Skin Alteration and Convective Solvent Flow Effects During Iontophoresis II. Monovalent Anion and Cation Transport Across Human Skin

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Total 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 as a result of applied field may also contribute to flux enhancement. The modified Nernst-Planck theory includes a solvent flow velocity term and predicts that the flux of uncharged molecules is enhanced or retarded depending on the polarity of the applied field. Polarity-dependent solvent flow velocity, as measured by the flux enhancement of mannitol, has been demonstrated in human epidermal membrane during iontophoresis. In the present study, the solvent flow velocity effects on the flux enhancement of a model cation (tetraethylammonium ion) and a model anion (salicylate ion) across human epidermal membrane were examined. The contribution of membrane alterations, due to the applied field, on overall ion flux was also considered. Solvent flow was found to have a small effect on the flux enhancement of both ions. However, membrane alterations were found to increase greatly the flux of the ionic species. Alterations in the epidermal membrane occurred at the highest voltage investigated (1000 mV) and appeared to reverse over time as indicated by the current and transport data.

KEY WORDS: iontophoresis; electroosmosis; human skin; solvent flow; Nernst-Planck theory.

INTRODUCTION

Iontophoresis is the process of increasing the flux of an ion across a tissue by the application of an external electric field (1–3). Recent efforts have focused on determining the physical chemical parameters governing ion transport during iontophoresis. In particular, the Nernst-Planck equation has been used as a starting point to model ion transport under the influence of an external electrochemical potential gradient (1,3,4). Modifications of the Nernst-Planck theory to incorporate a convective solvent flow velocity term (5,6) have enabled an evaluation of the current induced solvent flow contribution to total flux. This model predicts that there will be an asymmetry in the enhancements of cations and anions and that uncharged molecules will be enhanced or retarded depending on the polarity of the applied electric field.

Experimental iontophoresis studies, in which glucose or mannitol was used as a model neutral solute, have demonstrated flux enhancement in the anode to cathode polarity and flux inhibition in the cathode to anode polarity with hairless mouse skin (4,7), Nuclepore membranes (6,11), and human epidermal membranes (8). The data from these experiments were used to deduce convective solvent flow and the flux enhancement factors for the tetraethylammonium ion and the salicylate ion were predicted for the Nuclepore membrane using the modified Nernst-Planck theory. These predictions were compared to the experimental results: The agreement between experiment and prediction was very good when the electrical double layer thickness, $1/\kappa$, was small compared to the pore radius, r_o. Studies in which mannitol was used as a measure of the solvent flow velocity through human epidermal membrane (8) demonstrated that similar polarity-dependent solvent flow occurred in human skin under an applied electric field. Membrane alterations were also found to occur during iontophoresis at 1000 mV in these experiments; these membrane changes reversed over time once the voltage drop was removed.

In the present study, the contribution of convective solvent flow to the total flux enhancement of a monovalent anion and cation across human epidermal membrane is examined. Mannitol has been used to deduce the solvent flow (8) contribution to the flux enhancement of the tetraethylammonium ion (cation) and the salicylate ion (anion). Membrane alterations due to the applied electric field have also been assessed via transport experiments and current measurements.

MODIFIED NERNST-PLANCK THEORY

All enhancement factor, E, defined as the ratio of iontophoretic flux at an applied voltage, $\Delta\Psi$, across the membrane to the passive flux, can be obtained for cations (E_+) and anions (E_-) (5):

$$E_+ = -K[1 - (\mathrm{Pe}/K)]/\{1 - \exp[K(1 - \mathrm{Pe}/K)]\} \quad (1)$$

$$E_- = -K[1 + (\mathrm{Pe}/K)]/\{1 - \exp[K(1 + \mathrm{Pe}/K)]\} \quad (2)$$

where

$$K = (zF\Delta\Psi)/(RT) \quad (3)$$

$$\mathrm{Pe} = (v\Delta x)/D \quad (4)$$

z is the valency of the permeant, F is the Faraday constant, R the gas constant, T the absolute temperature, v the average solvent velocity, Δx the membrane thickness, and D the diffusion coefficient. Equations (1) and (2) give iontophoretic flux enhancements due to both a direct field (Nernst-Planck) effect and a solvent flow effect. The Peclet number (Pe) characterizes the effect of convective solvent flow on the flux of the permeant, while K involves the direct field effects. Equation (1) for cation enhancement reflects convective flow from anode to cathode. Similarly, Eq. (2) for anion enhancement reflects flow from cathode to anode. Predictions from Eqs. (1) and (2) are consistent with published results for cations and anions (6,11,12).

Assuming that the direct electric field effect applies only for charged permeants, any enhancement in the flux of un-

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charged solutes can be assumed to be due to convective flow only. The enhancement factor (due only to the solvent flow) can be obtained by letting $K = 0$ (i.e., $z = 0$) in Eqs. (1) and (2) (5):

$$\text{anode to cathode: } E = \text{Pe}/[1 - \exp(-\text{Pe})] \quad (5)$$

$$\text{cathode to anode: } E = -\text{Pe}/[1 - \exp(\text{Pe})] \quad (6)$$

Equation (5) predicts enhancement factors greater than 1 for the anode to cathode polarity, while Eq. (6) predicts $E < 1$ for the cathode to anode polarity. This is consistent with published experimental enhancement values (6–8,11).

If a charged solute and a neutral probe solute have approximately the same diffusion coefficient and if the electrical double layer thickness ($1/\kappa$) is small compared to pore dimensions, then under the same experimental conditions, the Peclet number for the two solutes can be assumed to be equal. From the experimentally determined enhancement factor for the charged solute and the Peclet number determined using the neutral solute, the asymmetry in cation and anion enhancement may be assessed.

MATERIALS AND METHODS

Materials

All permeants were obtained from New England Nuclear (Boston, MA): [1-^{14}C]mannitol (sp act, 55.0 mCi/mmol), [7-^{14}C]salicylic acid (sp act, 56.1 mCi/mmol), and [1-^{14}C]tetraethylammonium bromide (sp act, 3.0 mCi/mmol). Phosphate-buffered saline (PBS), ionic strength 0.1 *M*, pH 7.5, was used in all experiments (6). 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). The epidermal membrane, which includes the stratum corneum and part of the epidermis, was heat separated from the dermis as described previously (8).

Methods

All experiments were conducted with the four-electrode potentiostat system (JAS Instrumental Systems, Inc., Salt Lake City, UT) which has been described previously (1). The experimental protocol involved four stages (8). 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 them 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, a second passive transport run was conducted (during the 8 hr directly after removing the voltage drop). Finally, in Stage IV, a third passive run was conducted (during the 16- to 22-hr period after termination of the voltage).

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. Permeability coefficients, *P*, were calculated for each stage from the slope of the cumulative disintegrations per minute (dpm) transported into the receiver chamber over time, as described previously (8).

In some experiments the current was periodically measured at an applied voltage drop of 250 mV 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 drop was applied across the membrane for a very brief time; it was assumed that this applied voltage of 250 mV did not cause any significant membrane alterations.

RESULTS AND DISCUSSION

Skin Selection Criteria

As in previous studies employing human epidermal membrane (8), there was considerable variability in the subsequently obtained passive permeability coefficients of the tetraethylammonium ion (TEA^+) and the salicylate ion: The passive *P* value of TEA^+ ranged from 1.4×10^{-8} to 2.3×10^{-7} cm/sec. A passive *P* value of the order of 10^{-8} cm/sec was considered to be representative of undamaged human epidermal membrane (13). This criterion seems to be consistent with a recent report by Kasting and Bowman (14).

Experimental Results at 250 mV

In previous work with human epidermal membranes (6), membrane alteration effects were observed to occur at 1000 mV but not at 250 mV. Therefore, the results of the TEAB and salicylate transport experiments at 250-mV runs are presented first, followed by the results of the 1000-mV experiments.

Typical results of transport experiments in which 250 mV was applied during Stage II are shown in Fig. 1 for TEAB and in Fig. 2 for salicylate. As can be seen, the data plots in each case are essentially linear and the permeability coefficients were calculated for all experiments from the best-fit slope for each stage. These *P* values are presented in Table I.

Comparison of the TEAB Stage II *P* values with the passive *P* values (Stage I, III, or IV) shows an average flux enhancement of about 10 (±5) due to the applied field, except in run 2-T. The passive *P* value in Stage I of run 2-T was considered acceptable, as was the current profile obtained in Stage II (see below); therefore, for no apparent reason, this run appears to be an outlier from the remaining experiments (Table I). The Nernst-Planck equation (without solvent flow effects) predicts an enhancement factor of 9.4 at 250 mV for a monovalent ion (1,4). If solvent flow effects were important at 250 mV, then the TEAB flux would be increased over and above the increase caused by the applied electric field since the solvent flow would be in the same direction as the field (anode to cathode in a net negatively charged, porous, membrane). The TEAB results indicate that solvent flow is a secondary effect at the low applied voltage.

Similar comparison of the Stage II *P* values of salicylate to the passive *P* values for each run indicates an average enhancement of the salicylate flux of about 6 (±3) due to the

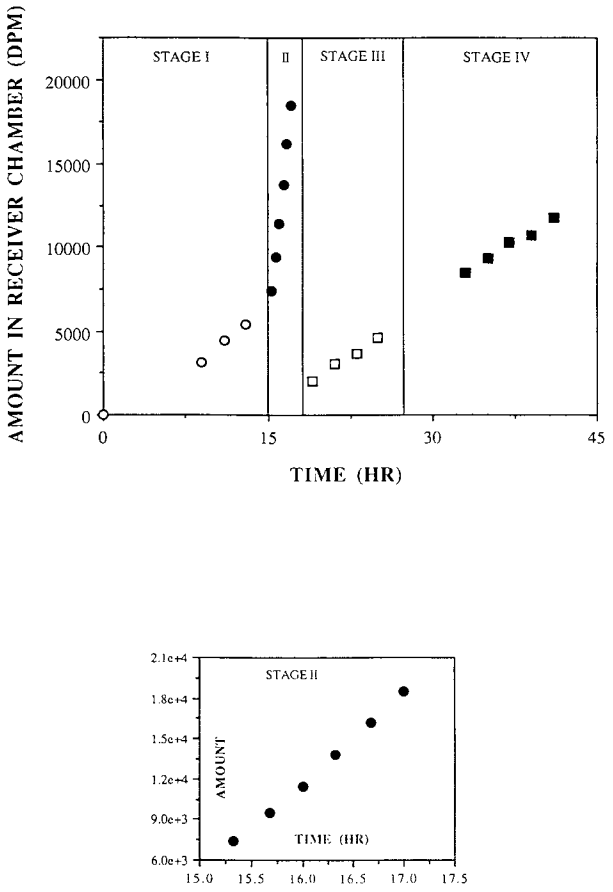

Fig. 1. Typical results of a TEAB experiment in which 250 mV was applied during Stage II (run 4-T). (○) Stage I; (●) Stage II; (□) Stage III; (■) Stage IV. Inset is an enlargement of Stage II.

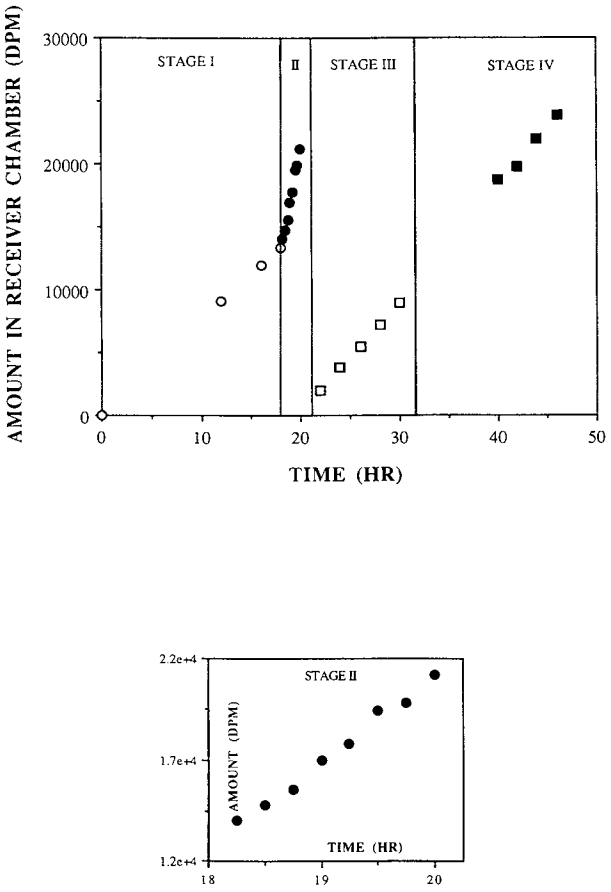

Fig. 2. Typical results of a salicylate experiment in which 250 mV was applied during Stage II (run 3-S). (○) Stage I; (●) Stage II; (□) Stage III; (■) Stage IV. Inset is an enlargement of Stage II.

applied field (versus 9.4 predicted by the Nernst-Planck equation). Solvent flow would oppose the electric field (cathode to anode polarity) in the salicylate runs. Thus, the small negative deviation from the Nernst-Planck prediction for the salicylate runs again suggests that solvent flow effects are secondary to the direct electric field effects.

Membrane alteration was assessed during the low-voltage runs by determining the passive permeability coefficient before (Stage I) and after (Stages III and IV) application of a voltage drop in Stage II. These passive P values for both TEAB and salicylate were, in general, found to be comparable for a given experiment, indicating that there were few or no irreversible changes taking place during these experiments. Additionally, the currents monitored during Stage II remained essentially constant, as shown in Fig. 3. This constant-current behavior is similar to that reported with the Nuclepore membrane (6) and human epidermal membrane (8) at low voltages and supports the idea of an unchanging membrane. Thus, both the transport data and the current data suggest that the epidermal membrane is not significantly altered by an applied field of 250 mV. Results reported (14) using a low constant current (corresponding to low voltages) seem to support the findings of this study. These authors found "little evidence for the production of irreversible or slowly reversible changes in the skin" at current values between 1 and ±10 μA.

Experimental Results at 1000 mV

Results of representative TEAB and salicylate transport experiments in which 1000 mV was applied in Stage II are shown in Figs. 4 and 5, respectively. As can be seen, the slopes in Stage I, III, and IV are linear, and thus, the P value

Table I. Individual TEAB and Salicylate Experimental Permeability Coefficients Determined for Runs in Which 250 mV Was Applied in Stage II

Run no.	Permeability coefficient, P ($\times 10^7$ cm/sec)			
	Stage I	Stage II	Stage III	Stage IV
	A. Tetraethylammonium bromide (TEAB)			
1-T	0.38	2.2	0.27	0.32
2-T	0.19	0.24	0.15	0.14
3-T	10	89	6.6	6.4
4-T	1.5	23	1.2	1.3
5-T	0.38	3.3	0.68	0.67
6-T	2.3	12	1.8	3.6
	B. Salicylic acid			
1-S	3.0	8.6	3.1	2.3
2-S	0.93	7.2	0.92	1.1
3-S	1.9	13	2.6	1.9

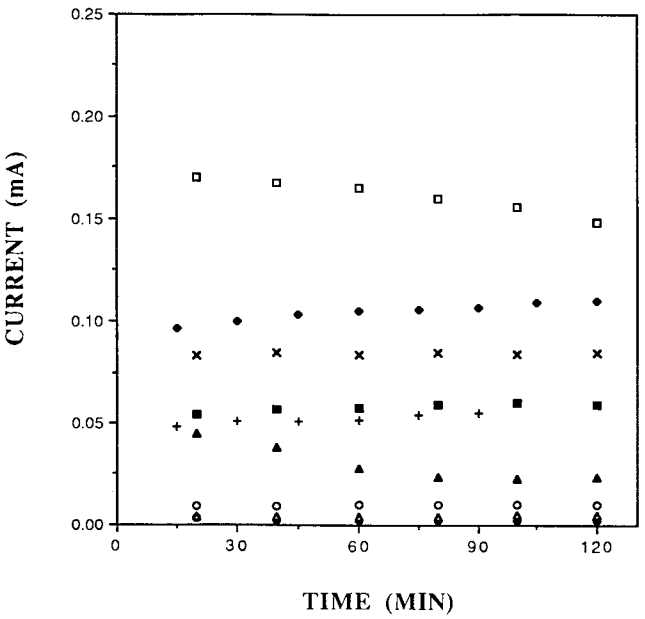

Fig. 3. Current profiles for individual experiments at 250 mV. Salicylate runs: (×) 1-S; (+) 2-S; (♦) 3-S. TEAB runs: (○) 1-T; (●) 2-T; (□) 3-T; (■) 4-T; (△) 5-T; (▲) 6-T.

was easily calculated from the best-fit slope in each period. Experimental protocol precluded obtaining data points during early times, and thus there were larger uncertainties in the Stage I passive P values. These P values are shown in Columns 2, 5, and 6 in Table II for each experiment.

In most of the 1000-mV runs, the amount of permeant (TEAB and salicylate) transported across the membrane into the receiver chamber during Stage II increased nonlinearly (Figs. 4 and 5). As in previous work using human epidermal membranes (8), this nonlinearity suggested that membrane alterations were taking place during the run. For this situation, 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. The Stage II P values are presented in Columns 3 and 4 in Table II for both TEAB and salicylate. It is clearly shown that the $P_{\Delta\Psi,f}$ value increased over the respective $P_{\Delta\Psi,i}$ value in each run. The increasing $P_{\Delta\Psi}$ value is indicative of membrane changes having occurred during the application of 1000 mV.

Figure 6 shows the current profiles during Stage II for the runs at 1000 mV. These data may be considered in two groups: runs in which the current was relatively stable, i.e., less than twofold changes (runs 7-T, 8-T, 4-S, and 5-S); and runs in which there were more than twofold changes in the current (runs 6-, 7-, and 8-S and 9-, 10-, and 11-T). The small changes in current during Stage II in the first group are reflected by the Stage II P values in Table II; TEAB runs (7- and 8-T) show a 70–80% increase in the current and about a 40% increase in the $P_{\Delta\Psi,f}$ value over the respective $P_{\Delta\Psi,i}$ value, while the salicylate runs (4- and 5-S), which have current changes of about twofold, show a similar increase in $P_{\Delta\Psi,f}$ over $P_{\Delta\Psi,i}$. The fact that these skin samples have relatively stable current profiles may indicate either a slowly changing membrane, a membrane which does not change, or a membrane which was rapidly altered at the outset of Stage II (prior to the first sample) by the applied field; once in the altered state, the membrane may then continue to change slowly over the remaining time course of Stage II.

The second group of experiments, in which there was a greater than twofold difference in the current, showed much larger increases (than in the first group) in $P_{\Delta\Psi,f}$ over $P_{\Delta\Psi,i}$ (Table II), with the greatest difference occurring in run 8-S: a 13-fold difference in the Stage II P values and about a 5- to 10-fold difference in the current. From the current and transport data, it appears that there were more extensive alterations during Stage II in these experiments.

In previous experiments using mannitol (8), the initial Stage II current of the 1000-mV runs (divided by 4) were found to correlate well with the Stage I passive P values. This was interpreted to mean that the membrane alterations did not occur rapidly but, instead, occurred continuously over the entire Stage II period. In the present work, the current was periodically measured at an applied voltage of 250 mV during Stages I, III, and IV of four salicylate runs. These current data are shown in Table III and are plotted along with their respective passive P values (Table II) in Fig. 7. The solid line in Fig. 7 is the best-fit line from mannitol transport experiments reported earlier (8). The same line appears to describe the present data also. The data scatter is

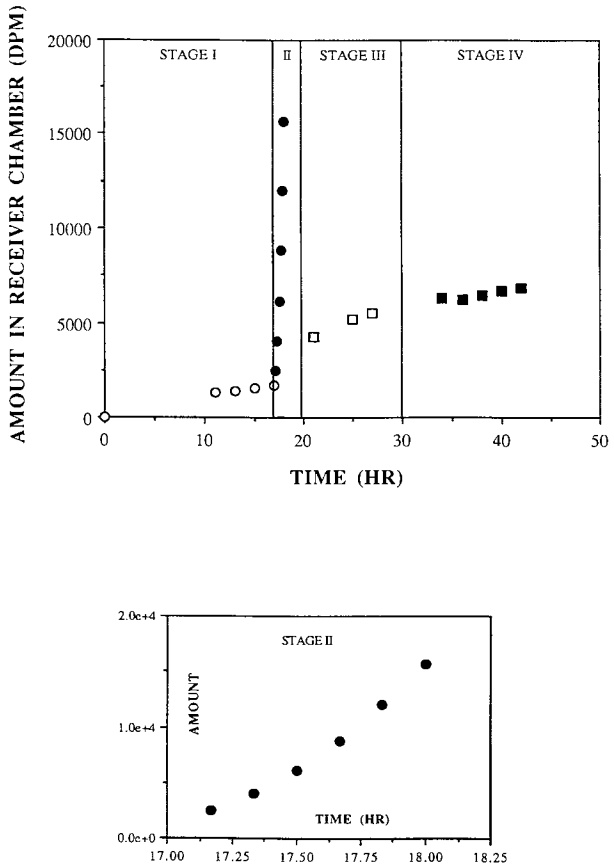

Fig. 4. Results of a representative TEAB experiment in which 1000 mV was applied during Stage II (run 10-T). (○) Stage I; (●) Stage II; (□) Stage III; (■) Stage IV. Inset is an enlargement of Stage II.

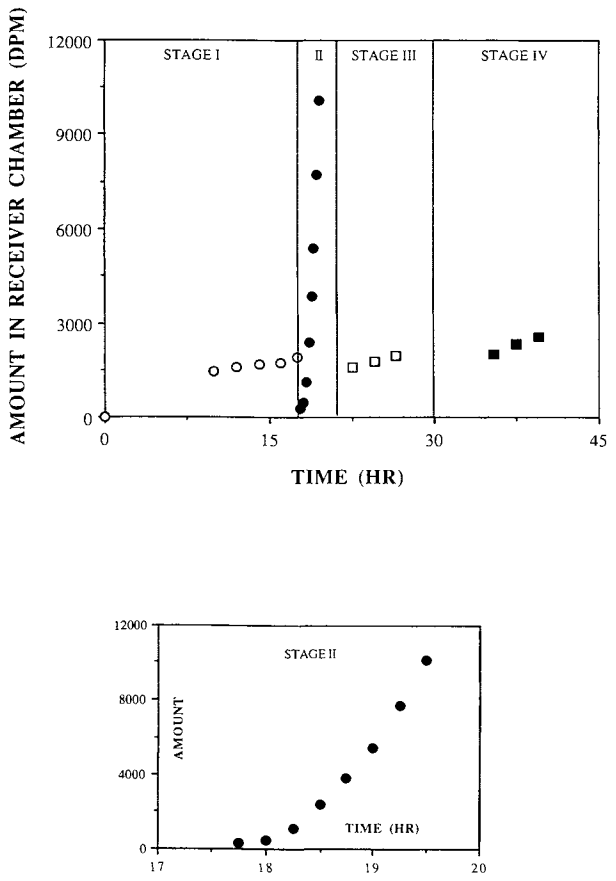

Fig. 5. Results of a representative salicylate experiment in which 1000 mV was applied during Stage II (run 8-S). (○) Stage I; (●) Stage II; (□) Stage III; (■) Stage IV. Inset is an enlargement of Stage II.

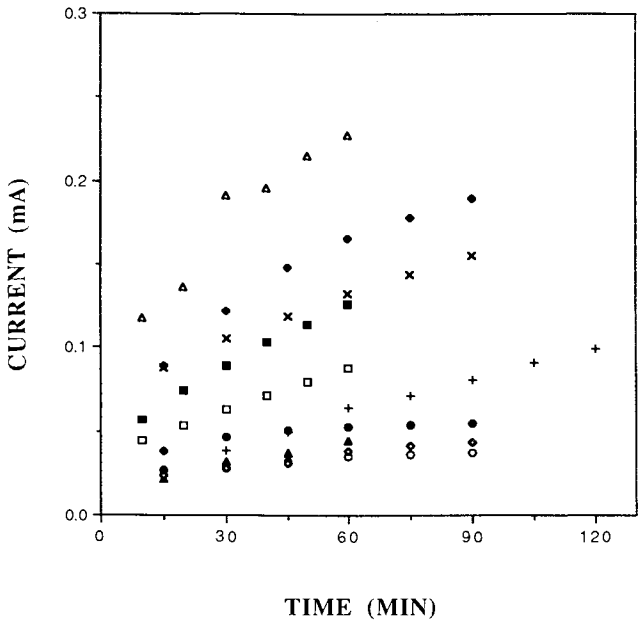

Fig. 6. Current profiles for individual experiments at 1000 mV. Salicylate runs: (▲) 4-S; (◇) 5-S; (◆) 6-S; (×) 7-S; (+) 8-S. TEAB runs: (○) 7-T; (●) 8-T; (□) 9-T; (■) 10-T; (△) 11-T.

believed to be due primarily to the error in the permeability coefficient determination. In Fig. 8, it is shown that the initial currents (measured 10–15 min after the start of Stage II and divided by 4) during the remaining runs at 1000 mV also fall on or near the best-fit line when plotted against their respective P values (Stage I), as do the data from studies with Nuclepore membranes (6). These results indicate that the membrane is not rapidly altered at the beginning of Stage II (at 1000 mV) but continues to change throughout the period.

Not shown in Fig. 6 is the current at time zero, i.e., when the voltage was first applied. It is evident, however, that a rapid increase in current occurred within the first 30 min of Stage II in many of the runs, suggesting that significant membrane changes occurred during this time period, in these instances. It is from the data within this time frame that the initial Stage II P value was calculated; thus, while it was thought that the $P_{\Delta\Psi,i}$ value might reflect a relatively unaltered membrane, it is clear that some membrane alteration effects were included in the $P_{\Delta\Psi,i}$ values.

Table II. Individual TEAB and Salicylate Experimental Permeability Coefficients Determined for Runs in Which 1000 mV Was Applied in Stage II

Run no.	Permeability coefficient, P ($\times 10^8$ cm/sec)				
	Stage I	$P_{\Delta\Psi,i}$	$P_{\Delta\Psi,f}$	Stage III	Stage IV
	A. Tetraethylammonium bromide (TEAB)				
7-T	4.6	150	190	4.3	4.6
8-T	4.8	230	320	4.2	4.8
9-T	1.4	290	440	12	1.8
10-T	2.2	290	620	6.5	2.9
11-T	9.8	610	1200	19	6.3
	B. Salicylic acid				
4-S	0.70	8.4	26	0.91	1.0
5-S	2.0	27	65	1.6	0.82
6-S	2.0	120	290	4.3	3.4
7-S	3.3	170	270	2.1	5.1
8-S	0.95	12	160	1.5	3.0

Reversibility of Membrane Alterations

The large changes seen during Stage II were not reflected in passive P values determined at long times after Stage II. In Table II, it can be seen that the Stage I and Stage IV passive P values are nearly the same in most experiments. The Stage III P value appears to reflect partly the

Table III. Current Measured at 250 mV During Stages I, III, and IV for Four Salicylate Experiments in Which 1000 mV Was Applied During Stage II

Stage	Current (mA)			
	Run 5-S	Run 6-S	Run 7-S	Run 8-S
I	0.0023	0.0078	0.0083	0.0023
III, initial	0.0083	0.041	0.039	0.022
III, middle	0.0026	0.017	0.014	0.0077
IV	0.0027	0.013	0.012	0.0056

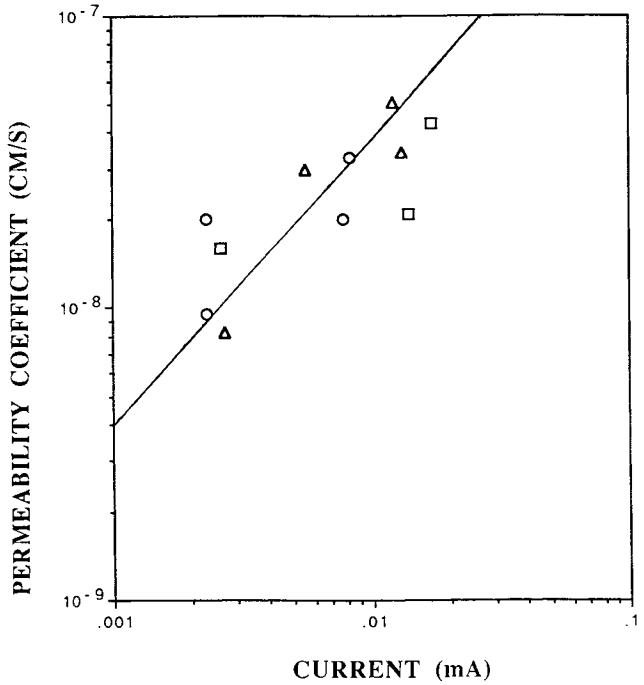

Fig. 7. Passive permeability coefficients of salicylate plotted against the current measured at 250 mV in each stage. (○) Stage I; (□) Stage III; (△) Stage IV. The solid line is the best-fit line from similar data using mannitol (8).

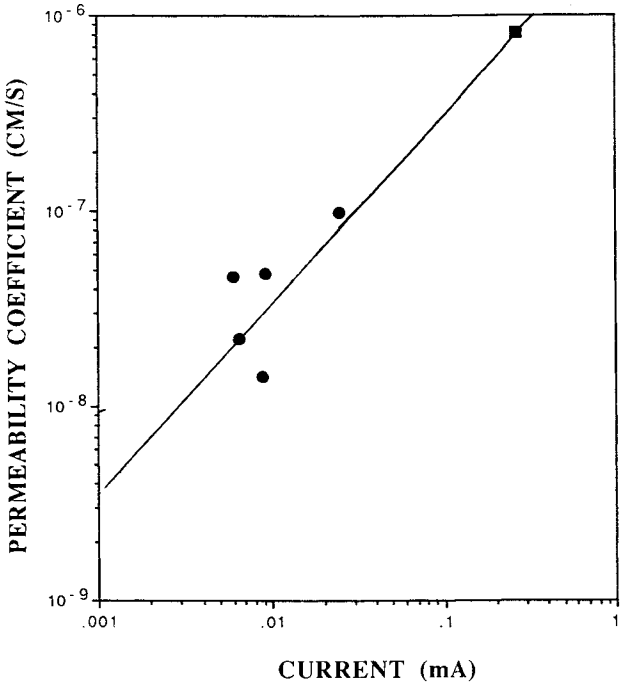

Fig. 8. Stage I passive permeability coefficients plotted against the initially measured current at 1000 mV (divided by 4) and compared to the best-fit curve from Fig. 7. (●) TEAB–human skin runs; (■) example of similar data from Nuclepore membrane studies (6).

large changes observed during Stage II, but in many cases, the Stage III P values are similar to those calculated for Stage I. Reversibility of electric field effects on human skin has been reported (8,15), and the present results support the view that alterations occurring during Stage II are reversible in 16–24 hr.

Current measurements (at 250 mV) during Stages III and IV provide additional insights into the recovery kinetics after membrane alterations have been induced in Stage II. The current values taken at different times during Stages III and IV are shown in Table III for four experiments. In these runs, the current showed a marked increase (over the baseline Stage I current) when measured just after Stage II. This is believed to be due to the membranes being in a perturbed state just after Stage II. The current decreased during Stage III in all experiments and was nearly back to its initial value by Stage IV. Thus, it appears that the membrane alterations reverse over time once the voltage drop is removed. The process of reversing occurred rapidly in some membranes (run 5-S), in which case the Stage III current is close to that measured in Stage I and the passive P values of Stages I and III are comparable (Table II).

Electroosmotic Flow Through Human Skin

In a previous study, mannitol was used as a measure of the solvent flow velocity across human epidermal membrane during iontophoresis (8). In this study, an enhancement factor for the transport of mannitol was calculated by taking the ratio of the Stage II permeability coefficient to the passive P value determined in Stage IV. The Peclet number (Pe), which embodies the solvent flow velocity [see Eq. (4)], was then determined using Eqs. (5) and (6). Assuming that the Pe value calculated from the mannitol transport enhancement is essentially the same as that for TEA^+ and the salicylate ion [this is believed to be a reasonably good assumption: at high ionic strengths, TEA^+, salicylate, and mannitol all have comparable passive permeability coefficients in porous membrane transport experiments (6)], enhancement factors may be predicted using Eqs. (1) and (2) for TEA^+ and salicylate ion, respectively.

Enhancement factors for TEAB and salicylate were determined by dividing the Stage II P value (at 250 mV) or the initial Stage II $P_{\Delta\Psi,i}$ value (at 1000 mV) by the passive Stage IV P value and these are presented in Table IV. The intrinsic passive P value for a solute in stratum corneum is measured by the Stage I P value. As shown in Tables I and II, the Stage IV permeability coefficient has returned to the intrinsic value. Because it was felt that the uncertainties in the slope determination for Stage I P values were generally greater

Table IV. TEAB and Salicylate Enhancement Factors

Applied potential (mV)	No. of expts.	Enhancement factor, E^a
A. Tetraethylammonium bromide (TEAB)		
250	6	8.1 ± 6.3
1000	5	88 ± 50
B. Salicylate		
250	3	5.7 ± 1.7
1000	5	23 ± 15

[a] Mean ± SD.

than those for Stage IV, the Stage IV P value was chosen as the reference passive P value for the calculation of enhancement. Also, initially it was thought that, by using the early time data from Stage II, the E values would not include the effects of membrane alteration. However, during that time, as noted above, the membrane appeared to have been changing somewhat.

In Fig. 9, the E values from Table IV are plotted against the predicted curves from Eqs. (1) and (2). As can be seen, the E values at 250 mV are close to the predicted values; however, the 1000-mV data show significant deviations. These deviations are believed to be due, at least in part, to the use of $P_{\Delta\Psi,i}$ values which insufficiently minimized the effects of membrane alterations in the calculation of E. In order to explore this question further, average E values were calculated from experiments in which the Stages I and IV P values were very close and in which the current remained relatively low and stable during Stage II (see Figs. 3 and 6). These E values are plotted against the predictions of Eqs. (1) and (2) in Fig. 10. Although the variability is high, it appears that if membrane alteration effects could be eliminated as a factor, the agreement between experiment and theory would be quite good.

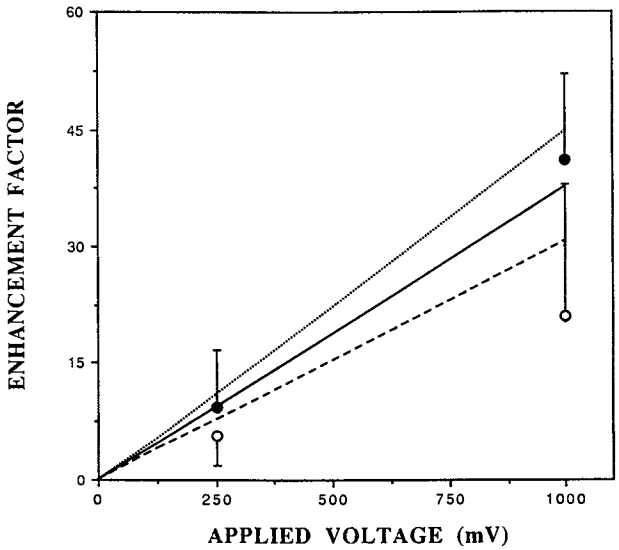

Fig. 10. TEAB (•) and salicylate (○) enhancement factors calculated from the experiments exhibiting the least amount of membrane alteration in the initial phase of Stage II. (· · · ·) predicted enhancement from Eq. (1) for monovalent cations; (- - -) predicted enhancement from Eq. (2) for monovalent anions. The solid line is the Nernst-Planck prediction without solvent flow.

CONCLUSIONS

Continuous membrane alteration occurs during *in vitro* iontophoresis of human epidermal membranes at high applied voltages (1000 mV) but does not occur when 250 mV is applied across the membrane. These changes appear to reverse over time once the voltage drop is removed. The membrane alterations caused an additional increase in the enhancement factors for the transport of ionic species over that expected from the combined applied field and solvent flow contributions. This phenomenon may be important for the delivery, by iontophoresis, of both charged and uncharged drug molecules.

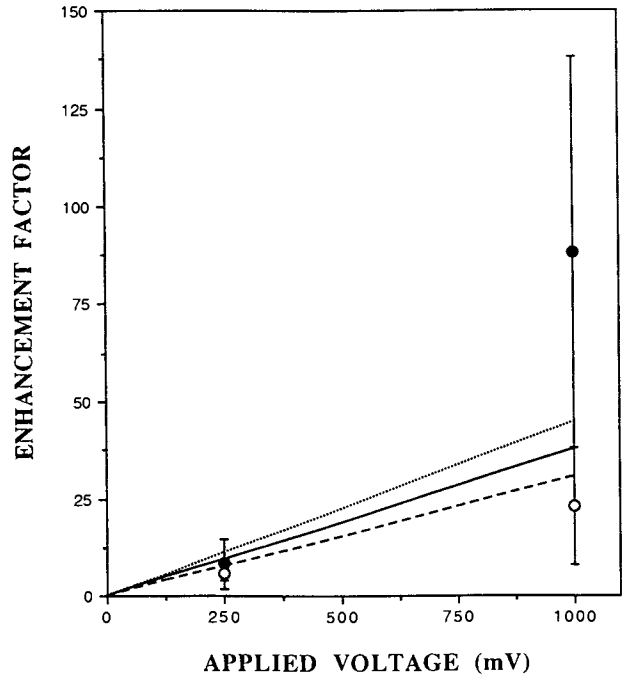

Fig. 9. TEAB (•) and salicylate (○) enhancement factors compared to the predicted curves from Eq. (2) for cations (· · · ·) and Eq. (3) for anions (- - -). The solid curve is the Nernst-Planck prediction without solvent flow. Mannitol flux was used as a measure of solvent flow (Pe) in Eqs. (1) and (2).

Solvent flow, as measured by an increase in mannitol flux, occurs in human epidermal membrane during the application of an electric field. The effect on ion transport is relatively small compared to the direct electric field effects. However, coupled with membrane alteration, the solvent flow effects may become significant.

The significance of this work lies in the determination of the underlying mechanisms of ion transport across human epidermis under the influence of an applied electric field. These *in vitro* results demonstrate that the overall flux of an ion during iontophoresis is due to direct field and electroosmotic effects, as well as membrane alterations by the field. The membrane alterations are reversible over time after the applied electric field is turned off. These results also describe the interaction among the solute charge, the membrane charge, and the polarity of the electric field. Thus, these results should be considered in the design of human *in vivo* iontophoresis studies and in future *in vitro* iontophoresis studies investigating transport mechanisms.

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NOMENCLATURE

D	Diffusion coefficient
z	Valence charge of permeant
F	Faraday constant
R	Gas constant
T	Absolute temperature
Ψ	Electric potential at any point x in the membrane
Δx	Membrane thickness
$\Delta\Psi$	Applied voltage drop
v	Average solvent velocity
E	Enhancement factor
E_+	Enhancement factor for a cation
E_-	Enhancement factor for an anion
K	Dimensionless constant
Pe	Peclet number
P	Permeability coefficient

REFERENCES

1. V. Srinivasan, S. M. Sims, W. I. Higuchi, C. R. Behl, and S. Pons. Iontophoretic transport of drugs: A constant voltage approach. In J. Kost (ed.), *Pulsed and Self Regulated Drug Delivery*, CRC Press, Boca Raton, FL, 1990, pp. 65–89.
2. R. R. Burnette and D. Marrero. Comparison between the iontophoretic and passive transport of thyrotropin releasing hormone across excised nude mouse skin. *J. Pharm. Sci.* 75:738–743 (1986).
3. J. C. Keister and G. B. Kasting. Ionic mass transport through a homogeneous membrane in the presence of a uniform electric field. *J. Membr. Sci.* 29:155–167 (1986).
4. S. M. Sims and W. I. Higuchi. 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.* 49:305–320 (1986).
5. V. Srinivasan and W. I. Higuchi. A model for iontophoresis incorporating the effect of convective solvent flow. *Int. J. Pharm.* 60:133–138 (1990).
6. S. M. Sims, W. I. Higuchi, and V. Srinivasan. Interaction of electric field and electro-osmotic effects in determining iontophoretic enhancement of anions and cations. *Int. J. Pharm.* 77: 107–118 (1991).
7. V. Srinivasan, W. I. Higuchi, and M. Su. 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. Control. Release* 10:157–165 (1989).
8. S. M. Sims, W. I. Higuchi, and V. Srinivasan. Skin alteration and convective solvent flow effects during iontophoresis. I. Neutral solute transport across human skin. *Int. J. Pharm.* 69:109–121 (1991).
9. D. E. Goldman. Potential, impedence and rectification in membranes. *J. Gen. Physiol.* 27:37–60 (1943).
10. S. G. Schultz. *Basic Principles of Membrane Transport*, Cambridge University Press, New York, 1980, pp. 21–29, 42–44.
11. R. Mathot, V. Srinivasan, W. I. Higuchi, and S. M. Sims. A model iontophoresis system for basic studies using Nuclepore membranes. *Proceedings of the 16th International Symposium on Controlled Release of Bioactive Materials*, 1989, p. 52.
12. V. Srinivasan, W. I. Higuchi, S. M. Sims, A. H. Ghanem, and C. R. Behl. Transdermal iontophoretic drug delivery: Mechanistic analysis and application to polypeptide delivery. *J. Pharm. Sci.* 78:370–375 (1989).
13. A. H. Ghanem, H. Mahmoud, Y. Seta, W. I. Higuchi, T. Kurihara-Bergstrom, and W. R. Good. Hairless mouse skin vs. human skin: Analysis via the parallel lipid pathway-pore pathway model. *Pharm. Res.* 6:112S (1989).
14. G. B. Kasting and L. A. Bowman. DC electrical properties of frozen, excised human skin. *Pharm. Res.* 7:134–143 (1990).
15. J. D. DeNuzzio and B. Berner. Electrochemical and iontophoretic studies in human skin. *J. Control. Release* 11:105–112 (1990).