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# MEASURING THE EFFECTS OF TOPICAL PREPARATIONS UPON THE HEALING OF SKIN WOUNDS

By SIDNEY GOFF, PH.D., and IRVING LEVENSTEIN, PH.D.*

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ABSTRACT

**Topically applied substances often come in contact with skin wounds. The effects of a number of such preparations on the healing of a standardized, surgically inflicted skin wound were studied. Using the mouse as the experimental animal, a direct relationship was found between lapsed time of repair and wound strength, as measured tensiometrically. The wound tensile strength of control (untreated) mice was compared with that of groups of animals treated with preparations which had been reported to have an effect on healing. Results are given on the effects of treatment with ointments containing fat soluble vitamins, *Aloe vera extract*, a fluorinated corticosteroid, the same corticosteroid in a cream base, an allantoin-coal tar mixture, a pantothenylol cream, and the steam distillate from *Artemisia tridentata*.**

**The determination of the tensile strength of segments of healing skin wounds appears to be a valuable tool in the study of the effects of topical agents.**


Cosmetic and drug preparations are frequently applied to skin area which have been cut or otherwise injured. This may be done intentionally for the purpose of the treatment, or it may be coincidental to the primary use of the preparation. Regardless of the reason, it is important to have some knowledge of the effects of these substances upon the healing of wounds. An investigation was undertaken in our laboratory to determine whether the effects of topically applied preparations on the healing of skin wounds could be measured quantitatively.

Research in skin physiology has made clearer many of the biochemical, physiological and histological changes that take place as injured tissue is

* Leberco Laboratories, Roselle Park, N. J.

restored to its normal state. There are shifts in the mucopolysaccharides, glycoproteins, amino acids and RNA concentrations (1–7). Cellular migrations and multiplications occur to replace lost tissue (8), and complex biophysicochemical reactions result in the formation of collagen fibers which help to restore tissue strength (3, 5, 9).

The effects of a wide variety of substances, administered both systemically and topically, on the reparative processes have also been studied. Nutrients, hormones, antimicrobial agents, botanicals and an array of pharmacologically active compounds have been evaluated. Among these, cortisone derivatives, ACTH, thyroxine, hyaluronidase, reserpine and 5-hydroxytryptamine have been reported to retard the healing of wounds (10–19). Promotion of healing has been ascribed to glycosides of *Centella asiatica*, extracts of *Aloe vera*, allantoin, protein hydrolysates, nitrofurans, pantothenates and hyaluronic acid (20–30).

Gross and histological observations, changes in the concentration of various tissue constituents, and the strength of the healing wound are the criteria most often used to determine healing rates and the effects of treatment upon them. As has been mentioned, many biological and biochemical mechanisms come into play during healing and may be considered as forces directed toward returning traumatized tissue to a normal condition. The strength of the healing wound is a general measure of the progress toward normalcy and may be thought of as the biological resultant of the various forces acting in and on the affected area. It is a comparatively simple measurement and represents a direct approach to the problem (9, 19, 31, 32).

Two general methods of skin wound tensiometry have been described and used. Prudden and co-workers (33–41), by measuring the pressure applied from within, required to disrupt abdominal incisions in the rat, have reported that the application of ground, acid-pepsin digested, bovine tracheal cartilage stimulated healing. The other technique consists of determining the force (usually as measured by weight) required to cause the separation of the edges of a standard segment of healing skin wound. This wound-strip method was used in the studies reported here.

Standardization of the method of injury has also been approached in a variety of ways. Burns, blows and cuts have all been used in the reports cited. A surgical incision of closely controlled length and depth was used in our work, since it could be reproduced with acceptable precision.

## Materials and Methods

### Test Animals

CF-1 male mice, 30-40 days old, and weighing 18 to 25 g. were used in all the experiments.

### Wounding Procedures

On the day prior to inflicting the wounds, the hair on the backs of the animals was removed with an electric clipper. With the animals under ether anesthesia, an incision approximately 25 mm. in length was made along the midline of the back, starting at the shoulders and proceeding caudally. In order to cut only the skin and not involve underlying tissue, an initial small slit was made in the skin with a sharp, fine pointed scissors. The skin was then raised by grasping it with forceps at a point just anterior to the slit and a scapel blade, cutting edge upward, was inserted into the opening and drawn through the skin to a point about 25 mm. from the point of origin. The edges of the wound were approximated and then closed with five equally spaced interrupted sutures (40 gauge stainless steel wire). Treatment was begun immediately. On the sixth day after the wound was inflicted, the sutures were removed.

### Tensile Strength Measurements

The mice were sacrificed and hair that had regrown was carefully clipped with a small scissors. A wide section of skin containing the healing wound was excised. Care was taken to include only the skin and closely adhering subcutaneous tissue. The skin section was then placed on a plate-glass block that was moistened with normal saline. This permitted the skin to assume what might be termed a standard dimension. A strip of skin along the posterior (caudal) end of the wound, perpendicular to the line of the wound, was trimmed off and discarded. Then a strip 8 mm. wide was cut off and used for the tensile strength measurements. The next 2–3 mm. section of the wound was removed and fixed in formalin for histological examination. Another 8 mm. segment was then obtained from the remaining (anterior) portion of the wound, for a second tensile strength determination. A modification of the Charney, Williamson, Bernhard (42) technique was used to determine the tensile strength of the healing wound segments. One end of the skin section was mounted in a clamp, and a lightweight basket lined with a polyethylene bag was attached to the other end. With the skin segment in a vertical position, weight in the form of dry, free flowing sand at a rate of 7 g./sec. was added to the basket until rupture of the wound occurred. The results are expressed as grams or weight required to separate the edges of the healing wound. Averages for each group were calculated and include the values obtained with both segments of wound from each mouse.

Four series of tests were conducted. The first was for the purpose of checking the basic method and to construct a time *vs.* wound tensile strength curve, with the animals under control conditions, i.e., no treatments applied to the wounds. Following this the experiment was repeated using available materials which had been reported to have an effect upon wound healing. The following preparations were evaluated:

| Series | | Treatment Material |
|---|---|---|
| II | (A) | Ointment containing Vitamins A and D |
| | (B) | Liquid containing 2% allantoin and 5% refined coal tar extract |
| | (C) | Cream containing 0.01% 6α,9α-difluoro-16α-hydroxy-prednisolone acetonide |
| III | (A) | *Aloe vera* extract in an ointment base |
| | (B) | Essential oil (steam distillate) from leaves of *Artemisia tridentata* |
| IV | (A) | Ointment containing 0.01% 6α,9α-difluoro-16α-hydroxy-prednisolone acetonide |
| | (B) | Cream containing 2% pantothenylol |

All test materials were applied once daily over the healing wound and surrounding skin until the day on which the tensile strength measurements were made.

### *Series I. Control Group*

Sixty mice were divided into six groups of ten animals each. After surgery, each animal was housed in an individual cage and given food and water *ad libitum.* The tensile strength of the healing wounds was determined 6, 9, 12, 15, 18 and 21 days after wounding, with one group of mice being sacrificed at each time interval.

Figure 1 shows the results obtained over the 21 days experimental period. There is a time-related increase in the tensile strength of the wounds. The ranges indicated above and below the central point at each interval represent the standard error calculated for that group of measurements. Through the 15 day determinations, the standard errors remained fairly constant at about plus or minus 20 g. At 18 and 21 days after surgery a wider range was observed as higher tensile strengths were reached and the standard errors increased to 40 to 50 g. A simple calculation showed that despite the absolute increases in standard error, these values generally represented between 5 and 10% of the group average. Results obtained indicate that with the basic techniques employed statistically acceptable measurements could be made and that the determination of increases in tensile strength may be a useful indication of the skin wound repair process.

### *Series II*

Ninety-six mice were used in these tests. They were divided into sixteen groups of six animals each and wounded and housed as in Series I. Five groups served as controls, and five groups received daily applications




Figure 1.—Tensile strength of untreated wounds.

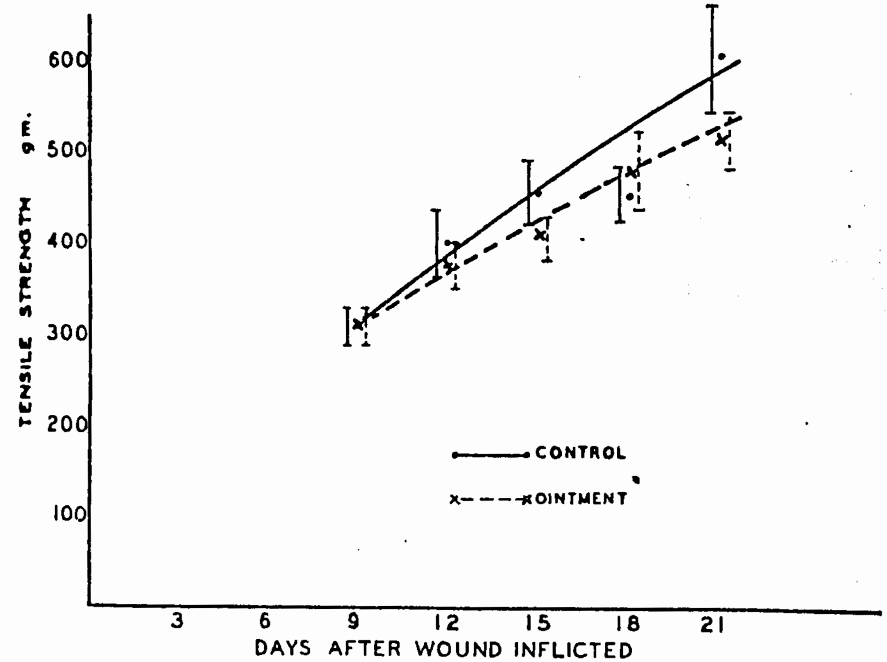


Figure 2.—Tensile strength of wounds treated with vitamin ointment.

of the oleaginous ointment containing fat soluble vitamins. Tensile strength measurements were made 9, 12, 15, 18 and 21 days after injury. Three groups were treated with the allantoin preparation and three with the corticosteroid cream. Animals receiving the latter two treatments were sacrificed 9, 15, and 21 days after wounding.

Figure 2 presents the tensile strength measurements made on control wounds and those treated daily with the oleaginous vitamin ointment. While some variations were observed, statistical analysis of the data indicated that the values at each time interval did not differ significantly. The wound tensile strength data which compares controls with the allantoin and corticosteroid treatments are given in Fig. 3. At 9 and 15 days the allantoin and untreated wounds were comparable in strength. The controls were significantly stronger 21 days after injury ($P < 0.05$). In agreement with the findings of other investigators (10–16) application of the corticosteroid cream appeared to interfere with the healing process. This is shown clearly in Fig. 3. The differences were highly significant ($P < 0.01$).

*Series III*

Seventy-four additional mice were divided into five groups of ten animals and three groups of eight animals. Three groups of ten were treated daily with the *Aloe vera* extract ointment. Two groups of ten and one of eight served as controls. The remaining two groups of eight were treated with an essential oil preparation obtained by steam distillation from the leaves of *A. tridentata*. Tensile strength determinations were made at 9, 15 and 21 days after surgery with the control and *Aloe vera* treated mice, and at 9 and 21 days with the *A. tridentata* extract treated animals.

The comparison of the strength of wounds treated with the *Aloe vera* extract ointment, the *Artemisia* oil and controls are shown in Fig. 4. Statistically significant differences ($P < 0.05$) in favor of the *Aloe vera* preparation are seen at 9 and 15 days. However, 21 days after the wounds were inflicted, the values are essentially the same. Application of the purported hypertrophying material (*A. tridentata* extract) had no effect at 9 days and an apparently inhibiting effect on healing after 21 days of treatment. In the latter case, the decreased tensile strength was significantly ($P < 0.01$) below the control value.

*Series IV*

Nine groups of ten mice each were used in this series of tests. Treatments consisted of an ointment containing 0.01% $6\alpha,9\alpha$-difluoro-$16\alpha$-hydroxyprednisolone acetonide and a material containing 2% pantothenylol in a water miscible cream base. Tensile strength determinations were made 9, 15 and 21 days after the surgical wounds were inflicted.



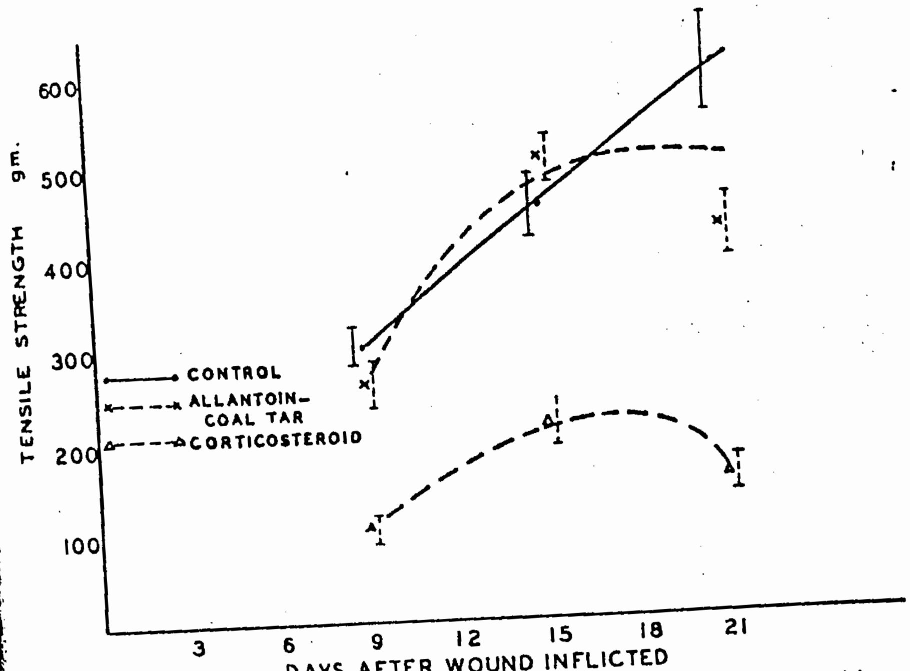


Figure 3.—Tensile strength of wounds treated with allantoin-coal tar combination or with corticosteroid.

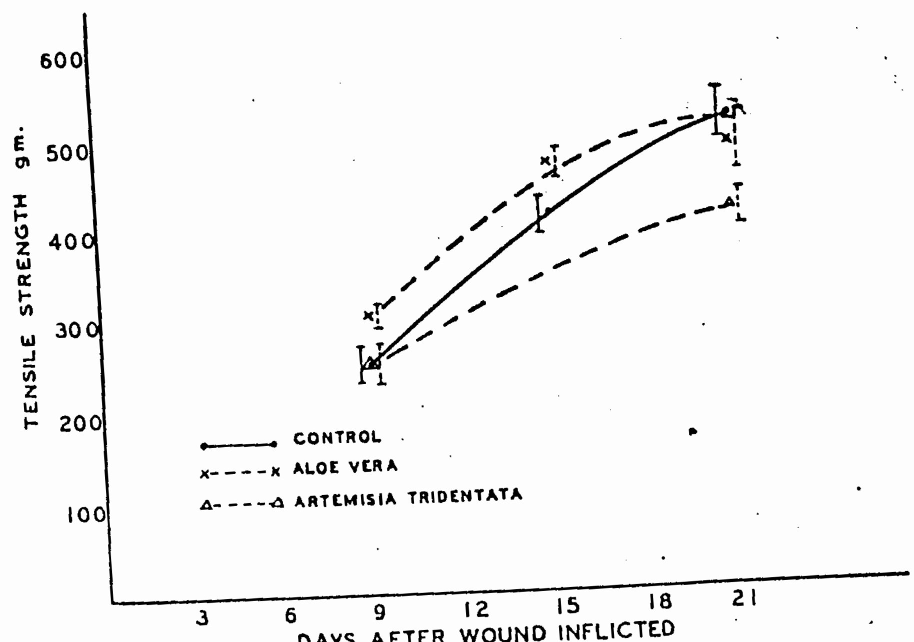


Figure 4.—Tensile strength of wounds treated with plant extracts.

The results are given in Fig. 5. Control values at each time interval again agree well with previous tests. At 9 and 21 days, the wounds treated with the corticosteroid ointment had significantly lower tensile strengths ($P < 0.01$) than the controls. While the values obtained at 15 days were also lower than the controls, the difference was not significant at the statistical 5% level.

Application of the cream containing pantothenylol showed some interesting results. At 9 days the wounds tested with this preparation demonstrated an average tensile strength that was almost identical with the controls. At 15 days, the treated wounds showed a significantly greater tensile strength ($P < 0.05$). However, at 21 days there was little additional increase in tensile strength over that achieved at 15 days, while the untreated wounds showed the expected increment. The difference at 21 days was significantly in favor of the controls ($P < 0.01$).

## Discussion

The results obtained in Series II, III and IV indicate that wound tensile strength measurements are able to detect the inhibitory or retarding action of a corticosteroid and some transitory degree of stimulation of healing by *Aloe vera* and pantothenylol preparations. Greater retardation with the corticosteroid in Series II as compared with Series IV may have been due to the greater absorption of the active compound from the cream base as compared with the ointment base.

Daily application of the oleaginous vitamin ointment and the allantoin-tar extract liquid appear neither to enhance nor inhibit the healing of the experimental wounds. While it is advantageous for a preparation to be able to promote healing, it is also important that products applied to the skin should not show an inhibitory or retarding effect on the healing of wounds. Permitting the natural processes of healing to proceed at their natural rates is a highly desirable attribute.

Studies correlating the histological changes found in healing with the tensile strength results obtained are now underway and will be reported in the near future.

A composite of the control values representing 274 determinations made on 147 animals permitted the construction of the curve shown in Fig. 6. The points plotted approach a smooth line. It should be especially noted that the composite averages at 9, 15 and 21 days post surgical injury (circled points on graph) fall in a straight line. This linear time *vs.* tensile strength relationship in the control animals indicates that the method proposed here has validity as a technique for evaluating the effects of topically applied material on the healing of skin wounds.



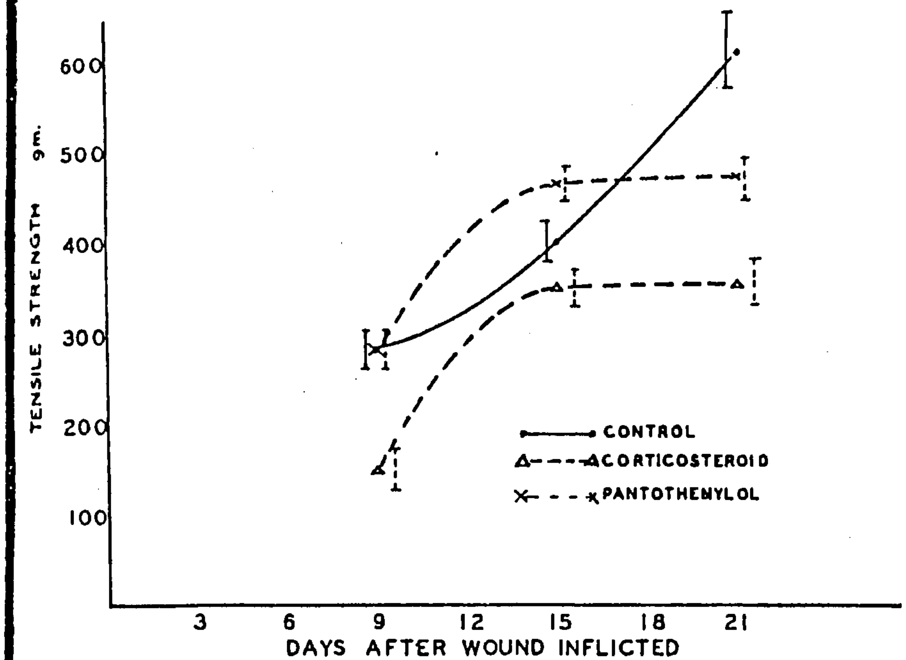


Figure 5.—Tensile strength of wounds treated with corticosteroid or with pantothenylol.

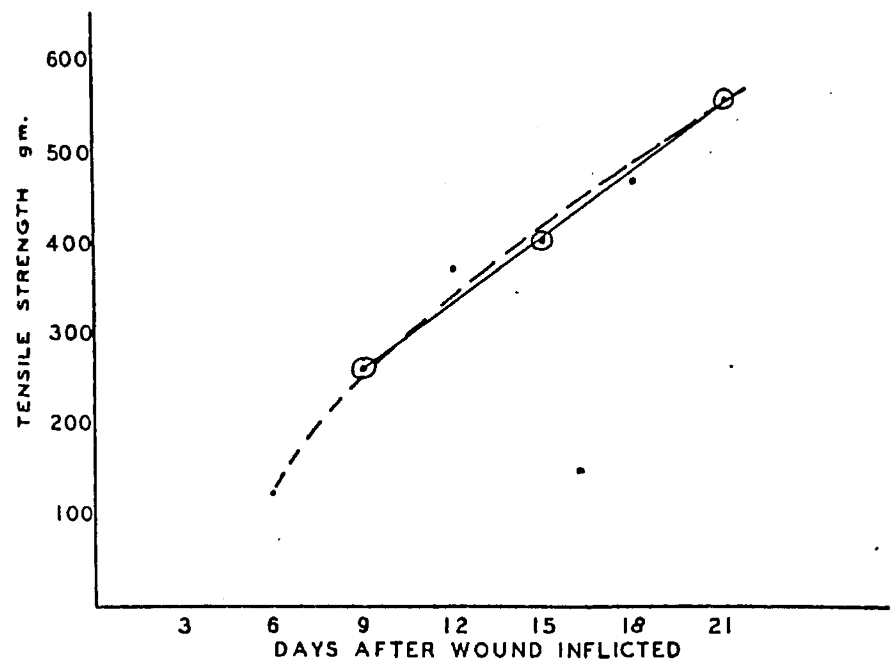


Figure 6.—Tensile strength of untreated wounds (composite of controls).

## Summary

I. Under controlled conditions, with the mouse as the experimental animal, it has been shown that the tensile strength of a standardized skin wound increased as the wound healed.

II. The effects of several preparations applied topically to the standard wound were demonstrated by tensile strength measurements.


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