ABSTRACT

THOMAS LEIGH RAYMOND. The Effects of Pulsed, High Frequency Direct Current Electrotherapy on Wound Epithelialization. (Under the Direction of GARLAND E. PENDERGRAPH, Ph.D. and GEORGE F. HAMILTON, M.S.)

Two groups of albino Guinea Pigs, each wounded on the lateral flanks with a hyfrecator, were used to demonstrate the effects of pulsed, high frequency, direct current electrotherapy on wound epithelialization. Only one of the groups received a daily regimen of direct current electrotherapy. Daily biopsies taken on both treated and non-treated groups to histologically analyze the epithelialization process showed that the treated group completed epithelial migration one twenty-four hour period earlier than the non-treated group. Also demonstrated was a noticeably thicker epidermis upon the completion of epithelialization. A third group was administered daily electrotherapy without prior wounding, producing no change in the intact epidermis.

THE EFFECTS OF PULSED, HIGH FREQUENCY DIRECT CURRENT ELECTROTHERAPY ON WOUND EPITHELIALIZATION

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ACKNOWLEDGEMENTS

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INTRODUCTION

The morphological systematics of the mammalian wound healing process have long been established. The mechanisms that initiate and maintain these processes are not known. Several authors have postulated theories of inhibition mechanisms and hormonal response, but no one theory has been universally accepted. The bioelectrical phenomenon in animals which has recently been studied in regard to tissue regeneration of several phyla, encouraged the present study.

Epithelialization

Although little is known of the control mechanism, the systematics of the epithelialization process have been well established (Weiss, 1959; Johnson and McMinn, 1960; Viziam, Maltosky, and Mescon, 1964; Peacock and Van Winkel, 1970). Payne and Hinshaw (1958) reported the histological sequence of events in mammalian wound epithelialization to be proliferation and migration, followed by differentiation.

Johnson (1964) in reporting on the reaction of epithelium to injury, indicated that epithelial reaction was interdependant upon cell migration, proliferation, and differentiation. The author states, "It is difficult to decide whether, when cells are mobilized, there is a primary change in their surfaces that decreases the intensity of adhesion, or if it is a change in an activating mechanism of movements." Giacometti (1967) distinquished an epidermal mechanism that controls the rate of wound healing, and that epipnephrine may play an indirect role once the process has begun. Ross (1969) reported that a phenomenon known as "contact inhibition" was one factor in the stoppage of epidermal

growth once contact had been made in the center of the area wounded. In explaining this phenomenon, the author proposed that as cells touch each other the distribution of surface charge changes, and this acts as a signal to halt growth.

Many parameters have been tested in attempts to accelerate cutaneous wound repair. Dyson and her colleagues (1968, 1970) demonstrated the positive effect of pulsed ultrasound therapy on the epithelialization of full thickness wounds in the rabbit ear. The authors postulated that the slight increase in temperature produced by this therapeutic regimen may enhance enzymatic activity. Furthermore the authors feel that the oscillating mechanical force created by ultrasound stimulates only the migratory phase of epithelialization. Acceleration of epithelialization in the rat by the slight increase in environmental temperature has been reported by Cuthbertson and Tilstone (1967). Two primary building materials of mammalian skin, collagen and n-acetyl glucosamine, have been demonstrated by Prudden (1969, 1970) to accelerate the healing of cutaneous wounds.

Bioelectric Phenomenon

Becker (1961) reported multiple studies of bioelectric phenomena in animals, such as the amphibian. In a study of amphibian limb regeneration he indicated that the direct or bioelectric field potential generated in an area is an environmental parameter for all cells of the organism which may act as a data transmission and control system influencing local growth processes. Becker (1967) also reported, that in amphibians the bioelectric field is a steady-state potential which is

reversed at sites of amputation and throughout the body under deep anesthesia. The author postulated that the rapid field reversal at the site of amputation may represent the cessation of a direct current electron flow in the associated nerve fiber. This nervous response to the induced trauma creates a local current of injury. Hence, the author is indicating that these electrical events occuring rapidly after induced trauma may potentiate some initial phase of the wound healing process. Lipman, Dodelson and Hayes (1966) reported that the epithelium of the toad bladder possessed a distinct negative surface charge, and that fixed charges in the vicinity of the cell surface may be of considerable importance in regulating ion movement across the cell and in promoting intercellular adhesions.

Neurotrophic Influence

The nervous system has long been suspected as a source tissue for the stimulation of the regenerative process. In amphibians, Singer (1958) reported that the initiation of limb regneration is entirely dependant on nervous innervation. Using the frog as a model, the author amputated the left forelimb and hindlimb, denervated the forelimb stump, and transplanted the sciatic nerve to the forelimb. As a result, regeneration was seen in the forelimb only. The mechanism of this relationship between the nervous system and regenerative capacity is not known. Two neurohumors, acetylcholine and norepipnephrine, have been shown by Needham (1964) to have no effect on initiating the regenerative process. Bodemer (1964) and Smith (1967) demonstrated successful partial limb regeneration in amphibians in response to electrical stimulation.

Bodemer's work involved direct augmentation of the stump nerve supply. Smith did not stimulate the nerve supply alone, but he did postulate the severed nerve ends in the experimental stumps to be the tissue reacting directly to the overall applied electrical stimulus. Thus these authors substantiated the theories of Becker relating the central nervous system to the bioelectrical factors of the wound epithelialization process.

Electrotherapy

Published successful experiments in wound healing acceleration by electrotherapy revealed little in the way of a detailed description of the current applied or the biological response mechanisms involved.

Cameron (1961) and Young (1966) reported the acceleration of wound healing in dogs in response to pulsed, high frequency treatment, but both lacked a detailed description of the applied therapy and sufficent data to provide statistical analysis of the results. Wheeler et al. (1969) reported the favorable effect of a low volt electrotherapeutic regimen upon human wound healing. Assimacopoulos (1968) reported a 25% acceleration in the healing of full thickness lesions in the rabbit ear augmented by continuous negative current therapy. In this study the output electrode was in direct contact with the wound surface and therapy was continued until healing was completed. The author postulated that the bioelectrical nature of collagen, as described by Edwards and Dunphy (1958), may have been augmented by the therapy applied to increase the rate of fibrogenesis. Clinical observations of response to a pulsed, high voltage regimen was reported by Zulli (1968). He noted a 30 to 50

per cent increase in the rate of the healing process in experiments on elderly patients with histories of slow healing.

MATERIALS AND METHODS

Experimental Animal

The experimental animal was a Camm Research Institute strain of male, English short hair Guinea Pig weighing between 800 and 1200 grams. They were maintained in wire bottom cages at a maximum of three animals per cage. During the course of experimentation, all animals under study were kept in isolation. All animals received Purina Guinea Pig Chow and water supplimented with L-Ascorbic Acid ad libitum. Water bottles were changed at a minimum of every other day. A high level of cleanliness and strict limitation of traffic through the laboratory were enforced to keep the possibility of wound infection to a minimum. Laboratory ambient temperature and relative humidity were recorded daily and maintained at approximately 25 degrees Centigrade and 60% saturation. All animals were numerically identified by marking the ears with a felt tipped pen.

Wounding

Using a 26 g needle each animal was injected with 3.5 mg per 100 g body weight of Pentobarbital Sodium (Nembutal, Abbott Laboratories) interperitoneally. As previously described by Hoar (1969), this procedure produced a satisfactory surgical plane for more than two hours. After attaining surgical plane the lateral walls were clipped of all hair from the distal end of the rib cage to the hind limbs producing a rectangular area of approximately one hundred square centimeters. The remaining stubble was removed with topical application of a hair dissolving cream, (Nair, Carter-Wallace). Wounds measuring approximately

2.2mm in surface diameter and 1mm in depth were inflicted with a hyfrecator (Birtcher Corporation, model no. 701). (see Plate 1). Desired wounding was produced at a setting of five units on low output for a period of fifteen seconds. The standard single probe supplied with the instrument was utilized in a technique of fulguration followed by dessication inclusive within the fifteen second period. Fulguration was used initially to establish a wound site on the skin surface after which the probe was placed directly on this site to allow the dessciating effect to complete the induced trauma. Individual histological analysis of entire wounds was made on a daily basis by removing the entire lesion with a rotating biopsy punch.

Electrotherapy

Immediately following wounding and daily for the duration of the experimental period, test animals were given electrotherapeutic stimulation using a Dynawave unit (Dynawave Corporation, Staunton, Va.). This instrument produces a high frequency, low amperage, pulsed, direct current output of square wave design. All animals in the experimental groups received a daily treatment of 150 volts, at 8 pulses per second, positive polarity, for 15 minutes. Cellulose sponges were soaked in tap water and placed over aluminum electrode plates. (see Plate 2). The animal served to complete the circuit of electricity from the applicator electrode to the dispersive electrode. The applicator electrode was placed on the wounded side of the animal in direct contact with the wound surfaces, and the dispersive electrode on the other side. Electrodes were provided to facilitate two animals receiving simultaneous

treatment. (see Plate 3, Fig. 1). Output current flow was alternated between the two sets of electrodes every twelve seconds, so that during a single therapeutic period each animal received current for a total of seven and one-half minutes. During electrotherapy the animals were restrained in a wooden block designed by the author. (see Plate 3, Fig. 2). The animals were kept under close observation during this period to insure that the electrodes remained in proper alignment and that contact was not interrupted. At the end of the treatment period, the animals were returned to their individual cages for anesthetic recovery.

Histology

To observe the characteristics and rate of the regenerating epithelium, daily biopsies were taken of individual wounds in both control and experimental groups beginning at day one post wounding and continuing for the duration of the experimental period. Biopsies were taken with a 4 mm rotating biopsy punch and curved iris scissors that had been previously sterilized in a 33 per cent ethyl alcohol solution. To insure removal of the entire lesion, material was taken to the subcutaneous level. This material was then placed in a normal saline solution (0.85 g/100 ml distilled water) to be transferred for sectioning. All material to be studied was quick frozen to minus twenty degrees Centigrade and 10 micron sections were cut on an A. O. Spencer Cryostat Microtome. Biopsy material was mounted in saline enabling sections to be cut across the entire wound perpendicular to the skin surface, allowing for the analysis of the proliferative, migratory and differentiating

stages of epithelialization. Sections were placed on slides coated with a thin film of albumin fixative and placed on a slide warmer to dry for 24 hours at 50 degrees Centigrade. All sections were stained with Harris Hematoxylin and Eosin Y. After staining slides were replaced onto the slide warmer for 24 hours at 50 degrees Centigrade. Coverslips were mounted with Permount mounting medium.

Photomicrographs

Photographs were taken with a Nikon SK-E microscope fitted with a Nikon AFM automatic 35mm camera. Black and white photographs were taken on Kodak Panatomic-X film, and color photographs were taken on Kodak Kodachrome II type A film.

PLATE I

Legend

Figure 1. Sample wound grid. Two recent biopsy sites seen at lower level.

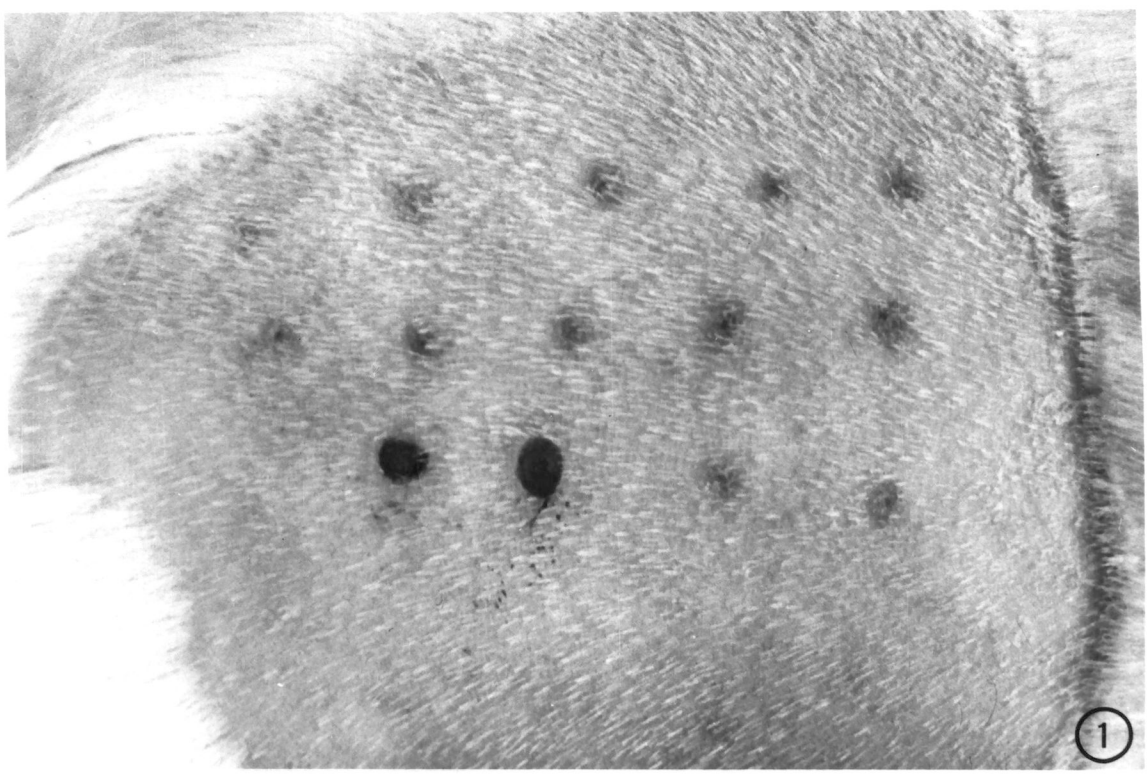


PLATE 1

PLATE II

Legend

Figure 1. Electrotherapeutic electrode assembly

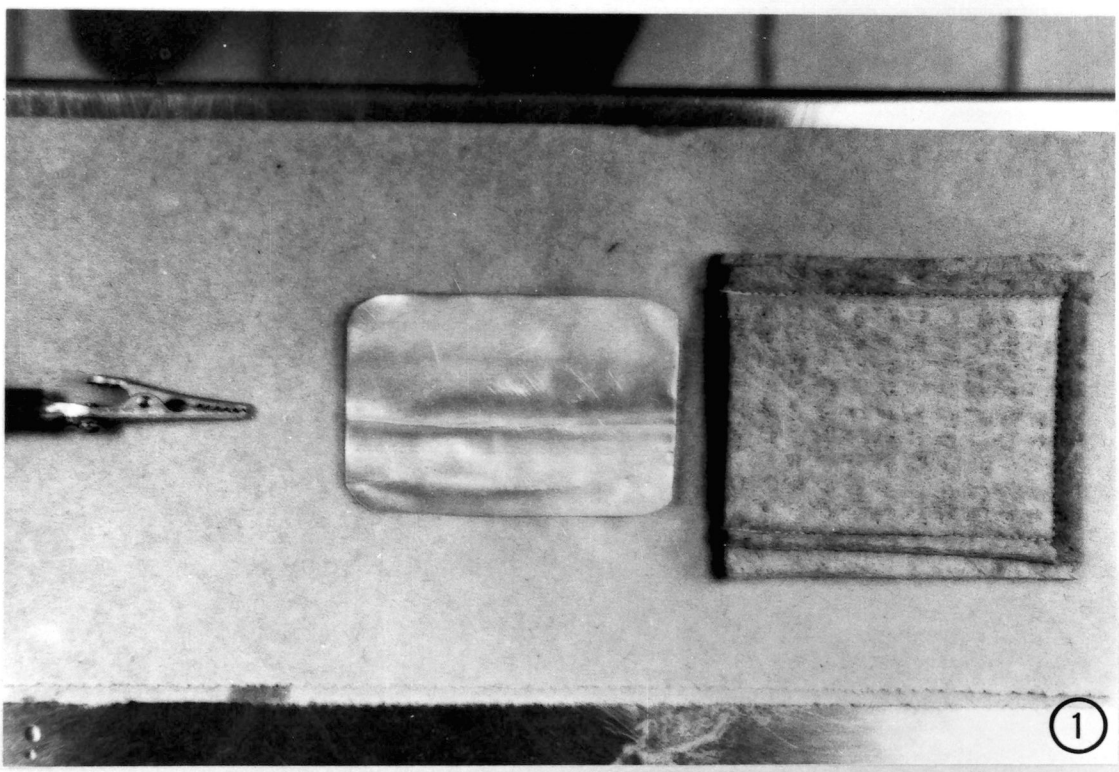


PLATE 2

PLATE III

Legend

Figure 1. Electrotherapeutic unit and electrode assembly for single animal treatment.

Figure 2. Restraining block and electrode assembly for simultaneous treatment of two animals.

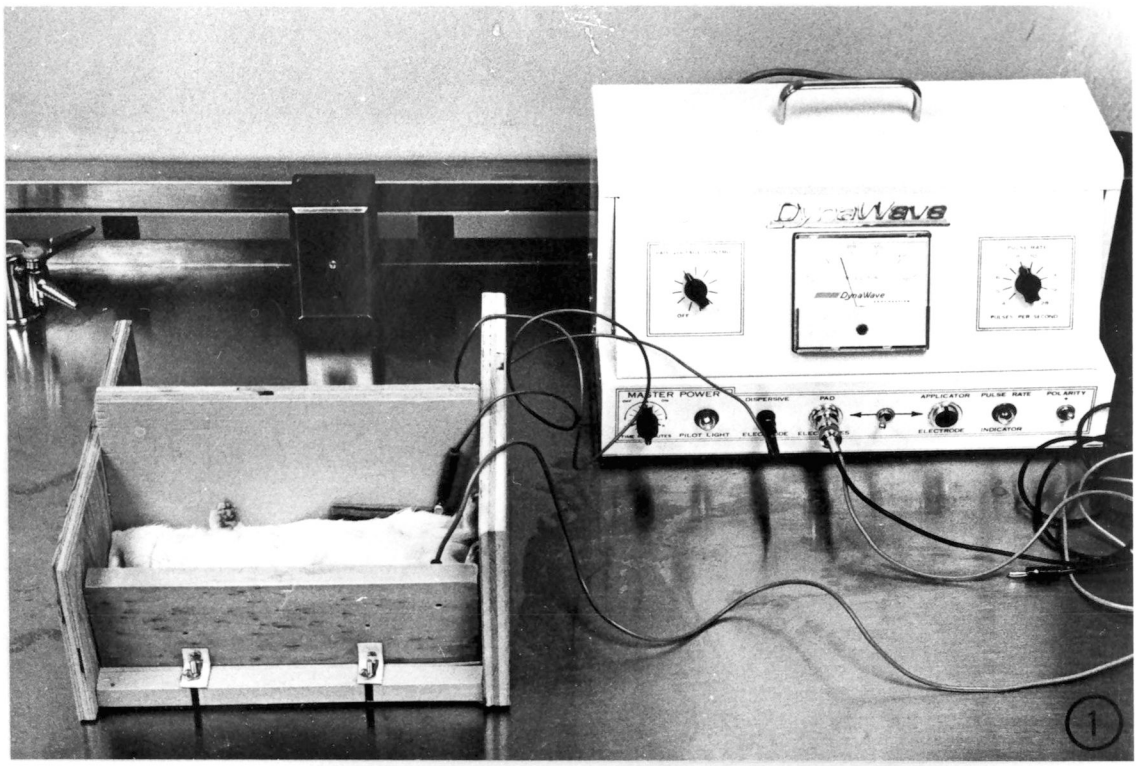

MASTER POWER
PILOT LIGHT
APPLICATOR
ELECTRODE
PULSE RATE
INDICATOR
POLARITY
1

PLATE 3


2

EXPERIMENTAL PROCEDURES AND RESULTS

Normal Epithelial Response

Experiment 1

Purpose: This experiment was conducted to establish the normal healing response in the experimental animal to the wounding model utilized.

Procedure: Six animals were wounded according to the procedure listed in Materials and Methods, and daily biopsies were taken for histological analysis of the healing response. After trial studies with the wounding model, an experimental period of eight days was established, over which biopsies were taken.

Results: Utilizing microscopic calibration, individual wounds were found to vary a maximum of 300 to 400 microns in surface diameter and depth. Epithelial migration was initiated by day two post wounding and completed by day seven. The leading edge of the migrating epithelium moved under the wound surface directly adjacent to the leukocytic barrier. (see Plates 5-7). Measuring from the basement membrane to the top of the stratum corneum, normal, unwounded Guinea Pig epidermis averages 45 to 60 microns in thickness. In this experimental group, the epithelium at the wound margin averaged 200 microns in thickness, or a four-fold increase over unwounded epithelium. (see Plate 4). By day eight a differentiated epithelium had covered the wound site and epithelialization was completed.

Acceleration of Healing

Experiment 2

Purpose: This experiment was conducted to ascertain if the electrotherapeutic model utilized could accelerate the epithelialization process.

Procedure: Six animals were wounded in the same manner as experiment 1 and given daily electrotherapy according to the specifications listed in Materials and Methods. Following treatment, daily biopsies were taken for histological investigation of the epithelial response to wounding and treatment.

Results: Epithelial migration was initiated by day two post wounding, and leading edges were joined or in close approximation by day six. (see Plates 9-12). In many cases this group demonstrated a noticebly thicker epidermis upon the completion of epithelialization in comparison to experiment 1. (see Plates 8 and 12).

Electrotherapy Control

Experiment 3

Purpose: This experiment was conducted to determine if the electrotherapeutic model utilized would have any effect on unwounded Guinea Pig Skin.

Procedure: Three animals received a daily electrotherapeutic regimen according to the specifications listed in Materials and Methods. Following treatment, daily biopsies were taken for analysis of any change that may have been produced.

Results: No change was seen in epithelial thickness and mitotic index. The normal average epithelial thickness of 45 to 60 microns was maintained without exception throughout the experimental period.

PLATE IV

Legend

Figure 1. Normal Guinea Pig skin demonstrating the orientation of hair follicles (HF), stratum corneum (SC), and underlying dermal tissue (D). (X230).

Figure 2. Regenerating epidermis at a wound margin. Mitotic figures are high in the basal layer (BL). Newly formed stratum corneum (SC) and underlying dermal tissue (D) are shown. (X230).

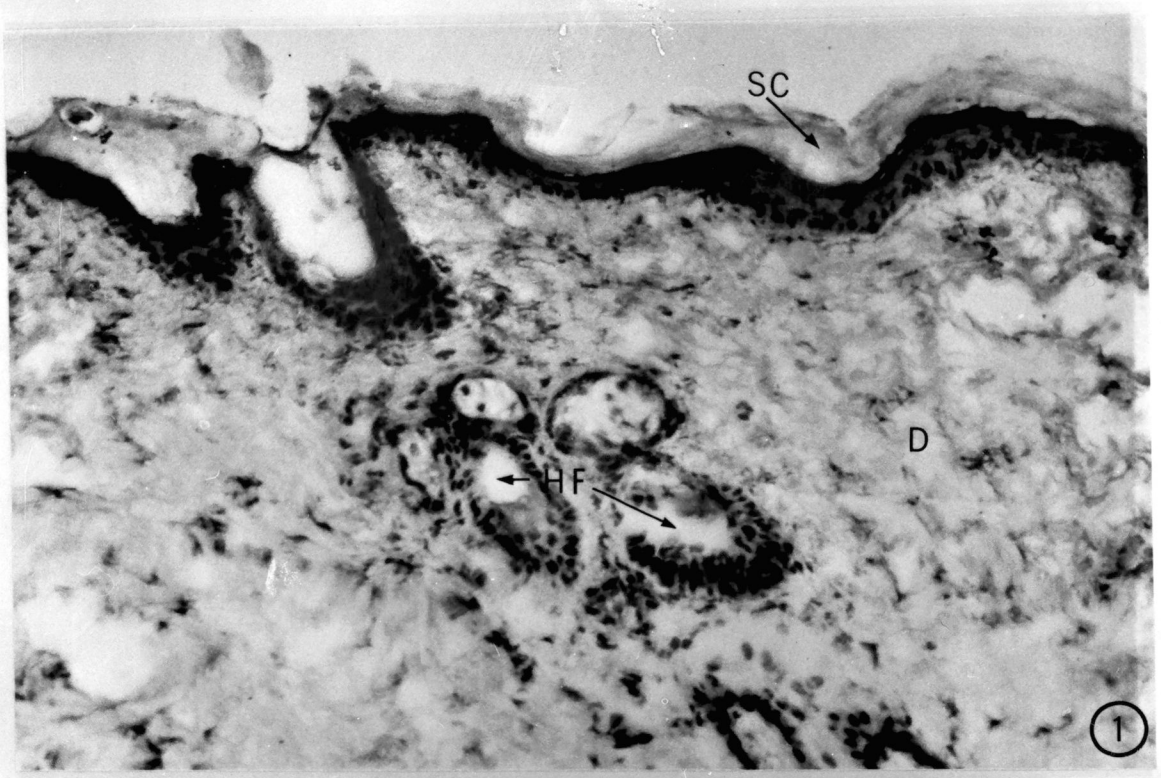


PLATE 4

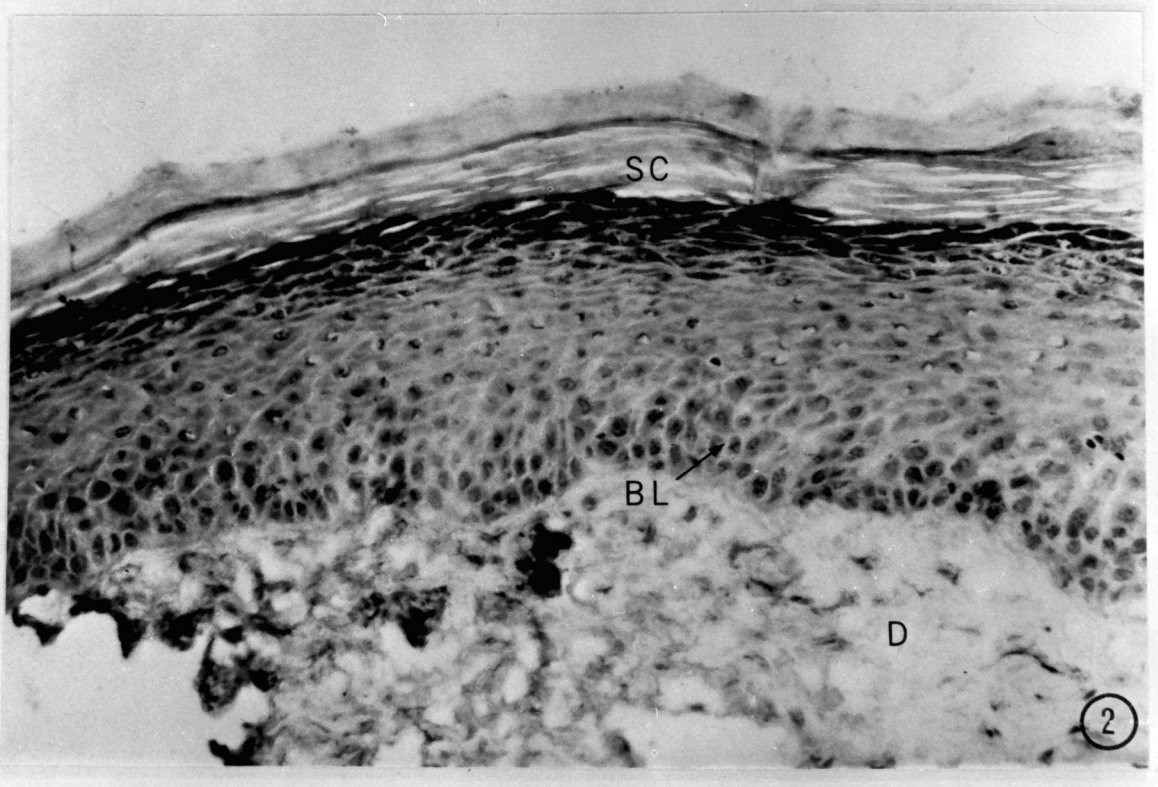
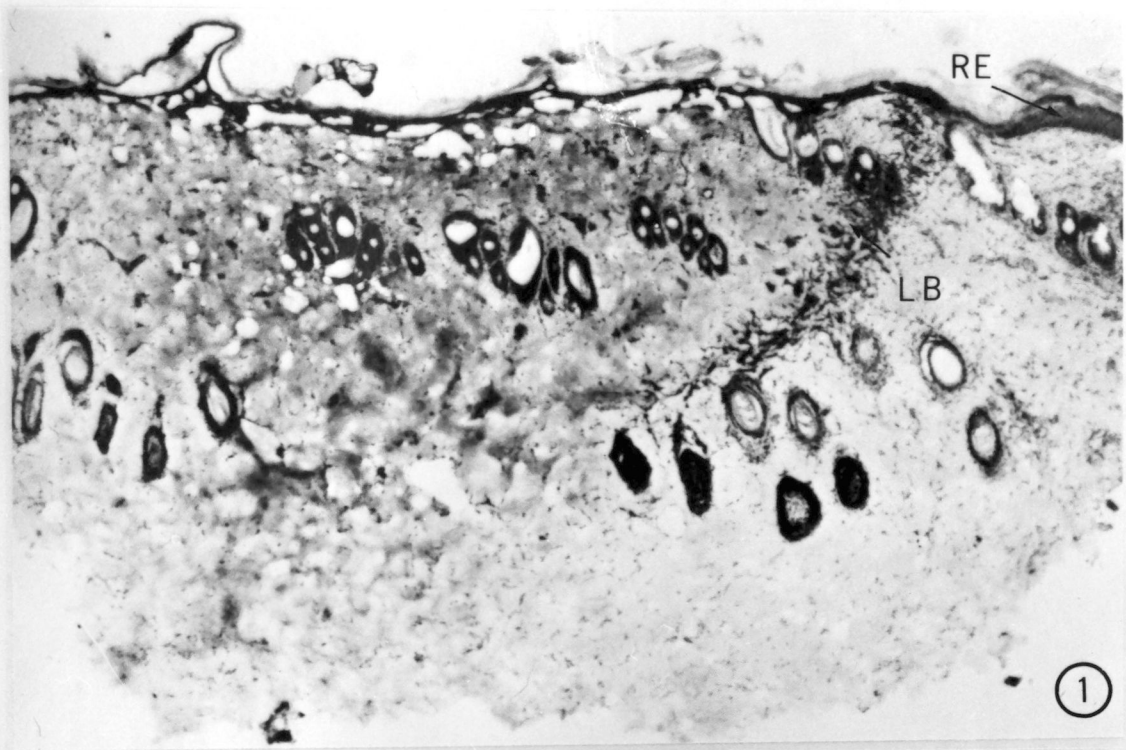


PLATE V

Legend

Figure 1. Experiment one, day one post wounding. Note leukocytic barrier (LB) formation, and proliferation of renewing epithelium (RE) at right margin. (X45).

Figure 2. Experiment one, day two post wounding. Note completed leukocytic barrier (LB). The initiation of epithelial migration is indicated by the epithelial tonque (ET) at the wound margin. (X45).



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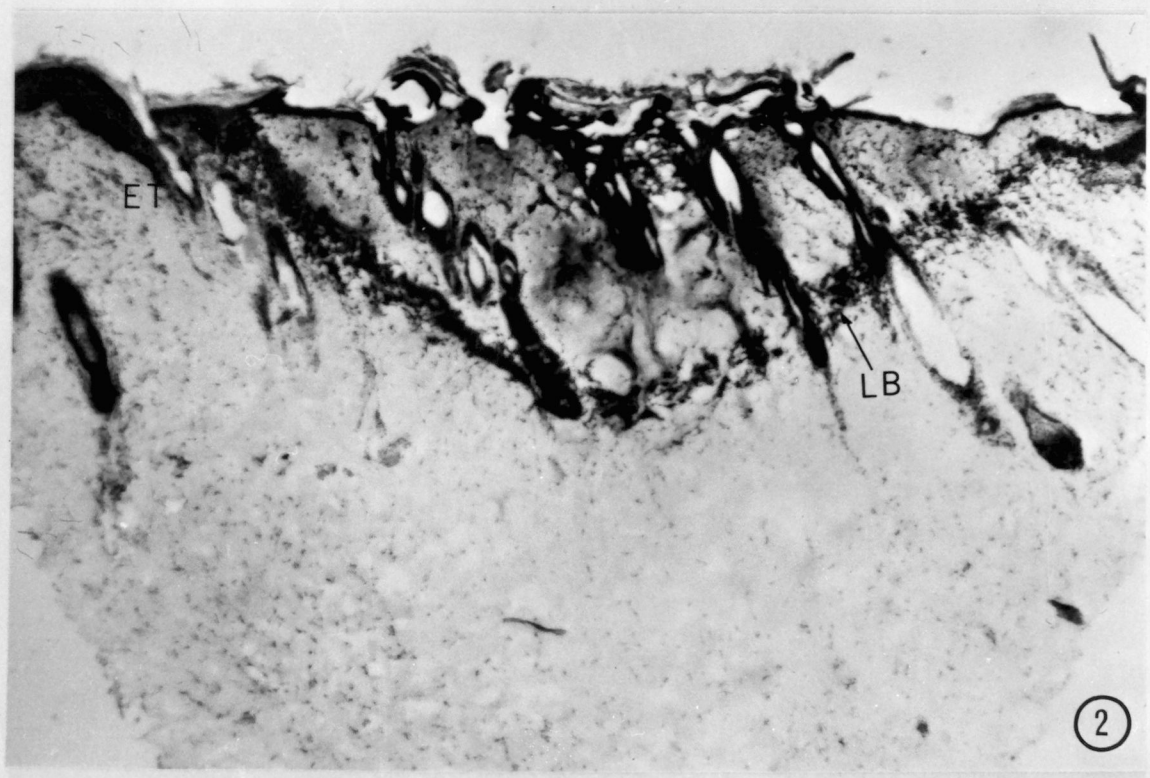


PLATE VI

Legend

Figure 1. Experiment one, day three post wounding. Mitotic proliferation of renewing epithelium (RE) is pronounced at the wound margin. (X45).

Figure 2. Experiment one, day four post wounding. Epithelial tonque (ET) seen adjacent to hair follicle. Leukocytic barrier (LB) well defined. (X45).

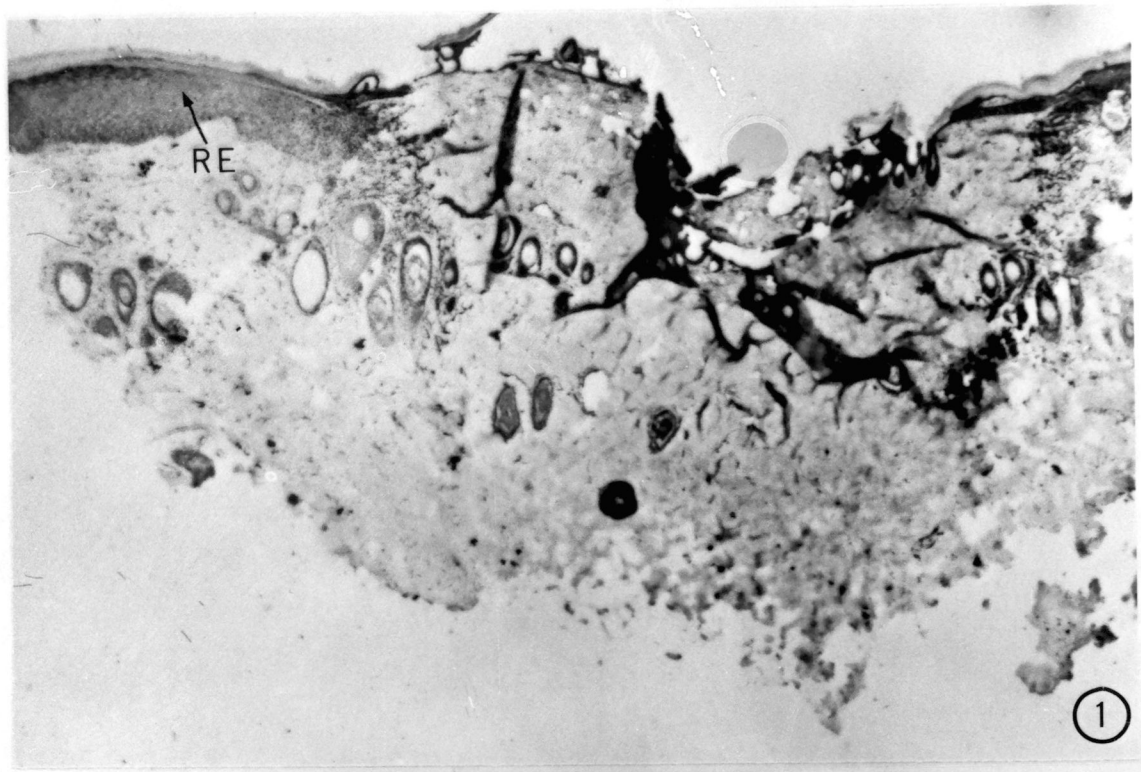


PLATE 6

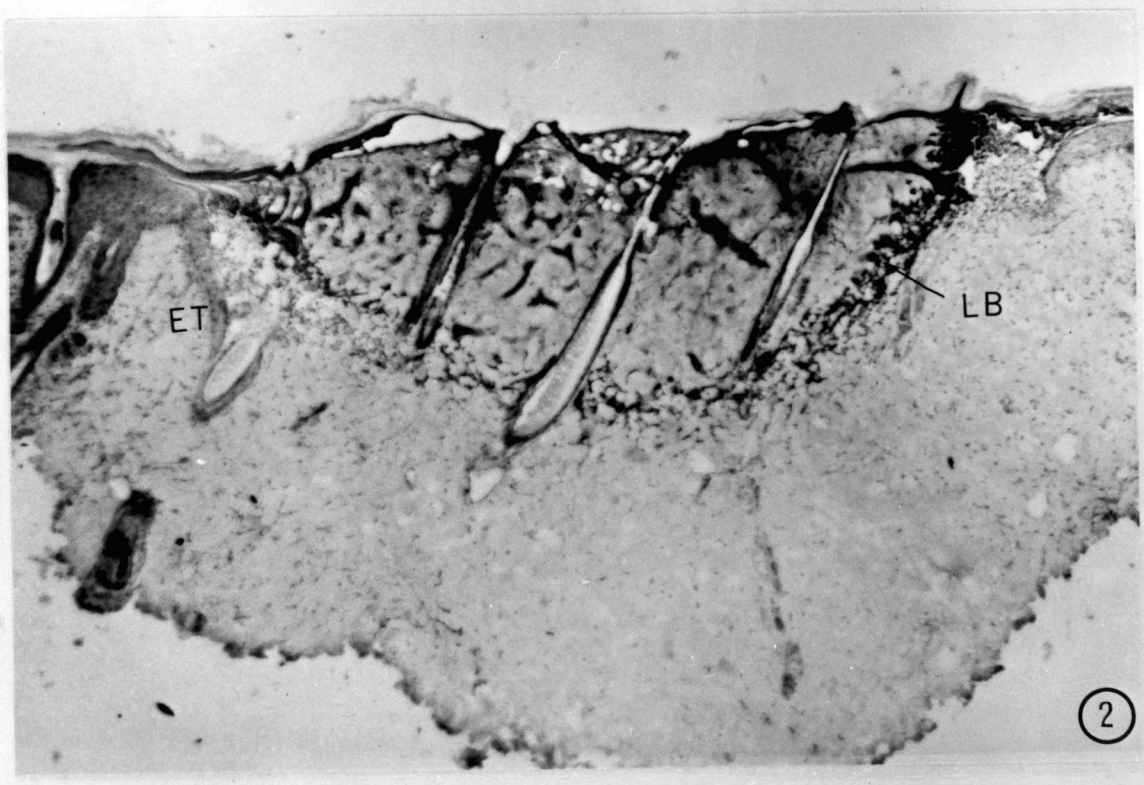
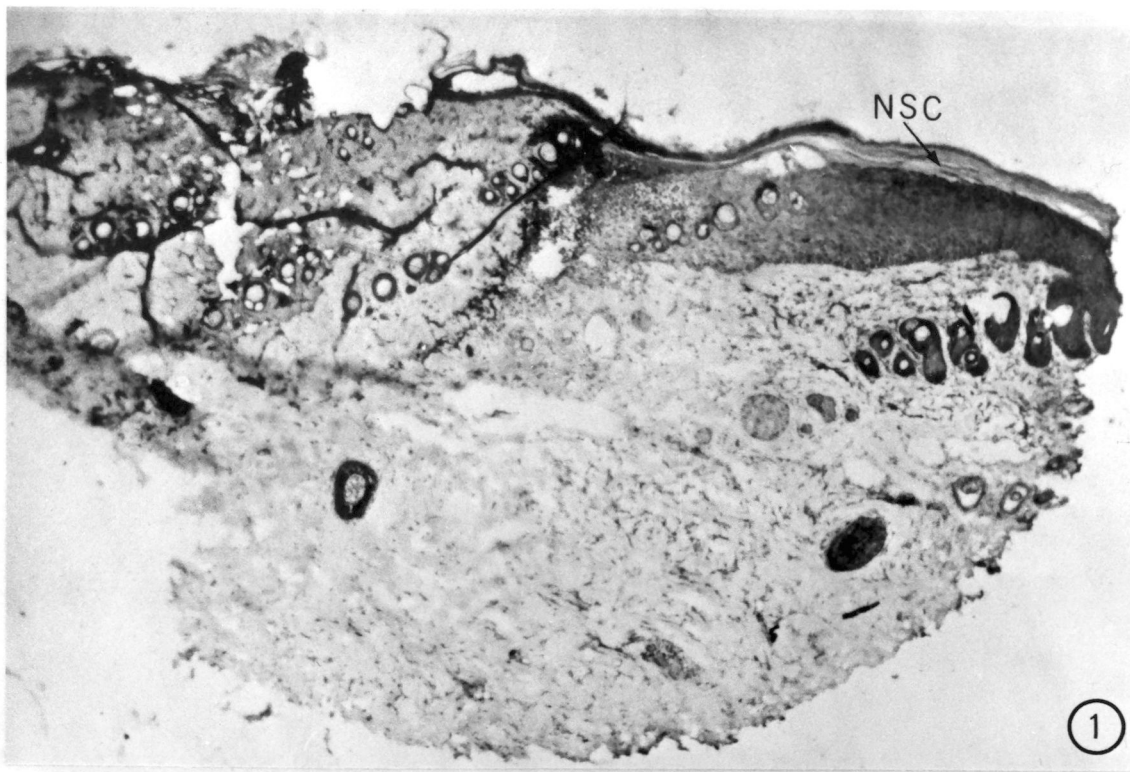


PLATE VII

Legend

Figure 1. Experiment one, day five post wounding. New stratum corneum (NSC) forming above proliferating epidermis. (X45).

Figure 2. Experiment one, day six post wounding. Epithelial tonque (ET) seen adjacent to leukocytic barrier. (X45).



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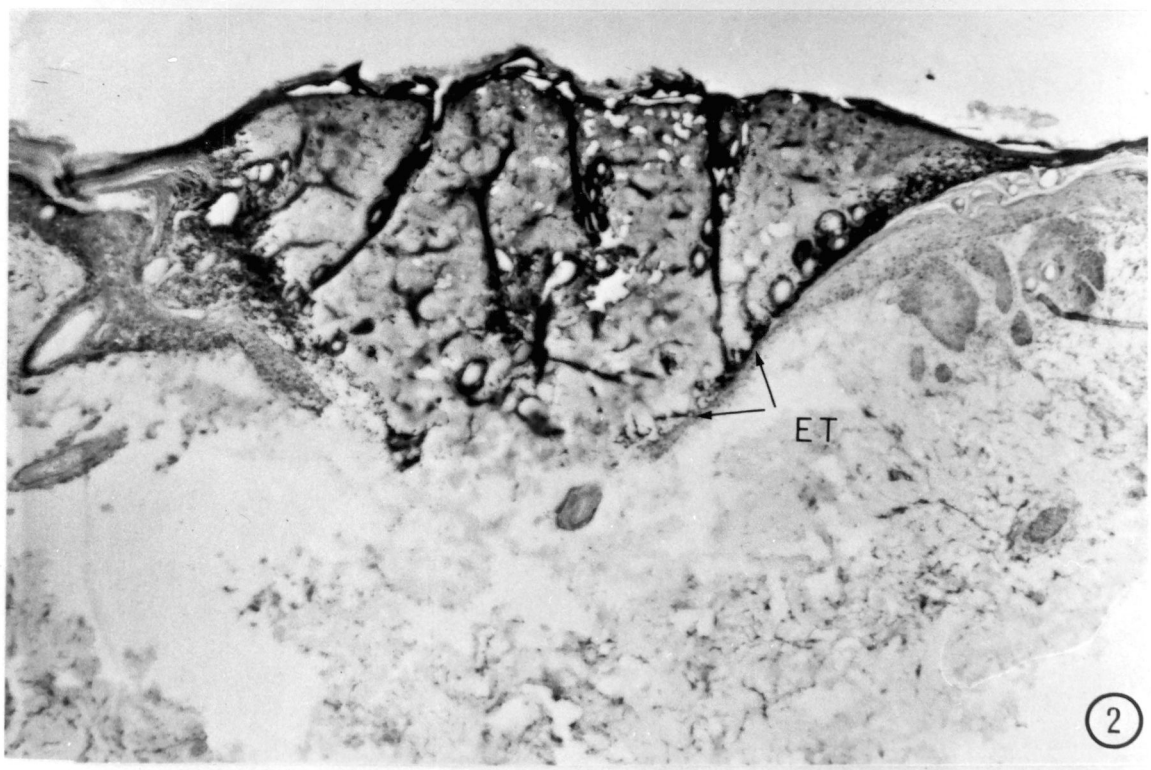
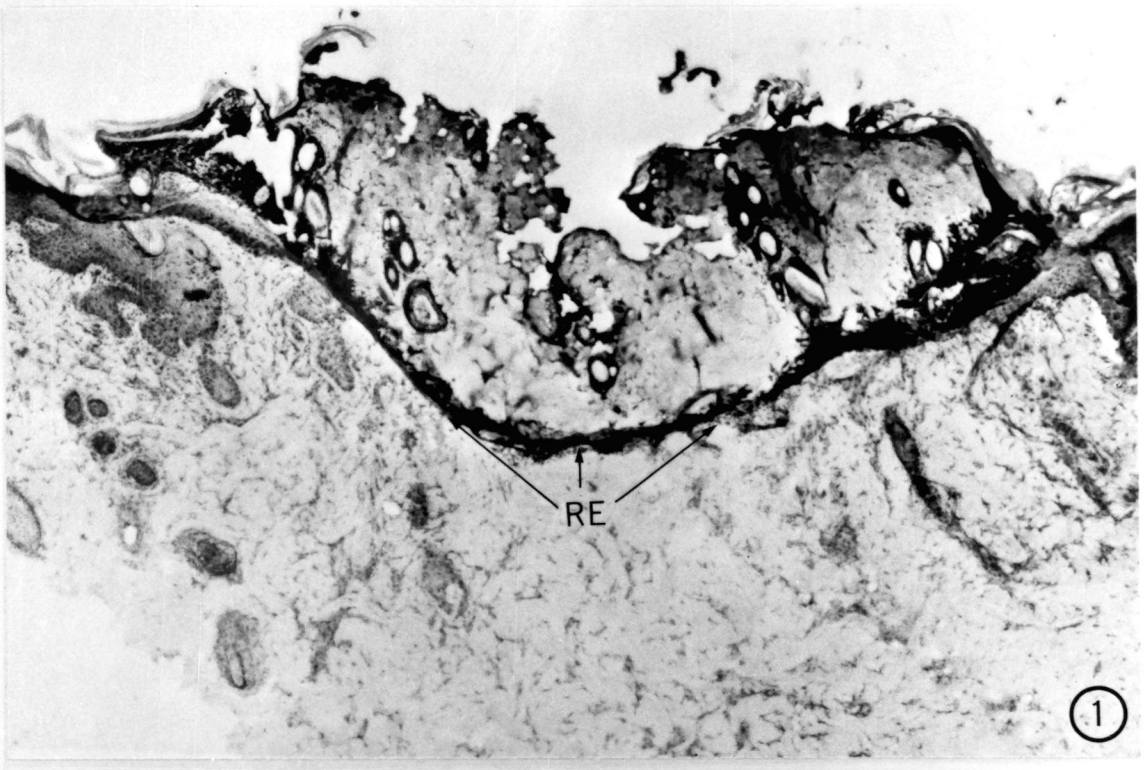


PLATE VIII

Legend

Figure 1. Experiment one, day seven post wounding. Regenerating epithelium (RE) has migrated across the entire wound floor.

Figure 2. Experiment one, day eight post wounding. Fibrous scar (FS) lifting off. Epidermal differentiation demonstrated here by newly formed stratum corneum (NSC) and rete peg (RP) formation. (X45).



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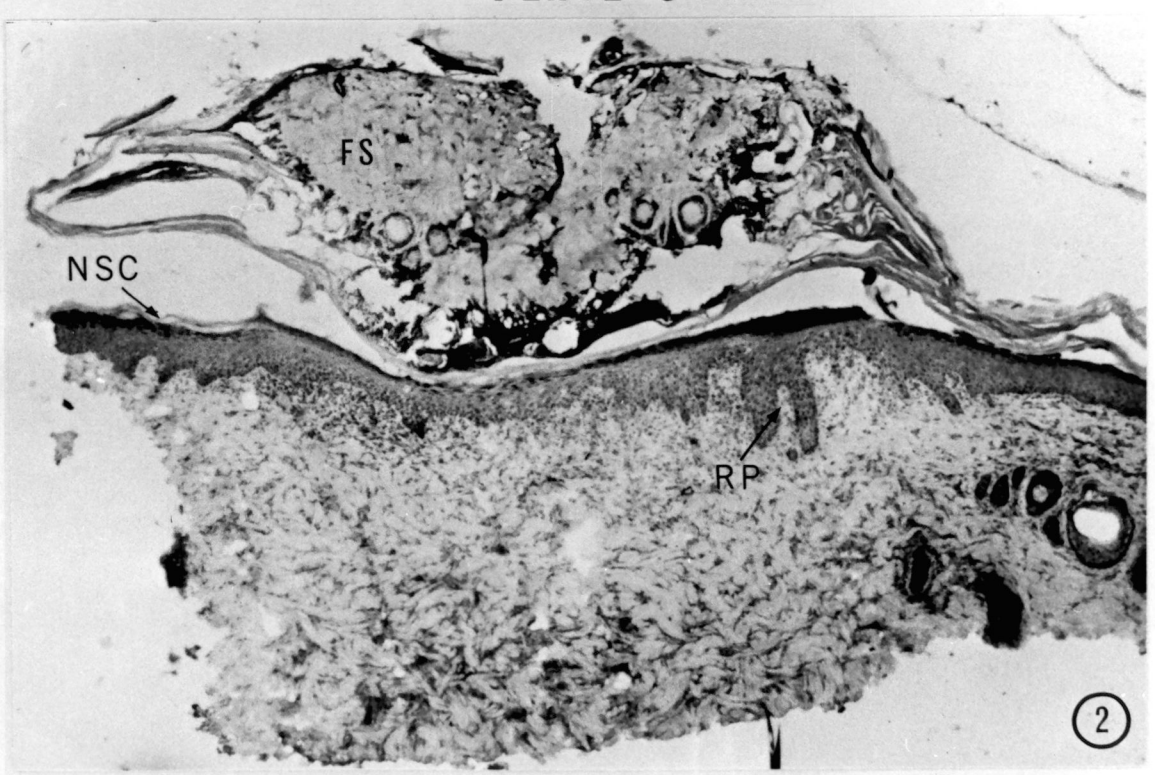
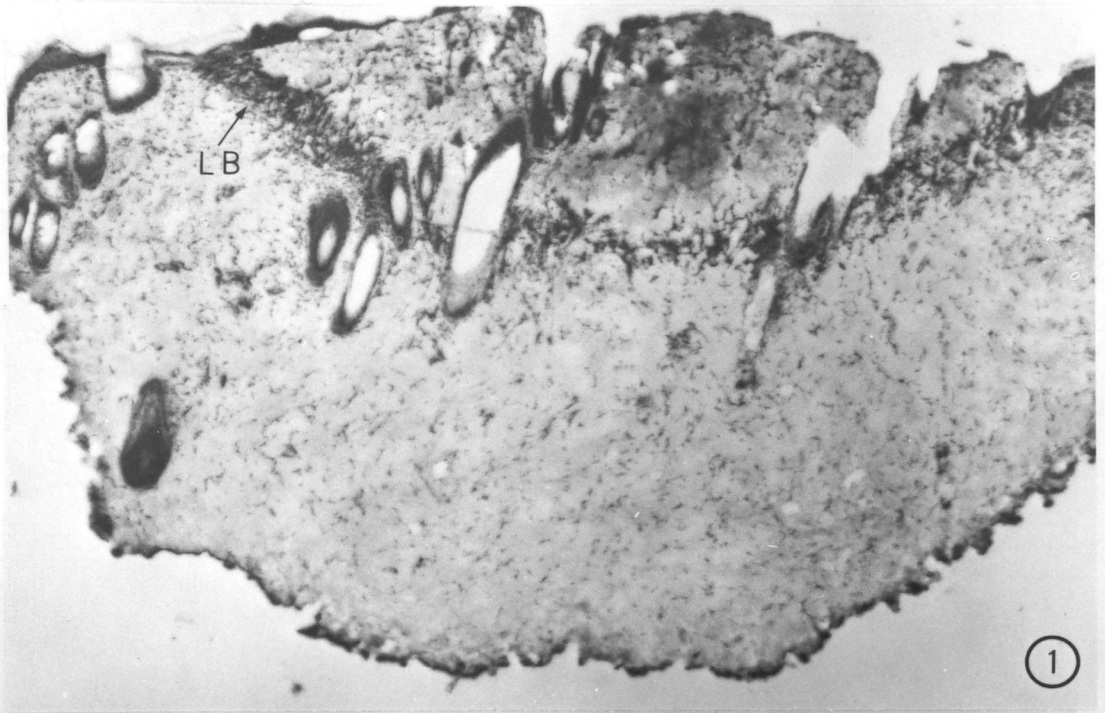


PLATE IX

Legend

Figure 1. Experiment two, day one post wounding. Leukocytic barrier (LB) complete. (X45).

Figure 2. Experiment two, day two post wounding. Regenerating epithelium (RE) seen in association with a hair follicle (HF). (X45).



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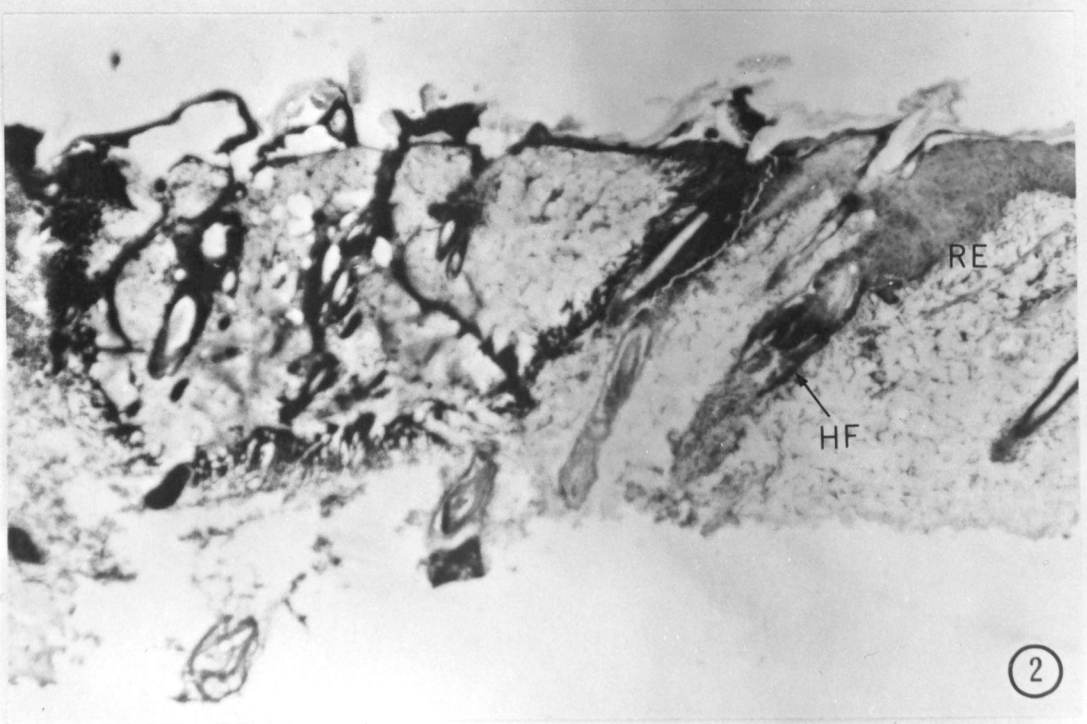
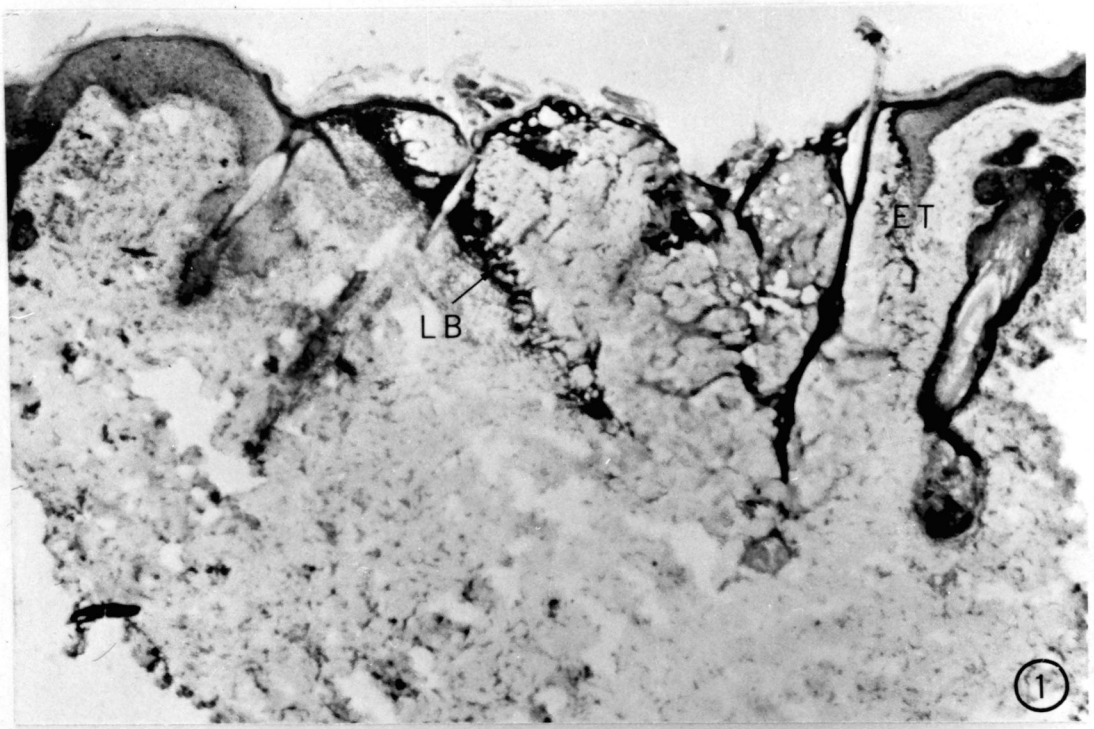


PLATE X

Legend

Figure 1. Experiment two, day three post wounding. Migrating epithelial tonque (ET) and leukocytic barrier seen. (X45).

Figure 2. Experiment two, day four post wounding. Hair follicle epithelium (FE) and a regenerating epithelial filament (RF) both contributing cellular material to the renewed epidermis. (x45).



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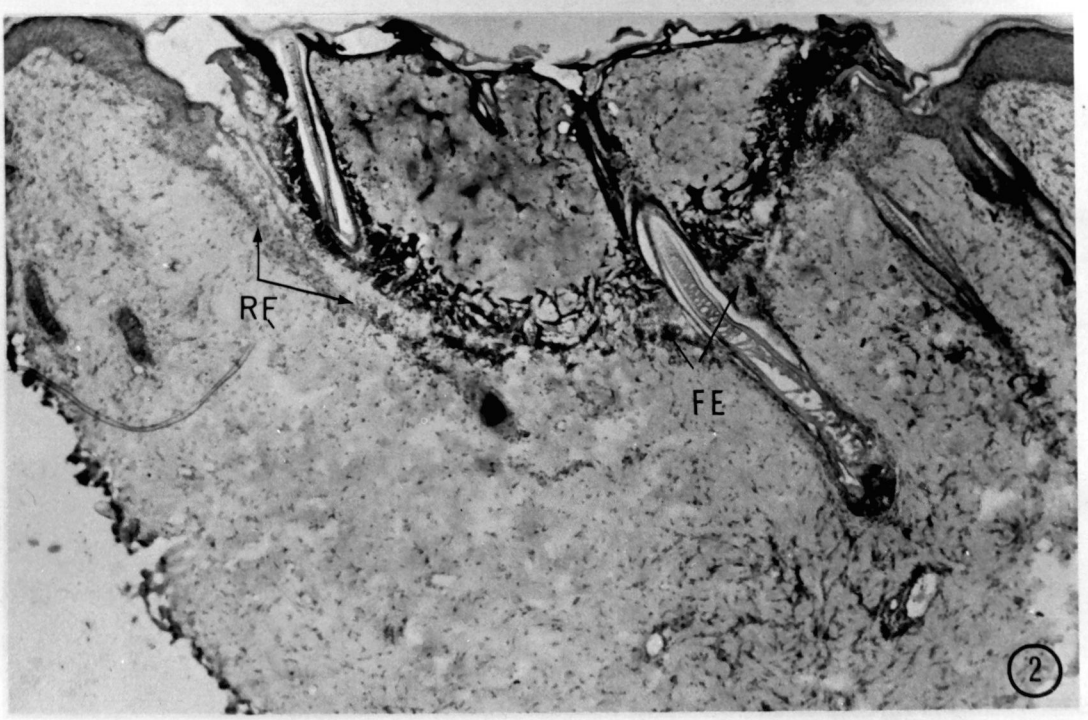
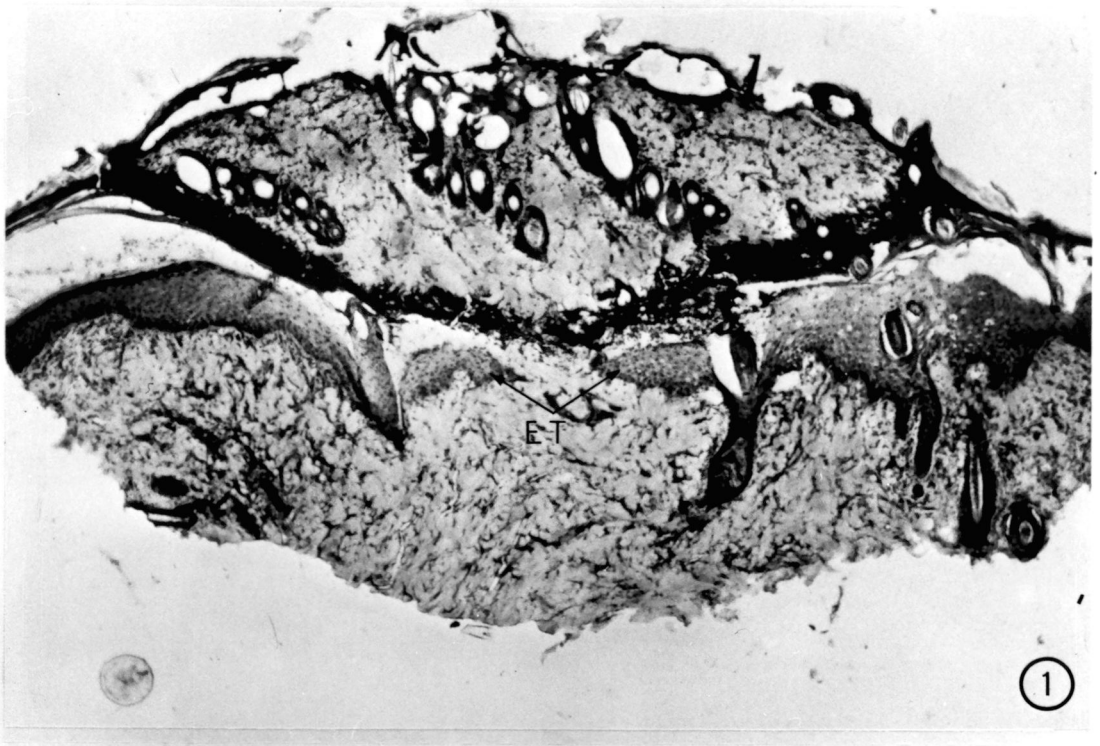


PLATE XI

Legend

Figure 1. Experiment two, day five post wounding. Epithelial tonques (ET) seen in close approximation below the wound surface. (X45).

Figure 2. Experiment two, day six post wounding. Epithelial migration complete, differentiation seen in rete peg (RP) formation. (X45).



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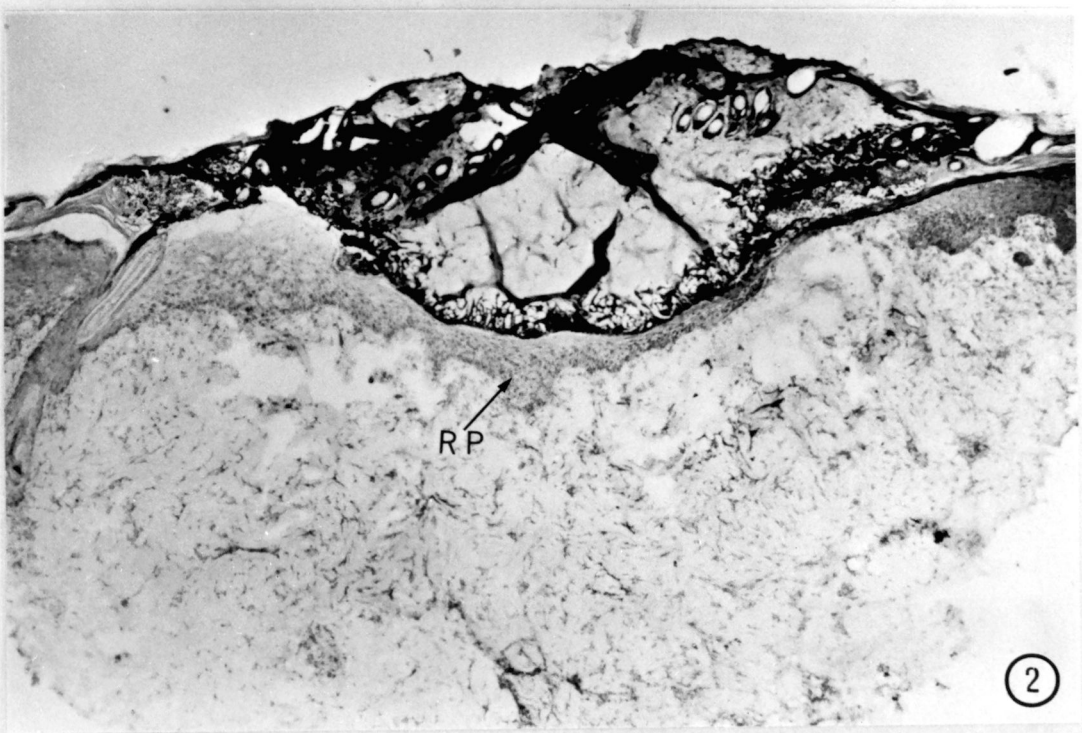


PLATE XII

Legend

Figure 1. Experiment two, day seven post wounding. A thickened epidermis is seen covering the wound floor. (X45).

Figure 2. Experiment two, day eight post wounding. Epithelialization complete; fibrous scar (FS) lifting off. (X45).

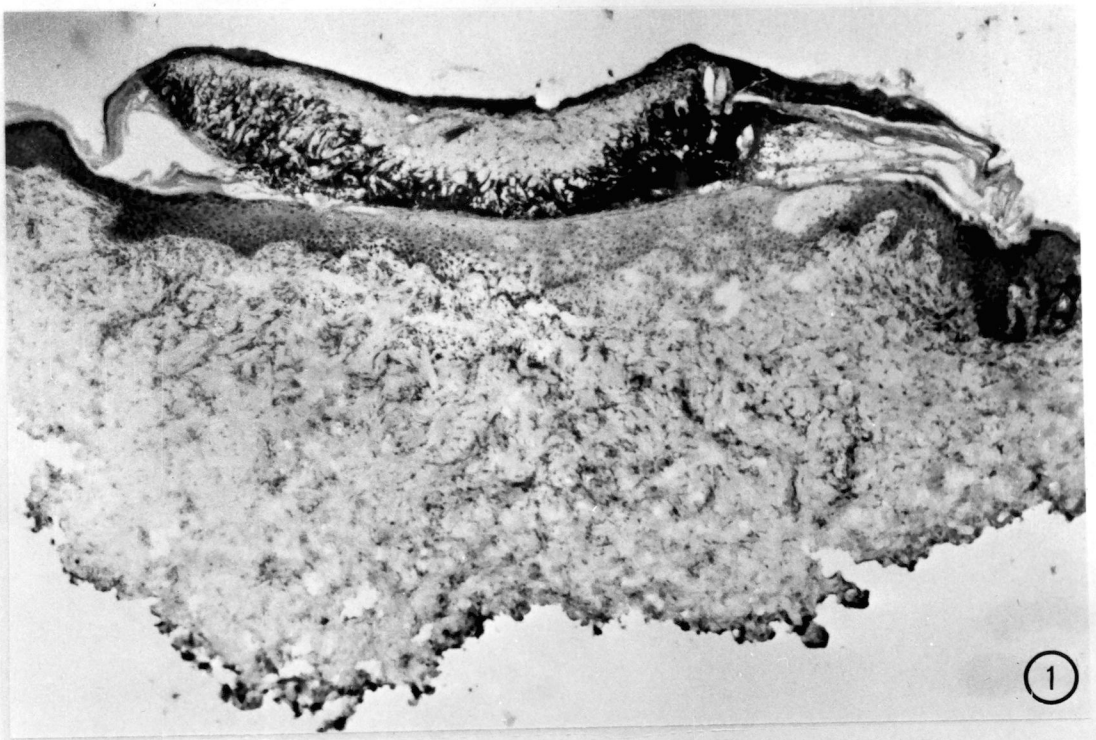
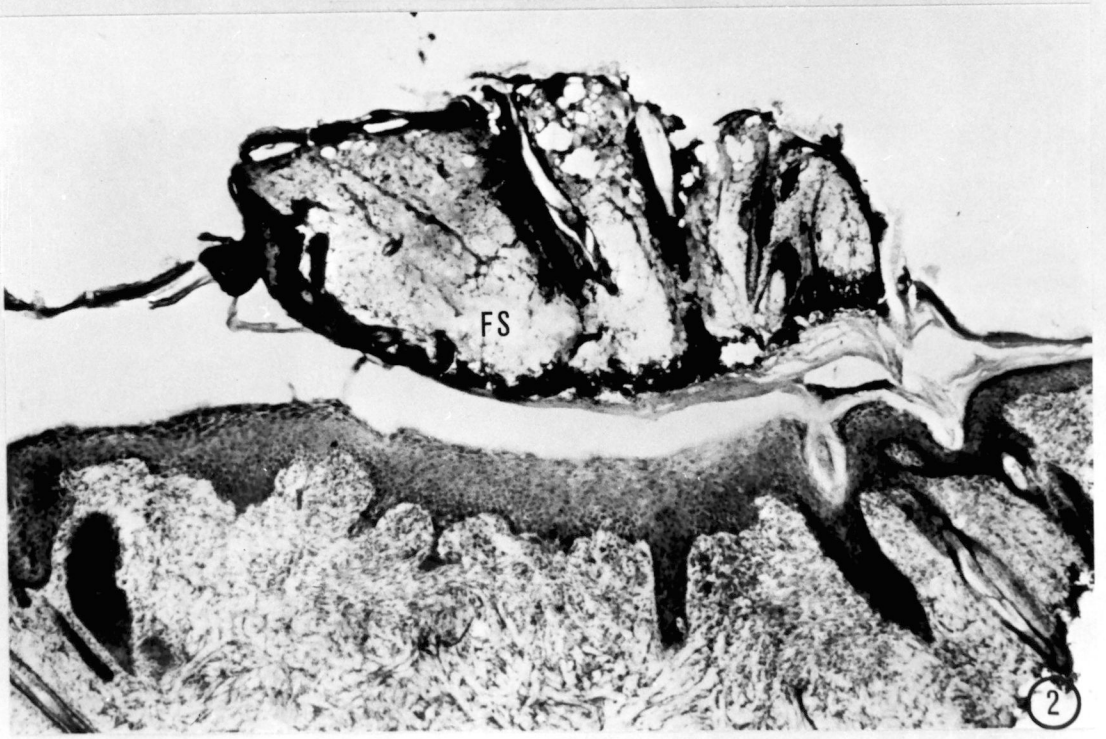


PLATE 12



DISCUSSION

One of the major objectives of this study was to develop an effective model for the study of the wound epithelialization process. Following the work of Jelenko (1969) several techniques were attempted on the Guinea Pig. Small wounds were utilized enabling entire lesions to be studied on a daily basis. The use of small wounds prevented having to inflict secondary wounds onto the primary site under study. Prudden (1970) reported that disrupting a primary site can greatly alter its healing rate. The level of wound individuality had to be kept to a minimum, for large variations in the size of the lesion creates equally varying distances for the regenerating epithelium to traverse. In addition the use of the hyfrecator for wound inflicition produced an immediate eschar that, along with general laboratory cleanliness, prevented bacterial infection.

In comparing the results of experiments one and two, the two parameters best demonstrated are epithelial migration and thickness. In experiment two, this migration was completed approximately twenty-four hours earlier and the epithelium appeared thicker. These results point toward the enhancement of both the proliferative and migratory phases of the epithelialization process. On the basis of previous studies most theories would apply the electrotherapeutic regimen to mitotic proliferation only. However, in the work on epithelialization enhancement by ultrasound therapy by Dyson et al. (1968,1970), it was postulated that the sheer mechanical force created by the therapeutic regimen was a source of migratory stimulation. In the present study epithelial migration may have been enhanced by the physical response to

the applied current by the peripheral musculature.

In regard to mitotic proliferation, the cutaneous nerve supply seems to present itself as the tissue most likely responding to the applied stimulus. Singer (1960,1964) and Schmidt (1968) have established the role of the nervous system in vertebrate limb regeneration, but the mechanism involved has yet to be identified. Undoubtedly some neurotrophic substance or ion balance system acts as a mitotic stimulator in the process.

The results of this study indicate that direct current electrotherapy may enhance the proliferative and migratory phases of mammalian wound epithelialization either by augmenting the release of a mitotic stimulator or by physically enhancing migratory streaming across the wound floor. Nevertheless, the results of this study are far from conclusive, and elaboration of these results should be pursued.

LITERATURE CITED

ASSIMACOPOULOS, D. 1968. Wound Healing Promotion by the Use of Negative Electric Current. Amer. Surg. 34:423-430

BECKER, R. O. 1961. The Bioelectric Factors in Amphibian-Limb Regeneration. Jour. Bone & Joint Surg. 43:643-656.

BECKER, R. O. 1967. The Electrical Control of Growth Processes. Orthopedics 95:657-669.

BODEMER, C. W. 1964. Evocation of Regrowth Phenomena in Anuran Limbs by Electrical Stimulation of the Nerve Supply. Anat. Rec. 148:441-458.

CAMERON, B. M. 1961. Experimental Acceleration of Wound Healing. Amer. Jour. Ortho. 3:336-343.

CUTHBERTSON, D. P. & W. J. TILSTONE. 1967. Effect of Environmental Temperature on the Closure of Full Thickness Skin Wounds in the Rat. Quart. Jour. Exper. Physio. 52:249-257.

DYSON, M. et al. 1968. The Stimulation of Tissue Regeneration by Means of Ultrasound. Clin. Sci. 35:273-285.

DYSON, M. & J. B. POND. 1970. The Effect of Pulsed Ultrasound on Tissue Regeneration. Physiotherapy 56:136-142.

EDWARDS, L. C. & J. E. DUNPHY. 1958. Wound Healing, I. Injury and Normal Repair. New Eng. Jour. Med. 259:224-233.

GIACOMETTI, L. 1967. The Healing of Skin Wounds in Primates, I. The Kinetics of Cell Proliferation. Jour. Invest. Derm. 48:133-137.

HOAR, R. M. 1970. Anesthesia in the Guinea Pig. Fed. Proc. 28:1517-1520.

JELENKO, C. 1969. Production of the Experimental Burn. Jour. Surg. Res. 9:159-165.

JOHNSON, F. R. & R. M. H. McMINN. 1960. The Cytology of Wound Healing of Body Surfaces in Mammals. Biol. Rev. 35:364-412.

JOHNSON, F. R. 1964. The Reaction of Epithelium to Injury. Sci. Basis Med. Ann. Rev. pp. 276-290.

LIPMAN, K. M., R. DODELSON, & R. M. HAYES. 1966. The Surface Charge of the Isolated Toad Bladder Epithelial Cell. Mobility, Effect of pH and Divalent Ions. Jour. Gen. Physio. 49:501-516.

NEEDHAM, A. E. 1964. Biological Considerations of Wound Healing in Advances in Biology of Skin, Vol. 5, Wound Healing, W. Montagna & R. E. Billingham eds., Macmillian, N. Y. pp. 1-26.

PAYNE, F. W. & J. R. HINSHAW. 1958. Histologic Studies of the Early Healing Processes in Moderate Second Degree Burns. Univ. of Rochester, Atomic Energy Project, VR-514.

PEACOCK, E. E. & W. VAN WINKLE. 1970. Surgery and Biology of Wound Repair. W. B. Saunders Co. Philadelphia.

PRUDDEN, J. F. 1969. The Acceleration of Healing. Surg., Gyn., & Obstet. 128 (37 ref.):1321-1326.

PRUDDEN, J. F. 1970. The Discovery of a Potent Pure Chemical Wound Healing Accelerator. Amer. Jour. Surg. 119:560-564.

ROSS, R. 1969. Wound Healing. Sci. Amer. 220(6):40-50.

SCHMIDT, A. J. 1968. Cellular Biology of Vertebrate Regeneration and Repair. Univ. of Chicago Press, Chicago.

SINGER, M. 1958. The Regeneration of Body Parts. Sci. Amer. 202(10):3-7.

SINGER, M. 1960. Nervous Mechanism in the Regeneration of Body Parts in Vertebrates, in Developing Cell Systems, D. Rudnick ed., Ronald Press, New York. pp. 155-133.

SINGER, M. 1964. The Tropic Quality of the Neuron: Some Theoretical Considerations, in Progress in Brain Research, Vol. 13, Mechanisms of Neural Regeneration, M. Singer & J. P. Schade eds., Elsevier Pub. Co., New York, pp. 228-232.

SMITH, S. D. 1967. Induction of Partial Limb Regeneration in Rana pipiens by Galvanic Stimulation. Anat. Rec. 158:89-98.

VIZIAM, C. B., A. G. MALTOSKY, & H. MESCON. 1964. Epithelialization of Small Wounds. Jour. Invest. Derm. 43:499-504.

WEISS, P. 1959. The Biological Foundations of Wound Repair. The Harvey Lectures, Academic Press, New York, 55:13-42.

WOLCOTT, L. E., P. C. WHEELER, H. HARDWICK, & B. M. ROWLEY. 1969. Accelerated Healing of Skin Ulcers by Electrotherapy. South. Med. Jour. 62:795-801.

YOUNG, H. G. 1966. Electrical Impulse Therapy Aids Wound Healing. Mod. Vet. Prac.

ZULLI, L. P. 1968. Pulsed High Frequency Electromagnetic Energy for Care of Foot Lesions. Jour. Amer. Podiat. Ass. 58:343-344.