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SOME ATTEMPTS TO MODIFY THE EARLY HEALING PROCESS OF
MODERATE SECOND DEGREE BURNS

by

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MODERATE SECOND DEGREE BURNS

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SOME ATTEMPTS TO MODIFY THE EARLY HEALING PROCESS OF
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and

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ABSTRACT

By the injection of hormones and other agents which have been reported to influence scar formation, attempts were made to interfere with normal wound healing after the production of moderate second degree burns in pigs. It was hoped that some information on the development or prevention of hypertrophic scars and keloids might be gained from this experiment. Systemic administration of Doca, cortisone and toluidine blue, and local injection of cortisone, histamine and hyaluronidase neither delayed healing nor altered significantly the histologic processes involved in the restoration of dermal tissues.

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It is generally agreed that hypertrophic scars and keloids are but different degrees of the same lesion. Delayed healing (due to infection, mechanical abrasion, embedded foreign bodies, and full thickness skin loss without graft coverage) almost invariably leads to hypertrophic scar formation and accounts for the majority of these unsightly defects. Certain individuals, however, respond to the most meticulous surgical incision or to the slightest puncture wound by the formation of a keloid, and this exaggerated accumulation of connective tissue may extend beyond the area of the initiating lesion. Because this untoward response to injury has been found in successive generations of the same family, a hereditary defect has been inferred. Because it may occur in one anatomical region and not in another of a susceptible individual, local factors have been implicated. Because it has been associated with a variety of experimentally induced hormonal imbalances, several hormones have been named the offending agents. The fact that the lesion is seen more frequently in the dark skinned races has raised the question of the role of melanin and of racial predisposition.

In this laboratory the histologic remodeling of dermal collagen following a second degree burn was recently described. The processes illustrated lead to the restoration of lost tissue by tissue very nearly resembling normal dermis. In the experiment reported here, local and systemic injection of hormones and other agents which have been reported to influence scar formation were investigated for their effects on these local wound healing phenomena. The dosage and route of administration of those which failed to alter significantly the method or time of tissue repair in one species of animal (the pig) are described, and some possible reasons for their ineffectiveness are discussed.

METHODS

I. Experimental Design

Young Chester White pigs averaging 10 kilograms in weight were used as the experimental animals. They were anesthetized by the intraperitoneal injection of Dial in urethane (Ciba) in a dose of 65 milligrams per kilogram of body weight. The hair on each side of the pig was clipped and the skin was washed with a detergent solution. The heat source was a modified 24 inch Army carbon arc searchlight (1) which produces burns 1.7 cm. in diameter. Since earlier work in our laboratory demonstrated that a radiant exposure of 10 cal/cm^2 delivered in 0.3 sec. will consistently produce moderate second degree burns, this radiant exposure-exposure time combination was employed. The burned areas were randomized so that there was equal probability of any one area being biopsied at any one of the selected times given below. These biopsies, which included normal tissue on each side of the burn, were fixed in 10% formalin and stained with hematoxylin and eosin and with the modified Verhoeff's stain (2). Intravenous Nembutal (from 0.65 to 130 milligrams) was used as the anesthetic agent when the biopsies were taken.

II. Agents investigated

A. Systemic Doca (desoxycorticosterone trimethyl-acetate - Ciba). A 50 mgm. dose of Doca in oil was injected intramuscularly 2 weeks prior to the burning. On the day of burning and for eight days thereafter an additional daily dose of 25 mgm. of Doca in aqueous suspension was injected intramuscularly. At no time did the animal exhibit abnormal salt and water retention as evidenced by abnormal weight gain. The diet was the routine one used in this laboratory. In this animal 20 burns were produced and biopsies were taken on the 4th, 7th, 14th and 21st days after burning. The burn sites

were inspected at these times for evidence of deviation from normal wound healing.

B. Local and systemic cortisone (Cortef - Upjohn)

(a) Local treatment: Forty-eight burns were produced on one pig; these were randomly divided into 3 groups of 16. One group, used as a "control," received no treatment. In the second group, each of the 16 sites was injected locally with Cortef, approximately 13 mgm. in 1 cc. of sterile normal saline being used at each treatment. The solution was injected into the subcutaneous fat at the burn site on the day following burning and for 4 consecutive days. Each burn in the third group was injected at similar times, but 1 cc. of normal saline only was used. Areas were biopsied on the 4th, 7th, 14th or 21st day following the burning.

(b) Systemic treatment: Sixty-four burns were produced on one pig. Two hundred mgm. of Cortef were injected intramuscularly (gluteal region) on the day of burning and on each subsequent day for 21 days. Areas were biopsied on the 7th, 14th or 21st day after burning.

III. Toluidine Blue - (Used in an attempt to neutralize any effect of natural heparin). A gelatin capsule containing 200 mgm. of crystalline Toluidine Blue was given orally for 8 consecutive days. Twenty burns were produced on the day treatment was initiated, and biopsies were taken on the 4th, 7th, 14th and 21st day.

IV. Histamine - 48 burns were placed on one animal, and these were randomly divided into three groups of 16 each. One group was used as a control; the second group was treated with local subcutaneous injection of 1 cc. of a 1:16,000 solution of histamine; and each burn in the third group was injected subcutaneously with 1 cc. of sterile normal saline. The injections were

given for 4 consecutive days, beginning on the day of burning. Four biopsies from each group were taken on the 4th, 7th, 14th and 21st day.

V. Hyaluronidase - 48 burns were produced on one animal and these were divided randomly into 3 groups of 16 sites. 150 TR units of hyaluronidase (Infiltrase - Armour Laboratories) were diluted in distilled water to 16 cc., and 1 cc. of this solution was injected subcutaneously under the burns of one group. One cc. of normal saline was injected under the burns in the second group, and the third group was not treated. The injections were again carried out for 4 consecutive days, beginning on the day of the burning, and the areas were biopsied on the 4th, 7th, 14th and 21st day.

RESULTS

I. Systemic treatment with Desoxycorticosterone-acetate (Doca).

The gross appearance of the healing burns is entirely normal. Good eschar formation is noted on the 4th day after burning, with partial separation on the 7th day and full separation by two weeks. The burned sites are completely covered with new epithelium at the end of two weeks. Contraction of the healing burn is evident at two weeks, and by the end of the third week the area is linear.

Histologically, the four day old burns exhibit good demarcation of the tissue to be sloughed. Epithelial proliferation from the normal epidermis at the burn margins and from the undamaged hair follicles and dermal glands is beginning. Lateral to the burn, in the most superficial dermis, the "spongy" layer is beginning to appear. This spongy layer or zone of degradation of collagen has been described in previous reports from our laboratory (3), and for a detailed description one is referred to these reports.

By the 7th day, the slough is nearly separated, and epithelialization is practically complete. The new dermis is hyperplastic, with thicker and deeper rete pegs evident, and the spongy layer is present just beneath the regenerating epidermis. Hyperplasia of the dermal glands is also noted.

On the 14th day, the regenerated epidermis is markedly hyperplastic, being about 2 to 3 times the thickness of the adjacent normal epidermis. The long rete pegs extend into the altered dermis and are thick and irregular. The spongy layer now is about 1/3 to 1/2 of the dermal depth. The hair follicles have regenerated, and small, new hairs are occasionally found. By the 21st day, the new epidermis has thinned and flattened out. Few rete pegs are seen, and none project deeply into the altered dermis. The spongy zone now involves practically the entire dermal thickness and is made up of a very loose network of fibroblasts, new collagen, capillaries and mononuclear leucocytes. By this time skin thickness is fully restored.

The subcutaneous fat appears to be unaltered in all of the sections studied.

II. Local and Systemic Treatment with Cortisone.

Grossly, wound healing progresses at a normal rate similar to that observed in the Doca treated pig. Histologically, in both the locally and systemically treated animals, wound healing follows the normal sequence of events as described in previous reports (3). In the locally injected burn sites, whether cortisone or saline was used, tiny, deep dermal and subcutaneous abscesses are evident in the majority of tissue sections. These apparently have no effect on the rate or manner of healing except that they are progressively encapsulated with fibrous tissue.

III. The Toluidine Blue treated animal also showed no apparent disturbance in wound healing, either grossly or histologically.

IV. Histamine and

V. Hyaluronidase. Local injections of both histamine and hyaluronidase produce no striking alteration in wound healing.

DISCUSSION

Although there is disagreement concerning histologic differences between keloids and hypertrophic scars, most authors agree that the distinction is a clinical one only. Numerous theories concerning the etiology of these lesions have been advanced, with hormonal factors, familial tendency, delayed epithelialization, excessive serous exudation in wounds, increased susceptibility by the individual, reaction to foreign bodies, skin pigmentation, a lack of tissue mast cells, and an increased heparin tolerance being considered as possibilities. Investigations of wound healing have also implied a role for such agents as hyaluronidase, heparin, and histamine.

Concerning keloid formation, Garb and Stone (4) believed hormonal factors to be very important since the lesion is most common during the second decade when increased hormonal activity is apparent. They listed of less importance race, familial tendency and delayed epithelialization. Marshall and Rosenthal (5) implicated excessive serous exudate in wounds because chronic edema leads to fibroplasia. Gathings (6) as well as Marshall reported on the use of kutapressin, a vasoconstrictor, for treatment of keloids. Gesschichter (7) found increased gonadotropic substance and estrogen in a keloid plaque of fibrosis over the stomach of one of four monkeys treated with estrogen. Trusler and Bauer (9) believed that in addition to the above factors the anatomical area may be of some importance. They cite the fact that keloids occur most frequently in incisions which cross normal flexor creases and are uncommon in areas where skin tension is constantly changing. Glücksmann (10) states that keloids or hypertrophic scars are due to a

spreading, proliferative, inflammatory process elicited locally in sensitive persons by the presence of various particles acting as foreign bodies. Bloom (11) reviewed the literature of familial cases and discussed the peculiarity of smallpox vaccination in regard to stimulation of keloids.

Iversen (12a) noted a high incidence of keloids in acromegalics, and Scow (13) reported on the stimulating effects of growth hormone on collagen and protein content of skin in thyroidectomized rats. Taubenhaus and Amromin (14) reported increased fibroplasia and formation of collagen fibrils in granulation tissue around turpentine abscesses after prolonged treatment of rats with desoxycorticosterone and after growth hormone. Pirani, Stepto and Sutherland (15) investigated the effect of desoxycorticosteroids on connective tissue by studying healing abdominal wounds in guinea pigs. Increased amounts of granulation tissue with a lag in the maturation process of both cellular and intercellular elements was noted. The effect on immature connective tissue was marked, but was less apparent or absent on mature connective tissue.

Robinson and Hamilton (16) noted increased heparin tolerance after severe burns and in keloid formers during the healing phase. The role of heparin as an inhibitor of excessive fibroplasia was implied.

Cannon (17) and Harkins (18) found that while keloids developed in third degree burns not grafted, none occurred in grafted areas.

Block and Tsuzuki (19) reported a high incidence of scar keloids and hypertrophic scars after healing of deep second or third degree flash burns. They felt that the mode of treatment, poor nutrition, high incidence of infection and delayed healing were important contributory factors.

Clarkson (20) in addition to the factors mentioned above considered the importance of skin pigmentation. (He also suggested the use of cortisone, after surgical removal of the keloid, as an adjunct to therapy.)

Cornbleet (21) recommended the use of local radiation and hyaluronidase prior to surgery to prevent recurrence of keloids. (It has also been reported (22) that hyaluronidase inhibits the formation of collagen fibers in fibroblast tissue cultures.)

Finally, Wichmann (23) reported a marked increase in mast cell count as healing progressed, with return to normal within three weeks. Robinson (16) and other workers reported a degranulation of mast cells in growing keloids and an almost complete absence of mast cells in scar tissue.

In this experiment the use of several agents which supposedly influence the formation or maturation of collagen had little influence on the healing of second degree burns in pigs. Only the animal treated with Doca showed much variation from average. Here, the "spongy layer" tended to involve most if not all of the dermis beneath the burned site, but this has been found in the past in some untreated animals.

There have been many reports of the inhibitory effects of cortisone on all components of the connective tissue and on wound healing (12c). In our experiment this was not demonstrated. Perhaps our experimental animal is little affected by this hormone. Ragan (24) reported that the effect of cortisone on wound repair is less in the mouse, guinea pig and rat than in the dog, rabbit and man. It was, nevertheless, surprising to find so little effect from the local injection of this hormone.

We can attach little significance to the findings in this experiment that toluidine blue, histamine, and hyaluronidase treatments had no effect on the histologic nature or rate of healing of the burns. One can only say that their use in this experiment shed no light on factors involved in abnormal scar formation.

The amount of the agent employed in this study was based on therapeutic doses for humans (Doca, cortisone, and toluidine blue), corresponded

to the minimum lethal pharmacological dose (histamine) or was chosen arbitrarily (hyaluronidase). However, there is no indication that either moderate increases in dosage or prolongation of treatment would have altered the results significantly.

In the pig, the type of burn produced in this experiment results in the early formation of an eschar. Epithelialization beneath the slough, which consists of the destroyed epidermis and superficial one-half of the dermis, is rapid. The resulting scar soon becomes pliable, and contraction of the damaged area occurs. Edema is minimal and disappears within a few days. There is no tendency to the formation of hypertrophic scars. Under these conditions, the use of the medications reported here offers little information concerning abnormal scar formation.

CONCLUSIONS

The histologic nature and rate of healing of moderate second degree burns in pigs was little altered by the systemic administration of either Doca or cortisone; by local injection of cortisone, histamine or hyaluronidase; or by the oral administration of toluidine blue. In the dosages given, these agents, which have been reported to affect wound healing directly or indirectly, did not alter the local tissue reactions during the first three or four weeks after injury.

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