of the wound was the main problem posed by this animal; as this increased the depth of the wound and greatly retarded the healing process. In order to solve this problem following measures were taken : –

(1) Restriction of neck movements by a plaster collar in the cervical region.
(2) Selection of the centres of the flank for the production of a wound, which is not easily approachable to the limbs and the mouth.
(3) Provision for a stiff dressing so that the wound is well protected.

In the present study change in the shape of the wound after production can be explained by the inherent tension along the Langer's lines or the so called "skin tension lines" (Barry, 1969). Immediate increase in the size of the fresh wound is probably due to retraction of the skin edges.

The increase in the size of wound in all the control animals and in 50% of the test animals in the first four days or the 'lag period', is difficult to explain. Mechanical fixation of the surrounding skin i.e. fixation of wire gauze to the skin may be a factor responsible for this. In this context the significant observation is that such an increase was completely absent in 50% of the test group. These animals had an actual diminution in size in the first four days while in rest of the animals of this group the increase in size was of a comparatively lesser magnitude. Hence zinc sulfate has caused shrinkage of the wound even during the 'lag period'. How zinc sulfate could effect this is not certain. Dunphy et al (1956) have shown that during 'lag period' mucopolysaccharide ground substance is deposited in the wound bed in association with the formation of new capillaries and fibroblasts. Zinc sulfate might be helping some of these complex processes.

Healing of an open wound with loss of substance includes two processes, contraction and epidermisation (Sabo, 1965). Contraction appears to be the main mode of healing in the area studied.

Zinc sulphate appears to increase the rate of contraction by accelerating either of the following three processes :

(a) Shrinkage of young granulation tissue (Carrel, 1910)
(b) Intussusceptive growth (Billingham & Medawar, 1955)
(c) Contraction of young fibroblasts (Abercrombic et al, 1956).

An absence of hypergranulation in the majority of the test animals again points to

the participation of zinc sulfate in new capillary formation and fibroblastic proliferation. The character of discharge from the wound was healthy in the majority of the test group animals. This could be due to normal healthy character of granulation tissue in this group. Occurence of moderate degree of infection in the early period in three of the animals in the control group was responsible for the unhealthy discharge but there was unhealthy exudate in four more animals of this group and this cannot be explained. The unhealthy character of the exudate persisted for not more than three to nine days while the hypergranulations persisted for, twelve to fifteen days. Perhaps, decreased capillary permeability (Cuthbertson, 1959) along with diminishing water content of the wound bed (Paul et al, 1948) are factors responsible for the absence of unhealthy discharge even though hypergranulation tissue persisted. Peries et al (1967) have also reported cleaner, plnker and healthier granulations with zinc sulfate.

The wounds in the test animals healed on an average about eight days earlier than those in the control group. In terms of percentage the healing was accelerated by about 21% in the test group. Strain et al, (1953) reported an increase in the healing by 30%.

The mechanism and site of action of Zinc have yet to be identified. The highest concentration of Zinc in man has been found in hair, bone, liver and skin (Lutz, 1926; Goldblum et al, 1953). Zinc is mainly an intracellularly occuring metal. It is an essential part (Under wood, 1962) and a cofactor to a number of enzymes. Zinc may be necessary to protect RNA by its inhibiting action on RNA—ase (Prasad, 1967). Savlov et al (1962) demonstrated that radiozinc concentrated in healing tissues with highest concentration during the first seven days. Probably zinc sulfate when administered systemically, acts through various enzymes and thus accelerates the process of healing.

Summary and Conclusion

(1) The dog, an easily available and cheap animal, has been utilised for conducting experiments on wound healing. Methods to prevent licking of the wound have been described.

(2) Zinc sulfate initiates healing during the 'lag period', accelerates the healing process and shortens the total time required for healing of granulating wound.

(3) The healthier granulation tissue and cleaner exudate was present in the wounds of animals fed on additional zinc sulfate.

(4) No significant toxic effects were observed after the systemic administration of zinc sulfate.

The systemic use of zinc sulfate appears to be promising for acceleration of healing process in healthy animals and needs further clinical trials.

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