Differences in Healing of Skin Wounds Caused by Burn and Freeze Injuries

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Circular, full-thickness dermal burn- and freeze-produced wounds were produced in rats to compare the difference in healing between the two types of thermal injuries. Contraction did not occur in wounds (n = 30) caused by freezing, while burn wounds (n = 30) contracted to less than one-third of original size by 21 days after injury. If the centers of the freeze-produced wounds (n = 12) were excised, contraction would then occur and proceed at the same rate as an open wound. Histologically, the degree of initial tissue destruction by the two types of injuries was similar. The burn wound contained only half the amount of collagen found in the freeze-produced wound. There was a greater and more rapid replacement of collagen in the burn wounds. With both injuries, the highest concentration of collagen was found on the fifteenth day and returned to normal by the twenty-eighth day. The burn wound contained three times the amount of collagen in normal skin, while the freeze-produced wound contained only 1¾ times the amount in normal skin. Contraction does not seem to occur in the healing of the freeze-injured skin because the slow removal and replacement of the residual matrix prevents contraction.

FULL-THICKNESS CRYOSURGICAL destruction of skin neoplasms can give excellent esthetic results,[6] but unsightly scars often result from full-thickness burns. Therefore, the healing processes associated with these two types of thermal injury may be different. Since all the cellular elements of the dermis are destroyed by both burning and freezing, the differences in healing could be due to the integrity of the remaining connective tissue matrix. In normal wound healing, inflammation, granulation tissue formation, contraction, and epithelialization all play a part.[3] Therefore, we studied the rate of contraction and the histologic and biochemical factors in the healing process following injuries in the rat skin produced by freezing and burning.

Materials and Methods

The backs of Sprague-Dawley rats (125–150 g) were shaved under ether anesthesia. A burn and a freeze-produced injury, each 1.8 cm^2, were produced on the backs of each rat. Both injuries were sited in corresponding locations on the backs of different animals for accurate comparison. The burn was created by applying for 20 seconds a lead disc at the temperature of boiling water. The freeze-wound was produced by applying for 30 seconds a brass disc equilibrated with the temperature of liquid nitrogen. All the animals were weighed daily and were randomly divided into three groups for separate study of wound closure, histologic appearance, and collagen content of the wounds.

Wound Closure (n = 18)

Four India ink tattoo marks equidistant from each other were made at each quadrant in the skin at the periphery of the wound edge. The degree of wound closure was assessed by measuring the dimensions between the tattoo marks across the wound. This measurement was carried out daily for the first week and at three to four day intervals for the next two weeks.

Photography at fixed focal length on alternate days was also used to assess the sizes of the wounds. A glass slide with a ruler marker attached was pressed onto each wound so that a flat surface area was presented for photography. Color transparencies of the wounds were projected onto standard graph paper so that the 1 cm scale on the slide corresponded to exactly 2 cm on the graph paper. The tracings of the wounds were then cut out and the wound areas represented by the paper weighed. As the thickness of the graph paper is even, the weight of the paper is a good representation of the area.

Histologic Appearance (n = 42)

Three rats were killed at 1 hour after injury, day one and at two to three day intervals from day 1 to 34. The

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wounds were widely excised to include normal skin at the periphery, fixed in 10% formalin, and sectioned and stained with hematoxylin and eosin. The sections prepared for histologic examination were coded, randomized, and revised by a pathologist unaware of the types of injury or the ages of the wounds.

Collagen Content (n = 24)

Six rats were killed at days 1, 8, 15, and 28 each. The wounds were narrowly excised to the muscle base, with care being taken to eliminate normal skin. The burn wounds and freeze-produced wounds were separately pooled, weighed and homogenized in 20 ml 0.5 M acetic acid. After addition of 20 mg of pepsin (Boehringer-Mannheim, Indianapolis, IN), the homogenates were stirred for 24 hours at 4 C, then centrifuged at 30,000 × *g* for 15 minutes.[7] Any insoluble collagen in the pellets were extracted by heating in sealed tubes to 65 C for 30 minutes in 10 ml of 5% trichloroacetic (TCA).[4] These TCA extracts were centrifuged at 30,000 × *g* for 10 minutes, the pellets were discarded, and 5% TCA solubilized material was analyzed for hydroxyproline content.

After the initial pepsin-soluble supernatants were adjusted to pH 8.0 with 1 M NaOH, crystalline NaCl was added to give a final concentration of 20% w/v.[9] The solutions were stirred at 4 C for one hour, then centrifuged at 30,000 × *g* for 15 minutes. The supernatants and pellets were separated. The pellets were taken up in 5 ml of 0.1 M acetic acid. Aliquots from both supernatants and pellets were dried by lyophilization, then hydrolysed in 6 N HCl in nitrogen at 108 C for 24 hours for subsequent hydroxyproline quantitation.[1]

Results

During the experimental period, all the rats had identical weight gains, with no evident infections. Measurements of wound size by tattoo marks and by photography showed good correlation, both giving similar results with less than 5% difference.

Following freeze injury, unlike burn injury, contraction did not appear to participate in the healing process. The burn wounds closed rapidly, reducing their size by 20% on the first day and 30% on the second day. After two weeks, they were only one-third of their original size. In comparison, the wounds produced by freezing increased in size by 20–25% during the first two days and returned to their original size in approximately two weeks (Fig. 1).

By histologic criteria, the degree of tissue destruction by the two injuries was similar. On the first day after injury epidermal necrosis was complete, dermal fibroblasts were pyknotic, and polymorphonuclear leukocytosis was present. Muscle necrosis with gross edema was evident in all sections. In the deep dermis, the epithelium of the hair follicles showed viable cells in both types of injury, especially at the extreme follicle base. By 9 days postinjury, the degree of inflammation and granulated tissue formation was the same for both types of injury. Although the dermis in both injuries was subsequently replaced by scar tissue, freeze-injured specimens showed a much wider area of residual scar at the end of the experiment on day 35 (Fig. 2).

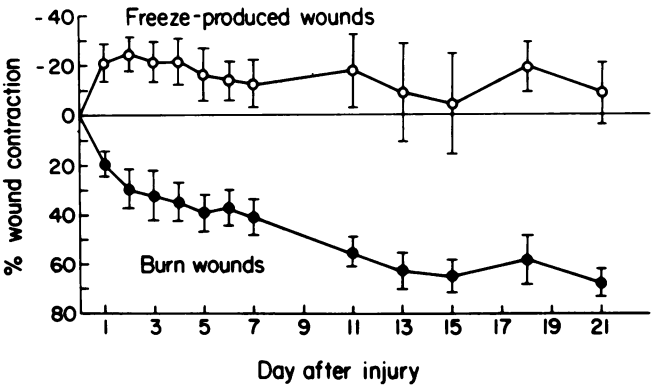
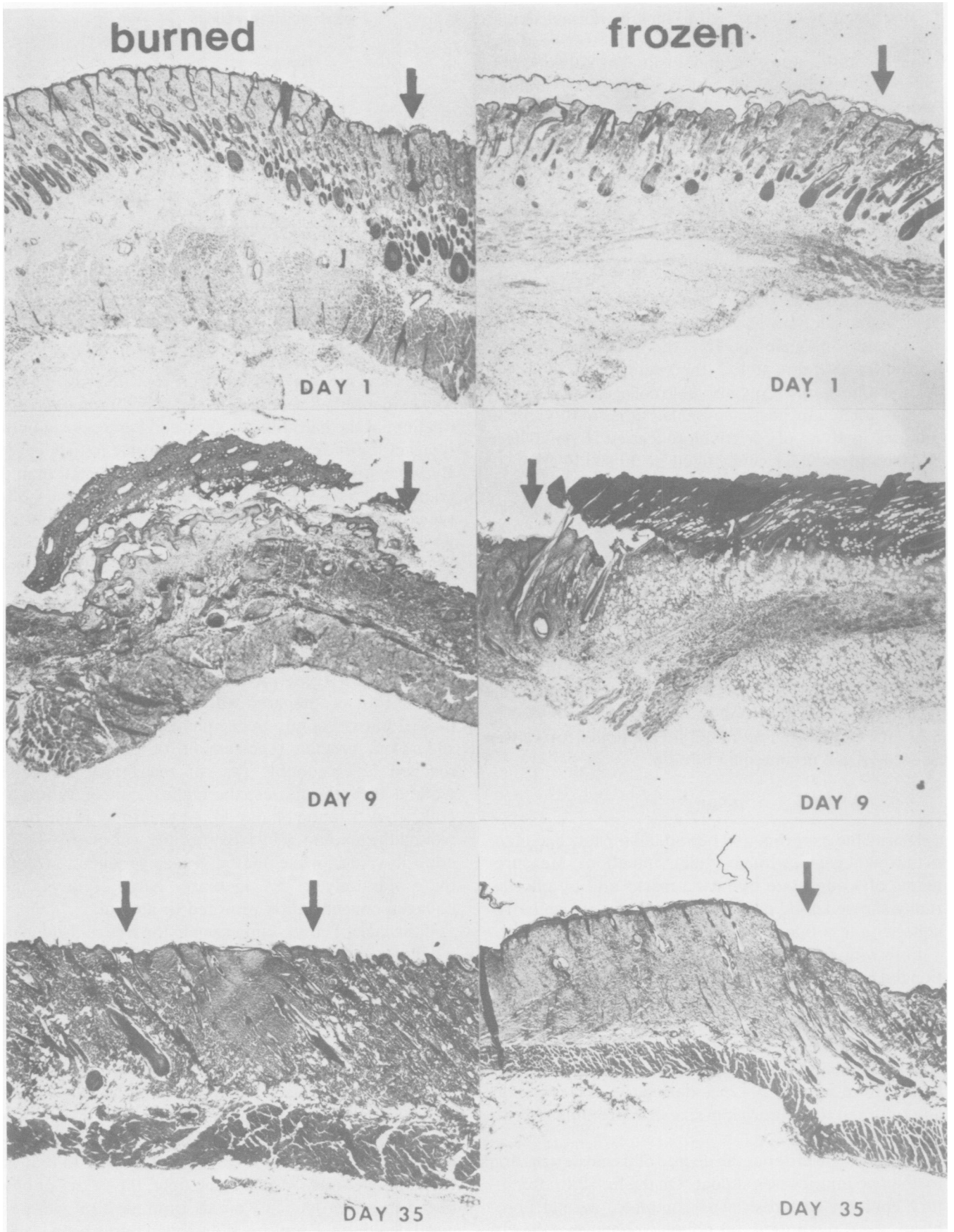


FIG. 1. Rate of wound healing in burns and in freeze-producing wounds.

Initially, burn wounds had less than half as much collagen as the freeze-produced wounds indicating a greater loss (Fig. 3). The amount of hydroxyproline present in the freeze-produced wound was 55% that of normal skin as compared with 21% that of normal skin in the burn wound. Subsequently the burn wound showed a greater replacement of collagen, which reached levels double that of the freeze-produced wound. In both injuries, the highest concentration of collagen was found on day 15. At that time, in the burn wound the amount of hydroxyproline was over 300% of control value, while in the freeze-produced wound there was only a 75% increase. After 28 days, the collagen concentration returned to normal.

If the lack of contraction seen in the freeze-produced wounds was due to the remaining matrix, then removal of the matrix should initiate contraction. To test this possibility, four wounds were produced in the shaved backs of each rat (n = 12). One was a burn wound and two were freeze wounds placed so that the corresponding sites in different animals were exposed to different injuries for accurate comparison. The fourth was an open wound produced one week after the thermal injuries by full-thickness skin excision, including the panniculus carnosus, of an area of size identical to that of the other wounds. At that same time, the center of one of the freeze-produced wounds from each rat was excised, leaving a 2 mm rim of damaged tissue as margin.


burned
frozen
DAY 1
DAY 1
DAY 9
DAY 9
DAY 35
DAY 35

Contraction occurred in the burn wounds, and no change occurred in the freeze-produced wounds. The wounds created by excision showed a 15% reduction of wound size by the second day, as compared with 25% in the burn wound. However, rapid contraction continued so that eight days later they became only one-third of their original size (Fig. 4).

The freeze-produced wounds with the centers excised one week after the initial injury behaved like the open wounds, with a 50% decrease of original wound size in five days. After three weeks, all the wounds healed.

Discussion

Although burn and freeze injuries destroy skin to a similar extent, leaving only some cells at the base of the hair follicles, after freeze injury the wounds do not contract. As shown here and elsewhere, burn wounds and open wounds drastically reduce their size after two weeks,[8] while the freeze-produced wounds remain at the original size reflected by the large size of the subsequent scar. Otherwise the normal components of repair—inflammation, granulation tissue formation, and epithelialization—occur to a similar extent after both injuries.

Freezing does not appear to interfere with the functions of the myofibroblasts believed to be responsible for wound contraction.[5] Excision of the center of the freeze-produced wound alters the behavior and causes it to contract like an open wound. In fact, these freeze-produced wounds, once their centers were excised, contract faster than open wounds made at the same time. Therefore, the mechanism for contraction appears to be already present, but merely prevented from operating by the freeze-produced matrix.

The condition of the residual matrix following injury seems to be a determinant of wound contraction and may explain the apparent difference in contraction between the two injuries. The rapid and complete destruction of the connective tissue matrix and cells in the burn wound make it behave like an open wound that is also denuded of cells and matrix and contraction occurs. On the other hand, freezing destroys cells, but relatively

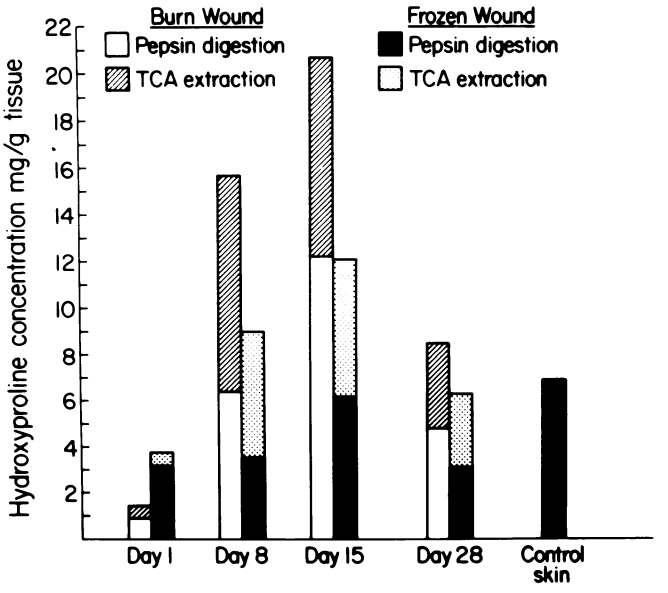


FIG. 3. Hydroxyproline concentration of burn and freeze-produced wounds.

spares the connective tissue matrix, which is then removed and replaced gradually, thereby simulating the effects of a full-thickness skin graft to an open wound. Skin grafted onto an open wound introduces both cells and matrix and prevents wound contraction.[2]

Changes in collagen concentration in these wounds support this explanation. In the burn wound there is a greater early loss of collagen followed by increased collagen deposition. In the freeze-produced wounds, the early loss of collagen and subsequent collagen deposition are much less pronounced.

The initial increase in sizes of the freeze-produced wounds may be attributed to the elasticity of the surrounding skin similar to gaping seen in open wounds.[10] Edema, which was evident at histologic examination, may also contribute to this phenomenon. The early rapid decrease in size of the burn wound is unlikely to be due to wound contraction, because contraction does not ordinarily occur during the first two days. In fact, the open wounds initially show less wound size reduc-

FIG. 2. Photomicrographs of burn wounds (left panels) and freeze-produced (right panels) dermal wounds at 1, 9, and 35 days following injury. Hematoxylin and eosin. 18×. The arrows indicate the interface between normal and injured areas. On day 1 following injury both the burned and frozen specimens showed epidermal loss, hair follicle necrosis, dermal and subdermal edema, and muscle necrosis. The differences in staining intensities of dermis and muscle in the normal regions to the right of the arrow are apparent. Inflammatory cells are present in the subdermis. On day 9 the burned area to the left of the arrow shows an eschar consisting primarily of a sloughed dermis in which the remnants of hair follicles can be seen. The epidermis has completely covered the wound, and subepithelial granulation tissue is present. The tissue at the far left of the day 9 burned specimen is the other interface of the burned-normal region. The day 9 frozen lesion contains a large eschar to the right of the arrow that includes most of the original injured dermis. Normal skin is to the left of the arrow. The hypodermal region contains early granulation tissue. The regenerating epidermis has extended under the eschar, but has not completely covered the wound. On day 35 the burn wound consists of a small dermal scar with absent or reduced hair follicles located between the two arrows. The frozen wound in contrast consists of a larger scar in surface area. The frozen scar contains reduced numbers of hair follicles and extends to the panniculus carnosus as in the burned specimen. However, the size of the wound extends beyond the picture to the left of the arrow.

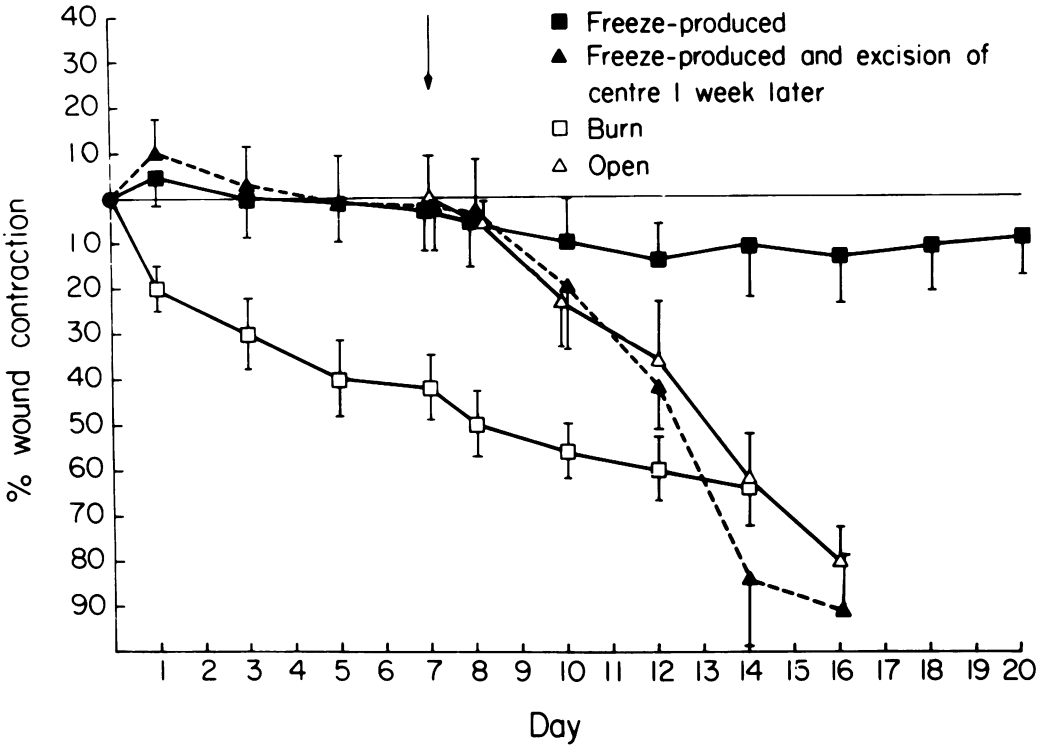


FIG. 4. Rate of wound healing between burn, freeze-produced, open wounds and freeze-produced wounds having had their centers excised one week after initial thermal injury. (Arrow denotes the time when open wounds and excision of the centers of the freeze-produced wounds were made.)

tion than the burn wounds. This early decrease in the size of the wound may be related to its heat-denatured connective tissue matrix, since heat denatured collagen fibers shrink.[11]

Although the scars from the freeze-produced wounds have larger surface area because contraction does not play a role in its wound closure and is not essential in its healing, the wound topographically is well preserved and appears like surrounding skin. Thus, inapparent scars may result, as seen in clinical practice following cryosurgery in man.

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