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HISTOLOGIC STUDIES OF THE EARLY HEALING PROCESS IN
MODERATE SECOND DEGREE BURNS

by

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ABSTRACT

The sequence of the histologic changes which occur during the repair of moderate second degree burns ~~has been~~ investigated. Between one and five days after injury, the burned tissue is effectively isolated by a leucocytic barrier. Epithelial coverage proceeds beneath the necrotic slough, and the new epithelial cells cover collagen fibers which are normal in appearance. Shortly after epithelial coverage, however, the collagen is degraded and the fibers disappear. At the same time, proliferation of capillaries and fibroblasts produces, beneath the new epithelium, a tissue which resembles granulation tissue. Later, new collagen fibers replace the capillaries, but the process is incomplete at the end of the fourteen day period included in this report.

These histologic changes lead to the restoration of normal dermal thickness and to the formation of a superficial layer which resembles the normal papillary layer of the skin.

Moderate second degree burns were produced on young Chester White pigs by radiant exposures of 10 cal/cm^2 . Injuries resulting from square pulse exposures of 0.3, 3.0 and 10.0 seconds duration and from simulated field pulse exposures of 1.8, 4.0 and 12.7 seconds duration were compared. Biopsies were performed four, five, nine, ten and fourteen days after injury.

Burns produced by the two shortest exposure times are covered with new epithelium faster than are those resulting from longer exposures. The short exposure burns exhibit an earlier, more massive leucocytic infiltration and

a narrower zone of reversibly damaged tissue. No variation either in the rate of epithelial coverage or in the mechanism of repair can be attributed to the shape of the energy pulse alone.

INTRODUCTION

In the years since the atomic bombings of Hiroshima and Nagasaki, many controlled, experimental data have been obtained on the flash burn, the injury which accounted for the majority of casualties among the survivors in those Japanese cities. In addition to variations produced by different radiant exposures (cal/cm^2), a number of factors such as exposure time (1, 2, 3, 4), skin temperature (5), wavelength (6, 7) and skin reflectance (8), have been found to affect the severity and, at times, the morphologic characteristics of the radiant energy burn.

This report deals with the effects of exposure time and energy pulse shape on the rate of epithelial coverage and on the histologic changes which occur during the first fourteen days after production of moderate second degree burns. The experiments for this study were done in conjunction with those for a recent report (4) on the effects of exposure time, energy pulse shape, and biopsy time on the microscopic pathology of and the severity of burns produced by a total exposure of $10 \text{ cal}/\text{cm}^2$.

METHODS

Young Chester White pigs, anesthetized with intraperitoneal Dial with urethane (Ciba), were the experimental animals. Their skin has been found by Moritz and Henriques (9, 10) and by workers in this laboratory to resemble human skin closely and to respond similarly to thermal energy. The hair was removed from both sides with electric clippers, and the skin was washed gently with a liquid detergent. The ambient temperature in the burning room was controlled in order to decrease variations in the skin temperature of the anesthetized animals (5).

The heat source was a modified 24 inch Army searchlight (2, 11). A rotating slotted wheel between the source and the animal provided the simulated field pulse (12, 13).

To produce moderate second degree burns consistently, a radiant exposure of 10 cal/cm² was chosen. This exposure was delivered in 0.3, 3.0, and 10.0 sec. with a rectangular pulse and in 1.8, 4.0 and 12.7 sec. with a pulse simulating that of the atomic bomb. Biopsies were taken at 4, 24, and 48 hours and at 4 or 5, 9 or 10, and 14 days. These treatments were randomly assigned exposure sites on an animal's side, and the treatment block was replicated 16 times. Biopsy times were also randomized to insure equal probability of any one of a particular type of burn being biopsied at any one of the chosen times. The three shorter biopsy times (4, 24, and 48 hours), from which the character and the severity of the damage were determined, provided the data for an earlier report (4).

Each biopsy included normal skin on both sides of the burn. They were placed in 10% formalin, and sections were stained by a method for differentiating burned and normal tissues (14). They were carefully examined for morphologic details of the healing process, and the rates of epithelial coverage were tabulated.

RESULTS

A. Histology of the Healing, Second Degree Burn

An earlier paper (4) describes in detail the histologic picture of the damage encountered in these burns. The findings are summarized here. With some variation in degree, irreparable epidermal damage occurs. Beneath the epidermis are varying depths of blue staining, damaged collagen fibers which frequently present a range of colors from dark blue-black superficially to faint blue deeper in the dermis. Underlying the differentially stained collagen

are normal appearing fibers with increased interfiber spacing (Figure 1); this represents a zone of edema. Unaltered collagen lies beneath this area. The hair follicles are damaged to a greater depth than is the collagen, and glandular structures well below the blue staining collagen may be disrupted.

The first histologic evidence of a local response to injury is the appearance of leucocytes in the normal tissue beneath the burn and in the sub-epidermal portion of the dermis immediately adjacent to the burn. The white blood cells are first apparent near small blood vessels. At 48 hours they are frequently in the zone of edema and they appear to have migrated there from the adjacent normal dermis. By four or five days there is usually infiltration of the light blue-stained collagen zone, and the tissue to be sloughed may be demarcated by a zone heavily infiltrated with leucocytes. With delineation of the tissue destined to be sloughed, undamaged epithelial cells from hair follicles and from the margins of the burn proliferate and send out thin sheets of cells to cover the tissue deep to the slough (Figure 2). The new epithelium overlies normal appearing, normal staining collagen fibers. This sheet of cells subsequently thickens by proliferation of the basal layer.

One of the most interesting occurrences in the healing burn is the degradation of undamaged collagen after epithelial coverage. The first evidence of this change is found just beyond the margin of the burn and in the most superficial portion of the dermis. Here, the overlying epithelium shows no evidence of damage. Leucocytic infiltration, capillary proliferation and disappearance of collagen fibers lead to a spongy tissue which resembles very early granulation tissue (Figure 3). Shortly after epithelial coverage of normal appearing collagen, a similar but more profound change occurs in the deeper dermal layers. The area of collagen resorption and capillary proliferation averages about one-half millimeter in depth (that is, about one-fourth the thickness of normal dermis) but it may involve the entire thickness of the

Fig. 1. Forty-eight hour biopsy of 0.3 sec. burn. Note three zones of dermal response: (1) slough infiltrated with leucocytes, (2) zone of edema (increased interfiber spacing), (3) unaltered zone.

X-20

Fig. 2. Five day biopsy of a 0.3 sec. burn showing thin sheets of epithelium advancing beneath the black staining slough. X-60

Fig. 3. Five day biopsy of a 0.3 sec. burn. The arrow indicates transition from regenerated epithelium on the left to unburned epithelium on the right. Note leucocytic infiltration and collagen resorption beneath normal epithelium at the edge of the burn. X-60

remaining, undamaged dermis. In the small area burns (diameter 1.7 cm.) reported here, new fibroblasts and fine fibrils make an early appearance. By the 14th day after burning the fibrils are coarser, fibroblasts and capillaries are less prominent, and the rete pegs of the epidermis are exaggerated and project deeply into the altered dermis (Figure 5).

In the four and five day biopsies, increased numbers of leucocytes and many fibroblasts appear among the normal collagen fibers deep to the zone of edema. They are first apparent near blood vessels and hair follicles. By the ninth or tenth day the leucocytic and fibroblastic response has become marked in the zone of edema as well. At this point it is difficult to distinguish the original, easily delineated zones of the dermis. Whereas, the uppermost collagen fibers disappear and are replaced by a dense network of fibroblasts and fine fibers, the deeper fibers sometimes appear to have been only partly resorbed and to have been enmeshed with increased numbers of fibrils, fibroblasts and leucocytes. The proliferation of fibroblasts and formation of new fibers in these two areas, the residuum of the original reticular zone, cause an increase in the thickness of the dermis. The moderate or severe second degree burn originally results in a thin dermis. The reparative process leads to restoration of dermal substance. In some of the burns the leucocytic and fibroblastic response extends into the superficial subcutaneous tissue which then is replaced by dense fibrous tissue similar to that in the overlying dermis (Figure 4). This makes a further addition to dermal thickness. Even though a considerable portion of the original dermis is lost in the slough, the healing burn at 14 days often consists of a thicker layer of collagen than does the skin immediately adjacent to it (Table I).

By 14 days, hair follicles are regenerated and often contain hairs. The glandular elements are either restored to their usual appearance, replaced

Fig. 4. Nine day biopsy of a 10.0 sec. burn. Note the massive replacement of subcutaneous fat by dense collagenous tissue.
X-60

Fig. 5. Nine day biopsy of a 0.3 sec. burn. Complete epithelialization. Note the newly formed papillary zone beneath the epidermis and the rete pegs extending into it.
X-60

TABLE I

COMPARISON OF THE THICKNESS OF THE DERMIS, OF THE SLOUGHED
TISSUE, AND OF NORMAL ADJACENT DERMIS IN HEALING SECOND
DEGREE BURNS 14 DAYS AFTER INJURY

Pulse Shape	Exposure Time (in sec.)	Average Thickness of Dermis Adjacent to Burn (in mm.)	Average Thickness of Slough 24 hrs. after Burning (in mm.)	Average Dermal Thickness in Burned Area at 14 days (in mm.)
Square	0.3	2.3	0.5	2.2
	3.0	2.3	0.8	2.3
	10.0	2.3	0.5	2.5
Simulated Field	1.8	2.3	0.7	2.4
	4.0	2.3	0.6	2.6
	12.7	2.3	0.5	2.5

entirely by connective tissue, or represented by solid nests of epithelial cells deep in the dermis.

B. Correlation of Variations in Healing Time with Exposure Time and Pulse Shape.

Table II presents the data on epithelial coverage.* All burns with 0.3 sec. square pulse exposures are epithelialized by ten days. Three-fourths of the 1.8 sec. field pulse exposure burns are covered by epithelium at this time. Only one-half or less of the remaining burns, those with longer exposure times, are covered completely by ten days, but all burns in this study are covered by 14 days. Figures 5, 6, and 7 illustrate the usual stage of healing at the ninth or tenth post-burn day for the square pulse burns (0.3, 3.0, and 10.0 sec.). It should also be noted that the early reconstruction of the subepidermal or papillary zone closely parallels epithelial coverage (Table II).

DISCUSSION

A. Correlation of the Type of Damage with the Method of Repair.

Hinshaw's Method for differentially staining burned tissue (a modification of Verhoeff's elastic tissue stain) has greatly facilitated microscopic evaluation of the depth of thermal injury. In order for this staining technique to have more clinical value, we should know what part of the differentially stained tissue the body will slough as healing takes place, what part will be absorbed and replaced, and what part, if any, is unaffected during the healing process. As one might suppose, direct measurement of the

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*Epithelial coverage refers to complete coverage of the surface by epithelium. This new epidermal layer may be either thicker or thinner than normal epidermis.

TABLE II

RELATIVE TIME REQUIRED FOR EPITHELIAL COVERAGE AND PARTIAL
RECONSTRUCTION OF THE PAPILLARY ZONE OF THE DERMIS

Pulse Shape	Exposure Time (in sec.)	Biopsy Time (in days post burn)	No. of Burns Showing Complete Epithelial Coverage	No. of Burns Showing Partial Formation of a New Papillary Zone
Square	0.3	4 to 5	1	0
		9 to 10	16	14
		14	15	15
	3.0	4 to 5	0	0
		9 to 10	8	3
		14	16	15
	10.0	4 to 5	0	0
		9 to 10	5	5
		14	16	15
Simulated Field	1.8	4 to 5	0	0
		9 to 10	12	9
		14	16	16
	4.0	4 to 5	0	0
		9 to 10	8	6
		14	15	13
	12.7	4 to 5	0	0
		9 to 10	4	0
		14	16	15

Fig. 6. Nine day biopsy of a 3.0 sec. burn. Partial epithelialization and fibroblastic proliferation in the underlying dermis. X-60

Fig. 7. Nine day biopsy of a 10.0 sec. burn. Partial epithelialization with very little fibroblastic proliferation in the underlying dermis. X-60

depths of the various types of damage and of the different healing zones should offer little clarification of this problem because of the shrinkage (dehydration) and swelling (edema) which occur independently in these various parts. It is necessary, then, that any correlation be based on an impression gained from careful study of a large number of serial biopsies. In this way it has been determined that the portion of the dermis which will be sloughed is that which, at 24 or 48 hours after burning, stains blue or blue-black. Some of the fibers which stain faint blue in biopsies made four hours after burning but which lose their color by 24 hours are not part of the slough.

The processes of degradation and repair which take place in deep, unburned collagen result in the reconstruction of a more nearly normal dermis. Normal dermal thickness and a superficial layer somewhat resembling the normal papillary layer are restored by the changes occurring in the more dense reticular layer and by capillary and fibroblast proliferation. Studies, aided by various histochemical techniques, on the healing process between two and six weeks after injury are now in progress. These will assist in the identification of the types of tissue which are broken down and then restored.

The zone of the dermis which shows increased interfiber spacing, termed the zone of edema, is originally not detectably different from normal dermis. The leucocytic and fibroblastic reparative response in this zone is usually somewhat delayed. However, nine or ten days following injury there is marked cellular proliferation here. It would seem that there is no stimulus for repair until enzymatic breakdown of relatively normal collagen has begun. After the reparative response begins, there is very active degradation of collagen fibers and proliferation of fibroblasts.

The unaltered dermis beneath the zone of edema responds promptly but mildly. There is less resorption of collagen fibers and only moderate fibroblastic proliferation.

Correlation can be made between the histologic findings described here and certain features which one may observe clinically in a healing burn or wound. The pink color of the recently epithelialized wound is evidence of the rich capillary bed, and the subsequent whitening of the scar reflects a decrease in the vascularity of this zone. The raised or rounded scar which may occur is evidence of tissue proliferation which should restore normal dermal thickness but which at times over shoots the mark. The final softening and flattening as well as the color and texture changes seen in the usual wound continue long after epithelial coverage is complete, and long after 14 days. The speed with which the changes noted in this experiment occur are probably due, at least in part, to three different factors: 1) the small size of the lesion, 2) the presence of numerous, deep hair follicles as a source for new epithelium, and 3) the use of very young pigs as the experimental animal. Later experiments, to be reported, indicate that diameter of the lesion has some influence on the rate of repair.

B. Discussion of Relative Healing Times

In the preceding report (4) dealing with the damage encountered in the burns of this experiment it was noted that at 48 hours the 0.3 sec. exposure burns had a layer of compressed, blue staining, damaged tissue 0.52 mm. thick and that burns with 10.0 sec. exposure times had a layer of similar damage at a depth of only 0.32 mm. One would think that the more superficial 10.0 sec. burn would heal first. Table II shows that this is not the case. The data accumulated in this and the preceding report suggest an explanation which may account in part for the more rapid epithelial coverage of burns produced by the short exposure time (0.3 sec.) than of those from longer exposure times (3.0 and 10.0 sec.).

Epithelial coverage occurs after the tissue destined to be sloughed has been demarcated from the underlying, normal appearing collagen; this

occurs by leucocytic infiltration of the area between the two. From examination of many histologic sections of these burns one gains the definite impression that the severe, superficial damage produced by a very short exposure time (0.3 sec.) calls forth an earlier, more massive leucocytic response than does the type of damage which results from longer exposure times. It has also been noted that the zone of edema which lies between severely damaged collagen and collagen showing no damage is not as wide in the burns produced by very short exposure times as it is in those resulting from longer exposures (4). Perhaps the very severely damaged tissue provides a strong stimulus for leucocyte migration and the close proximity of the source of supply for leucocytes (vessels in normal tissues) and the damaged tissue enhances delineation of the slough in burns resulting from short exposure times.

The differences in epithelial coverage rates of the 0.3 sec. square pulse burns and the 1.8 sec. field pulse burns on the one hand, and of the remaining burns from longer exposure times on the other, are apparently due to differences in exposure time rather than to differences in the shape of the pulse which produced the burn. This lack of a detectable influence of the pulse shape on both the healing rate and the mechanism of repair agrees with our previous observations that pulse shape alone does not affect the type of damage produced by radiant exposures of 10 cal/cm².

SUMMARY AND CONCLUSIONS

1. The histology of the early healing process in moderate second degree burns, produced by 10 cal/cm², was studied in biopsies made four, five, nine, ten and 14 days after burning. They were stained by Hinshaw's modification of Verhoeff's elastic tissue stain. Comparison of healing rates for burns produced by 0.3, 3.0, and 10.0 sec. square pulses and 1.8, 4.0 and 12.7 sec. simulated field pulses were made.
2. An illustrated description of the healing process which correlates type of damage with method of repair is given.
3. After the damaged tissue is separated from the remaining dermis by leucocytic infiltration, new epithelium covers normal appearing, normal staining collagen fibers. However, as epithelial coverage takes place, normal collagen is degraded, and proliferation of capillaries and fibroblasts occurs in this area. This process may extend through the entire thickness of the remaining dermis.
4. At a later time, this spongy tissue, which resembles early granulation tissue, contains new collagen fibers. The dermis is gradually restored to a more nearly normal appearance. These changes, along with fibroplasia in the superficial connective tissue, restore dermal substance. Another result of the reparative process is the formation of a subepidermal layer which resembles the original papillary layer.
5. Burns produced by 0.3 sec. (square pulse) and 1.8 sec. (simulated field pulse) exposure times, were covered by epithelium faster than were those produced by 3.0, 4.0, 10.0, and 12.7 sec. exposures. An earlier and more massive leucocytic response, and a narrower zone of reparable damage are probably factors in the more rapid epithelialization of the short exposure burns.

6. Neither the epidermis nor the dermis has a normal histologic appearance 14 days after injury.
7. Variation in pulse shape alone was found to affect neither the method nor the rate of healing.

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