

The Microclimate Chamber: The Effect of Continuous Topical Administration of 96% Oxygen and 75% Relative Humidity on the Healing Rate of Experimental Deep Burns

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The healing rate of small experimental burns continuously treated topically with 96% O₂ and 75% relative humidity was followed for 25 days. Serial image photographic magnifications (tenfold throughout) of the wounds enabled precise measurements of their size by means of a polar planimeter. Healing rate was expressed as decreased percentile of wound size on a given day compared to the initial area. The mean percentages of healing \pm SEM of the humidified O₂ treated wounds on postburn days 6, 11, and 16 were 31.25 \pm 6.15, 82.09 \pm 3.52, and 98.29 \pm 1.46, respectively, and those for the control wounds were 7.08 \pm 2.20, 47.68 \pm 3.39, and 84.41 \pm 1.38, respectively. Analysis of variance revealed highly significant differences in the healing rate between O₂-humidity-treated and control wounds ($p < 0.005$). The results indicate that topical treatment with 96% O₂ and 75% relative humidity improved healing of experimental burns in guinea pigs.

A recent review of the literature indicates that microclimate factors, such as gas composition, relative humidity, and temperature, which are in contact with the surface of burn wounds, may have a significant influence on the healing process of burns (15). However, we found no adequate methodology for studying these variables reported in the literature. Therefore, a simple system was devised to control the gas composition and the relative humidity delivered to the surface of experimental burn wounds. The system enables continuous delivery and monitoring of a controlled oxygen concentration and relative humidity at the wound surface, while permitting evaluation of the wound-healing process in unrestrained guinea pigs breathing room air. Employing this technology to control the microclimate factors, the effect of continuous application of humidified oxygen on the healing rate of deep burns in unrestrained guinea pigs was investigated over an interval of 25 days.

MATERIALS AND METHODS

Animals. Hartley-derived female guinea pigs weighing 650 to 700 gm were included in two sequential exper-

iments (four animals in each). The animals were housed in individual modified metabolic cages in order to apply the swivel apparatus, which enabled continuous delivery of humidified O₂ to the burn wound in unrestrained animals breathing room air. They were fed regular guinea pig chow and water ad libitum.

Burns. The animals were clipped and depilated 24 hours before the burn injury. Under general anesthesia (IM ketamine HCl, Ketalar, Parke-Davis, Division of Warner-Lambert, Morris Plains, NJ, 50 mg/kg body weight), two pairs of round (1.2-cm in diameter), deep, symmetrical burns were inflicted by a means of a modified soldering iron (450°C for 3 seconds) on the back of each animal at a constant distance from the midline and at an identical topographic location (Fig. 1). Preliminary studies in our laboratories showed such burns to be identical in their depths, involving the full thickness of the dermis. The anterior pair of burn wounds served as dual controls for the uniformity of the depth of the wounds as well as the healing rate. The right wound of the posterior pair was treated by the humidified O₂ and the contralateral injury served as the control.

The Microclimate Chamber. The chamber is composed of the following parts: 1) a polyethylene cylindrical chamber, which is impermeable to oxygen (Fig. 2-1) (internal diameter, 2.7 cm; volume, 10.3 cm³) which was attached to the animal's back by its pliable flange (Fig. 2-2) with a cyanoacrylate glue, reinforced by 4-0 Dermalon sutures; 2) a screw mount top (Fig. 2-3) which fits snugly on the chamber; and 3) a polyethylene tube (Fig.

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FIG. 1. Two pairs of deep burns are inflicted. The right posterior wound is treated with humidified O_2 ; the contralateral serves as control. The anterior pair of burns serve as dual controls.

2-4), which is inserted in the center of the screw mount top, to deliver the humidified gas from the swivel apparatus. The tubing is secured by means of a stainless-steel spring tether (Fig. 2-5).

The microclimate chamber and the swivel apparatus were attached to the treated wounds. To the contralateral control wound a chamber was also attached without its screw mount top and exposed to ambient air (temperature, $24^\circ C \pm 1$; oxygen, 21%; and relative humidity, $30\% \pm 2$). The anterior pair of control burn wounds were exposed without chambers.

Controlling and Monitoring the Microclimate Factors in the Chamber: Oxygen. Figure 3 illustrates the gas delivery system to the unrestrained animal. A constant flow of humidified oxygen was maintained by connecting the outflow tubing of a Bard-Parker humidifier (M-H8294-005213, Rutherford, NJ) to the top of the swivel. The cannular feedthrough swivel (18 gauge, Tech. Serv., Inc., Beltsville, MD. Cath. 192-03) was suspended over a rectangular hole in the roof of the metabolic cage. A stainless-steel tether spring linked the animal to the swivel (Fig. 3), protected the polyethylene tubing, and rotated the swivel when the animal turned.

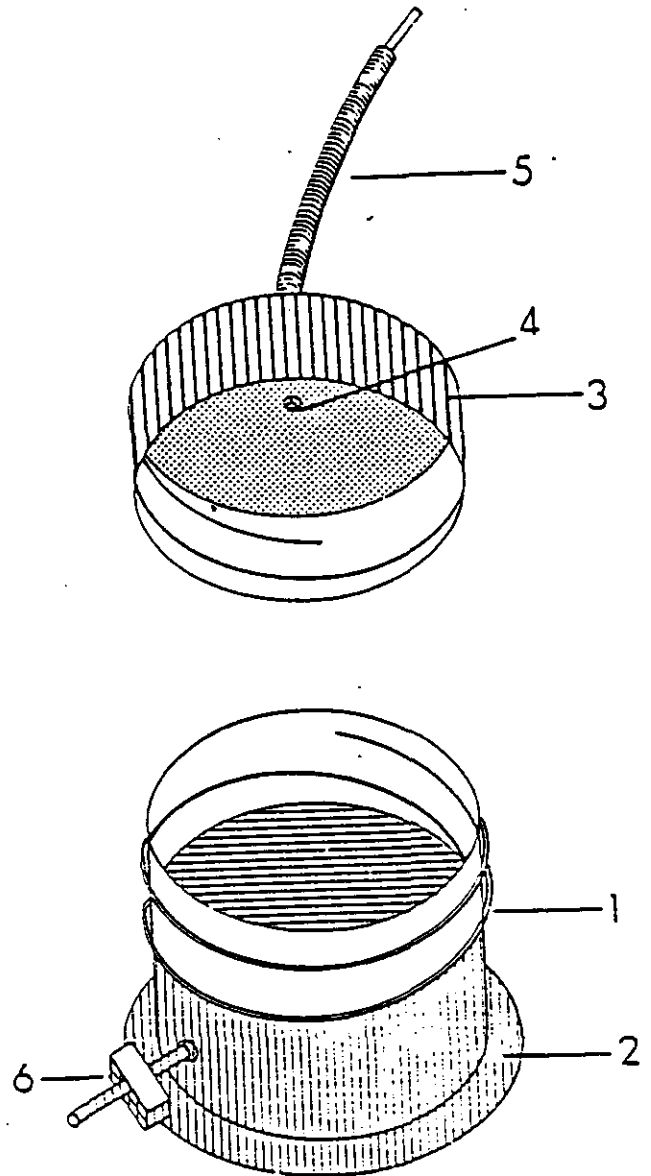


FIG. 2. The microclimate chamber: 1) The polyethylene cylindrical chamber. 2) The pliable flange. 3) Screw mount top. 4) Polyethylene tubing. 5) Stainless-steel spring tether. 6) Outflow and sampling vent.

This arrangement allowed the guinea pig to move freely in the cage.

During the 25 days of this study, pure oxygen was continuously administered to the chamber through the humidifier and maintained on the treated wounds at a constant level of 96% O_2 . Higher concentrations of O_2 were precluded due to the mixture of water vapor in the gas phase. The gaseous inflow rate to the system was kept constant at 1.5 L/min. This relatively high gas flow was necessary to reach the desired humidity in the chamber and to overcome the resistance to flow in the swivel apparatus and the tubing. Randomized monitoring of oxygen concentration was carried out by connecting the probe of an Oxycheck 2000 Critikon model to the outflow and sampling vent of the chamber (Fig. 2-6).

Humidity. The humidity in the chamber was adjusted by manipulating the gas flow rate and the temperature

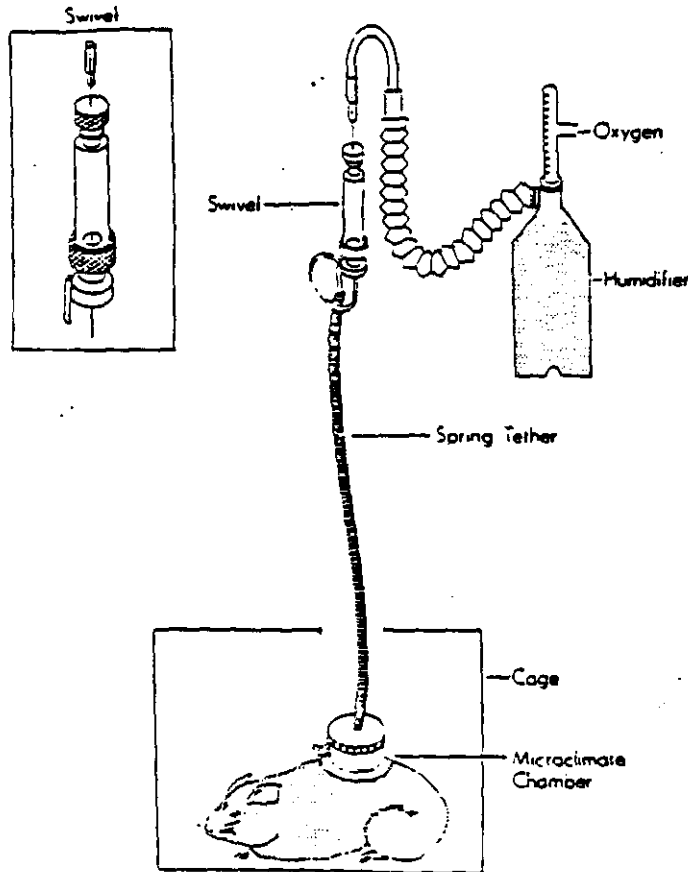


FIG. 3. The gas and humidity delivery system to the unrestrained guinea pig.

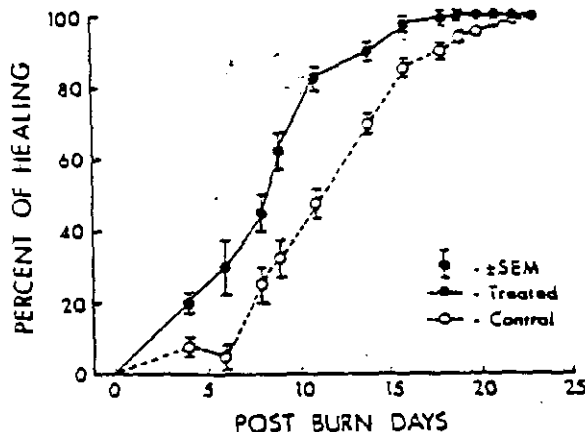


FIG. 4. Healing rate of the humidity- O_2 -treated and control wounds during the 25 days of the study.

of the humidifier. An indicator for measuring the humidity (Bachrach Instrument, Pittsburgh, PA, Pat. 2U66558) was placed at the outflow and sampling vent for randomized measurements of the relative humidity. In this study, the relative humidity in the microclimate chamber was kept at a constant level of 75% during the 25 days of observation.

Assessment of the Healing Rate. Removal of the screw mount top permitted tissue biopsies for histologic sections, biochemical analysis, and bacteriologic sampling of the burn wound. In this study, the healing rate

was evaluated by measuring the changes in size of the open wound area as a function of time, as described by the following formula, $RH = \frac{So - Sn}{S} \times 100$, where RH = wound healing rate expressed in per cent; So = the initial size of the wound immediately following the burn; and Sn = the wound size on a given day.

In order to precisely visualize the wound boundaries, the eschars from the four wounds were removed on the sixth postburn day. Based on our preliminary observations of the healing rate of this burn wound model, serial photographic tenfold magnifications of the wounds were made on postburn days 4, 6, 8, 9, 11, 14, 16, and 18 through 25, employing our previously described technique (16). The wound areas were measured by a polar planimeter.

Analysis of the Data. Analysis of the healing rate was carried out using a Digital Minicomputer (PDP 11/60, Digital Electronic Corp., Maynard, MA). Analysis of variance of a two-factor experiment with repeated measures on one factor was employed to evaluate the difference between the humidified- O_2 -treated wounds and the controls during the 25 days of this study. Student's paired *t*-test was employed to analyze the difference of healing rate in both groups on postburn day 11.

RESULTS

Computer-analyzed percentages of wound healing as a function of time were obtained for each guinea pig for the humidified- O_2 -treated wound and its control. The mean percentages of healing \pm standard error of the mean at postburn days (PBD) 4 through 23 are shown in Table I. The humidity- O_2 -treated wounds healed 99% of their initial size by PBD 19 and the controls required an additional 4 days to close. All wounds closed by PBD 23.

Figure 4 represents the S-shape of healing rate of both treated and control wounds during the 25 days of the study. The graph of the control wounds is shifted to the right by a lag time of 4 to 5 days. Three distinct healing rate periods were observed: 1) PBD 1-6, in which the healing rate was low; 2) PBD 6-16, represented by a rapid healing; and 3) PBD 16-23, in which both wounds approached completion of their healing and the rate slowed down.

Figure 5 represents the slope of the healing rate over the same intervals in both control and treated burn wounds. The O_2 -humidity-treated wounds showed a more rapid rate of healing immediately following the burn injury, and the wound closure rapidly increased after PBD 6 when the eschar was removed and healing attained a peak by days 8 to 9. Then healing slowed down until complete wound closure was achieved. In contrast, the control group healed relatively slowly following the burn, with a decrease in the rate at the time of removal of the eschar. The control wounds then moderately ac-

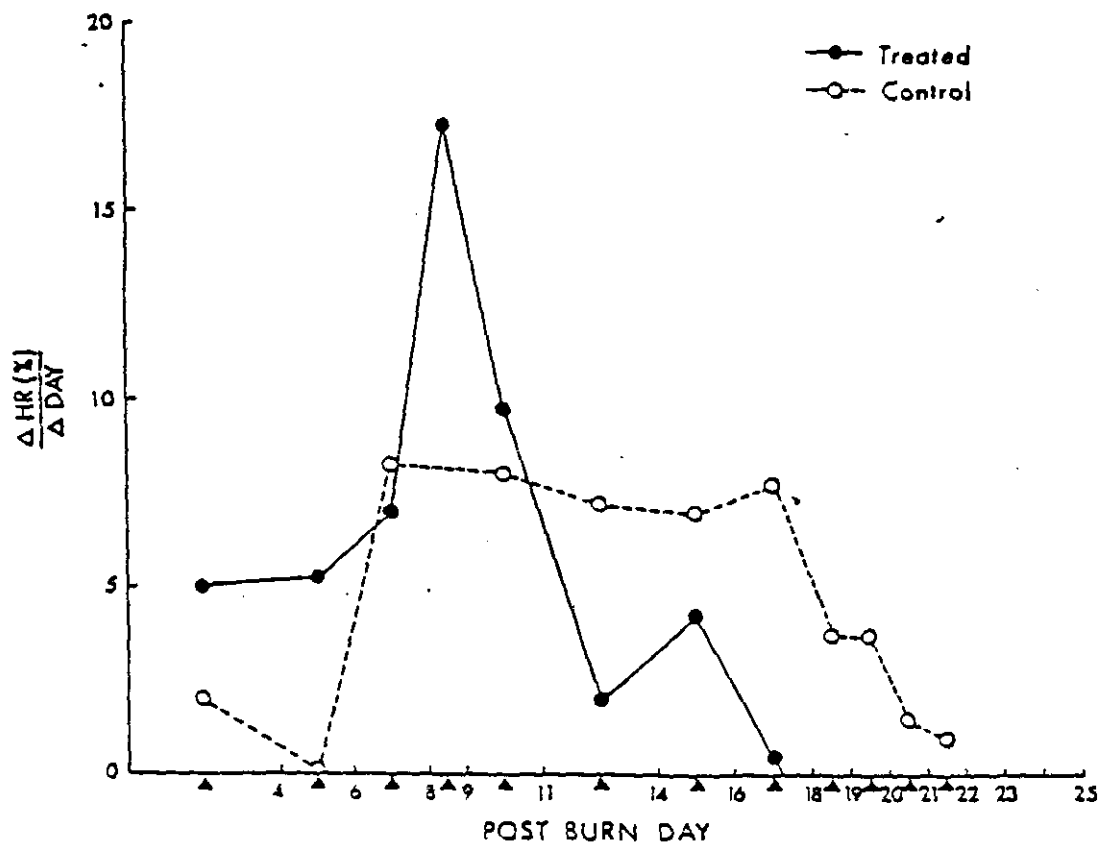


FIG. 5. Slope of healing rate. Note the peak on PBD 8 to 9 of the treated wounds and the almost constant rate of healing of the controls for 10 days following PBD 6.

celerated their healing and remained at about a constant rate during the next 10 days. Subsequently the healing rate of the control wounds also slowed when the wound was nearly completely closed. The uniformity of the wound injury was demonstrated in the other pair of dual control burn wounds, which healed at identical rates (Fig. 6).

Statistical Analysis. The difference in healing rate (assessed by wound size reduction) between the humidity-O₂-treated and control wounds was significant ($p < 0.005$). This improved healing remained significant even when the treated group of wounds was compared to the control of the successive experiment and vice versa (Table II). No significant difference was found comparing the controls in both experiments, nor did healing rate in the treated groups differ in the two tests. Student's paired *t*-test revealed significant differences ($p < 0.001$) between the healing rate of the treated and control wounds on PBD 11 (Fig. 7).

DISCUSSION

Experimental and clinical data suggest that burn tissue PO₂ is an excellent index of peripheral perfusion following burn injury. Remensnyder (21) showed a significant diminution of tissue oxygenation in the unstable zone of the burn edema in the first 45 minutes following burn in rat cremaster muscle. Steep gradients of O₂ tensions existed over very short distances, and the hypoxic areas

corresponded to the zones of vascular stagnation and thrombus formation. Hunt et al. (13) determined tissue PO₂ levels under unburned skin of human burned patients and suggested a microcirculatory impediment to O₂ diffusion into tissue in these patients. The burned patients exhibited normal arterial responses to O₂ breathing, but the tissue O₂ tensions responded more slowly and only marginally to an increment of arterial PO₂. In patients with greater than 30% burns, it took several weeks before a normal state of tissue oxygenation was re-established. In contrast, the same authors observed that a normal state of peripheral oxygenation was reached in injured but not burned patients as early as 4 to 5 days. Nieminen et al. (19) showed that thermal injury resulted in a rapid, progressive decrease of tissue PO₂ both in the burned areas and in the distant tissues, with the greatest decrease in the burn site. The minimum PO₂ levels were observed within 3 to 6 hours following the burn injury. The PCO₂ values increased markedly in both areas immediately after the trauma and gained their maximum within 1 to 3 hours postburn. These authors reported that the highest accumulation of CO₂ occurred at the burn site. Six hours following the burn injury, tissue CO₂ levels were found to be approaching normal. Another study demonstrated that PO₂ levels at the burn site were very low and did not exceed 10 mm Hg for several days (22). On the other hand, increased oxygen supplied through the circulation to open wounds accel-

TABLE I
Mean percentages of healing \pm SEM of the humidity-O₂-treated and control wounds during 23 days following the burn injury

Postburn Day	n	Treated	Control
4	8	20.53 \pm 2.00	8.05 \pm 1.23
6	8	31.25 \pm 6.15	7.08 \pm 2.20
8	8	45.69 \pm 5.00	24.32 \pm 3.58
9	8	63.4 \pm 3.90	32.74 \pm 5.30
11	8	82.09 \pm 3.52	47.68 \pm 3.39
14	8	88.78 \pm 2.40	68.96 \pm 2.30
16	8	98.30 \pm 1.46	84.82 \pm 1.38
18	8	99.01 \pm 0.99	92.62 \pm 2.00
19	8	100	96.53 \pm 2.00
20	8	100	97.94 \pm 1.60
21	8	100	98.89 \pm 1.10
22	8	100	99.66 \pm 0.34
23	8	100	100

TABLE II
Differences between groups of burn wounds*

Combinations of Groups	p
Ic and IIc vs. Ic and IIc	<0.005
Ic vs. IIc	NS
Ic vs. IIc	NS
Ic vs. IIc	<0.005
IIc vs. Ic	<0.005
Ic vs. Ic	<0.001
IIc vs. IIc	<0.001

* Analysis of the data shows a highly significant difference between the two groups of burn wounds: Ic, IIc—treated wounds in experiments I and II, respectively; Ic, IIc—control wounds in experiments I and II, respectively; NS—No significant difference.

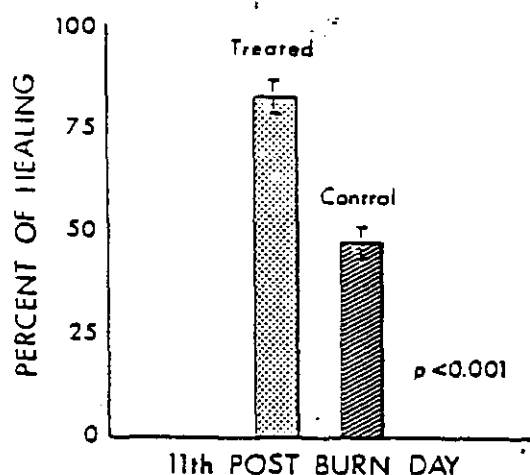


FIG. 7. Mean percentage of healing \pm SEM of the treated wounds on PBD 11 showing highly significant difference compared to that of controls.

erated the healing process, both in the granulation tissue, as well as the epidermis (22); however, in another report healing was faster only when inspired air consisted of 40% oxygen (18). Healing was delayed when inspired O₂ was at 100% (22). On the contrary, epithelial repair rates were improved when direct access of pure oxygen was allowed to the healing surface of the wound (8). This

discrepancy has been attributed to the fact that most studies have been carried out by modifying the environment of the whole experimental animal, and the animal was forced to breathe a high PO₂ atmosphere, resulting in a marked vasoconstriction of the small vessels in the skin due to increased secretion of epinephrine (2, 14, 23) and due to the stimulation of the sympathetic autonomic system (3, 7, 10, 12). Second, the oxygen supply to the wound might further be decreased due to pulmonary damage (4-6, 20), which leads to eventual lowering of the PO₂. Other studies demonstrated that when pure oxygen was directed to wounds covered with dressings permeable to O₂, such as Teflon or polyethylene, the epidermal PO₂ increased instantly to 685 and 628 mm Hg, respectively (22). The present controlled study showed that deep burn wounds, subjected topically to a continuous flow of humidified oxygen, without modifying the general environment of the animal, healed completely by PBD 19, and the control wounds required 4 to 5 additional days to heal. Examination of the slope of healing rate shows that the humidity-O₂-treated wounds had a healing rate higher than the control through the first 6 days following the burn injury. Evidently the eschar was not a complete barrier to the oxygen treatment. After removal of the eschar on PBD 6, both the control and treated wounds exhibited an increase of the healing rate; however, the humidified-O₂-treated wounds demonstrated a conspicuous peak on PBD 8 and 9. On the other hand, the control wounds maintained almost a constant rate of healing for the 10 days following the removal of the eschar. This trend could be attributed to the penetration of oxygen through the wound surface in the presence of a high level of humidity in the treated wounds, and, to a lesser extent, in the control wounds exposed to normal atmospheric oxygen concentration and a relatively low humidity. Silver (22) postulated that the energy requirements of the epidermal cells during migration under the eschar or a scab derive mainly from glycolytic activity. This is felt mainly due to the poor access of oxygen to the epidermal cells. In the present study, however, because of the higher availability of oxygen to the treated wounds, the aerobic metabolism of the epidermal cells may have been increased, resulting in a more rapid rate of healing. Our observations confirm those of Winter (24), who stated that oxygen supply was a major factor in determining the rates of both mitotic activity and epithelial migration. Moreover, the additional effect of high levels of humidity, which prevented desiccation of the tissue in the treated wounds, may have further enhanced the rate of epithelization. This result is in accordance with the observation of others (25, 26) who reported that vapors and humidity were essential for mitosis and migration of the epithelial cells, as well as the viability of the deeper tissues.

In view of increased healing rates in both O₂-humidity-treated and control burn wounds after removal of the eschar proved to be a factor in wound closure. We assume

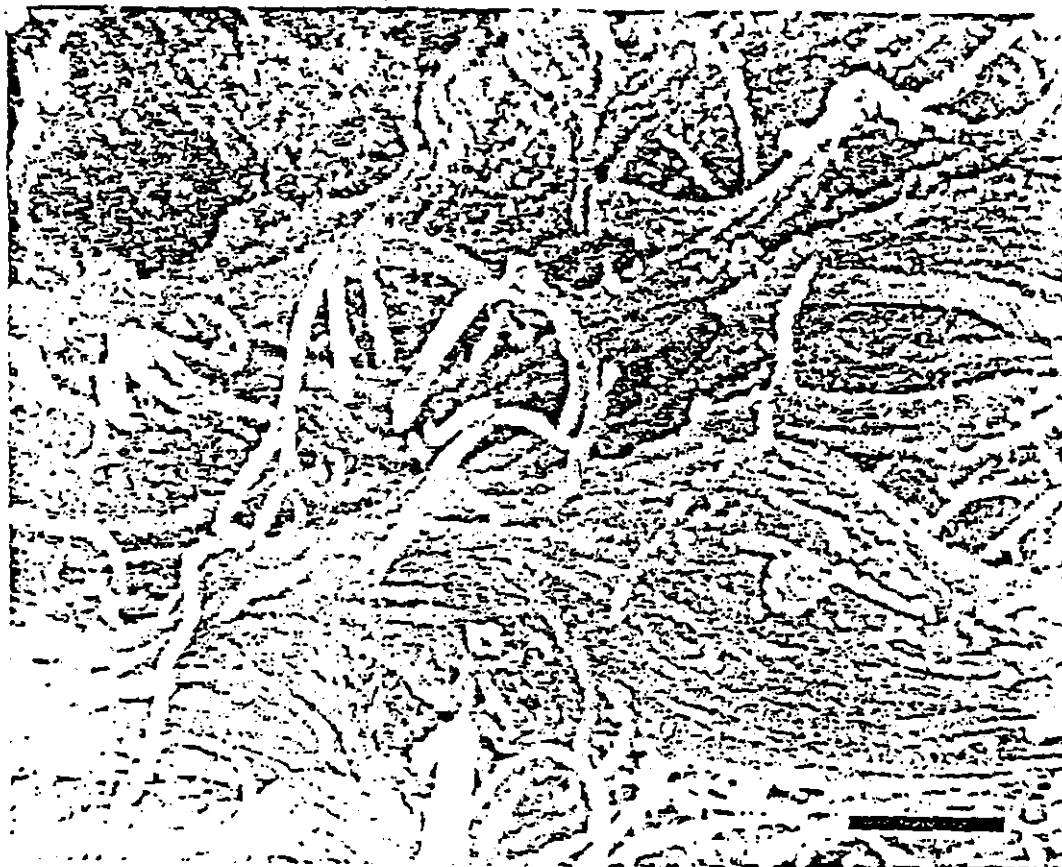


FIG. 8. Scanning electron micrograph of dermis from a humidified-oxygen-treated burn wound on PBD 25. Collagen is dense with many bundles of fibers interwoven to form a meshwork. Bar represents 1 micron (gold/palladium alloy).

that the eschar partially prevented both oxygen access to the wound and mechanical migration of the epidermal cells. In the present study, it was difficult to separately assess the contraction rate from that of epithelization, since the model consisted of small wounds 1.2-cm in diameter.

The contraction process of open wounds depends upon a variety of factors, such as location of the wound, the site and mobility of the skin, and underlying tissues of the particular animal. In the present study, both the humidity- O_2 -treated and control wounds were identically located and bore the same mechanical device, which to some extent could prevent mobility of skin and underlying tissues. However, this effect may have been abolished by attaching the flange of the microclimate chamber at a distance of 7 to 8 mm from the boundaries of the wound. Moreover, by attaching a chamber to the control wounds, identical mechanical conditions were created as in the O_2 -treated wounds.

Contraction has generally been attributed to the myofibroblasts present in the granulation tissue, which lead to a centripetal mechanical pull of the granulation mass (9). Grillo et al. (11) reported that square open wounds in guinea pigs, measuring 1.8 to 2.0 cm, were reduced in area by 30% on the fifth day; by 55% on the eighth day; and by 70% on the tenth day. Completion of contraction was achieved by postinjury day 15. In the present study,

the areas of the O_2 -treated burn wounds, as per cent \pm SE on PBD 6, 8, 11, and 16, were 31.25 ± 6.15 , 45.69 ± 5.0 , 82.09 ± 3.52 , and 98.3 ± 1.46 , respectively, and those of the control wounds were 7.08 ± 2.2 , 24.32 ± 3.58 , 47.63 ± 3.39 , and 84.82 ± 1.38 , respectively. The difference in contraction between the control wounds in this study and that of Grillo's report with open surgical wounds resides most probably in the nature of the injury as well as the different sizes and the mechanical and microclimate conditions to which they were subjected. However, the slope of healing rate of the control burn wounds is similar to that of open skin wounds in rats, as demonstrated by Abercrombie et al. (1).

Another aspect in the healing process of this burn wound microclimate is the formation of collagen fibers. We have observed (17) that topical application of 96% O_2 and 75% relative humidity was followed by markedly promoted collagen maturation in experimental deep burns. Scanning electron micrographs of the oxygen-humidity-treated wounds on PBD 25 showed more collagen fibers organized in bundles having a distinct orientation (Fig. 8). In contrast, the control burn wounds were found to be in an earlier stage of collagen maturation as demonstrated by relatively fewer collagen fibers embedded in a rich ground substance (Fig. 9). Moreover, the individual fiber in the treated wounds was 1,300 to 1,500 Å in diameter, while the control wounds consisted

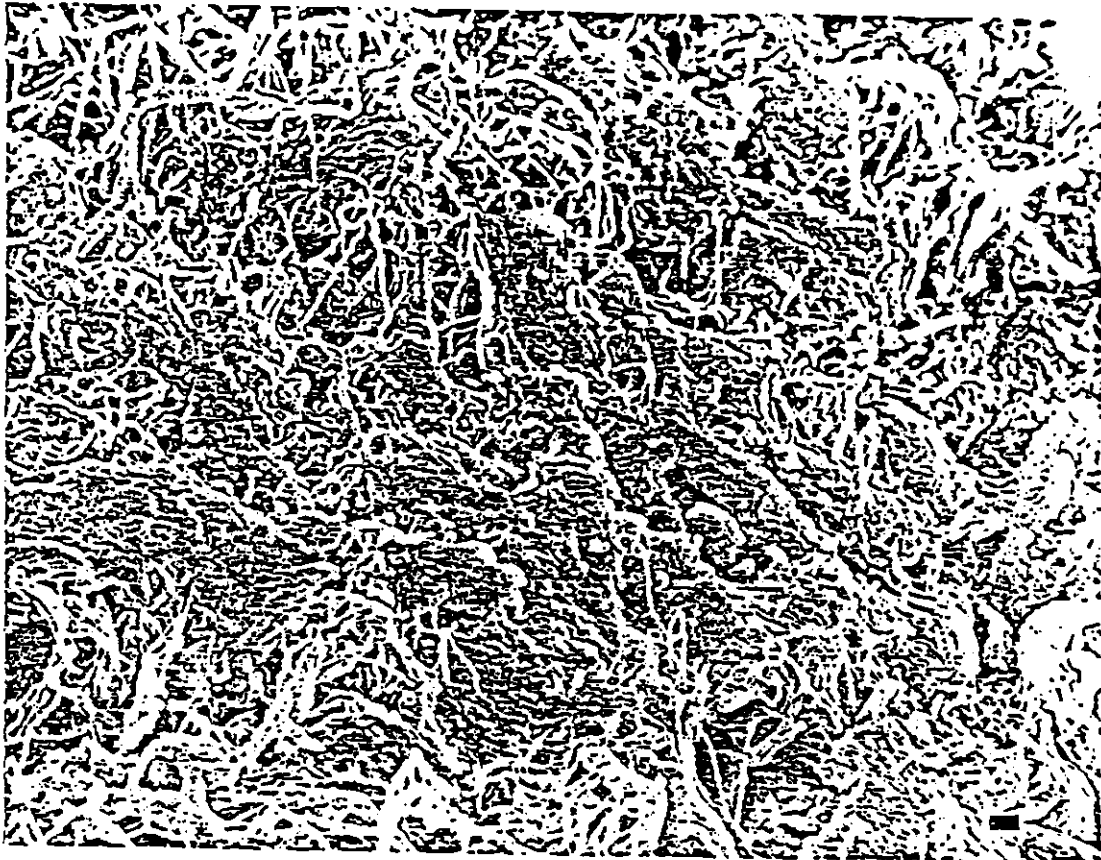


FIG. 9. Scanning electron micrograph of dermis from a control burn wound on PBD 25. Individual collagen fibers are embedded in an abundant amorphous matrix. Bar represents 1 micron (gold/palladium alloy).

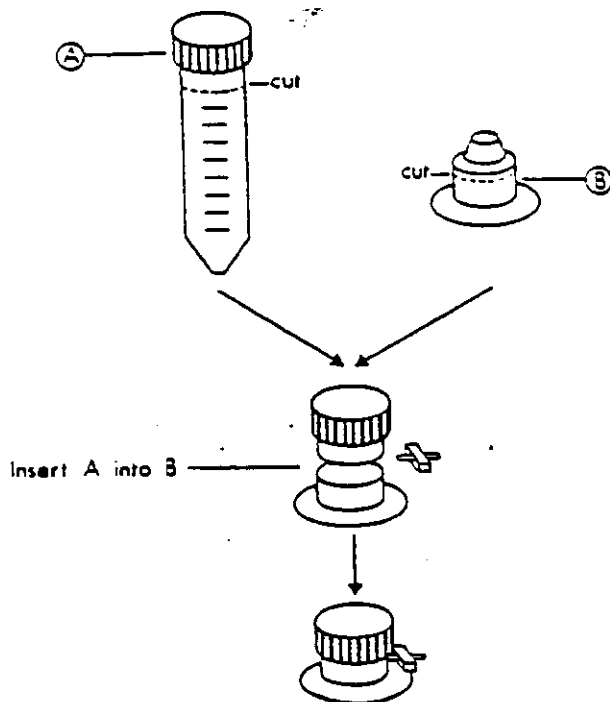


FIG. 10. Assemblage of the microclimate chamber.

of individual fibers of 700 to 800 Å in diameter, haphazardly oriented, with a poor tendency to form bundles (17).

Topical treatment of deep burns with 96% O₂ and 75% relative humidity promoted healing in this study. We conclude that this mainly affected the contraction, and, to a lesser extent, the epithelization process. However, further studies are necessary to more clearly define the role of topical oxygen in the wound healing process of experimental burns.

Acknowledgments

The authors are indebted to Ilan Amir, D.Sc., from Technion, Haifa, Israel, who originated the consensus; and to Ms. Kathryn A. Kenrich for the illustrations.

Addendum

Assembling the microclimate chamber is carried out from standard laboratory equipment at a negligible cost. Gluing parts A (1050 centrifuge tube, Denville Scientific, Denville, NJ) and B (microfilter cover, Gelman, Ann Arbor, MI, Mod. 4192) and the outflow vent (18-gauge polyethylene tubing, 1 cm long) is accomplished by a cyanomethacrylate glue (Fig. 10).

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DISCUSSION

DR. THOMAS K. HUNT (University of California, San Francisco, 94143): Doctor Kaufman and his associates have presented a new means of influencing the environment of healing burn wounds, and have shown that increasing humidity and oxygen leads to enhanced healing. The design of the study is tight, with controls in the same animals—a system of strictly local therapy. However, I have some questions about the suitability of the method and completeness of the design of the study.

In finding that humidity enhances epithelial repair, the authors have joined Winter, Maiöach, and at least a dozen others in showing that humidity effects epithelialization. In showing that oxygen affects epithelialization, they joined Otkina, Winter, Niinikoski, myself and Pai, Sir Peter Medawar, and at least six others that I know of who have shown that effect independently.

I say this not to imply that their report is not original, because it is. In fact, this is the first study on the healing of burn wounds that I am aware of done by controlling the local environment, and their results clearly imply that means of significantly enhancing or effecting repair in burn wounds are or will soon become available. Clearly, more investigation is warranted.

I am enthusiastic about the study, but I interpret some parts of the data in a slightly different manner. First, I suspect that the use of the microclimate chamber has not only affected the pattern of healing but probably has effected the death of some tissue, because some treated wounds were already 'healed' at 4 days, well before significant repair could be started. I therefore tend to suspect that the humidity and oxygen has perhaps affected the severity of the burn, since it is applied immediately after the injury. That, in itself, may be an important finding.

Second, I believe the data on fibroplasia and collagen synthesis are soft. It is difficult to get quantitative data on biopsy models in wounds. I am intrigued by the difference in the appearance of the fibroblasts, especially their endoplasmic reticulum. However, the amount of collagen produced is literally impossible to judge from a biopsy model and the authors have added no new wrinkles.

I would like to know how you assured yourself that the sampling for the collagen studies was uniform in all animals.

In your data you mentioned contraction, and you talked about the total unhealed area of the wounds, which comprise the standard basic data. The area of epithelialization is also important and can be calculated by the same means that you used. Is it possible to tell us about the area of epithelialization and how rapidly it increased?

I wonder how you assured yourself of the uniformity of the burn wounds, and if you used a blind evaluation. In other words, you did not mention to us whether the pictures you took and measured were evaluated blindly. If I missed it I apologize.

As a last comment, I would like to add that many control preparations need to be studied before the distinction between the contributions of humidity and oxygen can be made. Perhaps either one could be zero, but prior experience indicates that both will be operative. The data here are presented early in what, I hope, will be a long series of similar studies.

DR. BASIL A. PRUITT, JR. (U.S. Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, TX 78234): I would like to question the adequacy of control groups. It seems to me that since you didn't have a non-flowing chamber that was capped, the effects of oxygen level and humidity are hopelessly confounded. That is, there are no study groups in which the wounds were exposed to ambient oxygen/high humidity or high oxygen/ambient humidity. It would also be helpful to know whether the chamber influenced the bacte-

rial density of the wounds, since we know that infection can cause progression of wound depth and adversely affect healing rate.

DR. ROBERT COLEMAN (315 East Grant Avenue, Roselle, NJ 07204): You just asked one of the questions I was going to ask about the incidence of infection. I have a further question.

In your paper you mentioned that temperature in both chambers was at 24°C. How did you determine if the temperature at the actual burn site was 24°, or was it significantly less? I am thinking somewhat of a cooling effect which is going to promote microvascular patency.

DR. GLENN D. WARDEN (University of Utah Medical Center, Salt Lake City, 84132): I would like to reiterate Doctor Pruitt's questions as to which factors you are really evaluating. Are you looking at humidity or oxygen as promoting a better healing environment? The improved healing may not be due to humidity or oxygen, but rather to keeping the wound moist. It is well known to burn surgeons that after eschar separation the wound must be kept in a moist environment or it will dry out and there will be decreased healing. Thus are you just keeping the wound moist in an optimum environment rather than improving healing due to humidity or oxygen?

DR. PAUL R. SCHLOERS (Department of Surgery, University of Rochester Medical Center, Rochester, NY 14642): This is an intriguing study, and would logically lead to the application of applying oxygen to burn wounds.

Gas is exchanged very rapidly across the burn wound. Carbon dioxide transfers may be high enough, as we showed many years ago, to produce respiratory alkalosis. This is more apt to occur in children with a high ratio of body surface area to weight, but it can occur in adults as well, as was presented at the Surgical Forum many years ago. I would encourage the addition of carbon dioxide to the gas mixture.

DR. THEODOR KAUFMAN (closing): I would like to express my thanks to the discussers.

Regarding the effect of CO₂ and so on, our basic idea is to start and look at how the topical effect of each gas composition will affect the healing process of the burn. Therefore, the effect of topical CO₂ would be one for future investigations.

Regarding humidity and oxygen, I agree with Doctor Warden that in this study we managed to introduce two factors at a time. However, when we designed the study we thought that introducing O₂ only on the surface might inflict some damage to the healing rate of the burn because of the desiccation effect of blowing the gas. Therefore our next study, which we are running now, looking at one factor at a time, means blowing only 100% nitrogen, maintaining the same relative humidity on the burn wound.

Regarding the question of temperature, the temperature was monitored only in the chamber and over the near area of the burn wound, but not in the burn wound itself.

We did not manage to look at the rate of infection. This is scheduled for investigation.

Regarding the control model, I agree with you, Doctor Pruitt, that the control model in this study is not a proper one, since we did not manage to have a blow of gas over the contralateral control wound. Now we are using nitrogen and 75% relative humidity in order to evaluate one factor at a time. Having these

data in our hands, we might further continue and look at how each gas composition will affect wound healing.

Regarding Doctor Hunt's questions, to evaluate the difference of the collagen structure, ten equal fields were randomly examined in each humidity-oxygen treated and control wound, respectively, for each animal. The following parameters were considered: ratio of collagen/amorphous matrix, distinction of each fiber, diameter of each individual fiber, and the tendency to form bundles and the orientation of the fibers. To evaluate the diameter of each individual fiber, the randomized measurements of 20 collagen fibers in each field were carried out.

Moreover, looking at the fibroblast structure, we looked at 15 fibroblasts and examined them randomly in each humidity-O₂ and control wound, respectively, for each guinea pig. To evaluate the effects of the topical microclimate factors on the ultrastructure of these cells, quantitative assessment of the rough endoplasmic reticulum, the abundance of ribosomes, and the width of the cisternae cavities were carried out for each cell. Student's *t*-test was employed to analyze the difference at 25 days in collagen fiber diameter between the humidified-O₂-treated and control wounds, as well as the test of significance of difference between proportions for the morphology of the fibroblasts.

In this study, our interest was focused on the morphology of collagen as well as that of the fibroblasts directly related to collagen synthesis. Sections from the deep dermis of the humidified-oxygen-treated burn wounds exhibited on the 25th postburn day an outstanding abundance of distinct collagen fibers with only a little (about 5%) amorphous matrix. The mean diameter of the fibers \pm standard error of the mean was $1.360 \pm 95 \text{ \AA}$. The fibers had already assembled to form bundles over 80% of the whole wound, and only in 20% of the areas were the distinct collagen fibers oriented haphazardly. Moreover, orientation of the bundles, as well as the single fibers towards one direction, was already established.

On the contrary, in the control burn wounds, the amorphous matrix was the dominant component, occupying about 90% of the areas, and abundantly coated the relatively few collagen fibers. Moreover, the individual fibrils showed a haphazard orientation, and their assemblage to form larger fibers was rather poor. The mean diameter of the fibers \pm standard error of the mean was $700 \pm 70 \text{ \AA}$.

In 69% of the fibroblasts in the oxygen-treated wounds, the TEM micrographs showed the cisternae of the rough endoplasmic reticulum to be highly aggregated, forming a lamellar system of relatively large cavities, uniformly spaced and roughly parallel to one another. Moreover, the outer surface of the membranes of the reticulum bore a large number of adherent ribosomes. On the contrary only 30.5% of the fibroblasts in the control burn wounds exhibited a similar pattern, while the rest consisted of a rather loose aggregation of the rough endoplasmic reticulum, and the cisternae appeared to be flatter and narrower.

The difference between the diameter of the collagen fibers in the oxygen-treated wounds and the controls was highly significant ($p < 0.001$), as well as the difference in the morphology of the fibroblasts ($Z > 1.96$).

Thank you.