International Journal of Pharmaceutics, 69 (1991) 109–121

ADONIS 037851739100121R

IJP 02324

Skin alteration and convective solvent flow effects during iontophoresis: I. Neutral solute transport across human skin

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(Received 22 August 1990)
(Modified version received 8 November 1990)
(Accepted 9 November 1990)

Key words: Iontophoresis; Nernst-Planck theory; Electro-osmosis; Convective solvent flow; Human skin

Summary

Overall flux enhancement of ions during iontophoresis is due primarily to the electrochemical potential gradient. However, secondary effects such as convective solvent flow and, in biological membranes, permeability increases due to the applied field, may also contribute to flux enhancement. The modified Nernst-Planck theory includes a solvent flow velocity term and predicts uncharged molecules are enhanced or retarded depending on the polarity of the applied field. In this study, mannitol was employed as a probe permeant and the mannitol flux was used as a measure of the solvent flow contribution during iontophoresis across human epidermal membrane. Membrane alterations due to the applied field were also assessed, as was the extent of reversibility of the membrane changes. Mannitol transport was enhanced in the anode to cathode polarity and retarded in the cathode to anode polarity. This was interpreted to mean that significant solvent flow across human skin occurred during iontophoresis. Solvent flow velocity was found to be proportional to the magnitude of the applied field and independent of the system polarity. Membrane alterations occurred at the highest voltage investigated in this study (i.e., 1000 mV). These changes appeared to reverse over time as indicated by the current and transport data.

Introduction

Iontophoresis is a process by which the transport of ions into or through skin is increased by the application of an external electric field across the skin (Keister and Kasting, 1986; Masada et al., 1989; Srinivasan et al., 1989a,b; Sims and Higuchi, 1990). Recent efforts have focused on understanding the underlying mechanisms of solute transport during iontophoresis. In particular, the Nernst-Planck equation has been used to model ion transport under an applied potential (Schultz, 1980; Masada et al., 1985; Keister and Kasting, 1986; Srinivasan et al., 1990). The Nernst-Planck model assumes the iontophoretic flux is controlled entirely by the primary driving force (the electrochemical potential gradient) and neglects flux contributions from secondary effects. Secondary effects include current induced convective solvent flow (Burnette and Marrero, 1986; Burnette and

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Ongpipattanakul, 1987; Mathot et al., 1989; Srinivasan et al., 1989a; Sims and Higuchi, 1990) and, in biological membranes, permeability increase due to the applied electric field (Burnette and Ongpipattanakul, 1988; Srinivasan et al., 1989a; Pikal and Shah, 1990; Sims and Higuchi, 1990).

Modification of the Nernst-Planck equation to include a linear convective flow term (Sims et al., 1990a; Srinivasan and Higuchi, 1990) has enabled an evaluation of the current induced solvent flow contribution. This new model predicts (1) an asymmetry in the enhancements of cations and anions and (2) uncharged molecules are enhanced or retarded depending on the polarity of the applied electric field.

Nuclepore® membrane, having net negatively charged pores, was used previously to examine the modified Nernst-Planck model (Sims et al., 1990a). Glucose, a neutral, polar solute, was employed as a probe permeant and its flux was used as a measure of the solvent flow velocity through the membrane under an applied field. Results of these studies showed that glucose flux was enhanced when the anode was placed in the donor chamber ('anode to cathode' polarity). When the polarity was reversed (cathode to anode), glucose flux was opposed by the solvent flow and resulted in flux inhibition. The solvent flow velocity was found proportional to the magnitude of the applied voltage at high ionic strength which indicated electro-osmosis as one mechanism by which solvent flow occurs. Also, by including the solvent flow contribution, the experimental enhancement factors for monovalent cations and anions were found to be in good agreement with the Nernst-Planck prediction.

In the present study, the convective solvent flow contribution to total flux enhancement during iontophoresis across human epidermal membrane is examined. Mannitol (a neutral, polar solute) has been used as a probe permeant and its flux provides a measure of the solvent flow component. In skin there is the possibility for voltage induced membrane alterations. Therefore, experiments were designed to determine if alterations occurred and the extent to which membrane changes were reversible.

Theory

The flux of uncharged permeants is assumed to be affected indirectly by the applied electric field via convective solvent flow (Sims et al., 1990a; Srinivasan and Higuchi, 1990). For a porous, net negatively charged membrane, the solvent flow will be from the positive electrode (anode) to the negative electrode (cathode) (Srinivasan et al., 1989a). Thus, depending on which electrode is in the donor chamber, the solvent flow will either enhance or impede the flux of an uncharged molecule across the membrane. An enhancement factor, E, has been defined as the ratio of the flux at an applied voltage, $\Delta\psi$, across the membrane, to the passive flux. For neutral solutes the enhancement factors are (Sims et al., 1990a; Srinivasan and Higuchi, 1990):

$$\text{anode to cathode: } E = P_e/[1 - \exp(-P_e)] \quad (1)$$

$$\text{cathode to anode: } E = -P_e/[1 - \exp(P_e)] \quad (2)$$

$$\text{where } P_e = (v\Delta x/D) \quad (3)$$

v is the solvent flow velocity, Δx is the membrane thickness and D is the solute diffusion coefficient. The notation 'anode to cathode' refers to the case where the anode is in the donor chamber of a two chamber diffusion cell (see Methods). Likewise, 'cathode to anode' refers to the case where the cathode is in the donor chamber. The Peclet number (P_e) characterizes the effect of convective solvent flow on the flux of the permeant. Eqn 1 predicts enhancement factors greater than 1 for the anode to cathode polarity while Eqn 2 predicts $E < 1$ for the cathode to anode polarity.

Some additional comment on the meaning of Eqn 3 in the context of the present research is instructive here. For charged membranes (which is the case of interest), Eqns 1–3 are most easily understood when pore sizes are large compared both to permeant molecule size and to the electrical double layer thickness. In this limiting case, solvent flow due to electro-osmosis may be estimated by determining the transport enhancement of a neutral molecule such as mannitol. For this situation, solvent flow should be essentially 'plug'

flow and mannitol should be effective as a 'marker' for the solvent. These ideal conditions were believed met in the recent studies (Sims et al., 1990a) with the Nuclepore® membrane: in 0.10 M ionic strength (electric double layer thickness around 10 Å) and membrane pore size of 150 Å diameter. When electrical double layer thickness is not small compared to pore size, plug flow no longer holds, radial diffusion may become important (Anderson and Quinn, 1974; Deen, 1987), and the meaning of v in relation to the probe permeant as a marker for solvent flow becomes less clear. Finally, when pore sizes are comparable to molecular dimensions, the use of a permeant such as mannitol as a marker for solvent flow should be highly questionable.

Materials and Methods

Materials

[^{14}C]Mannitol (MW = 182.2) was obtained from New England Nuclear having a specific activity of 55.0 mCi/mmol. Phosphate-buffered saline (PBS), ionic strength 0.1 M, pH 7.5, was used in all experiments (Sims et al., 1990a). Sodium azide (0.1%, Baker) was added to the buffer as a bacteriostatic agent.

Human skin was obtained from Ohio Valley Tissue and Skin Bank (Cincinnati, OH). The epidermal membrane was heat separated from the dermis by placing the full thickness skin in a 60 °C water-bath for 1 min. The epidermal membrane, which includes the stratum corneum and part of the epidermis, was then peeled away from the underlying dermis and stored at −20 °C until used. The area available for diffusion was about 0.7 cm^2 and the thickness of the epidermal membrane was about 100 μm.

Methods

All experiments were conducted with the four-electrode potentiostat system (JAS Instrumental Systems, Inc., Salt Lake City, UT) which has been previously described (Srinivasan et al., 1990). This system is able to maintain a constant, known voltage drop across a membrane positioned between the donor and receiver chambers of a two-chamber diffusion cell. After visual inspection for absence of holes, the epidermal membrane was mounted between the half-cells with the stratum corneum facing the donor chamber. An additional check for small holes in the membrane was made by tilting the cell toward the donor side and adding 2 ml of PBS to the receiver chamber. The cell remained in this position for 15–20 min. If buffer was then found in the donor chamber, the skin was discarded and the procedure repeated with a new piece of skin. Once an intact skin sample was selected, the Luggin capillaries, filled with the same buffer as used in the donor and receiver chambers, were inserted. The receiver chamber was filled with 5 ml of PBS. [^{14}C]Mannitol (tracer level) was pre-mixed in PBS prior to pipetting 5 ml of the solution into the donor chamber.

A typical experiment involved four experimental stages. In stage I a passive transport run was carried out by taking 1 ml samples from the receiver chamber at predetermined time intervals and replacing with 1 ml of fresh PBS. At the end of stage I, the entire contents of the receiver chamber were removed and replaced with fresh PBS. During stage II a fixed voltage drop was applied across the membrane. The current was continuously monitored during this period and 1 ml samples were taken from the receiver chamber and replaced with fresh PBS. At the end of stage II, the voltage was turned off and both donor and receiver chambers were flushed and refilled with their respective solutions. In stage III (during the 8 h directly after turning off the voltage drop), a second passive transport run was carried out. Finally, in stage IV (during the 16–22 h period after termination of the voltage), a third passive run was conducted.

The samples from each of the runs were mixed with 10 ml of scintillation cocktail (Opti-Fluor, Packard Instrument Co.) and were assayed on a Beckman Liquid Scintillation Counter, Model LS-7500. The data were plotted as Q, the cumulative disintegrations per minute (dpm) in the receiver chamber, as a function of time, t. The permeabil-

ity coefficient, P, was calculated for each stage from:

$$P = \frac{1}{A\Delta C}\frac{dQ}{dt} \quad (4)$$

where dQ/dt is the steady-state slope, A is the area for diffusion, and ΔC is the concentration difference across the membrane. Receiver solution samples were also checked by HPLC to ensure no significant radiochemical impurities were present.

In some experiments the current at an applied voltage drop of 250 mV was periodically measured during stages I, III, and IV. This was done as an independent measure of membrane alteration (by comparing this current prior to and directly after stage II) and of reversibility over time (by showing this current returning to its initial value determined in stage I). The voltage was applied across the membrane for a very brief time.

Results and Discussion

Skin sample selection criteria

Skin samples were selected on the basis of visual examination and through the tilting process described in Methods. Membranes which showed no gross holes by either of these methods were used in this study. Subsequent mannitol transport studies showed wide variability in the passive permeability coefficient (95% of the skin samples had passive P values in the range 3×10^{-7}–3×10^{-9} cm/s). A passive permeability coefficient of the order of 10^{-8} cm/s was considered to be representative of undamaged human epidermal membrane (Ghanem et al., 1989). This criterion seems to be consistent with the recent report of Kasting and Bowman (1990). In some cases, however, skin samples having 10-fold higher permeability coefficients were used. Since these membranes did not have visible holes, the results have, with some reservations, been included herein.

125 mV and 250 mV experimental results

Because membrane alteration effects occurred at 1000 mV but not at the lower voltages, the 125 mV and the 250 mV data will be presented first, together, and the 1000 mV results are presented later.

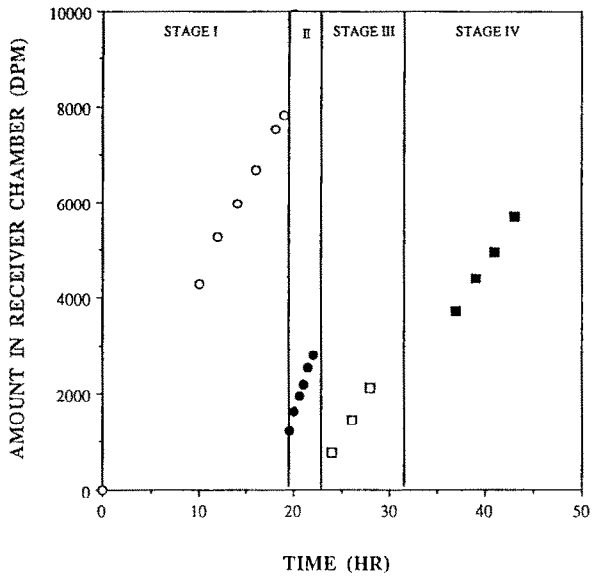

Fig. 1. Typical results of a mannitol transport experiment during which 125 mV was applied across the membrane in the anode to cathode polarity (run no. 4-1AC). (○) Stage I; (●) stage II; (□) stage III; (■) stage IV.

Typical results of mannitol transport experi-

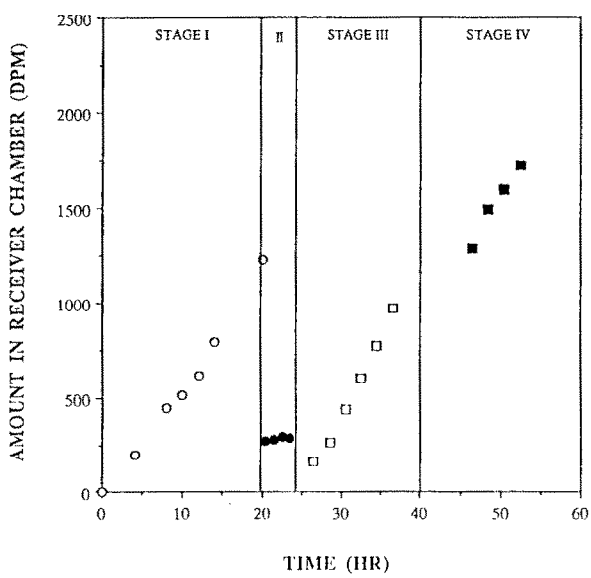

Fig. 2. Typical results of a mannitol transport experiment during which 250 mV was applied across the membrane in the cathode to anode polarity (run 3-2CA). (○) Stage I; (●) stage II; (□) stage III; (■) stage IV.

ments are shown in Fig. 1 for an anode to cathode polarity (at 125 mV, anode in the donor chamber) run and in Fig. 2 for a cathode to anode polarity (at 250 mV, cathode in the donor chamber) run. As can be seen, the data plots are essentially linear in all cases and, permeability coefficients were calculated for all of the experiments from the best-fit slope (dQ/dt) for each stage using Eqn 4. These P values are presented in Table 1.

The electrode polarity effects are seen by comparing the stage II P values with the passive P values in Table 1. For the anode to cathode situation, the stage II P values are always larger than the passive P values and this effect is greater at 250 mV than at 125 mV. On the other hand, for the cathode to anode situation, the stage II P values are smaller than the passive P values, and at 250 mV the stage II slopes of the data plots were all indistinguishable from zero. This is clearly demonstrated in Fig. 2. For a net negatively charged membrane, the solvent flow opposes the concentration gradient in the cathode to anode polarity (Srinivasan et al., 1989a; Pikal and Shah, 1990; Sims et al., 1990a). Since mannitol is uncharged, this results in an inhibition of the flux compared to the passive mannitol flux (stages I, III, or IV). In the opposite polarity, i.e. anode to cathode, the solvent flow is in the same direction as the concentration gradient and the result is enhancement of the flux relative to the passive flux. The results of these studies support the idea of human skin having a net negative charge (Burnette and Marrero, 1986).

Membrane alteration was assessed during the low voltage runs by determining the passive permeability coefficients before (stage I) and after (stages III and IV) application of a voltage drop in stage II. These passive P values, in general, indicated that there were little or no irreversible changes taking place in these experiments. Additionally, the currents monitored during stage II remained essentially constant at both 125 and 250 mV as shown in Fig. 3. This constant current behavior has also been observed with Nuclepore® membranes in similar experiments (Sims et al., 1990a). Nuclepore® membranes remain unaltered by an applied electric field under the conditions employed herein. The constant current therefore implies that the epidermal membrane did not change during the application of low voltage in stage II. Thus, both the transport data and the current data suggest that the epidermal membrane

TABLE 1

Mannitol permeability coefficients from individual experiments at 125 mV and 250 mV across human epidermal membrane

Applied potential (mV)	Run No.	Permeability coefficient, P ($\times 10^8$) (cm/s)			
		Stage I	Stage II	Stage III	Stage IV
(A) Anode to cathode					
125	1-1AC	1.3	1.6	1.5	1.5
125	2-1AC	0.9	1.4	1.9	1.3
125	3-1AC	64	90	58	33
125	4-1AC	5.5	8.8	5.0	4.8
250	1-2AC	7.9	42	11	18
250	2-2AC	1.6	2.9	2.0	2.3
250	3-2AC	0.7	5.8	2.2	2.1
(B) Cathode to anode					
125	1-1CA	1.3	1.2	1.4	1.5
125	2-1CA	4.5	2.3	5.4	5.9
125	3-1CA	2.8	0.9	3.8	2.1
250	1-2CA	1.4	[a]	2.0	1.9
250	2-2CA	5.9	[a]	6.3	4.9
250	3-2CA	1.7	[a]	2.0	1.6

[a] Permeability coefficient during stage II in the cathode to anode polarity at 250 mV was indistinguishable from zero.

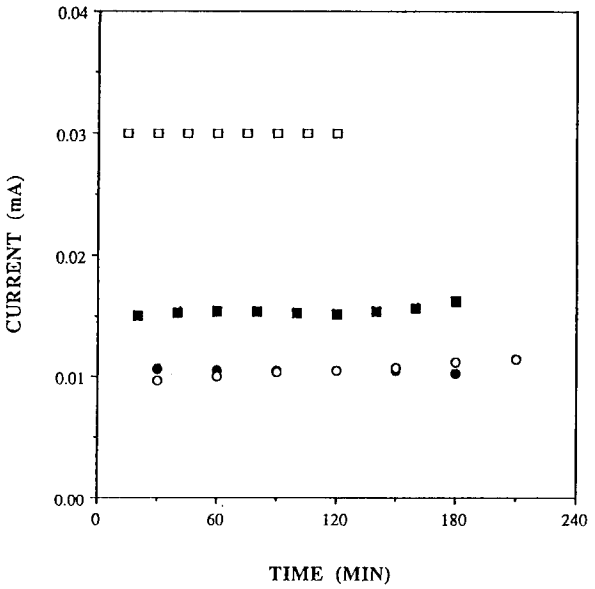

Fig. 3. Current-time profiles during stage II for representative experiments at 125 and 250 mV. (●) 125 mV, anode to cathode (run 4-1AC); (■) 125 mV, cathode to anode (run 2-1CA); (□) 250 mV, anode to cathode (run 2-2AC); (○) 250 mV, cathode to anode (run 3-2CA).

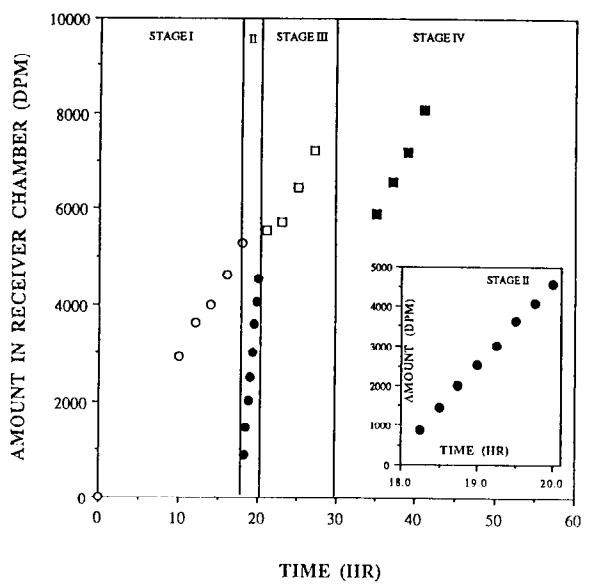

Fig. 4. Representative results of a mannitol transport experiment in which the skin was not altered during the application of 1000 mV in the anode to cathode polarity (run 4-3AC). Inset is an enlargement of stage II. (○) Stage I; (●) stage II; (□) stage III; (■) stage IV.

is not significantly altered by the applied field at either 125 or 250 mV. Results reported (Kasting and Bowman, 1990) using a low constant current (corresponding to low voltages) seem to support the findings of this study. Observations of the current-voltage relationship for various skin samples by Kasting and Bowman showed a 'stable voltage' for current values between 0 and $\pm 10\ \mu A$ with 'little evidence for the production of irreversible or slowly reversible changes in the skin.'

1000 mV experimental results

Anode to cathode polarity The results of the runs at 1000 mV were significantly different from those at the two lower voltages. There was no 'typical' experiment. However, there appeared to be two extreme cases: in the anode to cathode polarity (anode in the donor chamber) the amounts of mannitol transported across the membrane into the receiver chamber during stage II either increased linearly or non-linearly, the latter suggesting membrane alterations taking place during the run. Representative plots of each of these are shown in Figs 4 (linear) and 5 (nonlinear). The

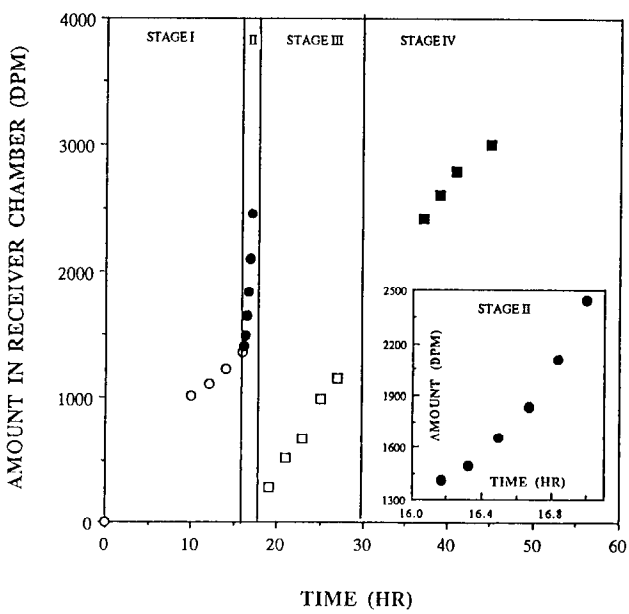

Fig. 5. Representative results of a mannitol transport experiment in which the skin was altered during the application of 1000 mV in the anode to cathode polarity (run 1-3AC). Inset is an enlargement of stage II. (○) Stage I; (●) stage II; (□) stage III; (■) stage IV.

stage II data plots have been enlarged to show that the slope changed with time in the case of 2-3AC but not for 4-3AC.

As in the case of the low voltages, the passive permeability coefficients were calculated from the best-fit slopes for stages I, III, and IV. Experimental protocol precluded the taking of early time points; thus, in some cases, the errors were rather large in the determination of the stage I passive permeability coefficients. The passive P values are shown in columns 2, 5, and 6 of Table 2A for each experiment.

The evaluation of the stage II P values was made difficult by the nonlinear behavior (flux increasing with time) in two out of the five mannitol runs. In these nonlinear cases, two P values were calculated from the stage II data. An initial P value, $P_{\Delta\psi,i}$ was determined from the initial slope of the amount in the receiver chamber over time and a final P value, $P_{\Delta\psi,f}$, from the limiting slope at the end of stage II. These two P values were the same in runs where the increase in the amount in the receiver over time was linear (Fig. 4). The stage II P values are presented in columns 3 and 4 of Table 2A. Runs 1- and 2-3AC show $P_{\Delta\psi,f}$ increasing 3–4 times over that of the respective $P_{\Delta\psi,i}$. The increasing $P_{\Delta\psi}$ value is indicative of membrane alteration having taken place during application of the field. Fig. 6 shows the current profiles during stage II for the runs at 1000 mV.

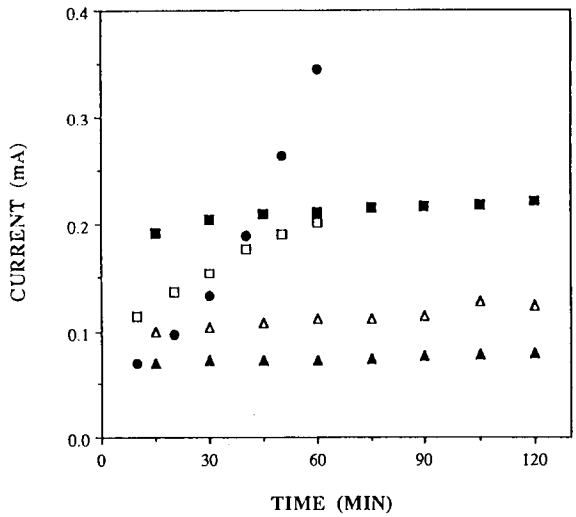

Fig. 6. Current-time profiles during stage II for each of the 1000 mV, anode to cathode, runs. (●) Run 1-3AC; (□) run 2-3AC; (■) run 3-3AC; (△) run 4-3AC; (▲) run 5-3AC.

As can be seen, for the two experiments (1- and 2-3AC) which showed significant increases in $P_{\Delta\psi}$ during stage II, there were also significant increases (factor of 2–4) in the current during stage II. The remaining experiments (3-, 4- and 5-3AC) showed relatively constant $P_{\Delta\psi}$ values as well as current profiles which indicated only small increases (15–20%) in current over time during stage II.

TABLE 2

Mannitol permeability coefficients determined from individual experiments at 1000 mV across human epidermal membrane

Expt	Permeability coefficients, P ($\times 10^7$) (cm/s)				
	Stage I	Stage II		Stage III	Stage IV
		$P_{\Delta\psi,i}$	$P_{\Delta\psi,f}$		
(A) Anode to cathode					
1-3AC	0.21	1.9	7.5	0.44	0.24
2-3AC	0.44	1.9	6.3	0.42	0.32
3-3AC	2.1	19	18	1.2	2.0
4-3AC	0.43	3.5	3.1	0.58	0.47
5-3AC	0.71	4.0	2.8	0.55	0.77
(B) Cathode to anode					
1-3CA	3.0	[a]	[a]	20	5.3
2-3CA	3.2	[a]	[a]	4.0	3.6
3-3CA	0.2	[a]	[a]	0.2	0.3

[a] Permeability coefficient during stage II in the cathode to anode polarity at 1000 mV was indistinguishable from zero.

Evidence for membrane alterations at high voltages (≥ 1.0 V) may be found in the work of Kasting and Bowman (1990). These authors carried out constant current studies and measured the voltage as a function of current. At low current/voltage, the current vs voltage profiles were linear; at high voltages (≥ 1.0 V) these profiles became distinctly nonlinear, in very good agreement with the present findings.

Cathode to anode polarity In the cathode to anode polarity (cathode in the donor chamber), the stage II P values were all found to be indistinguishable from zero. Solvent flow in the direction opposite to that of the electrochemical potential gradient appeared to completely inhibit the transport of mannitol across the membrane. Passive permeability coefficients in stages I, III, and IV were calculated in the same manner as above and are shown in Table 2B.

Reversibility of membrane alteration The large changes seen during stage II (experiments 1- and 2-3AC) were not reflected in passive permeability coefficients determined at some long time after stage II. It is seen in Table 2 that the stage I and stage IV passive P values are essentially the same in nearly all experiments. Reversibility of electric field effects on human skin has been reported (DeNuzzio and Berner, 1990), and the present results support the view that membrane alterations which take place during stage II are reversible in 16–22 h.

Current measurements (at 250 mV) during stages III and IV provide additional insights into the recovery kinetics after significant membrane alteration during stage II. The current values taken at different times during stages III and IV are shown in Table 3 for three experiments. These data may be interpreted as follows. The current values at the beginning of stage III are large compared to those for stage I in the cases of 1-3CA and 2-3CA because it is believed (based on current measurements during stage II) that, in both of these cases, there were significant membrane changes during stage II, and, at the beginning of stage III, the membranes are still in the perturbed state. In experiment 2-3CA the recovery back to the stage I state is faster than for experiment 1-3CA, and, by the middle of stage III, experiment 2-3CA is nearly fully recovered (i.e., 0.18 mA vs 0.13 mA for stage I), while the recovery for 1-3CA is slower (i.e., 0.35 mA at midpoint of stage III vs 0.14 mA for stage I). For both 1-3CA and 2-3CA, however, the current values during stage IV indicate essentially full recovery. In the case of experiment 5-3AC, the current value at the beginning of stage III indicates that little or no membrane alteration had taken place during stage II, and this is in agreement with the 5-3AC current pattern for stage II (see Fig. 6, which shows constancy of the current during stage II).

TABLE 3

Current measurements at 250 mV during stages I, III, and IV for three runs in which 1000 mV was applied during stage II

Stage	Run No.	Current (mA)		
		5-3AC	1-3CA	2-3CA
I		0.019	0.14	0.13
III, initial		0.023	0.64	0.82
III, middle		0.020	0.35	0.18
IV		0.019	0.16	0.13

Possible mechanism(s) of membrane alteration

Reversible pore formation has been suggested as a mechanism by which an electric field increases the permeability of some lipid bilayers (depending on their chemical composition) (Kinosita and Tsong, 1977; Benz and Zimmermann, 1981; Chernomordik et al., 1983; Glaser et al., 1988), erythrocyte cell membranes (Kinosita and Tsong, 1977; Serpersu et al., 1985), and mesophyll protoplast cell membranes (Zimmermann and Vienken, 1982). The mechanism of pore formation is still unclear but several theories have been offered. It has been suggested that a high electric field intensity causes local compression of the membrane (electromechanical stress) which leads to thinning of the membrane (Benz and Zimmermann, 1980; Zimmermann and Vienken, 1982). Reorganization of the membrane structure may then occur to form the transient pores, e.g. by inducing a looser packing of the lipids (Sugar, 1979). Teissie and Tsong (1981) have suggested a 'gating' mechanism in which the polar head groups

of the phospholipids act as electrical dipoles. These dipoles are thought to be reoriented by an externally applied electric field. The existence of spontaneous pores within the membrane due to structural defects has also been advocated (Teissie and Tsong, 1981). These pores are thought to be stabilized due to lowered activation energy for pore formation by an electric field. More recently, Glaser et al. (1988) have used planar lipid bilayers to show pore formation may involve at least two steps. First, the formation of hydrophobic pores and secondly, inversion of the pore edge to form a hydrophilic pore. It is interesting to note that all of these mechanisms are voltage dependent, i.e. both the magnitude and duration of the applied voltage determines the size, quantity, and reversibility of the pore formation.

While the present studies with human skin do not allow for the determination of the mechanism of membrane alteration, there is some basis to believe reversible pore formation may be occurring when an electric field is applied across the epidermal membrane.

Membrane resistance as a measure of the passive permeability coefficient

In some of the experiments the current was periodically measured at an applied voltage of 250 mV during stages I, III, and IV. Membrane resistance was then calculated using Ohm's law and the measured current values. Fig. 7 shows these resistance values plotted against their respective passive P values. Data presented here involved only those experiments where there was little or no membrane alteration during stage II; therefore, it is believed that current membrane resistance values are paired with the appropriate passive P values.

Fig. 7 shows that there is a clear linear relationship between the resistance and the passive permeability coefficient. The data scatter is believed to be primarily due to the error in the P value determinations. This linear relationship demonstrates that the transport pathway for a highly polar molecule such as mannitol is the same as the pathway for electrical conduction, i.e., a pore pathway is the pathway for both processes. It is interesting to note that Kasting and Bowman

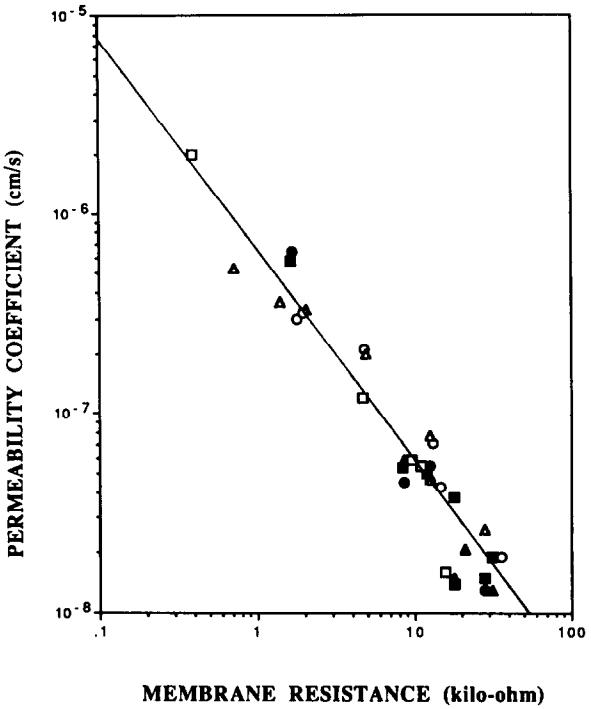

Fig. 7. Passive permeability coefficients vs the membrane resistance calculated from the current measured at 250 mV in each stage. Open symbols: 1000 mV applied in stage II. Closed symbols: 125 mV applied in stage II.

(1990) have shown a similar type of plot; these authors plotted the passive P values of Na^+ vs membrane resistance and obtained an essentially linear relationship.

In Fig. 8, the results of additional experiments are presented to show that the linear relationship seen in Fig. 7 is rather general for the present apparatus. As expected from Ohm's law, it is seen that resistance values determined at 1000 mV (but before there is any membrane alterations) and at 125 mV also fall on or near the best-fit line when plotted against their respective passive P values (stage I). Also, the data point taken from a recent study with the synthetic Nuclepore® membrane falls on the line (Sims et al., 1990a). Finally, one example (taken from experiment 2-3CA, Table 3) is presented showing that, when there is significant membrane alteration (during stage II) the pairing of the passive P value with the membrane resistance can be a problem: it is seen here that the resistance calculated from the current at the

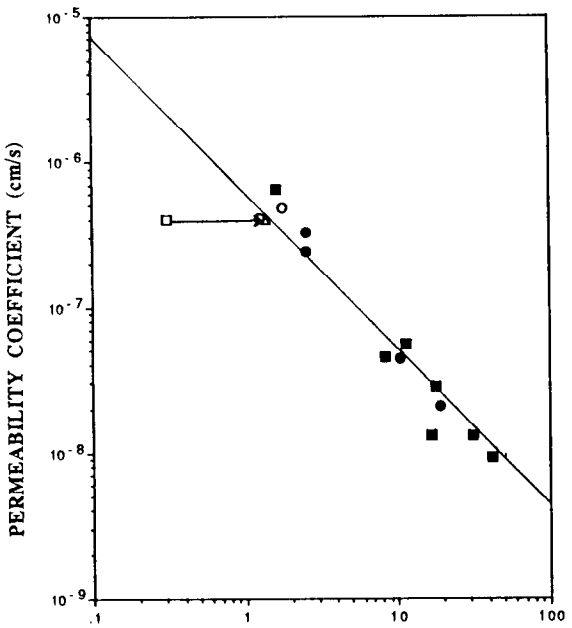

Fig. 8. The stage I passive permeability coefficients are plotted against the membrane resistance calculated from the initial current measured during (●) 1000 mV runs and (■) 125 mV runs. The solid line is the best-fit line from Fig. 7. (□, △) Expt 2-3CA (see Tables 2 and 3). (○) Passive P value from a Nuclepore experiment vs the current at 250 mV (Sims et al., 1990a).

beginning of stage III gives a data point that falls far away from the best fit line; however, if the resistance calculated from the current value taken from the midpoint of stage III is used, this data point falls much closer to the best-fit line.

The preceding discussion points out that the membrane resistance may be a reliable measure of the passive permeability coefficient associated with the pore pathway. It may be used to monitor membrane changes during iontophoresis. Also, the resistance may be used to help preselect skin samples for dermal and transdermal transport research purposes.

Convective solvent flow through human skin

An enhancement factor, E, has been defined (Srinivasan et al., 1990) as the ratio of the flux

TABLE 4

Mannitol enhancement factors

Applied voltage (mV)	n	Enhancement factor, E [a]
(A) Anode to cathode		
125	4	1.3 ± 0.4
250	3	2.1 ± 0.7
1000	5	7.0 ± 2.9
(B) Cathode to anode		
125	3	0.6 ± 0.2
250	3	[b]
1000	3	[b]

[a] Mean ± S.D.

[b] Permeability coefficient during iontophoresis (stage II) was indistinguishable from zero.

Mannitol enhancement factors as a function of applied voltage, polarity, and reference passive permeability coefficient. E_1 is referenced to the stage I passive P value, E_2 to the stage III passive P value and E_3 to the stage IV passive P value.

with an electric field to the flux without the field (passive flux) and is given as:

$$E = P_{\Delta\psi}/P \tag{5}$$

$P_{\Delta\psi}$ is the stage II permeability coefficient and P is the appropriate passive P value from stage I, III, or IV. The average mannitol enhancement factors for the 125 and 250 mV cases were calculated using Eqn 5 and the stage II and IV P values (see Tables 1 and 2). In the 1000 mV cases, the initial stage II P value, $P_{\Delta\Psi,i}$, was used in Eqn 5 to calculate the E value. Thus, the E values, presented in Table 4, are not expected to include contributions from membrane alteration effects or, at least, the effects of membrane alterations were expected to be minimal for these E values. The stage IV passive P value was chosen as the reference P value (denominator in Eqn 5) because it was felt that the uncertainties in the slope determination for stage I P values were generally greater than those for stage IV. As can be seen, qualitatively, the results follow the predictions of Eqns 1 and 2, i.e. $E > 1$ in the anode to cathode polarity and $E < 1$ in the opposite polarity.

The Peclet number, P_e, which includes the solvent flow velocity term (see Eqn 3), may be

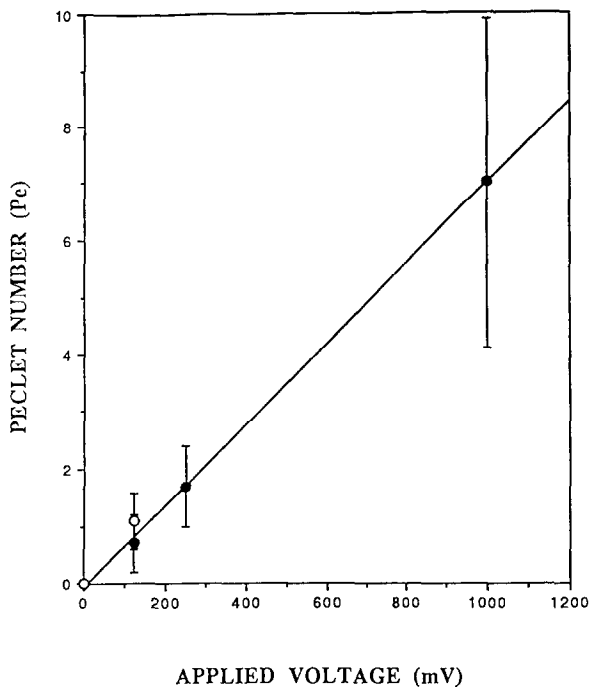

Fig. 9. Peclet number calculated from mannitol enhancement factors using Eqns 1 and 2 in the anode to cathode polarity (●) and cathode to anode polarity (○).

calculated from the E values in Table 4 using Eqns 1 and 2. As can be seen in Fig. 9, the P_e values calculated using the results in the anode to cathode polarity are proportional to the applied voltage. In the cathode to anode experiments, the stage II P values for the 250 and 1000 mV runs were very low and could not be distinguished from zero; however, the P_e value for the 125 mV case was found to fall within experimental error on the best-fit line for the anode to cathode results, this in accordance with theoretical expectation, i.e., the magnitude of the electro-osmotic velocity should be independent of the system polarity. It should be mentioned that the very low flux results for the 250 mV and the 1000 mV experiments in the cathode to anode case are consistent with predictions based on the anode to cathode data: very low P values of the order of 10^{-9} and 10^{-10} cm/s for the 250 and 1000 mV cases, respectively, were expected.

Electro-osmosis has been suggested as the mechanism for convective solvent flow in skin (Burnette and Marrero, 1986; Burnette and Ongpipattanakul, 1987; Pikal and Shah, 1990; Srinivasan and Higuchi, 1990). The classical theory of electro-osmosis in porous membranes was developed by Smoluchowski (1921) for the case when the pore radius, r, is much larger than the thickness of the electrical double layer, $1/\kappa$ (Bockris and Reddy, 1970; Hunter, 1981; Hiemenz, 1986). Combining the Smoluchowski equation with the constant field assumption of Goldman (1943) results in an expression for the electro-osmotic velocity (Sims et al., 1990a):

$$v = (\sigma/\kappa\eta)(\Delta\psi/\Delta x) \quad (6)$$

where σ is the surface charge density of the 'pore wall', κ is the Debye-Hückel reciprocal length, η is the bulk viscosity, and $\Delta\psi/\Delta x$ is the electric field. Eqn 6 has been derived assuming a low surface potential and is expected to be strictly valid when $1/\kappa \ll r$ (in the present studies, $1/\kappa$ is around 10 Å).

In human stratum corneum the pore size is unknown and it is likely that a range of pore sizes exists. Thus, in considering electro-osmosis in skin, it is necessary to keep in mind that there may be pores smaller than or comparable in size to the electric double layer thickness. Therefore, in order to develop the more general theory applicable to stratum corneum, the situation where $1/\kappa$ is of comparable magnitude to the pore radius must be considered and the Poisson-Boltzmann equation must be solved numerically. It is believed, nevertheless, that the direct proportionality of v with respect to $\Delta\psi/\Delta x$ is general and this relationship is seen with the present data (Fig. 9). The theoretical considerations have been found useful in qualitatively describing the solvent flow velocity through skin during iontophoresis and implicate electro-osmosis as one mechanism by which solvent flow occurs.

The volume flow across the epidermal membrane is of practical importance. In these studies, using tracer levels of mannitol, the volume flow when 1000 mV was applied, anode to cathode, in stage II was found to be in the order of 1 μl h^{-1} cm^{-2} for tissue samples having passive P values of the order of 10^{-8} cm/s. Studies in progress (Sims et al., 1990b) in our laboratory are showing

that solvent flow considerations will probably impact in only a secondary manner in the iontophoretic transport of ionic species.

Conclusions

Human epidermal membrane may undergo continuous membrane alteration at high applied voltages (1000 mV) but not at 250 mV or lower. These changes appear to completely reverse over time once the voltage drop is removed under the present experimental conditions. This phenomenon may be significant for the delivery, by iontophoresis, of both charged and uncharged drug molecules.

Significant solvent flow, as measured by an increase in mannitol (a probe permeant) flux, occurs in human skin under the influence of an electric field. The mechanism of solvent flow includes electro-osmosis, and the classical electro-osmotic theory may be used to interpret the data on the transport of an uncharged molecule across skin.

Acknowledgements

This research was supported by NIH Grant GM43181. The authors wish to thank TheraTech, Inc. of Salt Lake City, UT, for the donation of the human skin.

References

Anderson, J.L. and Quinn, J.A., Restricted transport in small pores. A model for steric exclusion and hindered particle motion. *Biophys. J.*, 14 (1974) 130–150.

Benz, R. and Zimmermann, U., The resealing process of lipid bilayers after reversible electrical breakdown. *Biochim. Biophys. Acta*, 640 (1981) 169–178.

Bockris, J.O'M. and Reddy, A.K.N., *Modern Electrochemistry*, Vols 1 and 2, Plenum, New York, 1970.

Burnette, R.R. and Marrero, D., Comparison between the iontophoretic and passive transport of thyrotropin releasing hormone across excised nude mouse skin. *J. Pharm. Sci.*, 75 (1986) 738–743.

Burnette, R.R. and Ongpipattanakul, B., Characterization of the permselective properties of excised human skin during iontophoresis. *J. Pharm. Sci.*, 76 (1987) 765–773.

Burnette, R.R. and Ongpipattanakul, B., Characterization of the pore transport properties and tissue alteration of excised human skin during iontophoresis. *J. Pharm. Sci.*, 77 (1988) 132–137.

Chernomordik, L.V., Sukharev, S.I., Abidor, I.G. and Chizmadzhev, Y.A., Breakdown of lipid bilayer membranes in an electric field. *Biochim. Biophys. Acta*, 736 (1983) 203–213.

Deen, W.M., Hindered transport of large molecules in liquid-filled pores. *AIChE J.*, 33 (1987) 1409–1425.

DeNuzzio, J.D. and Berner, B., Electrochemical and iontophoretic studies in human skin. *J. Controlled Rel.*, 11 (1990) 105–112.

Ghanem, A.H., Mahmoud, H., Seta, Y., Higuchi, W.I., Kurihara-Bergstrom, T. and Good, W.R., Hairless mouse skin vs. human skin: Analysis via the parallel lipid pathway-pore pathway model. *Pharm. Res.*, 6 (1989) 112S.

Glaser, R.W., Leikin, S.L., Chernomordik, L.V., Patushenko, V.F. and Sokirko, A.I., Reversible electrical breakdown of lipid bilayers: formation and evolution of pores. *Biochim. Biophys. Acta*, 940 (1988) 275–287.

Goldman, D.E., Potential, impedance and rectification in membranes. *J. Gen. Physiol.*, 27 (1943) 37–60.

Hiemenz, P.C., *Principles of Colloid and Surface Chemistry*, Dekker, New York, 1986.

Hunter, R.J., *Zeta Potential in Colloid Science, Principles and Applications*, Academic Press, San Diego, 1981.

Kasting, G.B. and Bowman, L.A., DC electrical properties of frozen, excised human skin. *Pharm. Res.*, 7 (1990) 134–143.

Keister, J.C. and Kasting, G.B., Ionic mass transport through a homogeneous membrane in the presence of a uniform electric field. *J. Membr. Sci.*, 29 (1986) 155–167.

Kinosita, K. and Tsong, T.Y., Voltage-induced pore formation and hemolysis of human erythrocytes. *Biochim. Biophys. Acta*, 471 (1977) 227–242.

Masada, T., Higuchi, W.I., Srinivasan, V., Rohr, U., Fox, J., Behl, C. and Pons, S., Examination of iontophoretic transport of ionic drugs across skin: baseline studies with the four-electrode system. *Int. J. Pharm.*, 49 (1989) 57–62.

Mathot, R., Srinivasan, V., Higuchi, W.I. and Sims, S.M., A model iontophoresis system for basic studies using Nuclepore membranes. *Proceedings of the 16th International Symposium on Controlled Release of Bioactive Materials*, 52 (1989).

Pikal, M.J. and Shah, S., Transport mechanisms in iontophoresis. III. An experimental study of the contributions of electroosmotic flow and permeability change in transport of low and high molecular weight solutes. *Pharm. Res.*, 7 (1990) 222–229.

Schultz, S.G., *Basic Principles of Membrane Transport*, Cambridge University Press, New York, 1980, pp. 21–29, 42–44.

Serpersu, E.H., Kinosita, D. and Tsong, T.Y., Reversible and irreversible modification of erythrocyte membrane permeability by electric field. *Biochim. Biophys. Acta*, 812 (1985) 779–785.

Sims, S.M. and Higuchi, W.I., Baseline studies on iontophoretic transport in hairless mouse skin: The effect of applied voltage drop and pH on the iontophoresis of a model weak electrolyte. *J. Membr. Sci.*, 49 (1990) 305–320.

Sims, S.M., Higuchi, W.I. and Srinivasan, V., Interaction of electric field and electro-osmotic effects in determining iontophoretic enhancement of anions and cations. *Int. J. Pharm.*, (1990a) submitted for publication.

Sims, S.M., Higuchi, W.I. and Srinivasan, V., Skin alteration and convective solvent flow effects during iontophoresis II. Monovalent anion and cation transport across human skin. *Pharm. Res.*, (1990b) submitted.

Smoluchowski, M., In Graetz, B. (Ed.), *Handbuch der Elektrizität und des Magnetismus*, Vol. 2, Leipzig, Germany, 1921, p. 366.

Srinivasan, V. and Higuchi, W.I., A model for iontophoresis incorporating the effect of convective solvent flow. *Int. J. Pharm.*, 60 (1990) 133–138.

Srinivasan, V., Higuchi, W.I. and Su, M., Baseline studies with the four electrode system: I. The effect of skin damage and water transport on the iontophoresis of a model uncharged solute. *J. Controlled. Rel.*, 10 (1989a) 157–165.

Srinivasan, V., Higuchi, W.I., Sims, S.M., Ghanem, A.H. and Behl, C.R., Transdermal iontophoresis drug delivery: Mechanistic analysis and application to polypeptide delivery. *J. Pharm. Sci.*, 78 (1989b) 370–375.

Srinivasan, V., Sims, S.M., Higuchi, W.I., Behl, C.R. and Pons, S., Iontophoretic transport of drugs: A constant voltage approach. In Kost, J. (Ed.), *Pulsed and Self Regulated Drug Delivery*, CRC Press, Boca Raton, FL, 1990.

Sugar, I.P., A theory of the electric field-induced phase transition of phospholipid bilayers. *Biochim. Biophys. Acta*, 556 (1979) 72–85.

Teissie, J. and Tsong, T.Y., Electric Field induced transient pores in phospholipid bilayer vesicles. *Biochemistry*, 20 (1981) 1548–1554.

Zimmermann, U. and Vienken, J., Electric field-induced cell-to-cell fusion. *J. Membr. Biol.*, 67 (1982) 165–182.