Characterization of Electric-Pulse-Induced Permeabilization of Porcine Skin Using Surface Electrodes

Stephen A. Gallo,* Allan R. Oseroff,# Patricia G. Johnson,* and Sek Wen Hui*
*Biophysics Department and #Dermatology Department, Roswell Park Cancer Institute, Buffalo, New York 14263 USA

ABSTRACT We measured the transient and long-term changes of permeability of full-thickness porcine skin after the application of a single or a train of electric pulses, as the basis for optimization of the electrical parameters for enhancing transdermal drug or gene delivery by electroporation. Two electrodes were attached to the stratum corneum of excised skin for transdermal electric pulse delivery and impedance measurement. Both transient and long-term permeabilization were found to be dependent on the electrical exposure dose, i.e., the product of pulse voltage and cumulative pulsing (exposure) time. Skin resistance dropped to about 20% of its prepulsing value when pulsed beyond a critical dosage of 0.4 V-s (with 20–40 V across each skin path), but recovered rapidly within seconds after the pulse. Long-term permeabilization of the skin required repeated pulsing with a minimum potential of 160 V (80 V across each skin path). The maximum long-term resistance drop, to 35% of the initial value, required a dose greater than 200 V-s, recovering slowly and seldom completely in tens of minutes to hours. The decrease and recovery of the resistance were dependent on the frequency and pulse length only for low-dose electrical exposure.

INTRODUCTION

Transdermal drug delivery has potential advantages over other delivery methods: convenience, noninvasiveness, and avoidance of degradation in the gastrointestinal tract or liver. The main barrier to the success of transdermal delivery has been the impermeability of the stratum corneum (s.c.), the outermost layer of the skin (Bronaugh and Maibach, 1989; Hadgraft and Guy, 1989). The stratum corneum consists of densely packed layers of flattened, dead, keratinized cells (Hadgraft and Guy, 1989; Madison et al., 1987) surrounded by lipid lamellae consisting primarily of ceramides, cholesterol, and fatty acids (Schurer and Elias, 1992; Hadgraft and Guy, 1989). The stratum corneum serves as a barrier to the outside world and prevents evaporation of water from underlying tissues. If the stratum corneum is disrupted, the barrier to molecular transport is greatly reduced. The skin can be electrically modeled as a resistor and a capacitor in parallel, and much of the skin's resistance is found to be in the stratum corneum (Yamamoto, 1976). We can monitor the skin's resistance experimentally and estimate, under different conditions, the permeability of the stratum corneum.

Disruption of the s.c. can be achieved by electroporation. Electroporation was first applied to permeate cell membranes for cell loading and transfection (Chang et al., 1992, and references therein). Short (microseconds to milliseconds) pulses of sufficiently high voltages are used to permeabilize the cell membrane, so that molecules may pass through and the membrane may reseal after the pulses. The thin layer of highly resistive stratum corneum over less resistive dermis and underlying tissue is electrically analogous to the highly resistive plasma membrane over conductive cytoplasm on the cellular scale. If an electric field is imposed across the skin, most of the potential drop is developed across the resistive stratum corneum, where a breakdown is likely to occur when the imposed electric field rises beyond a certain critical strength. It has been shown that cell membranes may be reversibly broken down by 30-μs pulses at about 0.8 V of imposed transmembrane potential (Stenger et al., 1991). The membranes of the cells in a centrifuged pellet also break down, at about 1 V of imposed transmembrane potential (Abidor et al., 1994b). Because the stratum corneum is equivalent to about 100 membrane layers (Chizmadzhev et al., 1995), we may expect that electroporation may occur at an imposed potential difference of $\sim$100 V.

Electroporation of the stratum corneum is different from iontophoresis, which is the movement of ions across the skin driven by a low electric field, primarily through hair follicles and sweat glands. Electroporation, on the other hand, can be described as the creation of aqueous pores in lipid bilayers by an electric pulse. Ions are suggested to move through the gaps or pores of the keratinocyte layer of the stratum corneum (Chizmadzhev et al., 1995).

The feasibility of transporting molecules through the stratum corneum has been explored by several groups (Prausnitz et al., 1993; Vanbever et al., 1994). They have reported extensive characterization of electric properties of the skin with respect to electroporation, using excised stratum corneum or skin mounted in a side-by-side flow-through chamber. Based on the transport data of Prausnitz et al. (1993), Chizmadzev et al. (1995) evaluated two alternative models for pulse-driven permeabilization of the stratum corneum. Recently, Pliquett et al. (1995) found conditions for permeabilization and recovery of the stratum corneum

Received for publication 26 April 1996 and in final form 24 March 1997.
Address reprint requests to Dr. Sek Wen Hui, Department of Biophysics, Roswell Park Cancer Institute, Elm & Carlton Sts., Buffalo, NY 14263-0001. Tel.: 716-845-8595; Fax: 716-845-8683; E-mail: roswhui@ubvms.cc.buffalo.edu.

0006-3495/97/06/2805/07 $2.00

by single and multiple pulses, using passive impedance measurement. Factors influencing the transdermal transport of metoprolol by electroporation have recently been delineated by Vanbever and Preat (1995). These studies on excised stratum corneum or skin mounted in dual-compartment chambers, together with the rich data on the mechanism and models for iontophoresis (Oh et al., 1993), provide the foundation for further studies of electroporation of the stratum corneum as a means of enhancing transdermal molecular transport.

Although studies have been conducted on excised skin, epidermis, and stratum corneum, there has been no report on electroporation experiments with full-thickness human skin, which we believe could provide relevant information concerning the use of our findings toward clinical applications. Pig skin, a good model for human skin with respect to the size and structure of the stratum corneum (Ferry et al., 1995), can be used at full thickness to simulate human, in vivo transdermal delivery. We examine the effect of electric pulses on the resistance of pig skin, to optimize electrical parameters for possible use in enhanced transdermal drug or gene delivery in humans.

MATERIALS AND METHODS

Fresh porcine skin was obtained from a local abattoir and immediately placed on ice. Full-thickness samples of the skin, including a fat layer, were used within 24–48 h after arrival. The skin was cut into a 200-cm^2 section, the surface was washed with deionized water and soap, and then much of the hair was cut off with scissors. Stainless steel electrodes or Ag/AgCl electrodes (Red Dot; 3M Health Care, St. Paul, MN) were used with a conductive paste (Redux Paste; Hewlett Packard, Waltham, MA) or phosphate-buffered saline solution (5 mM NaP_i with 0.9% NaCl). To simulate the conditions of in vivo application, we used an electrode configuration similar to that used in iontophoresis as applied to human subjects, namely, two electrodes attached to the same side of the skin surface, separated by 7.4 cm. A slight pressure was applied evenly to the electrodes to ensure a good contact. The skin was wiped periodically with a paper towel to reduce surface current between electrodes.

The skin with electrodes attached was placed on a sheet of Saran Wrap over ice. A pulse generator (model 345; Velonex, Santa Clara, CA) was used to produce single or multiple unipolar square pulses at 1–10-ms widths and 2–200 Hz, with negative voltages ranging from 5 to 500 V. The electrical exposure dose was calculated as volts × frequency × pulse width × duration of treatment (V-s). Initial and final resistances were measured using a continuous low-voltage (300 mV), 1000-Hz bipolar square wave generated by a Dynascan frequency generator (3300 pulse generator; Dynascan, Chicago, IL). A load resistor (4–10 kΩ) was placed in series with the skin, so that the voltage drop could be measured across the whole circuit (V_o) or just across the skin (V_s) by a recording digital oscilloscope (model 99 Scopemeter, Series II, Fluke Corp. Everett, WA). The schematic is shown in Fig. 1. With this configuration, two stratum corneum layers were exposed to the pulse voltage. Data were used only if the initial skin resistance was equal to or greater than 5 kΩ.

Two types of experiments were performed:

Single-pulse experiment

The voltages V_o and V_s were measured during the application of a single pulse to a skin area that had not been pulsed previously. The resistance of the skin R_s may be approximated from the formula below (Abidor et al., 1994a), when the capacitive load of the stratum corneum is small compared to the resistive load:

$$R_s = (V_s R_L)/(V_o - V_s)$$

where R_L is the load resistor in series with the skin. Because the electric relaxation time of the skin, manifested as the charging time of the skin capacitor given by the oscilloscope trace, was usually shorter than the applied pulse width, the R_s value could be approximated by using V_s at the end of the pulse when the membrane capacitor was fully charged. If the resistance (R_o) is measured before the pulse with the bipolar square wave, the value of R_s/R_o during the pulse then represents the transient resistance drop. A drop in skin resistance was defined as transient if the resistance after the pulse (R_p) recovered to 90% or more of the prepulse resistance R_o.

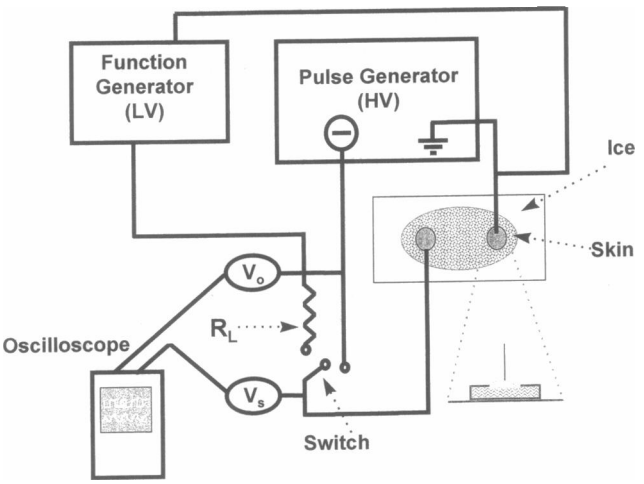

FIGURE 1 Schematic diagram of electrical setup. The low-voltage function generator was used to measure the pre/post-pulse resistance from oscilloscope measurements of V_o and V_s. The high-voltage pulse generator was used to apply the permeating pulses and to measure the resistance during the pulse.

Multiple-pulse experiment

A train of pulses of given amplitude and width were applied to the skin at a given frequency. After the pulse train application, the resistance R_p was measured with the square wave. The recovery process was monitored every 10 min for 30 min. The resistance drop was defined as "long term" when it gradually regained some of the initial resistance only after a period of tens of minutes.

RESULTS

All pulse current goes through the stratum corneum to the more conductive epidermal and dermal layers and underlying tissues, and emerges again through the stratum corneum at the second electrode. The pulse voltage traverses two strata cornea plus their more conductive substructures. This configuration involves multiple factors, such as the stratum corneum/epidermis/dermis/fatty tissue interfacial impedances, as well as the internal resistance of these layers. We have measured the impedance of these layers and found that their total contributions are less than 20% of that of the s.c. (data not shown). Therefore, most of the voltage develops across the stratum corneum.

Single pulses were applied to the skin to measure transient permeabilization, in which the resistance recovers within seconds. Pulses of 1, 4, and 10 ms and 10–300 V were used. The resistance during the pulse, R_s, normalized by the prepulse resistance R_o, was plotted against the exposure dose VT (Fig. 2). VT is the initial voltage across the skin multiplied by the exposure time of the skin to the pulse. The quantity can be considered an electrical exposure dosage. In the single-pulse experiment, the exposure time is the pulse width.

The normalized resistance decreased rapidly with increasing VT and leveled out at a critical VT value of about 0.4 V-s. At the maximum permeabilization, the resistance is ~10–20% of the initial (prepulse) resistance. The breakdown was found to be very sensitive to initial resistance: large resistance drops correlated well with larger initial resistances (data not shown). To gain more insight into the mechanism of transient permeabilization, normalized resistance-versus-voltage plots were made separately for each of the three pulse lengths applied (Fig. 3). For each of the pulse widths, there is an initial steep decrease in resistance as a function of applied voltage. The plot suggests that the voltage at which the minimum resistance is reached is roughly inversely related to the pulse width.

Long-term resistance drop is achieved after prolonged multiple pulse application. There are two more experimental variables to be considered in the multiple pulse experiments, namely, the frequency f and the total electrode contact time t, in addition to the voltage V and the pulse width τ used in the transient permeabilization experiments. In the multiple-pulse experiments, we have

$$\tau f t = T$$

where T represents the cumulative time during which the skin experiences a pulsed electric field. The influence of the independent parameters V, τ, f, and t on long-term permeabilization was investigated separately.

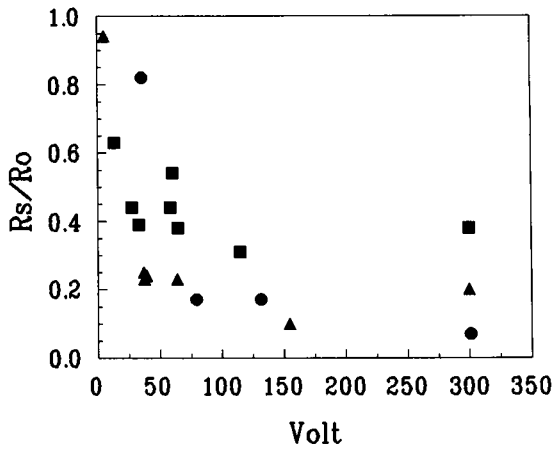

FIGURE 3 Normalized skin resistance versus voltage. Single pulses were applied, and resistances were measured during the pulse. Three plots represent the three pulse lengths used: ■, 1 ms; ●, 4 ms; ▲, 10 ms.

A plot of R_p/R_o versus voltage V for 1- and 10-ms pulse widths, at a common frequency of 10 Hz and an exposure dose VT = 100 V-s, is shown in Fig. 4. The trend of decreasing resistance with increasing voltage is observed for both pulse widths. There is only a slight displacement of the voltage dependence of long-term permeability between the curves for τ = 1 ms and τ = 10 ms, both of which reach their basin values at $V > 160$ V. The basin values of R_p/R_o, 0.4 and 0.22, respectively, for 1- and 10-ms pulses, differ, indicating that permeabilization by shorter pulses cannot be compensated by higher pulse voltages at this exposure dose (VT) range. At higher VT (>600 V-s) range, the responses to pulses of all widths equal to or greater than 1 ms are essentially the same (results not shown).

The dependence of long-term permeabilization on the frequency f of the applied pulse train was also studied. Fig.

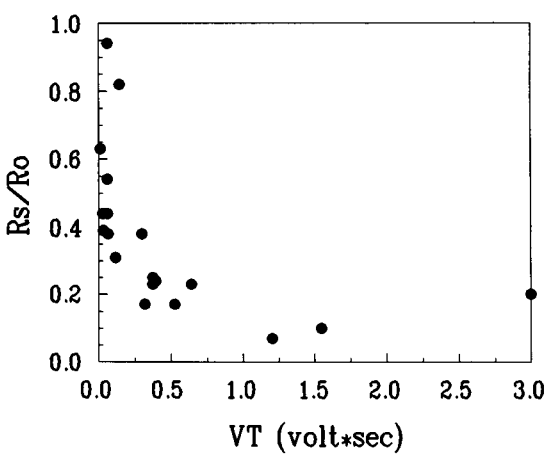

FIGURE 2 Normalized skin resistance versus VT (voltage × exposure time). Single pulses were applied, and resistances measured during the pulse were normalized by the prepulse value. All resistances after the pulse recovered to approximately the initial resistance value.

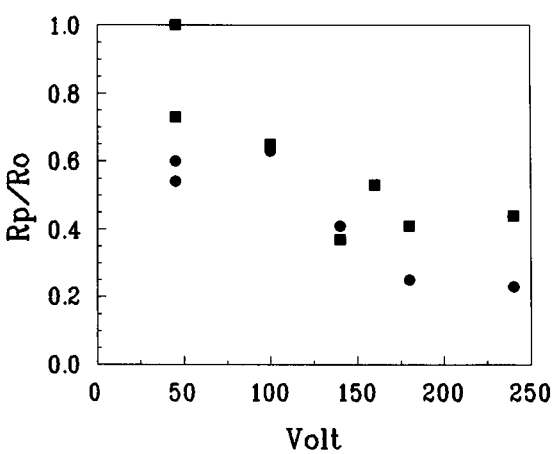

FIGURE 4 Normalized post-pulse skin resistance versus voltage after the application of trains of 1-ms (■) and 10-ms (●) pulses. The applied exposure dose was 100 V-s, and the frequency was 10 Hz, for both cases. Both voltage and contact time were varied to keep VT constant.

5 shows a slight frequency dependence of R_p/R_o at $f < 50$ Hz, for $VT = 100$ V-s. The frequency sensitivity is lost at $VT > 300$ V-s.

If multiple pulses in a train are required to cause long-term permeabilization, the cumulative effect of the pulses is implied. Because the transient permeation was found to be a monotonically decreasing function of $VT = V\tau$ (Fig. 2), and that molecular transport was found to be proportional to the cumulative electric exposure VT (Liang et al., 1988), we plotted the long-term resistance drop ($\%R_{drop}$) against VT as a trial case (Fig. 6). To reduce the small influence of τ and f at low VT range, we analyzed only those data points with $\tau = 1$ ms and $f = 2$ and 10 Hz at $VT < 300$ V-s. The observed R_p/R_o difference of ± 0.1 due to the frequency difference between 2 and 10 Hz is less than the variation of measurement from sample to sample (~0.1–0.2). There is a considerable amount of scatter in this graph, but an underlying trend was detected, similar to that seen in Fig. 2, in which a rapid decrease and then a leveling of normalized resistance as a function of VT were observed. However, superimposed on these data were points that did not follow the trend.

An attempt was made to isolate the parameter(s) contributing to VT that could account for two apparently different R_p/R_o response patterns. The monotonically decreasing data may be fitted with an exponential or a quadratic polynomial function. As an example, the % normalized resistance drop, $(R_o - R_p)/R_o$, was plotted against log(VT) in Fig. 7. Linear correlation coefficients were calculated for groups of data points above different voltage thresholds as a means of locating any threshold voltage below which data are poorly correlated (i.e., scattered). Fig. 8 shows a clearly defined threshold at 160 V. Below that value the correlation coefficient decreases from ~0.75 to a low of 0.4. Thus pulse trains of lower voltages (<160 V) apparently have VT-dependent characteristics different from those of higher

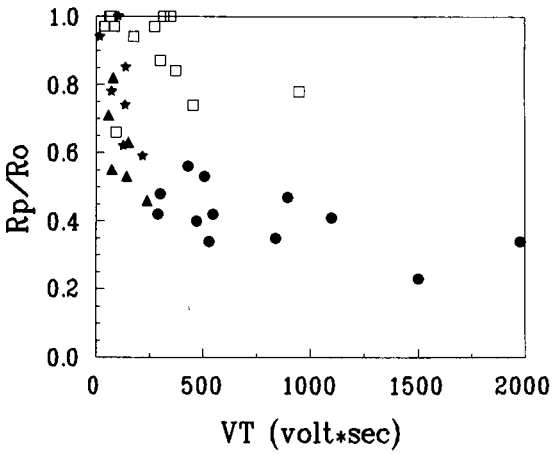

FIGURE 6 Normalized post-pulse skin resistance versus VT for all voltages. ●, ▲, ★, Applied voltages above 160 V; □, applied voltages below 160 V. Data below a VT of 300 V-s are represented according to frequency: ▲, 2 Hz; ★, 10 Hz; all with 1-ms pulse widths. ●, Data above 300 V-s.

voltages. The normalized post-pulse resistances from pulse trains of voltages higher than 160 V (Fig. 6, *closed symbols*) follow a trend similar to that shown in Fig. 2 for transient permeabilization, that is, an abrupt drop of resistance at lower VT, followed by a leveling off at $VT > 200$ V-s to ~50% of the initial resistance. Those data from pulse trains of voltages lower than 160 V do not show any apparent VT dependency, and most resistances remain more than 70% of the initial (Fig. 6, *open symbols*).

To determine whether the normalized post-pulse resistance is a simple function of exposure time alone, the data above 160 V were plotted against exposure time (Fig. 9). The relation bears a resemblance to the VT plot for voltage > 160 in Fig. 6, indicating that above the threshold voltage of 160, much of the VT dependency is due to exposure time dependency.

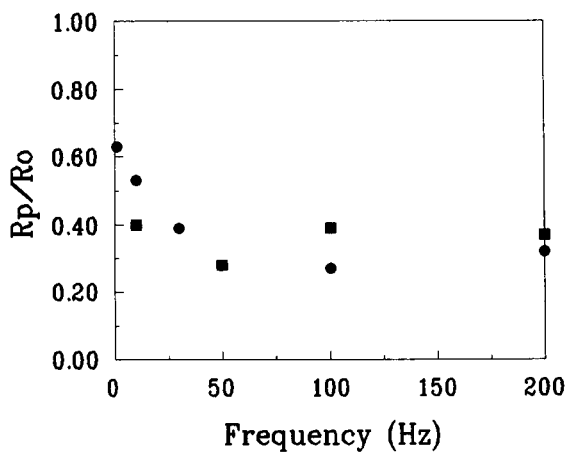

FIGURE 5 Normalized post-pulse skin resistance versus frequency of applied pulses. The VT is held constant, with 240 V, 1-ms width pulse. ●, VT at 100 V-s; ■, VT at 300 V-s. The contact time is varied with frequency to keep VT constant.

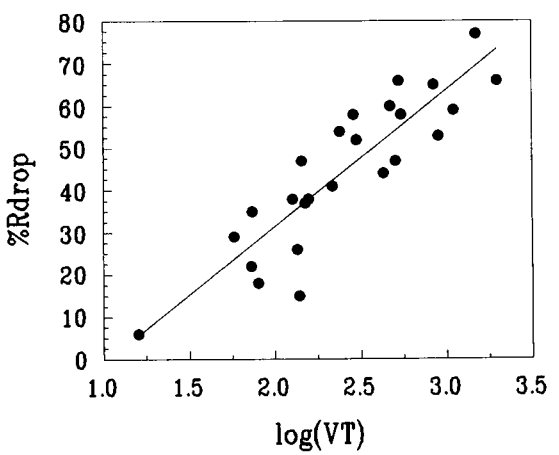

FIGURE 7 Normalized resistance drop versus log(VT) for multiple-pulse experiments. The percentage drop is defined as the fraction of resistance drop caused by pulse application (×100). Only data above 160 V are used. The correlation coefficient of linear fitting is 0.73.

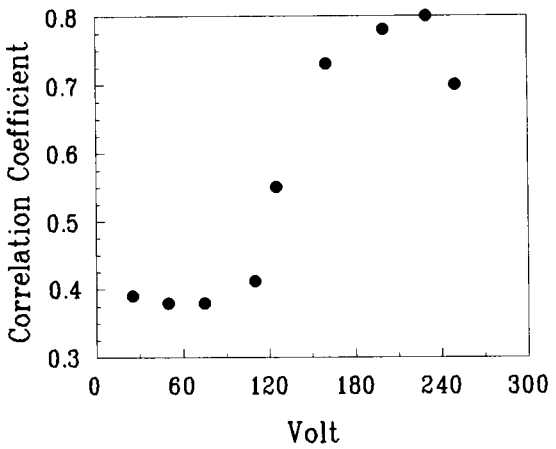

FIGURE 8 Correlation coefficient of log(*VT*) plots versus threshold voltage. Plots like those in Fig. 4 were contain only those points with voltages above a designated voltage threshold. The linear fitting correlation coefficient of these plots is plotted against threshold voltage, giving the voltage-dependent scatter.

DISCUSSION

Electric impedance measurements reflect the transport of small ions through the skin barrier, which to some degree approximates the transport of larger charged molecules (Prausnitz et al., 1993; Pliquett et al., 1995). Electric impedance measurements are easier to make and quantitate, and will be used as a guide to later investigation of optimizing electrically enhanced permeabilization of skin.

Fig. 3 suggests that the thresholds for maximum transient resistance drop occur at 40 V and 80 V, respectively, for a 10-ms and 4-ms pulse. For the more scattered 1-ms pulse, the approximate voltage at which the resistance reaches maximum permeabilization is 120 V. The actual potential across each stratum corneum is therefore 20 V and 40 V, respectively, for 10-ms and 4-ms pulses. If the stratum corneum is assumed to consist of some 100 lipid bilayers, the thresholds of 0.2 V and 0.4 V agree with the reported reversible bilayer breakdown voltages of 200–400 mV (Benz et al., 1979; Chernomordik et al., 1987).

More interestingly, the threshold voltage seems to decrease with increasing pulse length. A similar time-voltage relationship was observed in lipid bilayer breakdown (Chernomordik et al., 1987). The contribution of the time factor may be regarded as the time required to create electropores or to open up existing pathways. It implies that there may be a lag time related to the elasticity and plasticity of the stratum corneum (the time required to enlarge existing openings in the stratum corneum), or that the pore formation is an adiabatic process such that energy is accumulated in the stratum corneum to overcome the pore-forming energy barrier. In either case, the transient permeabilization of the skin during the pulse application does not seem to be a simple electrical breakdown event of the lipid multilayer capacitor in the stratum corneum. Not only the electrical relaxation (charging time, in microseconds), but the mechanical relaxation time (resilience of the stratum corneum multilayers, in milliseconds) is also involved.

Recovery from the transient permeabilization during a single pulse is very rapid. Usually the skin resistance regains more than 90% of its prepulse value within seconds, i.e., as fast as we can switch to the bipolar low-voltage square wave generator to measure the post-pulse impedance. At present, we have no means of measuring the recovery within 1 s of the termination of the pulse. This is different from the recovery profile of human stratum corneum reported by Pliquett et al. (1995), who found that the extent and time of recovery are pulse voltage dependent, lasting tens of seconds to hours, and plateauing at <50% recovery with pulse voltages greater than 130 V across one stratum corneum. This discrepancy may be due to the fact that, in their case, the stratum corneum is heat-stripped, hydrated, and mounted in a chamber, whereas in our setup, the stratum corneum is still attached to the epidermis and underlying tissue, and is relaxed.

For the multiple pulse experiments, recovery is delayed and often incomplete. This delayed recovery or long-term permeabilization requires a large number of pulses (at least several hundred) and is slightly pulse frequency dependent at low *VT* (Fig. 5). For pulses with an amplitude greater than 160 V, the extent of long-term permeabilization increases with *VT* (Fig. 6) in a manner similar to that of the transient, reversible permeabilization (Fig. 2). However, the threshold value of *VT* for maximum permeabilization is about 200 V-s, much greater than that for transient permeabilization by a single pulse (0.4 V-s). The nearly 3 orders of magnitude difference in threshold indicates that the mechanisms for these two processes are fundamentally different. In addition, although pulses of amplitude less than 160 V may cause some long-term permeabilization, the effect is likely to be a different phenomenon, as suggested by its poor correlation with *VT* (Fig. 6).

The high voltage permeabilization requirement of a minimum of 160 V (80 V for each stratum corneum) is close to

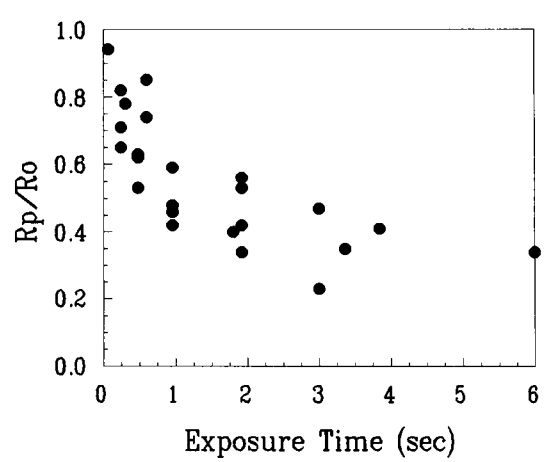

FIGURE 9 Normalized post-pulse resistance versus exposure time for data above 160 V.

the 75-V threshold for calcein transport (Prausnitz et al., 1993) and metoprolol transport (Vanbever and Preat, 1995) by multiple pulses. The agreement between our results on intact skin, with electrodes on the same side of the skin, and those using isolated stratum corneum (Prausnitz et al., 1993) and skin (Vanbever and Preat, 1995) mounted in vitro on perfusion chambers, argues for the validity of the assumed serial transdermal circuit of a large resistor for stratum corneum and small resistors for the dermis and underlying tissues.

The high-voltage, long-term permeabilization has been suggested to be associated with a structural alteration caused by repeated breakdown of the stratum corneum after prolonged pulsing. As reported by Pliquett et al. (1995), recovery of the stratum corneum immediately after each pulse is not complete, and subsequent pulses applied during this period may cause additional permeabilization, leading to cumulative effects. At our experimental frequencies of 1–200 Hz, which are much higher than that used by Pliquett et al. (1995), the cumulative effect would be expected to be much more pronounced. Although frequencies above 50 Hz induce greater permeabilization than those below, the difference in resistance drop at low *VT* between 1 and 50 Hz is only 0.3 (Fig. 5), and at high *VT* the difference is negligible. This small difference imposes no significant consequence in the general trend of resistance drop, even at the sensitive, low-*VT* range (Fig. 6). The saturating effect above 50 Hz implies that the skin may recover in 20 ms at low *VT*. The same holds true for pulse length dependence (Fig. 4). At low *VT*, longer pulse lengths have a greater effect than shorter ones, but the difference in resistance drop is only 0.2, and again diminishes at higher *VT*. In the low-*VT* range, the dependence of long-term permeabilization on both the frequency and pulse length may be related to the kinetics of recovery, as in the case of transient permeabilization. At higher exposure doses, the permeabilization is semipermanent and dependent on the extent of permeabilization, which is proportional to the cumulative exposure *VT*, independent of pulse width and frequency.

The type of structural alteration after long-term permeabilization is not known. The so-called long-term permeabilization undergoes a slow and incomplete recovery over hours in the excised porcine skin we used. The recovery process could be much faster in living skin. It has been speculated that semipermanent pores are created at the stratum corneum. These types of alterations would indeed lead to an increase in conductivity. Heating could play a role in structural alteration. In Fig. 4, larger pulse lengths and higher frequencies at low *VT* might deliver more heat against thermal diffusion, thereby raising the local temperature more effectively. However, the effect is small compared to the cumulative long-term permeabilization and structural alteration at higher *VT*. At this stage, we cannot be certain of the nature of alteration without additional structural data. We are currently conducting structural studies of pulsed skin areas.

There are several factors that must be taken into consideration in our setup. The electric impedance we measured consists of several elements in addition to that of the stratum corneum. These include the polarization at the surface of the metal/gel electrodes, the impedances at the electrode-skin interface, the stratum corneum-epidermis interface, the epidermis-dermis-fatty tissue interfaces, and the conductivities of these different tissues. The relative importance of these items varies from skin to skin, and from area to area. To model these elements in an equivalent circuit and to derive the values of the resistive and reactive elements in the model circuit from our limited data is not practical. Despite these variables, the relative resistance drop measurements derived from different skins, using different electrodes, over a period of time and under different electric parameters, still fall along a distinct pattern (Figs. 2 and 6). It suggests that the other factors may not be very significant.

The results of this study help to characterize the effects of electroporating human skin. Because of the realistic nature of the setup, these electrical characterizations can be used to augment human in vivo transdermal drug or gene delivery.

This work is supported by a grant from the Roswell Park Alliance Foundation and partially by grants P01 GM 30969 (to SWH) and 5P01 CA 55791 (to ARO) from the National Institutes of Health. SAG was supported in part by a training grant fellowship 5T32CA61800 from the National Cancer Institute.

REFERENCES

Abidor, I. G., L. H. Li, and S. W. Hui. 1994a. Studies of cell pellets. I. Electrical properties and porosity. *Biophys. J.* 67:418–426.

Abidor, I. G., L. H. Li, and S. W. Hui. 1994b. Studies of cell pellets. II. Osmotic properties, electroporation, and related phenomena: membrane interactions. *Biophys. J.* 67:427–435.

Benz, R., F. Beckers, and U. Zimmerman. 1979. Reversible electrical breakdown of lipid bilayer membranes: a charge-pulse relaxation study. *J. Membr. Biol.* 48:181–204.

Bronaugh, R. L., and H. I. Maibach. 1989. Percutaneous Absorption: Mechanisms, Methodology, Drug Delivery. Marcel Dekker, New York.

Chang, D. C., B. M. Chassy, J. A. Saunders, and A. E. Sowers, editors. 1992. Guide to Electroporation and Electrofusion. Academic Press, New York.

Chernomordik, L. V., S. I. Sukharev, S. V. Popov, V. F. Pastushenko, A. V. Sokirko, I. G. Abidor, and Y. A. Chizmadzhev. 1987. The electrical breakdown of cell and lipid membranes: the similarity of phenomenologies. *Biochim. Biophys. Acta.* 902:360–373.

Chizmadzhev, Y. A., V. G. Zarnitsin, J. C. Weaver, and R. O. Potts. 1995. Mechanism of electroinduced ionic species transport through a multilamellar lipid system. *Biophys. J.* 68:749–765.

Ferry, L. L., G. Argentieri, and D. H. Lochner. 1995. The comparative histology of porcine and guinea pig skin with respect to iontophoretic drug delivery. *Pharm. Acta Helv.* 70:43–56.

Hadgraft, J., and R. H. Guy. 1989. Transdermal Drug Delivery: Developmental Issues and Research Initiatives. Marcel Dekker, New York.

Liang, H., W. J. Purucker, D. A. Stenger, R. T. Kubiniec, and S. W. Hui. 1988. Uptake of fluorescence-labeled dextrans by 10T ½ fibroblasts following permeation by rectangular and exponential-decay electric field pulses. *Biotechniques.* 6:550–558.

Madison, K. C., D. C. Swartzendruber, P. W. Wertz, and D. T. Downing. 1987. Presence of intact intercellular lipid lamellae in the upper layers of the stratum corneum. *J. Invest. Dermatol.* 88:714–718.

Oh, S. Y., L. Leung, D. Bommannan, R. H. Guy, and R. O. Potts. 1993. Effect of current, ionic strength and temperature on the electrical properties of skin. *J. Controlled Release.* 27:115–125.

Pliquett, U., R. Langer, and J. C. Weaver. 1995. Changes in the passive electrical properties of human stratum corneum due to electroporation. *Biochim. Biophys. Acta.* 1239:111–121.

Prausnitz, M., V. Bose, R. Langer, and J. C. Weaver. 1993. Electroporation of mammalian skin: a mechanism to enhance transdermal drug delivery. *Proc. Natl. Acad. Sci. USA.* 90:10504–10508.

Schurer, N. Y., and P. M. Elias. 1992. The biochemistry and function of stratum corneum lipids. *Adv. Lipid Res.* 24:27–56.

Stenger, D., K. Kaler, and S. W. Hui. 1991. Dipole interactions in electrofusion: contributions of membrane potential and effective dipole interaction pressures. *Biophys. J.* 59:1074–1084.

Vanbever, R., N. Lecouturier, and V. Preat. 1994. Transdermal delivery of metoprolol by electroporation. *Pharm. Res. (NY).* 11:1657–1662.

Vanbever, R., and V. Preat. 1995. Factors affecting transdermal delivery of metoprolol by electroporation. *Bioelectrochem. Bioenerg.* 38: 223–228

Yamamoto, T. 1976. Electrical properties of the epidermal stratum corneum. *Med. Biol. Eng.* 14:151–158.