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• R. J. BRENNAN, M.D.

A comparative study of the immediate and delayed histopathological changes of the skin in untreated and treated thermal burns.

Aloe Cream Ointment

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It is well known that approximately one week after a minor thermal burn, an eschar begins to separate. However, there is little if any description in the literature of the successive microscopic changes as they develop during the first 48 hours. The extent of the damage ordinarily is determined at the time of injury. Our study of the timing of subsequent histopathological changes in the early hours and days following thermal burns suggests that early medical treatment could modify some of these early changes and prevent some of the later changes.

The purpose of this study was to determine, with serial biopsies, the progressive skin changes in thermal burns from the time of burning to the end of the pathological and healing process. The available literature primarily is concerned with the terminal effects of burns without particular study of the timing and characteristics of the early histopathological changes. This is reflected in the common classification of burns. According to the general classifications, first degree burns involve the epidermis, dissecting mostly the outer layers with vesication, hyperemia and slight edema of the dermis. Second degree burns more seriously involve the dermis with damage to the capillaries, hair follicles and sweat glands, with considerable edema of the dermis. In third degree burns the damage involves the entire thickness of the skin.

The process of gradual formation of the eschar has been followed in these experiments, examining the gross and microscopic changes step by step after establishing a definite procedure to provide (1) a controlled procedure of burning, (2) a close examination of the initial damage to the skin structures, (3) the timing of subsequent histopathological changes in the early hours and days following burning, (4) an insight into factors to be corrected and possibly prevented by the early treatment of thermal burns and (5) a uniform method to evaluate the efficacy of early therapeutic measures.

This work has been done at the laboratories of Viamax, Inc., Miami, Florida.

Experimental Procedure

Albino rabbits weighing six to seven pounds were kept on a standard diet to assure a homogeneous group. In this preliminary experiment six animals were then selected and the skin of the back was epilated over four by four cm area 24 hours before producing a thermal burn. The burning instrument was a steel plate 43 mm in diameter, four mm thick, with a steel handle measuring 12 cm in length and nine mm in diameter arising from the center. The handle was insulated with glass wool. To heat the instrument, a propane gas reducing flame 25 mm long was used. The plate was held at the tip of the reducing flame for one minute. Between successive burns the plate was immersed in water until cooled to touch. The heated plate was applied to the unanesthetized skin for two seconds using slight pressure to assure contact of the entire burning surface of the plate to the epilated skin. All animals thus received identical burns—and on two sides. Biopsy specimens were taken from the burned areas at intervals of one-half hour, one hour, two hours, six hours, 24 hours, 48 hours, four days, six days, 10 days, 12 days, 14 days, 18 days, 25 days, 29 days and 35 days, which was grossly the end of the pathological process.

Experimental Findings in the Untreated Group

When no treatment was given, immediately after the burning, the skin appeared greyish-white with smooth edges. In the first six hours the color remained the same but the burned area became diffusely edematous with slight elevation of the edges. After 24 hours the burned area was brownish and the surrounding unburned skin appeared erythematous. After 48 hours the burned area was dry and brown and the surrounding unburned skin was markedly congested. During the following days the ther-

Figure 1

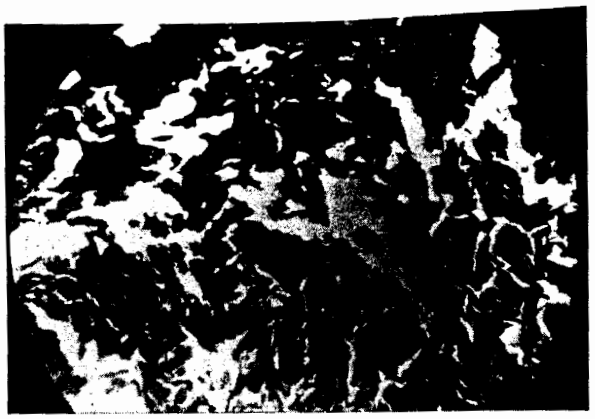

Figure 2

mally injured skin became dryer and harder until, on the tenth day, the edges began to curl and dissect from the underlying tissue. The complete eschar separated on the thirteenth or fourteenth day. The burned areas in all animals were healed by firm, pearl white adherent scars by the twenty-ninth day.

The microscopic examination of the histopathological changes showed that during the first 30 minutes after the burning the epidermis became partially dissected and the upper layers of the dermis showed diffuse thermal coagulation. Red thrombi were found in the deeper layers of the dermis, while a perivascular inflammatory reaction began to develop (Figure 1). Numerous spaces retaining fluid, following thermal coagulation and development of edema were present in the dermis after one hour. After two hours there was evidence of severe damage of the follicular structures, while numerous thrombosed capillaries were present in the dermis. After six hours the entire dermis appeared markedly dehydrated and numerous round cells infiltrated the deep dermis. After 24 hours the coagulated upper layers of the dermis tended to dissect in a large eschar from the deeper layers of the dermis where a marked inflammatory reaction was present (Figure 2). After 48 hours the process of dissection of the upper layers of the dermis was definitely more advanced than after 24 hours. At this time the deeper layers of the dermis showed polymorphonuclear infiltration, microscopic debridement and in some areas entire loss of structure. Microscopically at four days the eschar formed by the upper layers of the dermis was entirely separated and showed a patchy irregular staining. After six days the deep dermis showed a loose structure with abundant perivascular infiltration and numerous capillary thrombi. At 10 and 12 days the fragments of amorphous material arising from the thermally damaged dermis were invaded by an abundance of leukocytes, the picture being partly one of dry necrosis of the eschar and partly one of microscopic colliquation and softening of the deep dermis. After 14 days the appearance of the dermis was that of an arborescent structure of dissociated collagen branches undergoing partial autolysis (Figure 3). After 18 days the image of arborization markedly changed, owing to a process of small fragmentation in which the tissue lost structure and stainability and underwent a process of debridement (Figure 4).

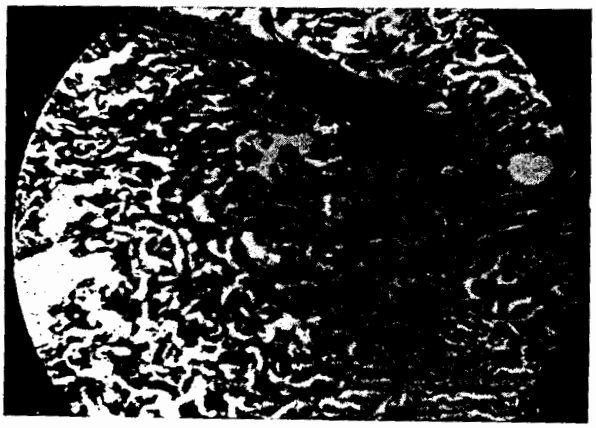

Figure 3

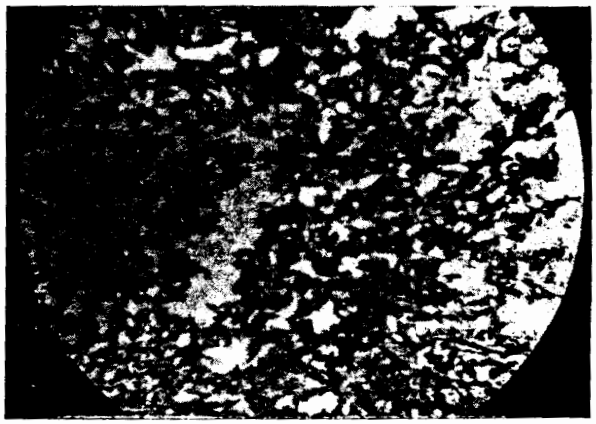

Figure 4

Preliminary Experiments on Medications

In our experiments on untreated thermal burns, gross and microscopic observations showed that the eschar forms and separates microscopically in 24 to 48 hours and the eschar separates grossly in 10 to 14 days. We therefore undertook a comparative study of the effectiveness of various methods of early medical treatment using several medications and the previously established controlled procedure of burning. Preliminary research work done by us on identical burns following the same method and procedure showed that aloe vera gel alone was not well suited for continuous dressing of the thermally injured skin and an ointment consisting of lanolin base alone was not effective in the treatment of these experimental burns. Preparations of aloe vera gel and lanolin base and preparations in lanolin base of aloe vera gel and a synthetic protecting and stabilizing colloid compound (preparation S) were tested for effectiveness following the same procedure. The ointment, consisting of aloe vera gel, preparation S. 30% in a specially prepared bland ointment base with 5% lanolin, used in Groups I and II of the following comparative experiments, was found to be the most effective in preventing the formation of the microscopic eschar.

Experimental Procedure in the Treated Groups

Twelve albino rabbits were selected for experiments with various medications and divided into four groups of three animals each. The skin of the back of each animal was epilated on both sides over a four by four cm area. Each animal was burned for two seconds on both epilated areas using the previously described steel plate with our controlled heating procedure. Each animal was treated on one side only. The other burned area served as a control and was thus available for gross and microscopic comparison. Biopsy specimens were taken from all burned areas at intervals of one-half hour, one hour, two hours, six hours, 24 hours, 48 hours, four days, six days, 10 days, etc., as in the previous experiments.

The first group was treated with Alo-Creme Ointment.* The second group was treated with Alo-Creme Ointment containing, in addition to the above mentioned components, 5% cystine. The third group was treated with 1% trinitrophenol butylaminobenzoate ointment. The fourth group was treated with petrolatum and gauze dressing. In every case the ointment was applied immediately after burning and twice daily thereafter.

*Alo-Creme Ointment manufactured by Alo-Creme Laboratories, Fort Lauderdale, Florida.

Experimental Findings in the Treated Groups

Group I—Treated with Alo-Creme Ointment: Six hours after burning, the treated area was greyish-white and edematous. After 24 hours, the treated area was more pliable than the untreated area of the same animal. There was definitely less erythema around the treated burned area than there was around the untreated control area. After 48 hours the untreated area was brown and definitely dryer than the burned area treated with Alo-Creme Ointment. During the following days the treated area remained soft and pliable while the untreated area became harder and dryer. Between the seventh and the fifteenth day, there was slight and continuous superficial debridement of the treated area without gross formation of an eschar, while after two weeks the untreated area showed a large eschar which gradually separated from the underlying tissue. The Alo-Creme treated lesions healed in two weeks without gross evidence of scarring. By the end of the fourth week, the untreated area of the same animal healed with a firm pearly-white scar.

Group II—Treated with Alo-Creme Ointment containing 5% cystine: During the first week the treated areas were similar to the treated areas of Group I. In the second week the debridement appeared to be greater in Group II than in Group I. The healing process took place without formation of a gross eschar and by the third week the treated areas gradually became softer, more pliable and less edematous.

Group III—Treated with 1% trinitrophenol aminobenzoate ointment: At 24 hours the treated areas were grossly similar to those of Groups I and II. During the next 48 hours the treated area became soft and edematous while the surrounding skin became markedly congested. At the end of the first week the periphery of the treated area showed multiple petechial hemorrhages while the center became white and gelatinous. There was a gradual worsening of the general condition of these animals and none survived the tenth day. Gross and microscopic hemorrhages of the liver and kidneys were found at autopsy.

Group IV—Treated with petrolatum and gauze: During the first three days there was a gradual development of congestion, edema and focal hemorrhages. By the end of the first week the treated area had gradually changed from greyish-white to brown. At this time there were several small abscesses in the skin. During the second week a purulent eschar separated leaving granulation tissue. By the end of the fourth week the treated area healed by the formation of a firm pearly-white scar similar to that of the untreated area of the same animal.

Figure 5

Figure 6

Histopathological Changes in Treated Animals

Group I: Thirty minutes after burn and treatment with Alo-Creme Ointment the epidermis was almost entirely missing and, where still present, formed several vesicles. The upper dermis did not stain as darkly as in the controls. There was no definite perivascular reaction and there were no thrombi evident in the deep dermis (Figure 5). A diffuse edema developed within an hour and a few isolated areas of perivascular reaction were present in the deep dermis at this time. After two hours the dermis showed a slight thermal coagulation as indicated by the stainability of tissue, while in the deep layers of it numerous capillaries appeared congested. At six hours there was not such evidence of dehydration of the dermis as in the controls. The framework of the collagen fibers showed edema.

After 24 hours there was little evidence of thermal necrosis of the upper dermis and there was no indication that the upper layer of the dermis would dissect from the deeper layers forming an eschar as in the controls and in the untreated animals. At this time the perivascular infiltration was marked and diffuse (Figure 6).

After two days the superficial dermis showed moderate infiltration with polymorphonuclear leucocytes and diffuse congestion and edema. There were no areas of focal necrosis found within the dermis. A slight debridement of the superficial dermis was evident at four days while the texture of the deep dermis was well preserved and the collagen fibers appeared practically normal. The capillaries were free from thrombi. After six days the epidermis showed regeneration from the basal layers in some areas. There was no evidence of thrombosis or necrotic dissection in the dermis. After 10 days the thermally injured dermis still showed some superficial debridement; the fibroblastic activity was increased in all layers of the dermis and numerous vessels showed perivascular round cells infiltration. Active epithelial regeneration was more evident at 12 and 14 days while the texture of collagen fibers of the dermis was more dense than normal. At this time there were no areas of necrosis or enzymatic lysis evident (Figure 7). At 18 days there was definite hyperplasia of collagen in the dermis without evidence of scarring. There were many fibroblasts in the deep dermis and several round cell clusters surrounded the capillaries (Figure 8).

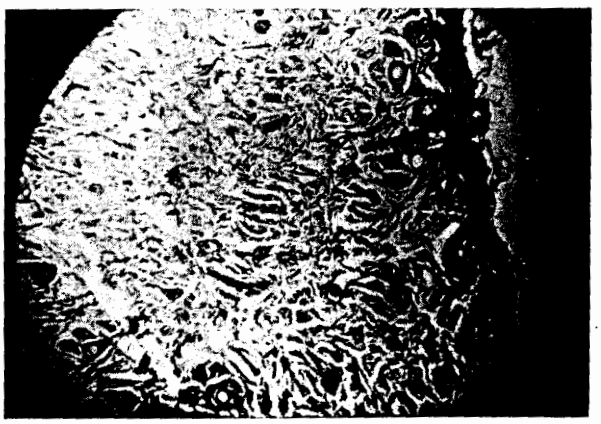

Figure 7

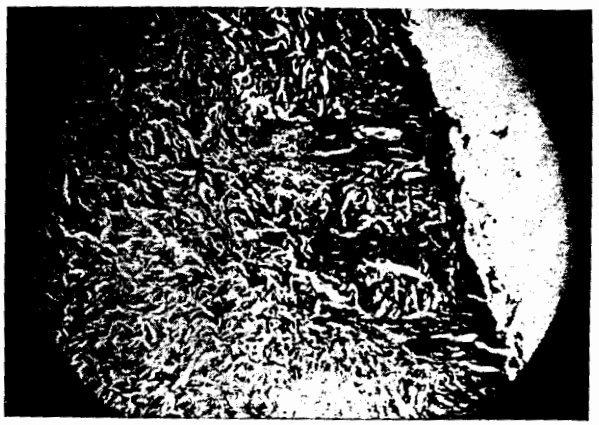

Figure 8

Group II: The microscopic findings in these animals treated with Alo-Creme Ointment containing 5% cystine did not show any appreciable difference from those of Group I during the first 48 hours. However, after four days there was more marked debridement of the upper dermis. At the end of the first week there was hyperplasia of the basal layer of the epidermis. During the second and third week there was intensive fibroblastic activity, some congestion and diffuse collagen hyperplasia in the dermis.

Group III: Marked edema, severe congestion and isolated capillary thrombosis developed during the first six hours. After 48 hours the upper dermis showed dissection from the deeper dermis. After four days there were multiple hemorrhages in the dermis. Capillary congestion and perivascular polymorphonuclear infiltration increased between the fourth and tenth days. At this time there was massive debridement of the superficial layers of the dermis which was infiltrated with polymorphonuclear leucocytes, while there was some evidence of fibroblastic activity in the deeper layers of the dermis.

Group IV: The microscopic appearance of the areas treated with petrolatum and gauze was similar to that of Groups I and II in the first two hours except that there was more dehydration and isolated capillary thrombi at two hours. After six hours the dermis showed leucocytic infiltration which became abundant after 24 hours. At this time there were many capillary thrombi and the upper layer of the dermis showed numerous dark staining areas of debridement. After 48 hours the microscopic demarcation of the eschar was complete. After one week there was intensive fibroblastic activity underlying the zone of demarcation. At the end of the second week the entire eschar was shredded and debriding in large pieces which were densely infiltrated with polymorphonuclear leucocytes. During the third and fourth weeks there was marked collagen hyperplasia, fibroplastic activity and scarring with little regeneration of the epidermis. There was little difference between this group and the controls.

Summary

Gross and microscopic observations in these experiments showed that in deep dermal burns an eschar forms and separates microscopically in 24 to 48 hours and grossly the eschar separates in 10 to 14 days if the skin is not treated with ointment after burning.

The study of the burned skin in the untreated group, showing this clear-cut separation and demarcation, suggest that early treatment should be directed toward the prevention of the changes which produce the formation of the eschar within the first 24 hours.

Group I—Treated with Alo-Creme Ointment: The skin burned and treated with Alo-Creme Ointment remained pliable and soft during the first week with slight and continuous superficial debridement of the upper dermis and without gross or microscopic separation of an eschar. These lesions healed in two weeks without gross evidence of scarring.

Group II—Treated with Alo-Creme Ointment containing cystine: Identical burns treated with Alo-Creme Ointment containing 5% cystine, showed during the second week more superficial debridement than observed in animals of Group I. There was no gross or microscopic separation of an eschar and no gross scarring occurred. There was little or no difference between this group and Group I.

Group III—Treated with trinitrophenol ointment: The appearance of the skin was comparable during the first 24 hours to that observed in Groups I and II. Then these lesions became grossly and microscopically hemorrhagic and the separation of an eschar was evident microscopically at 48 hours. None of the animals survived the tenth day and hemorrhages were found in the skin at the end of the first week.

Group IV—Treated with petrolatum and gauze: During the first three days there was a gradual development of congestion, edema and focal hemorrhages of the skin area in these burns. Microscopically an eschar did develop and separate during the first 48 hours. By the end of the first week there were numerous hemorrhages and several small abscesses. At the end of the second week the entire dermis was debriding in large masses and the lesions healed by scarring during the third and fourth week.

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