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New Method for Assessing Epidermal Wound Healing: The Effects of Triamcinolone Acetonide and Polyethelene Film Occlusion

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Epidermal healing of superficial, excised wounds in domestic white pigs was evaluated visually and histologically after separation of the epidermis and dermis. The visual determination of epidermal healing correlated well with the histologic studies of surface re-epithelialization. Wounds healed 40% faster when occluded with polyethelene film. Topical triamcinolone acetonide treatment delayed healing (62% slower than control).

Epidermal wound healing is affected by topically applied agents and changes in the physical environment. Winters [1] showed that polyethelene film occlusion increased the rate of histologically confirmed epidermal wound healing in domestic white pigs and Hinman and Maibach [2] showed similar results in man. In histologic studies on the healing of subepidermal suction blisters in mouse skin, Krawczyk [3] found that epidermal regeneration was more rapid when the blister roof was left intact than when it was removed. Similarly, Rovee [4] found that polyethelene film occlusion increased the rate of gain in the breaking strength of guinea pig foot pad epidermis previously wounded by incision. Several studies have shown, on the other hand, that topical corticosteroids slow healing of linear incisions in human skin [5], retard the return of normal epidermal barrier properties to human skin stripped with pressure sensitive tape [6], and decrease the rate of epidermal repair when applied beneath plastic film for several weeks before wounding [7].

We have used a new method of assessing the effects of polyethelene film occlusion and topical corticosteroids on epidermal repair in superficial wounds made on white domestic pigs.

MATERIALS AND METHODS

Wounding Method

White domestic pigs (12–18 lb) were shaved with standard animal clippers and the remaining hairs removed with a barber's clippers. The skin on both sides of the animal was prepared for wounding by washing with sterile saline. Antiseptics were not used because of their potential effect on the healing process.

Each pig was anesthetized with sodium pentobarbital (15 mg/kg) i.p. and approximately 22, rectangular 7 mm × 10 mm wounds, 0.3 mm deep were made in the paravertebral and thoracic areas with a Castro-Viejo dermatome. Roughly 15 mm separated the wounds. The pigs were housed separately after wounding.

Treatment

Each wound was assigned to one of these treatments: (1) control—no treatment; (2) occlusion only-covered daily with polyethelene film taped to the skin 10 mm from the edge and further covered by a thick plastic sheet loosely taped over several adjacent wounds treated with occlusion; (3) petrolatum—white petrolatum U.S.P. 0.1 ml applied once daily to the wound or crust; or (4) triamcinolone acetonide—triamcinolone acetonide 0.1% ointment, 0.1 ml applied once daily to the wound or crust. Treatments were initiated shortly after the wounds were made (Day 0).

Each animal had untreated control wounds. Animals treated with triamcinolone acetonide ointment always had vehicle (petrolatum) treated wounds. To avoid the spread of topical agents from one wound to another and to allow application of the protective plastic sheet over occluded wounds, treatment assignments were made for wounds in the same region of the animal. To correct for this nonrandom assignment, regions used for each treatment varied for each animal.

Sampling and Direct Evaluation

Beginning on Day 2 after the wounds were made (Day 0), several wounds from each treatment group were excised with a Castro-Viejo dermatome. By using a standard blade, 22 mm wide, the wound and surrounding nonwounded skin were removed. The dermatome was set to a depth of 0.4 mm. This excised skin, which contained the wound site, was incubated in 2 N NaBr at 37°C for 3 hr allowing separation of the epidermis from the dermis [8] (Fig 1). When no defect was seen in the separated epidermis, the wound was considered healed. Representative samples of the macroscopic findings are shown in Fig 2. Occasionally an intact crust (scab) attached to the epidermis prevented absolute visual confirmation of epidermal integrity in untreated control wounds. Microscopic serial sections of epidermal specimens showed that crust tended to adhere to the dermal portion of the specimen unless separated by an epidermal sheet, so that such specimens were, in fact, healed.

HISTOLOGIC EVALUATIONS

Serial sections of the following specimens were examined by light microscopy after routine fixation and staining with H & E.

1. Tissues removed in wounding.
2. 9-mm punch biopsies of untreated wounds on Days 0, 1, 2, 3, 4, 5, and 6.
3. Dermatone excisions containing untreated wounds and unwounded adjacent skin on Days 0, 1, 2, 3, 4, 5, and 6.
4. Separated, intact (healed) epidermal specimens which had a crust over the wound site.

Additionally standard dermatome specimens containing the wound site and adjacent normal skin were taken on Days 4 and

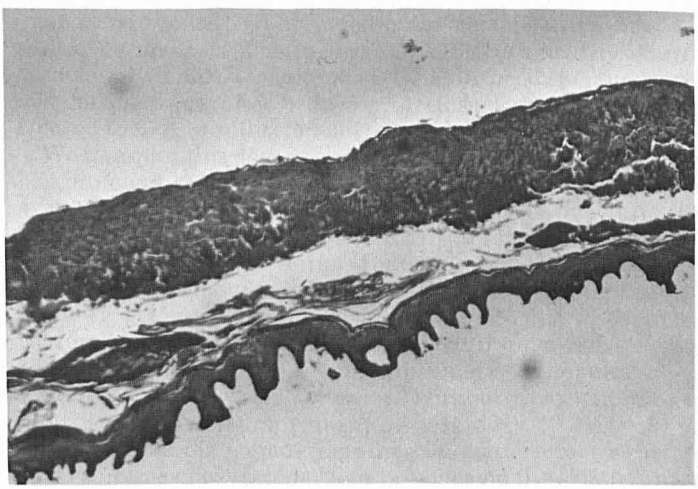

FIG 1. Epidermis and crust obtained after NaBr incubation and separation of the full thickness dermatome specimen (reduced from × 40).

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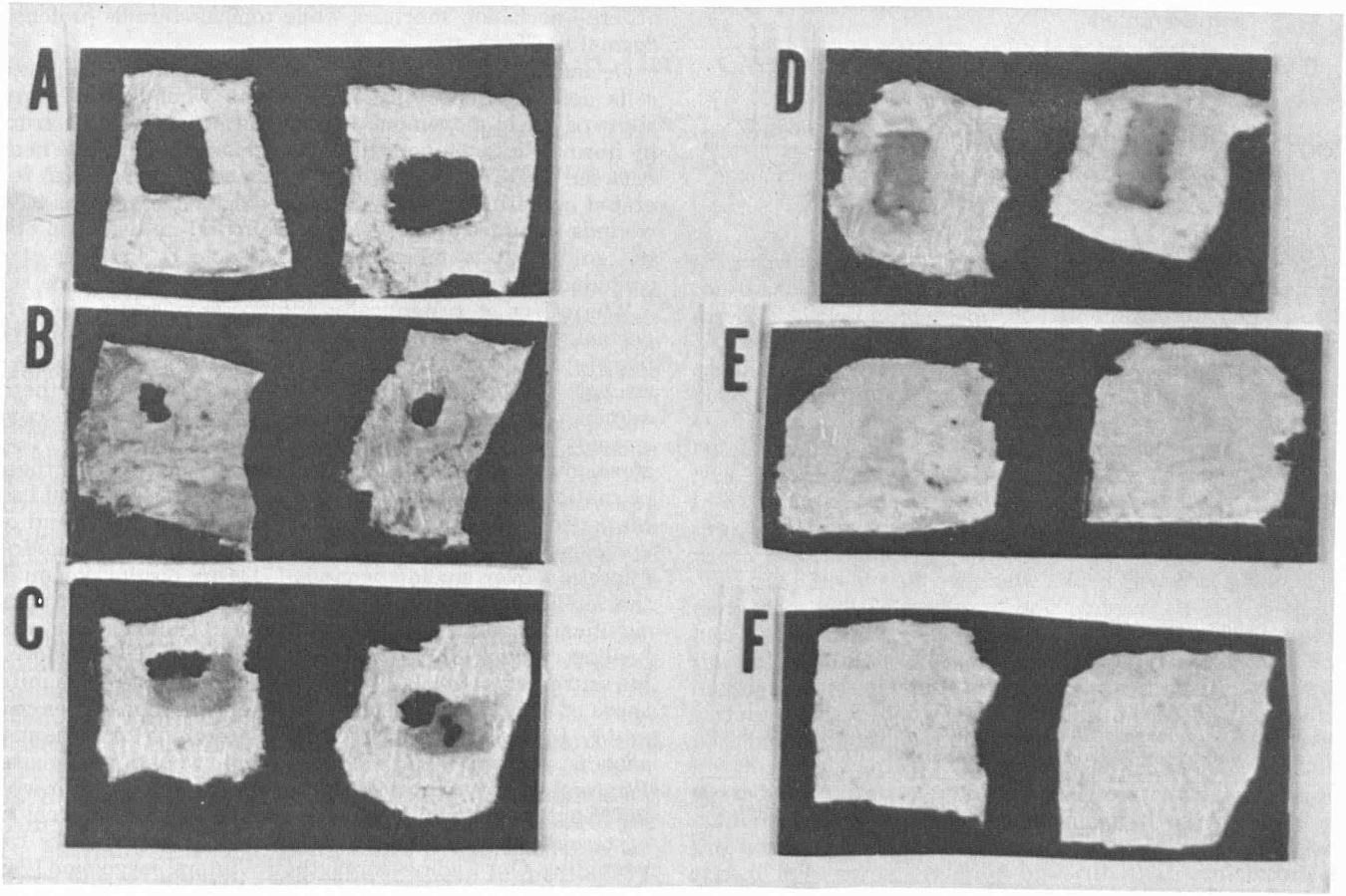

FIG 2. Separated epidermal specimens containing wound sites. *A*, Day 3—not healed; *B*, Day 4—not healed; *C*, Day 5—not healed with crust; *D*, Day 5—healed with crust; Day 5,—healed, no crust; *F*, Day 6—healed.

TABLE I. *Number of specimens and percent of wounds healed*

Treatments	Day 2[a]	Day 3[a]	Day 4[a]	Day 5[a]	Day 6[a]	Day 7[a]
Untreated Control wounds	0/16 (0%)	0/26 (0%)	9/27 (33%)	15/16 (94%)	10/10 (100%)	2/2 (100%)
Occluded Wounds	0/6 (0%)	12/14 (86%)	10/11 (90%)[c]	6/6 (100%)	8/8 (100%)	N.S.[b]
U.S.P. white Petrolatum	N.S.[b]	0/7 (0%)	3/14 (21%)[d]	7/13 (53%)	4/7 (51%)	5/5 (100%)
Treated wounds Triamcinolone Acetonide Ointment 0.1%	N.S.[b]	0/2 (0%)	1/10 (10%)[e]	2/10 (20%)[e]	1/8 (12%)[e]	5/9 (55%)

[a] Day 0 = Day of wounding.
[b] N.S. = No Specimens.
[c] P = .004.
[d] P = .04.
[e] P < .001.

5. They were bisected through the rectangular wound site, and one-half of each specimen was then incubated in 2 N NaBr to separate the epidermis for macroscopic evaluation of healing. Serial section of the the other half of each specimen was then evaluated histologically for healing.

RESULTS AND DISCUSSION

Histologic Results

The wound surface was composed of the upper part of the reticular dermis while hair follicle epidermis and apocrine gland duct epidermis remained within the dermis. On Day 1 the dermis was covered with a crust containing many erythrocytes and a "tongue" of epidermal cells extended onto the dermis beneath the crust from the wound edges and remaining adenexal epithelium. Progressive re-epithelialization of the wound surface from the wound edges and follicular and apocrine duct epithelium continued until the wound surface was completely covered with epidermis on the 5th day or 6th day after wounding. The new epidermal cells separated the crust from the dermis.

Macroscopic and microscopic evaluations of healing on sister halves on the specimens correlated well. When the macroscopic evaluation showed the wounded epidermis was not intact, the epidermal portion of the sister half had microscopic defects.

Treatment Results

Preliminary results showed no regional differences in the healing rate of untreated wounds. The results are presented in Table I, and presented graphically in Figure 3. From this graph the time needed for 50% of the wounds in each treatment group

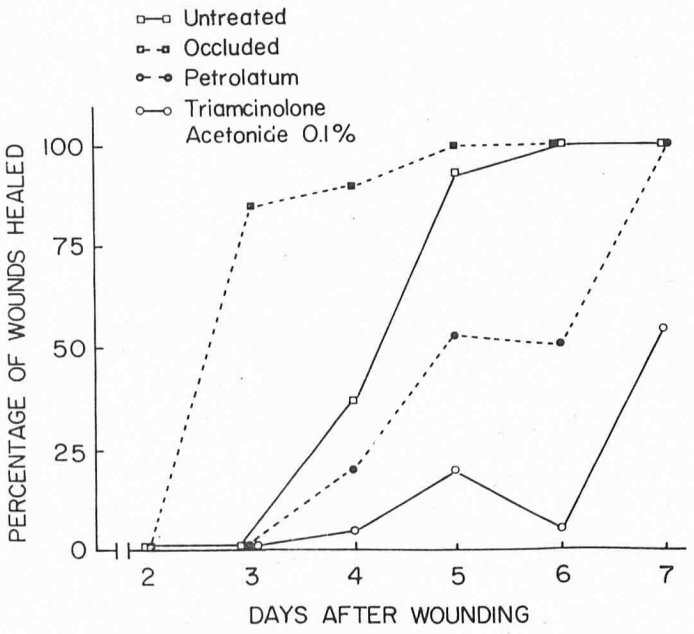

FIG 3. Percentage of wounds healed.

TABLE II. *Comparison of the time for 50% of the wounds in each treatment group to heal*

Agent	HT_{50} (Days)	Relative rate of healing compared to control
Occlusion	2.6	+40%
Untreated control	4.3	0
Triamcinolone Acetonide ointment 0.1%	6.9	−62%

[a] HT = Healing Time.

to heal (HT_{50}) can be estimated. In Table II the HT_{50}'s are listed and compared.

Polyethelene film occlusion of the wound shortened the time necessary for epidermal healing, while treatment of the wound with a topical corticosteroid increased the time needed for healing. These findings confirm the earlier work done in domestic pigs, other animals and humans.

Epidermal healing has 3 principal phases thought to occur in sequence: (1) migration, (2) proliferation, and (3) maturation [9]. Preliminary autoradiographic studies with our system support the idea that the proliferation phase begins after the migratory phase and suggest that our results apply to the migration phase of epidermal wound healing.

The evaluation of epidermal wound healing by inspecting separated epidermis at various times after wounding correlates well with histologic studies done by us and others. The migrating epidermal cells come from the intact epidermis of the wound edge and the remaining portions of the follicles and other adenexal structures within the dermis of the wounded area. In Winter's histologic studies of untreated wounds in domestic pigs, migration of epidermis was complete by the 5th or 6th day after wounding. The same results were obtained in our system—the separated epidermal specimens were intact (healed) by Day 5 or 6 (Table I).

Local factors, however, have a profound effect on the rate of healing—as shown in our studies and those of others—occlusion shortens, while topical steroids prolong epidermal healing time.

Occlusion is thought to enhance the migration of epidermal cells across a defect by keeping the wound surface moist, allowing direct movement over the surface without obstruction by fibrin. Winters found that in wounds occluded while healing, between 62% and 100% of the surface was covered with regenerated epidermis; by Day 3 the average coverage was 93%. In wounds occluded while healing, we found no defect in 86% of the epidermal samples on Day 3 (Table I). In these studies occluded wounds healed 40% faster than control (Table II).

The effect of systemically administered corticosteroids on dermal repair as reflected by the tensile strength of wounds has been evaluated and both increase or decrease the tensile strength depending upon the time after wounding they are administered. The effect of systemically administered corticosteroids on epidermal repair has not been determined. Topical steroids are reported to decrease epidermal DNA synthesis in psoriatic epidermis [10], delay the return of epidermal barrier properties after pressure sensitive tape stripping [6], and cause epidermal atrophy and a subsequent decrease in epidermal migration over shallow wounds [7]. Our results demonstrate that daily topical application of corticosteroids after wounding significantly retards epidermal healing (Table I). The relative healing rate for steroid treated wounds being 62% below that for untreated controls (Table II). These results are similar to those of Winters but the experimental conditions differ considerably. In our studies, triamcinolone acetonide 0.1% was applied without occlusion and only after the wound was made. In Winters' studies topical steroids were not applied after wounding. Epidermal atrophy was induced by applying topical corticosteroid beneath an occlusive film for several weeks before wounding. Our findings strengthen Winters' suggestion [7] that the retardation of epidermal repair by topical corticosteroids is related to their effect on the migratory ability of epidermal cells and not to epidermal atrophy.

It is a widely held clinical impression that petrolatum is occlusive or semiocclusive and consequently might be expected to aid epidermal healing. However, in our studies topically applied petrolatum did not significantly alter epidermal wound healing.

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