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Theoretical Model for the Electrical Resistance of Skin

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피부의 전기적 저항에 대한 이론적 모델

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The kinetic change of electrical resistance of hairless mouse skin as a function of ionic strength of the bathing medium was determined from impedance measurements. After increasing (decreasing) the ionic strength of the bathing medium, resistance decreased (increased) continuously with time, finally reaching an equilibrium value. We have modelled this process, using nonsteady-state diffusion kinetics. The results show semi-quantitative correlation between theoretically derived and experimentally obtained values. Overall, this work provides further mechanistic insight into ion-conduction through the skin.

Keywords—Resistance, Diffusion, Ionic strength, Pore

Recently, transdermal iontophoresis has received great interest because of its potential to provide a safe, economical, and convenient way to deliver both charged and uncharged drug molecules in a controllable manner.[1] The potential to deliver peptide drugs by iontophoresis has drawn particular attention[2-5] because these usually charged molecules are very potent but difficult to deliver by conventional methods. In order to optimize or maximize the benefits from iontophoresis, it is important to have a detailed understanding of the electrical properties of the skin.

A simple but practical representation of the electrical properties of the skin consists of a parallel combination of a resistance (R) and a capacitance (C).[6] The capacitance of the skin is believed to originate from the lipid matrix-keratin cell complex; the resistance appears to be primarily associated with the pore pathways through the skin.[7-9] Thus it is expected that the ionic content of these conducting pathways will influence R mostly.

In previous studies,[10] we measured the impedance of hairless mouse skin *in vitro* as a function of frequency to determine how the R and C of the membrane were affected by changes in ionic strength (I) and temperature (T). R was inversely dependent upon the I of the bathing medium, but C was, in general, unchanged. Changes in R following alterations in I were rapid and the equilibrium R values were reversible. The activation energy for ion conduction through the skin (calculated from the dependence of R upon T) was low (3.4 Kcal/mol) suggesting that the pore is quite aqueous in nature.

In this paper we followed the change in R after the change in I of the bathing medium by rapid impedance measurement at single frequency. The results provide evidence that the change in R (or equivalently the

conductance G=1/R) with I is a diffusion controlled process. Thus, when a bathing medium at low I is replaced by a solution with higher I, ions diffuse into pores in the skin (absorption), with the result that R of skin will decrease continuously until the concentration inside the pore reaches an equilibrium value. Conversely, ions will move out of the pores when the bathing medium is replaced by a solution with lower I and R will increase continuously until it reaches an equilibrium value (desorption).

Based on these observations, we have modelled the dependence of R, as a function of both I and time, using the nonsteady-state diffusion equation. Theoretical values of time-dependent resistance were obtained in terms of the nonsteady-state ion concentrations in the pore of the skin and were compared with some preliminary experimental data. Good correlations between the measured and calculated resistance values were found. These results provide further mechanistic insight into ion-conduction through the skin.

Experimental

Impedance Measurement

In the previous study on the effect of current, ionic strength and temperature on the electrical properties of skin,[10] we determined the resistance of skin by measuring the impedance as a function of frequency. This procedure took 2 to 3 minutes depending on the range of frequencies. This method cannot be used to study the time course of resistance after the ionic strength is changed, due to the rapid change in resistance in a time period of seconds. In order to follow the rapid change in resistance, we measured impedance (Z) and phase shift (q), using a lock-in amplifier, at a single frequency, instead of sweeping the frequencies.

Skin impedance measurements were made using excised full-thickness hairless mouse skin of 8-12 week old females (Simonsen, Gilroy, CA). Design of diffusion cell and the prepapration of Ag/AgCl electrodes are described in our previous paper.[10] Skin was mounted on a thermostatted (20°C) diffusion cell and equilibrated overnight at a set ionic strength. At the beginning of the experiment (t=0), ionic strength was suddenly changed to a new value by replacing the bathing solutions on both sides of the skin. After that point, skin impedance was measured continuously.

The electric circuit employed for the impedance measurements included a 1MΩ resistor in series with the skin. At an applied voltage of 1V (peak-to-peak), a sinusoidal current was applied via a signal generator (Hewlette Packard 8116A, Mountain View, CA) to the signal electrode on the epidermal side of the skin (the dermal-side electrode was grounded). Since the skin impedance was routinely 100KΩ or less, the current was determined primarily by the 1MΩ resistor in series, and the current was constant to within 10%. The potential difference and the shift in phase (θ) across the skin were then measured by the sensing electrode using a lock-in amplifier (Stanford research Systems SR530, Sunnyvale, CA). The fixed frequency used in all the measurements was 70 Hz. After the impedance (Z) of skin was determined from the potential difference, R was calculated by Z/cosq, assuming that the skin can be represented by a simple RC circuit. The contribution of the bathing medium to impedance was ignored in the calculation, because its magnitude is usually less than 1% of the impedance of skin.

Model for the Resistance

Although there is evidence that paracellular transport takes place at least for some substances such as mercuric chloride[11] and calcein,[12] we focus only on the transport of ions into and out of pores. These pores are thought

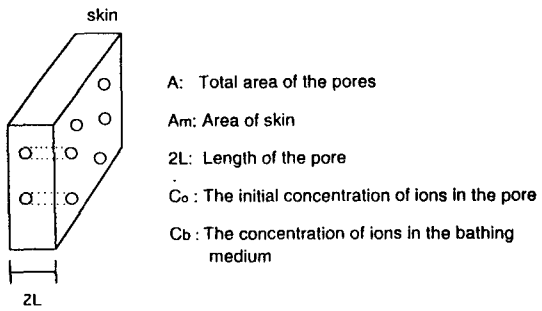

Figure 1—Simplified view of skin for modelling purpose. The pores represent the pathways of current or ions in skin. C_o is the initial concentration of ions in the pore and C_b is the concentration of ions in the bathing medium.

to be present mainly at the appendages, such as hair follicles and sweat glands.[7-9] In hairless mouse skin the pores are associated with hair follicles, because there are no sweat glands. Fig. 1 shows a simplified view of the skin for our modelling purpose. A is the total area of pores and Am is the area of the skin in contact with the bathing medium. The length of the pore is given by 2L. C_b is the concentration of ions in the bathing medium and C_o is the initial concentration in the pore. The concentration of ions within the pore as a function of both position (x) and time (t) is given by C(x,t). We have made some assumptions to model the resistance of skin: 1) resistance is determined solely by pores. Any contributions to the current flow other than aqueous pores are thought to be negligible; 2) the resistivity (r) of the solution is inversely proportional to the concentration of conducting ions, so that $\rho(x,t) = b/c(x,t)$ when b is in a constant scaling factor; 3) the concentration of ions in the bathing medium does not change with time; 4) at equilibrium, i.e. after the resistance of skin reaches a stable value, the concentration of NaCl ion in the pores is the same as that of the bathing medium; 5) the distribution of pores in skin is homogeneous and the radius and length of each pore are the same. The validity of these assumptions will be discussed in the Discussion section.

The concentration of ions C=C(x,t) at point x in the pore and time t is given by the non-steady state diffusion equation[13]

$$C = C_o + (C_b - C_o)\left(1 - \frac{4}{\pi}\sum_{n=0}^{\infty}\frac{(-1)^n}{(2n+1)}\right.$$
$$\left.\mathrm{Exp}\left(\frac{-D(2n+1)^2\pi^2 t}{4L^2}\right)\cos\left(\frac{(2n+1)\pi x}{2L}\right)\right. \quad \text{(Eq. 1)}$$

Where $-L<x<L$ and D is the diffusion coefficient of the NaCl. Using this profiles the pore conductance G(t) can be expressed as follows:

$$G(t) = \frac{1}{R(t)} = \frac{A}{\int_{-L}^{L}\rho(x,t)dx} = \frac{A}{2L\beta\int_0^1 \frac{dy}{C(y,t)}} \quad \text{(Eq. 2)}$$

In the above equation, β is the scaling factor for resistivity and it can be considered as the inverse of molar conductivity (κ). The variable y is x/L and it represents the position in pore normalized by the half-length of the pore. The units of β, A, C and L are Ωmol/cm^2, cm^2, mol/cm^3 and cm, respectively. Hence the right-hand side of Eq. 2 has the unit of $1/\Omega$ Numerical evaluation of Eqs. 1 and 2 was carried out by a FORTRAN program. The series in Eq. 1 converged quickly after about 10 or 20 terms. For Eq. 2, a simple trapezoidal integration was used with 50 equally spaced nodes.

Results and Discussion

In the development of the theory, several assumptions were made. These assumptions are primarily based on the literature or observations we have made in our previous study.[10] Assumption 1) is based on the literature, which strongly indicates that current flow occurs mainly through pores present at appendages, such as hair follicles and sweat glands.[7-9] The literature also indicates that the activation energy for ion conduction is very low

(~3.4 Kcal/mol), which is indistinguishable from ion conduction in water (3.6 Kcal/mol).[10] This suggests that the pores are aqueous in nature. Due to the high Born energy requirement, it is expected that the flow of ions through the lipid domain of stratum corneum is highly unfavorable. The assumption that resistivity is inversely proportional to concentration (assumption 2) is not absolutely correct; however, it is a reasonably good approximation in the range of concentration studied. Assumption 3) is true for most practical purpose because the volume of bathing medium is much larger than that of the skin. Assumption 4) is based on the finding that the pores are aqueous in nature, as discussed above.[10] Even if assumption 4), made above, is not adequate, we can assume that the concentration of the ions in pore is probably linearly related to the bathing medium concentration. In this case, we simply need to incorporate some partition coefficient into the model which correlates C_b to Co. A partition coefficient of one is chosen in the model. Assumption 5) cannot be realistic. Hence the L and A in the model should be treated as an average of a wide distribution of pores of different L and A.

The pores discussed here also should include those at non-appendageal region because there are evidences that small currents also flow through the skin at some locations where no appendageal structures are apparent. Cullander and Guy[6] used vibrating probe electrode to rapidly detect and vectorize current flow through hairless mouse skin and found that, although the largest currents flow through the hair follicles, small currents also flow through non-appendageal region. Similar results were obtained by Scott and co-workers, using a scanning electrochemical microscope to detect the flux of Fe^{+2} and Fe^{+3} through hairless mouse skin.[14] They also observed that the fraction of the current passing through these non-appendageal routes is dependent upon the magnitude of applied current, increasing as the magnitude of current density increases.

Examination of Theoretical Trends

Fig. 2 shows the change in calculated G as a

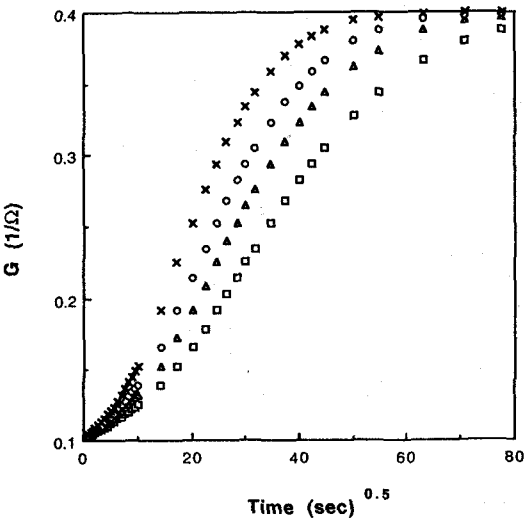

Figure 2—Calculated conductance as a function of time with various D/L^2 values when C_o=0.1M and C_b=0.4M.
Key: □: 0.0002, △: 0.0003, ○: 0.0004, x: 0.0006

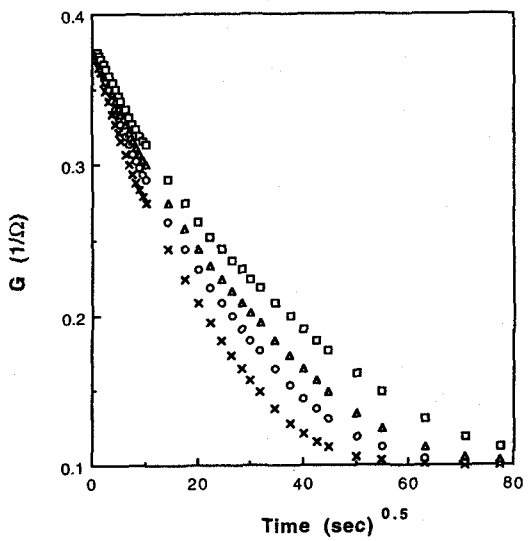

Figure 3—Calculated conductance as a function of time with various D/L^2 values when C_o=0.4M and C_b=0.1M.
Key: □: 0.0002, △: 0.0003, ○: 0.0004, x: 0.0006

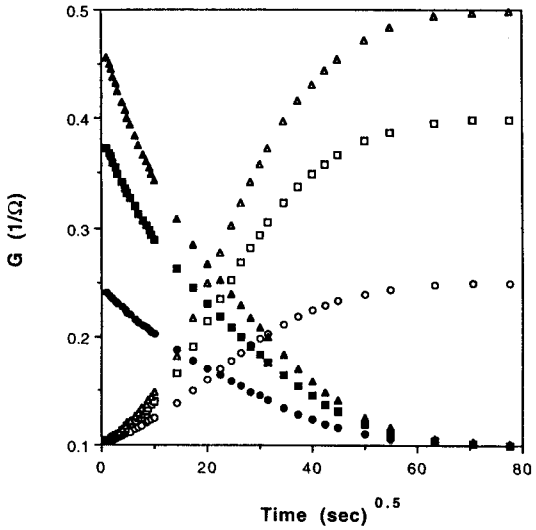

Figure 4—Calculated conductance as a function of time when D/L^2=0.0004.
Key: ○; 0.25M (out), ●; 0.25M(in), □; 0.4M(out), ■; 0.4M(in), △; 0.5M(out), ▲; 0.5M(in)
(In) and (out) refer to ionic strength within the pores and in the bathing medium, respectively. The concentration of the counter-medium in all cases listed above is 0.1M.

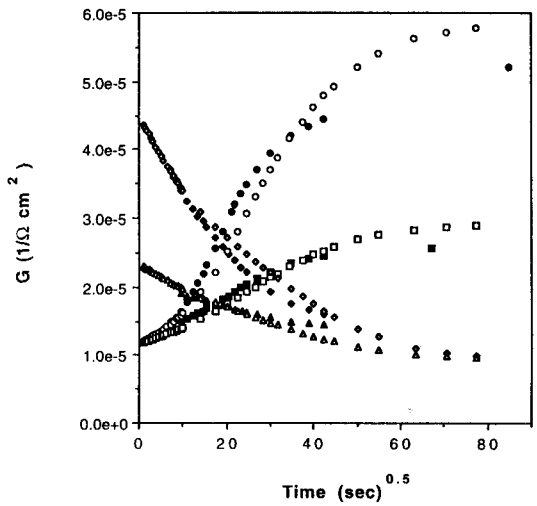

Figure 5—Plot of experimental data (filled symbols) and fit of theoretical values (empty symbols). All experimental data are obtained from the same skin. The D/L2 value used is 0.0003.
Key: ○; 0.5M (out), ◇; 0.5M (in), □; 0.25M (out), △; 0.25M (in)

function of time for four different D/L^2 values, when C_o and C_b are 0.1M and 0.4M, respectively. Conductance increases with time (absorption) and it approaches 0.4. G approaches 0.4 more rapidly as D/L^2 increases. Fig. 3 shows the change in calculated G as a function of time for those D/L^2 values used in Fig. 2, when C_o and C_b are 0.4M and 0.1M, respectively. Conductance decreases with time (desorption) and it approaches 0.1 at a rate which increases with increasing D/L^2. When D/L^2=0.0006 (1/sec), for both the absorption and desorption cases (Fig. 2 and Fig. 3), it takes about an hour to reach the equilibrium value 0.4 and 0.1. The changes in G with time for D/L^2=0.0004 are shown in Fig. 4 for the absorption and desorption at three concentrations. G increases/decreases to the value of the concentration of the bathing medium and this change reaches the equilibrium value after about 2 hours. A, b and L are chosen to be 1 in all calculations above.

Comparison of Theory with Experiment

Experimental data and the theoretical fit are shown in Fig. 5. All the experimental data used in this figure are obtained from the same skin. The experiment was carried out in this manner: 1) after the freshly excised skin was bathed in 0.1M NaCl solution overnight, the change in G with time was measured in 0.25M solution. 2) after the G value reached a stable value (probably an equilibrium value), the bathing medium was replaced by 0.1M solution and the change in G with time was measured. 3) the concentration of the bathing medium was changed again to 0.5M after G in 0.1M solution reached an equilibrium value. 4) after the G value in 0.5M solution reached a stable value, the change in G was studied again in 0.1M solution. The dimension of G is $1/(\Omega m^2)$, because the resistance obtained by experiment is normalized by the area of the skin (A_m) in contact with the bathing medium. Theoretical values of G calculated for D/L^2=0.0003 are used in the fit after division by some constants (8600 or 10500 (Ω mol/cm)). These constants

correspond to $2L\beta/A$ in Eq. 2 and a more precise form should be $2LA_m\beta/A$ due to the normalization by A_m. Fig. 6 shows experimental data for a skin and the theoretical fit when C_b is 0.4M and C_o is 0.1M. The theoretical value of G calculated for $D/L^2=0.0005$ are used in the fit after division by a constant (6900).

Fig. 5 show good correlation between theory and experiment. All the experimental data in Fig. 5 are obtained from the same skin. In all fits, the same D/L^2 value was used, because the pores in skin involved in current flow should be the same entity irrespective of the concentration of the bathing medium. The scaling factor ($2LA_m\beta/A$) used for absorption and desorption processes are somewhat different from each other. Ideally, this scaling factor also should be one value, as is the case for D/L^2. If a value in between these two, such as 9500 is used for both cases, then the fit will not be as good as shown in Fig. 5. However, the fit still predicts the shape of experimental data quite well. Fig. 6 shows remarkable overlap between the predicted value and the experimental data.

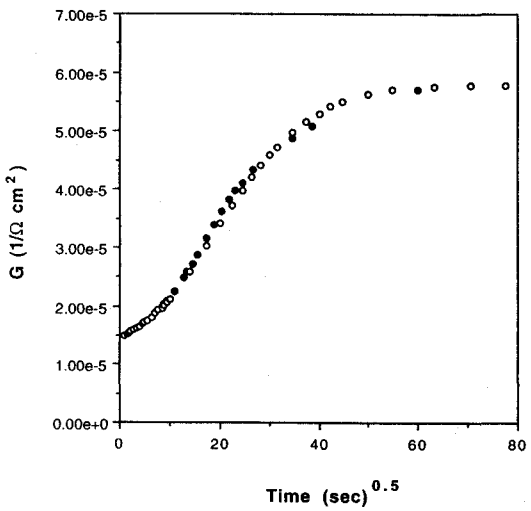

Figure 6—Plot of experimental data (filled symbols) and fit of theoretical values, when NaCl concentration within the pore is 0.1M and that in the bathing medium is 0.4M. 0.0005 and 6900 are the D/L^2 value and scaling factor, respectively (see text).

The D/L^2 value and scaling factor from the fit of the experimental data can be used for a somewhat crude estimation of the pore length (L) and the area of pore (A), if we know D value. The D/L^2 and $2LA_m\beta/A$ values used in the fits range between 0.0003-0.0005 (1/sec) and 6900-10500 (Ωmol/cm), respectively. If we choose the average value 0.0004 and 8700 for D/L^2 and $2LA_m\beta/A$, respectively, and if D is assumed to be 1.5×10^{-5} cm^2/sec, which is the diffusion coefficient of NaCl ion in aqueous bulk solution,[15] then L is 0.19 cm. A also can be estimated from $2LA_m\beta/A=8700$ if we know β. β can be approximated as $1/\kappa$, where κ is the molar conductivity of NaCl in aqueous solution (126 Ωmol/cm^2).[16] Using the parameter values above, A is estimated to be 27 μm^2.

Considering the depth of penetration of hair follicle in hairless mouse skin is ~ 0.1 cm and the pathway of pores is probably quite tortuous, L values estimated above (0.19 cm) seems reasonable. More accurate estimation of L and A can be made after determination of D by experiment. D and L can be determined after measuring the permeability (KD/l, K=partition coefficient and l=length of pore) and the lag time (l^2/6D) for ion diffusion, experimentally.

One aspect of the fit that should be addressed is that the experimental R values used here are calculated from raw data (impedance and phase shift), based on the simple parallel RC circuit which does not represent the skin exactly. Skin may be modeled by different circuits with much complexity. Hence different R values may be obtained for the same raw data and fits to the theory with different degree of goodness may result as a consequence. we think that, despite its simplicity, parallel RC circuit is a quite good model for all practical purposes and the general feature of R calculated from different models with different complexity can be well-represented by the R value based on this circuit.

Conclusion

Semi-quantitative correlation between theory and experiment has been observed. Although the model may be too simple to represent the complex biological system correctly, it provides useful mechanistic insight into the electrical resistance of skin. The results further support the observations that current is mainly flowing through the shunt pathways (pores) of the skin.

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