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The Effect of Occlusive Dressings on Collagen Synthesis and Re-epithelialization in Superficial Wounds

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The effects on superficial wounds in domestic pigs of (1) two different occlusive dressings, (2) non-occlusive wet to dry gauze dressings, and (3) air exposure were compared. Collagen synthesis and re-epithelialization were increased in the wounds treated with occlusive dressings. Re-epithelialization was increased beneath both the oxygen-impermeable and the oxygen-permeable dressing. When they were removed the wet to dry gauze dressing and one of the occlusive dressings often damaged the new epidermis.

INTRODUCTION

The effect of occlusive dressings on the rate of epidermal resurfacing is well established. In 1962 Winter [1] showed that an occlusive plastic dressing increased the rate of superficial wound re-epithelialization nearly 50%. Hinman *et al.* [2] found occlusive dressings produced a similar enhanced rate of epidermal repair in incisions made on human volunteers. These observations have subsequently been confirmed for a variety of occlusive and semiocclusive dressings in several types of wounds in both animals and man [3, 4]. Most investigators believe that the increased rate of re-epithelialization produced by occlusive dressings is related to their ability to keep the wound moist and to prevent desiccation of the wound bed. However, some studies have also related the increased rate of re-epithelialization to the ability of an occlusive dressing to transmit oxygen and to increase wound bed oxygen concentrations [5, 6].

The effect of occlusive dressings on dermal repair has received less attention. Winter found that connective tissue regeneration began 3 days earlier in wounds beneath a polyethylene film [7]. New vessel growth began in 2 days, many fibroblasts were present by the fifth day after wounding, and new collagen bundles were seen on the seventh day. More recently Linski *et al.* [8] reported that compared to untreated air-exposed incisions, occlusive film-treated guinea pig incisions had a decreased number of inflammatory cells, a decreased influx of "fibroblasts," and a decreased breaking strength. When treated with an occlusive film, incisions in humans had less clinical inflammation and subsequently a finer, less pigmented, more attractive scar. Although the mechanism involved is unknown, the dermal effects described by Linsky *et al.* were speculatively related to the film's ability to decrease the early inflammatory response to wounding.

In order to investigate more closely what effect if any occlusive dressings have on dermal repair and epidermal resurfacing, we chose to study the effects of an oxygen-permeable and an oxygen-impermeable dressing on dermal collagen biosynthesis and epidermal resurfacing of superficial wounds.

MATERIAL AND METHODS

Experimental animals. In all studies young Yorkshire pigs weighing approximately 12 kg were used. The experimental animals were fed

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a basal diet *ad libitum* and housed individually in our animal facility with controlled temperature (19–20°C) and light (12 hr LD).

Wounding. Each animal was clipped with standard animal clippers and the skin on both sides of the animal was prepared for wounding by washing with a nonantibiotic containing soap and water. The animals were anesthetized (Nembutal sodium 60 mg/2.3 kg ip) (Abbot Laboratories), and between 90 and 130 rectangular wounds measuring 7 × 10 mm and 0.3 mm deep were made in the paravertebral and thoracic area with an Electrokeratome (Storz Instruments). The electrokeratome was fitted with a razor blade modified so that the cutting edge was reduced to 7 mm. The wounds were separated from one another by at least 15 mm of normal skin.

Treatments. The wounds were blotted with sterile towels and treated in one of the following ways. (1) An adhesive 0.07-mm transparent occlusive, oxygen-permeable, vapor-permeable membrane polyurethane film (PUF)[2] was applied within 5 min after wounding and left in place until the wound was harvested. (2) An adhesive 1.5-mm tan, opaque, occlusive, oxygen-impermeable hydrocolloid dressing (HCD)[3] was applied within 5 min after wounding and left in place until the wound was harvested. (3) Wet to dry gauze dressing (WDG). Saline-soaked sterile gauze was wrapped about the animals and over the wounds within 5 min of wounding. The dry gauze was soaked off daily and replaced by new saline-soaked gauze. Wounds were harvested after removing the soaked gauze, and (4) untreated, air exposed.

A nylon mesh customized pig jacket was worn by the animals to prevent dislodgement of the wound dressing.

Determination of collagenous and noncollagenous protein synthesis. Several wounds were evaluated each day after wounding (Day 0). The wounds to be evaluated had the dressing removed and were completely excised with an electrokeratome fitted with a 22-mm blade set to cut at a depth of 0.5 mm. The wounds remaining for sampling on later days were left unmolested. The excised skin containing the wound site was incubated in 0.25% Trypsin at 4°C for 12 hr. After incubation the epidermis was separated from the dermis, and the 7 × 10-mm dermal wound area was dissected out and assayed for collagen synthesis. The dermal wound area from each treatment was incubated in 20-ml flasks containing 3 ml of HEPES buffered Krebs ringer medium supplemented with ascorbate (50 μg/ml). Tissues were finely minced and 5 μCi of [^{14}C]proline (Amersham) was added. The flasks were incubated in a shaking water bath at 37°C for 3 hr. The entire incubation mixture was cooled at 0–4°C, after adding the following protease inhibitors: 1 m*M* phenylmethysulfonylfluoride, 5 m*M* *N*-ethylmaleimide, and 10 m*M* disodium ethylenediaminetetraacetate (EDTA). After rapid freezing and thawing the mixture was homogenized (Tekmar Tissuemizer) and treated with protease-free ribonuclease (20 mg/ml) for 5 min at 37°C to cleave radioactive prolyl transfer RNA. The homogenate was then chilled to 4°C and trichloroacetic acid (TCA) was added to give a final concentration of 5%. Precipitated protein was separated by centrifugation at 10,000*g* and unincorporated radioactive proline in the supernatant was discarded. The precipitate was then resuspended in 5 ml cold 5% TCA and recentrifuged. The TCA from the protein pellet was extracted by 2 ethanol/ether (3:1 v/v) washes and the protein was desiccated to a powder. The dried protein was dissolved by homogenization in 0.2 *N* NaOH at 37°C (15 mg/ml) and the collagen was digested by bacterial collagenase and separated from noncollagen protein. The procedure used was essentially as described by Peterkofsky and Diegelmann [9] with the following modifications: an aliquot (0.25 ml) of the dissolved substrate was transferred to each of three 15-ml conical centrifuge tubes and partially neutralized by the addition of 0.15 ml of 0.1 *N* HCl. The incubation mixture was buffered by the addition of 0.2 ml *N*-2-hydroxyethylpiperazine-*N'*-2 ethanesulfonic acid (HEPES)

[2] PUF, Op-Site, Smith and Nephew Research, England.
[3] HCD, DuoDerm, Squibb, Princeton, N. J.

(100 μM, pH 7.2). $CaCl_2$ (0.25 μM) was added to stabilize the collagenase and *N*-ethylmaleimide (1.25 μM) added to inhibit any possible trace amounts of an SH-containing protease. The reaction mixture was adjusted to pH 7.2 and duplicate tubes received 0.01 ml of purified bacterial collagenase (50 μg) (Advanced Biofactures) in 0.05 *M* Tris–HCl buffer (pH 7.6) containing 5 m*M* $CaCl_2$. The third tube served as an enzyme blank and received only the Tris–$CaCl_2$ buffer. The amounts of collagenous (collagenase-digested) and noncollagenous (collagenase-resistant) proteins were measured after dialysis by liquid scintillation as described previously [10].

Epidermal assessment. The excised wound which provided the dermal sample also provided the epidermal sample. After incubation with 0.25% Trypsin the epidermis and dermis were separated. The epidermal sheet was examined macroscopically and classified as healed or not healed based on the presence or absence of macroscopic defects in the epidermal sheet. This method has been reported previously [3].

STATISTICAL METHODS

The Student's *t* test was performed on the individual analytical data from replicated assays for each analysis [11]. Probit analysis was done by Zars method [12].

EXPERIMENTAL DESIGN

Four experiments were done: (1) the wounds on each of two animals were treated with HCD, WDG, and air exposure, (2) the wounds on each of six pigs were treated with PUF, HCD, or air exposure, (3) the wounds on five pigs were treated with PUF or air exposure, and (4) the wounds on two pigs were treated with PUF or air exposure but the PUF was changed daily.

RESULTS

Clinical

The HCD and PUF adhered easily to the skin surrounding the wound. Both stayed on the wound sites until removed. Within 24 hr the portion of the HCD directly over each wound appeared darker and was softer than the adjacent portion of the HCD. Clinical inspection of the HCD after removing it from the wounds and H & E sections of the dressing and subadjacent tissues showed that the HCD "dissolved" over the wound producing the soft discolored areas in the HCD and allowing removal of the HCD without trauma to the wound bed (Fig. 1). A small amount of wound fluid was present beneath the PUF on Days 0, 1, 2, and 3. On Days 4, 5, and 6 fluid was undetectable beneath the PUF and occasionally when the PUF was removed some adherent new epidermis was stripped away (Fig. 2). Crusts developed on the air-exposed and WDG-treated wounds. Crusts did not develop in the HCD- and PUF-treated wounds. The WDG frequently stripped away adherent epidermis and crust despite resoaking.

Collagen Synthesis

The relative collagen synthesis in wounds treated with HCD, PUF, and air exposure (Experiment (2) Table 2) are seen in Table 5. The wounds treated with PUF and HCD had significant increases in collagen synthesis on all 5 days compared to the air-exposed wounds. There were no significant differences between the values of the HCD- and PUF-treated wounds or for the values on different days after wounding.

Re-epithelialization

The re-epithelialization results for Experiments 1, 2, 3, and 4 are presented in Tables 1, 2, 3, and 4. These data were subjected to probit analysis to generate curves from which the time needed for 50% of the wounds to be healed (HT_{50}s) were determined. The HT_{50}s were compared to determine the relative rate of re-epithelialization (Table 6).

DISCUSSION

In these studies we compared the effects on superficial wounds of (1) two different occlusive dressings, (2) nonocclusive wet to dry

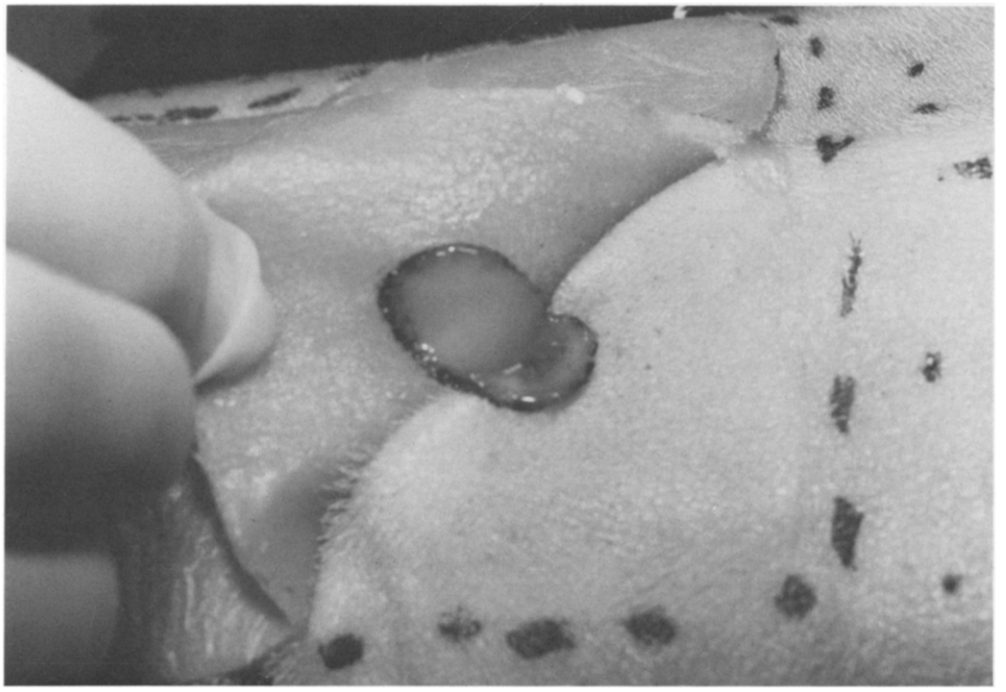

FIG. 1. HCD being removed from the wound and adjacent nonwounded skin. The area covered by the HCD is outlined with a dashed line drawn on the skin. The HCD which was directly over the wound is more gelatenous than the HCD which was over nonwounded skin. Some of the gelatinous HCD remains on the wound bed.

gauze dressings, and (3) air exposure. The two dressings studied are quite dissimilar physically and chemically. However, they are considered occlusive because they keep the wounded tissues moist and prevent crust formation. PUF is designated semiocclusive by its manufacturers because it is permeable to moisture vapor. This property is important in allowing PUF to remain adherent to normal skin despite insensible water loss and sweating. In addition, oxygen and other gases are easily transmitted through the PUF. In contrast HCD is not permeable to water vapor and oxygen. Moisture is absorbed into the HCD over normal skin while on the wound bed the HCD mixes with the wound fluid producing a tan gelatinous material on the wound bed.

As in earlier studies [3] we found that occlusive dressings increased the rate of re-epithelialization. Both of the occlusive dressings produced an increased re-epithelialization compared to air exposure and GWD dressings. HCD produced a significantly greater number of resurfaced wounds on the early days (Table 2, Days 3 and 4) and had a lower HT_{50} than PUF. The tendency we noted for PUF to strip new epidermis from the wound surface when it is removed has been reported [13]. As illustrated by the actual decreased percentage of re-epithelialized wounds on Day 4 in Table 4 the rewounding occurred on the later days of the study when the wound surface was dry. The occasional rewounding by PUF probably produced a lower number of re-epithelialized wounds on the later days of the studies and accounts for the 21% relative rate of healing in these studies compared to a relative rate of 40% we obtained in earlier studies with a nonadherent occlusive plastic film dressing [3] and compared to the 36% obtained with HCD. Unlike earlier reports [5, 6], we did not find a more rapid re-epithelialization beneath the oxygen-permeable film. Although we did not

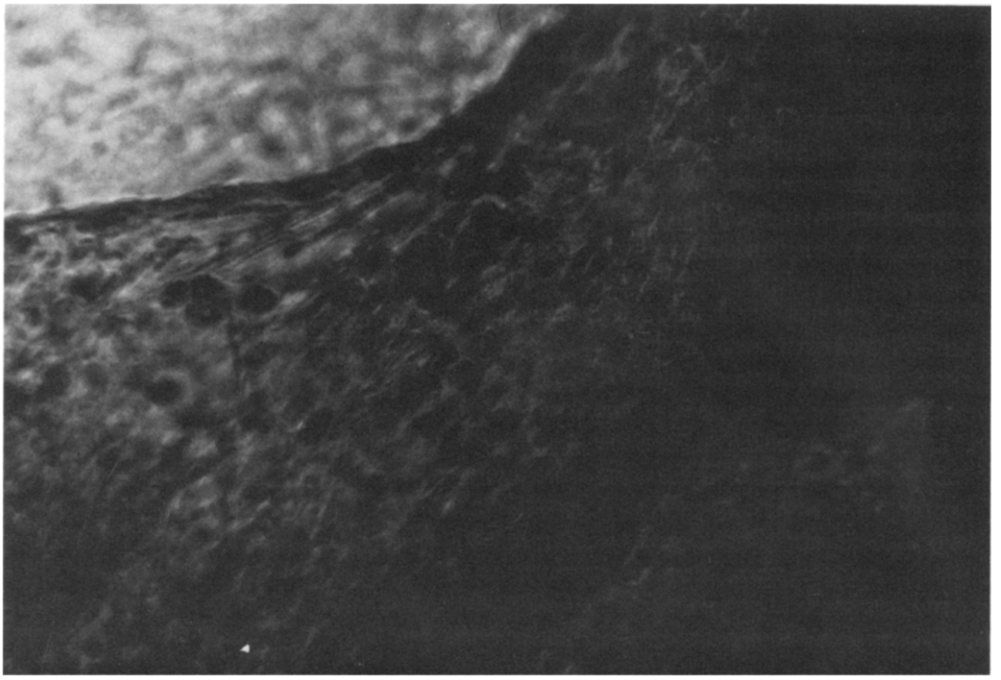

FIG. 2. Photomicrograph 100× PUF with adherent sheet of new epidermis on Day 5.

measure the oxygen tension beneath the dressings we did review the unpublished data on HCD and agree that HCD does not seem to transmit oxygen when dry or when hydrated. The rate of environmental oxygen permeability is 41× greater through PUF than the HCD [14]. Other investigators have been unable to relate the increased re-epithelialization rate produced by occlusive dressings to the dressings oxygen permeability (personal communication). It is possible that wounds in normal skin receive ample oxygen via the blood. Alternatively HCD might contain "stimulating" substance(s) which compensate

TABLE 1

PERCENTAGE OF WOUNDS RE-EPITHELIALIZED[a]

	Days after wounding			
Treatment	2	3	4	5
Air exposed	0	0	80	90
Gauze[b]	0	0	25	70
HCD[c]	25[d]	91[d]	100[e]	100

[a] Number of wounds re-epithelialization/number of wounds sampled. Between 6 and 14 wounds were sampled for each treatment daily. The mean number was 9. Two animals were studied.

[b] Gauze, wet to dry saline soaked (Kling).

[c] HCD, Hydrocolloid dressing.

[d] $P < 0.05$ compared with air exposed.

[e] $P < 0.05$ compared with gauze treatment.

TABLE 2

PERCENTAGE OF WOUNDS RE-EPITHELIALIZED[a]

	Days after wounding				
Treatment	2	3	4	5	6
Air exposed	0	0	66	86	100
PUF[b]	6	30[d]	70	83	100
HCD[c]	16	53[d,e]	96[d,e]	100	100

[a] Number of wounds re-epithlelialized/number of wounds sampled. Between 15 and 30 wounds were sampled for each treatment daily. The mean number was 24. Six animals were studied.

[b] PUF, Polyurethane film.

[c] HCD, Hydrocolloid dressing.

[d] $P < 0.05$ compared to air exposed.

[e] $P < 0.05$ compared to PUF.

TABLE 3

PERCENTAGE OF WOUNDS RE-EPITHELIALIZED[a]

	Days after wounding				
Treatment	2	3	4	5	6
Air exposed	0	0	23	72	90
PUF[b]	0	29[c]	59[c]	84	100

[a] Number of wounds re-epithelialized/number of wounds sampled. Between 5 and 31 wounds were sampled for each treatment daily. The mean number was 19. Five animals were studied.

[b] PUF, Polyurethane film.

[c] $P < 0.05$ compared to air exposed.

for its oxygen impermeability. In unpublished studies of HCD in a granular form we have failed to detect a stimulatory activity *in vitro* but this possibility cannot be totally excluded. Since in this study only one of two variables could be controlled, the hypothesis that oxygen permeability is related to re-epithelialization was not supported but was not disproved. GWD dressings are used primarily because of their debridementing activity and its tendency to rewound is not surprising.

As reported earlier [15], the dermis of these superficial wounds quickly began to synthesize more collagen than the nonwounded skin. To our knowledge, this is the first report of occlusive dressings affecting collagen synthesis in wounded tissue. The increase occurred by the first day and continued through the 6 study days beneath both the oxygen-permeable and impermeable dressings. An increased relative collagen synthesis is not inconsistant with the reported decrease in tensile strength and decreased number of cells induced by occlusive dressings. Tensile strength is related to ma-

TABLE 4

PERCENTAGE OF WOUNDS RE-EPITHELIALIZED[a]

	Days after wounding			
Treatment[b]	1	2	3	4
Air exposed	0	0	0	69
PUF[c]	0	25[d]	92[d]	75

[a] Number of wounds re-epithelialized/number of wounds sampled. Between 4 and 14 wounds for each treatment were sampled daily. The mean was 9. Two animals were studied.

[b] PUF was changed daily.

[c] PUF, Polyurethane film.

[d] $P < 0.05$ compared to air exposed.

TABLE 5

EFFECT OF OCCLUSION ON RELATIVE COLLAGEN PRODUCTION DURING WOUND HEALING[a]

Day after wounding	Air exposed	PUF[b]	HCD[c]
1	49.1 ± 2[d]	59.2 ± 3[e]	60.2 ± 4[e]
2	33.4 ± 2[d]	51.6 ± 5[e]	55.2 ± 4[e]
3	43.5 ± 4[d]	57.2 ± 2[e]	55.1 ± 3[e]
4	42.0 ± 3[d]	64.6 ± 4[e]	63.9 ± 5[e]
5	44.1 ± 3[d]	54.8 ± 3[e]	54.6 ± 4[e]

[a] Relative collagen production (%) = [collagen (dpm)]/[noncollagenous (dpm) × 5.4 + collagen (dpm)] × 100.

[b] PUF, Polyurethane film.

[c] HCD, Hydrocolloid dressing.

[d] Values are expressed as mean ± SEM from 18 independent observations. Three wounds sampled from each treatment group daily on each of six wounds.

[e] Values with different superscripts are statistically different, $P < 0.05$.

TABLE 6

POOLED HT_{50}S AND RELATIVE RATE OF HEALING

Treatment	HT_{50}[a] (days)	Relative rate of healing[b] compared to air exposed (%)
Gauze[c]	4.1	−5
Air exposed	3.9	0
HCD[d]	2.5[f,g]	+36
PUF[e]	3.1[h]	+21

[a] HT_{50} = Healing time 50, days needed for 50% of wounds to be 100% healed.

[b] Relative rate of healing = (air exposed HT_{50} − treatment HT_{50})/air exposed HT_{50} × 100.

[c] Gauze, wet to dry saline soaked (Kling).

[d] HCD, Hydrocolliod dressing.

[e] PUF, Polyurethane film.

[f] $P < 0.001$ compared to air exposed.

[g] $P < 0.05$ compared to PUF.

[h] $P < 0.05$ compared to air exposed.

turity (intermolecular cross-linking), not to the amount of collagen synthesized. The number of fibroblasts present and their synthetic activity are not directly correlated. For example, after wounding the rate of collagen synthesis by a fibroblast increases.

The biologic implications of increased collagen synthesis in superficial wounds is not clear. We could not detect any undesirable consequence such as hyperplastic scarring. Alternatively we cannot define a beneficial effect attributable to the increased collagen synthesis. It is possible that the increased rate of re-epithelialization and the increased collagen synthesis are related. We have reported that re-epithelialization can be affected by interfering with collagen synthesis in wounds [15] and alternately we have found hydrocortisone application to wounds decreased wound collagen synthesis but did not affect re-epithelialization (16). The exact relationship, if any, between the rate of epidermal resurfacing and dermal collagen synthesis remains to be determined.

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REFERENCES

1. Winters, G. D. Formation of scab and the rate of epithelialization of superficial wounds in the skin of the young domestic pig. *Nature (London)* **193:** 293, 1962.
2. Hinman, C. D., Maibach, H., and Winters, G. D. Effect of air exposure and occlusion on experimental human skin wounds. *Nature (London)* **200:** 377, 1963.
3. Eaglstein, W. H., and Mertz, P. M. New method for assessing epidermal wound healing: the effects of triamcinolone acetonide and polyethelene film occlusion. *J. Invest. Dermatol.* **71:** 382, 1978.
4. Rovee, D. T., and Miller, C. A. Epidermal role in the breaking strength of wounds. *Arch. Surg.* **96:** 43, 1968.
5. Winter, G. D. Oxygen and Epidermal Wound Healing. Oxygen Transport to Tissue III. *Advances in Experimental Medicine and Biology,* Vol. 94. New York: Plenum Press, 1978.
6. Silver, I. A. Oxygen tension and epithelialization. In H. I. Maibach and D. T. Rovee, (Eds.) *Epidermal Wound Healing.* Chicago: Year Book Medical Publishers, 1972. P. 291.
7. Winter, G. D. Epidermal regeneration studied in the domestic pig. In H. I. Maibach and D. T. Rovee (Eds.), *Epidermal Wound Healing.* Chicago: Year Book Medical Publishers, 1972. P. 85.
8. Linsky, C. B., Rovee, D. T., and Dow, T. Effects of dressings on wound inflammation and scar tissue. In G. Hildick-Smith (Ed.), *The Surgical Wound.* Philadelphia: Lea & Febiger, 1981. Pp. 191–205.
9. Peterkofsky, B., and Diegelmann, R. Use of a mixture of proteinase-free collagenases for the specific assay of radioactive collagen in the presence of other proteins. *Biochemistry* **10:** 988, 1971.
10. Peterkofsky, B., and Prockop, D. J. A method for the simultaneous measurement of the radioactivity of proline-^{14}C and hydroxyproline-^{14}C in biological materials. *Anal. Biochem.* **4:** 400, 1962.
11. Steel, R. G. D., and Torrie, J. H. Comparison involving two sample means. *Principles and Procedures of Statistics.* New York: McGraw-Hill, 1960. Pp. 91–104.
12. Zar, J. H. *The Normal Distribution in Biostatistical Analysis.* Englewood Cliffs, N. J.: Prentice-Hall, 1974. Pp. 70–85.
13. James, J. H., and Watson, C. H. The use of opsite, a vapour permeable dressing, on skin graft donor sites. *J. Plastic Surg.* **28:** 107, 1975.
14. Pawelchek, J., Ph.D., Princeton, NJ on a file Convatec, Squibb Corp.
15. Alvarez, O. M., Mertz, P. M., and Eaglstein, W. H. The effect of proline analogue 1-azetidine-2-carboxylic acid (LACA) on epidermal and dermal wound repair. *Plastic Reconstruct. Surg.* **69:** 284, 1982.
16. Levendorf, K. D., Alvarez, O. M., Mertz, P. M., and Eaglstein, W. H. Topical steroids alter relative collagen synthesis during wound healing. In preparation.