Convective Solvent Flow Across the Skin During Iontophoresis

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Enhanced flux of neutral solutes during transdermal iontophoresis is attributed largely to electroosmotic volume flow. In this study, the iontophoretic fluxes of tritiated water (^3H_2O) and ^{14}C-labeled mannitol through hairless mouse skin (HMS) were determined. The following questions were addressed: (i) What is the variability of water flux during iontophoresis? (ii) To what extent is the iontophoretic flux of a neutral solute correlated with water flux? (iii) Does the intrinsic permeability of the skin to neutral solutes change following iontophoresis? (iv) What is the effect of low pH on electroosmotic volume flow? and (v) Does the skin remain permselective after removal of the stratum corneum? Transport of both water and mannitol reached steady-state levels during 10 hr of constant-current iontophoresis (0.36 mA/cm^2). Anodal fluxes exceeded cathodal values. Cathodal mannitol flux was retarded, relative to passive transport, by net volume flow in the opposite direction, such that transport of this molecule increased significantly after the termination of current passage. Anodal equivalent volume flows for water and mannitol, respectively, were 2.7 (±1.3) and 1.23 (±0.59) μL/hr cm^2, indicating that only ~50% of the water flux participated in the electroosmosis of mannitol. The passive permeability of water and mannitol after 10 hr of iontophoresis was, respectively, 6 and 30 times greater than the pretreatment values. At pH 7, the cationic permselectivity of HMS was marginal [the Na^+ transport number (t_{Na^+}) was determined to be 0.46] and less than that reported for human skin. Lowering the pH values of the solutions on either side of the skin to slightly less than 4 reversed the direction of net volume flow; cathodal flux was greater than anodal flux. When the donor solution was at pH 3.8 and the receptor was pH 7.4, the flux profile was complicated and net volume flow was not obvious. Finally, it was found that electroosmosis from anode to cathode was retained even following removal of the stratum corneum by tape-stripping.

KEY WORDS: electroosmosis; hairless mouse skin; iontophoresis; mannitol; skin permeability; transdermal delivery; water flux.

INTRODUCTION

While the major driving force for the iontophoretic flux of charged molecules is electromigration, the enhanced flux of polar neutral solutes is, in large part, the result of electroosmotic volume flow (1–4). Burnette and Ongpipattanakul (2) measured the flux of sodium and chloride ions during iontophoresis and demonstrated the permselectivity of human cadaver skin to cations. They also showed that mannitol flux from the anodal compartment was an order of magnitude higher than that from the cathodal chamber. Pikal and Shah (3) demonstrated that net volume flow through hairless mouse skin was from anode to cathode. Although all these data suggest that the electroosmotic volume flow occurs from anode to cathode (due to permselectivity of the negatively charged skin), the kinetics of this process have not been documented.

In this study, iontophoretically driven fluxes of water and mannitol through hairless mouse skin have been measured to gain more insight into the electroosmotic volume flow phenomena. Passive transport after termination of iontophoresis was followed to examine the effect of current on the intrinsic permeability of the skin. Anodal and cathodal iontophoretic flux of mannitol at low pH was also determined to examine whether the skin's permselectivity is reversed under these conditions. Finally, the iontophoretic flux of mannitol was measured after tape-stripping the skin to evaluate the contribution of the viable portion of the skin to the electroosmotic volume flow across HMS *in vitro*.

MATERIALS AND METHODS

Materials

Flow-through iontophoretic diffusion cells were used in conjunction with Ag–AgCl electrodes, as described previously (4,5). The cells permit the positioning of both anode and cathode on the epidermal side of a single piece of skin. The electrode chambers are physically and electrically isolated from one another by an insulating central compartment. Constant current was passed between the electrodes from a custom-built power supply (Professional Design and Development Services, Berkeley, CA) interfaced to a Macintosh IIfx computer (Apple Computer Inc., Cupertino, CA) running Labview software (National Instruments Inc., Austin, TX). The lower receptor chamber was continuously perfused throughout the experiments and was well stirred. Full-thickness hairless mouse skin, freshly excised from 8- to 12-week-old females (Simonsen, Gilroy, CA), was used in this study. *N*-2-Hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), NaCl, and D-mannitol were obtained from Aldrich Chemical Company (Milwaukee, WI). Tritiated water, ^{14}C-mannitol, and ^3H-mannitol were purchased from NEN Research Products (Wilmington, DE).

Iontophoretic Flux Measurements

Transport of water and mannitol was determined under three pH conditions: (a) receptor and electrode solutions buffered at pH 7.4; (b) receptor solutions at pH 7.4, electrode solutions at pH 3.8; and (c) receptor and electrode solutions at pH 3.8. The buffer employed was 25 m*M* HEPES with 133 m*M* NaCl; titration with 1 *N* NaOH or with 1 *N* HCl, respectively, was used to bring the pH to 7.4 or 3.8. To avoid air bubbles in the flow-through system, the buffer was degassed prior to use by vacuum filtering through a 0.45-μm membrane (Millipore, Bedford, MA).

The receptor phase flow rate was controlled at ~3 mL/hr using a peristaltic pump (Manostat, New York, NY). The appropriate electrode solutions contained 0.5–1 μCi of either ^3H_2O or radiolabeled mannitol (^{14}C or ^3H); in the case of mannitol, its initial concentration was set at 1 m*M* using

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unlabeled chemical. All flux measurements were made in triplicate.

Hairless mice were sacrificed by asphyxiation in a dry ice chamber. The excised dorsal skin from each mouse was divided into two pieces: one was used for the anodal delivery of either water or mannitol; the other, for the corresponding cathodal delivery experiments. For the studies involving iontophoretic delivery through stripped skin, the mouse skin was excised after the stratum corneum had been removed by repeated (20 times) adhesive tape-stripping using Scotch tape (3M Co., St. Paul, MN).

Freshly excised skin was mounted in the diffusion cell, the upper and lower halves of which were clamped together. Appropriate buffer solutions were then placed in the electrode and receptor chambers, and the system was allowed to equilibrate for 2 hr. Then the electrode solutions were replaced with fresh solutions, one of which now contained the radiolabeled marker. Perfusion of the receptor phase was commenced and a constant current (0.2 mA) was delivered to the electrodes for the next 8–12 hr at a current density of 0.36 mA/cm^2. After termination of current the post-iontophoretic passive transport of the permeant was monitored for a further 10 hr. The voltage across the electrodes in each of the six cells (which were connected in series) was measured immediately after initiation, and just before termination, of current passage. Typical values observed at these two times were 2.1 (±0.46) and 1.24 (±0.25) V, respectively. When stripped skin was used, the values were 10-fold less.

At the end of each experiment, the absence of significant pH changes in the electrode solutions was verified by measurement with pH indicator strips (Baxter Healthcare Co., McGaw Park, IL). The samples of the receptor phase were collected hourly on a fraction collector (Isco Inc., Lincoln, NE). The collection vials contained 0.2 mL dimyristoylstearate to prevent evaporation of water. After the addition of 5 mL of scintillation cocktail (Ready Gel, Beckman Instruments Inc., Fullerton, CA) to each sample, transport was determined by measuring radioactivity in a liquid scintillation counter (Beckman LS 6000, Beckman Instruments Inc., Fullerton, CA). The disintegrations per minute were converted to molar flux, by taking into appropriate consideration the flow rate and the receptor phase volume.

Transport Number Determination

The transport numbers of Na^+ and Cl^- were measured under the conditions employed in the iontophoretic experiments. Transport numbers were determined by (a) the Hittorf method or (b) measurement of membrane potential (6). In the first approach, freshly excised skin was clamped between the two halves of a side-by-side diffusion cell (Crown Glass Co., Somerville, NJ). The two chambers were filled with 3 mL of a 133 m*M* NaCl solution, the pH of which was adjusted to 7.4 by titration with 1 *N* NaOH; to simplify analysis of the data, we chose to eliminate the need for buffering ions. The anode was placed in the chamber facing the epidermal side of skin; the cathode, in the chamber facing the dermis. After 1 hr of equilibration, a current of 0.4 mA (0.51 mA/cm^2) was passed for 4 or 8 hr. No significant pH changes occurred during the experiment. At the end of current flow, the Cl^- concentration in both chambers was determined either electrochemically or by measurement of osmolality (Advanced Instruments, Inc., Needham Heights, MA). The latter approach was more reproducible and was used routinely, therefore. Because electroneutrality must be maintained, changes in Cl^- concentration exactly mirror changes in $[Na^+]$. The Na^+ transport number (t_{Na^+}) was calculated from (6)

$$t_{Na^+} = i_{Na^+}/i_T \quad (1)$$

where i_{Na^+} is that part of the total current (i_T) carried by Na^+.

In the measurement of membrane potential, the anodal chamber (facing the stratum corneum side of skin) contained 200 m*M* NaCl, while the cathodal chamber held 100 m*M* NaCl. The electromotive force (e.m.f.) was measured using a digital voltmeter (Keithley Instruments, Cleveland, OH) until a steady-state value was achieved. The e.m.f. is the sum of the electrode potential (V_e) and the membrane potential (V_m). The former was independently determined when the two solutions were connected via a salt bridge. Hence, V_m was obtained from

$$V_m = \text{e.m.f.} - V_e \quad (2)$$

The Na^+ transport number was then calculated using

$$t_{Na^+} = [FV_m]/[2RT\ln(C_2/C_1)] + 0.5 \quad (3)$$

where F is Faraday's number (96,500 *C*/mol), C_2 = 200 m*M*, C_1 = 100 m*M*, and T is the absolute temperature (3,6).

RESULTS

Voltage Change During Iontophoresis

In constant-current iontophoresis, the potential difference between the electrodes fluctuates as the total resistance of the system changes. Thus, if current passage causes skin resistance to decrease, for example, then (in accord with Ohm's law) the applied voltage must also decrease in order that the current flowing in the circuit remains constant. The Labview interface to the iontophoretic power supply used in this study permitted the temporal change in applied voltage to be monitored. A typical result is shown in Fig. 1, which reports the change in voltage supplied across six iontophoresis cells in series during the course of an experiment. We observe that the resistance drops rapidly in the first 30 min of iontophoresis and then remains essentially constant for the remainder of the experiment. Contributions to the total resistance of the circuit are the skin, electrolyte solutions, and electrodes. The latter two of these, it is easy to show (by measurement in the absence of skin), do not change appreciably under the conditions of the iontophoresis experiment. Thus, the voltage change observed in Fig. 1 must be due to a decrease in skin resistance. The results suggest that, on average, the potential difference across a single piece of skin falls from ~1.5–2 V at the beginning of iontophoresis to a steady value (achieved within about 30 min) of <~1 V, which is maintained throughout the remainder of the iontophoretic period.

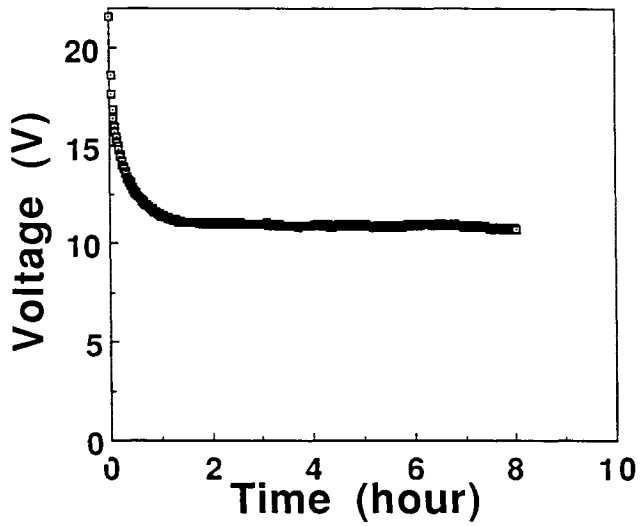

Fig. 1. Change in the voltage applied across six iontophoresis cells in series during passage of a continuous current of 0.2 mA (0.36 mA/cm^2) for 8 hr.

Iontophoresis of Water and Mannitol

The iontophoretic fluxes of water and mannitol from donor solutions at pH 7.4 into a receptor phase at the same pH are shown in Figs. 2a and b, respectively. Anodal, cathodal, and passive fluxes are compared in these figures. The anodal iontophoretic transport of water was not significantly greater than the cathodal (Fig. 2a, Table I); after termination of current passage, the anodal flux decreased appreciably, whereas the cathodal flux remained elevated. The transport of water post-iontophoresis was considerably higher than the passive level observed following exposure of the skin to the same aqueous donor and receptor phases for the same period of time (Fig. 2a). One infers from this observation, therefore, that iontophoresis alters skin barrier function in some way. In order to separate out the electroosmotic contribution to the iontophoretic flux, at the time of current termination, from the enhanced "passive" permeability of the membrane, the transport rate at 20 hr is subtracted from that at 10 hr (Table I). For water, then, we find a large positive electroosmotic flow from the anode (consistent with the skin's expected permselectivity) and a negligible contribution from the cathode. The results for mannitol dramatically emphasize these points. Anodal flux is ~100 times greater than passive transport and decreases rapidly after current termination. Cathodal mannitol flux exceeds the passive level slightly but then increases after the current is stopped. Notably, after both anodal and cathodal iontophoresis, the new passive permeation rates, that are ultimately achieved, are identical (Fig. 2b). Net volume flow from anode to cathode is clearly demonstrated by these data. During cathodal iontophoresis, the electroosmotic flow of solvent retards the net mannitol transport which would otherwise be achieved through the iontophoretically modified barrier.

When the pH of the donor and receptor solutions was buffered at slightly less than 4, the iontophoretic flux of mannitol was greater from the cathode than from the anode (Fig. 3a). After current termination, the cathodal flux decreased

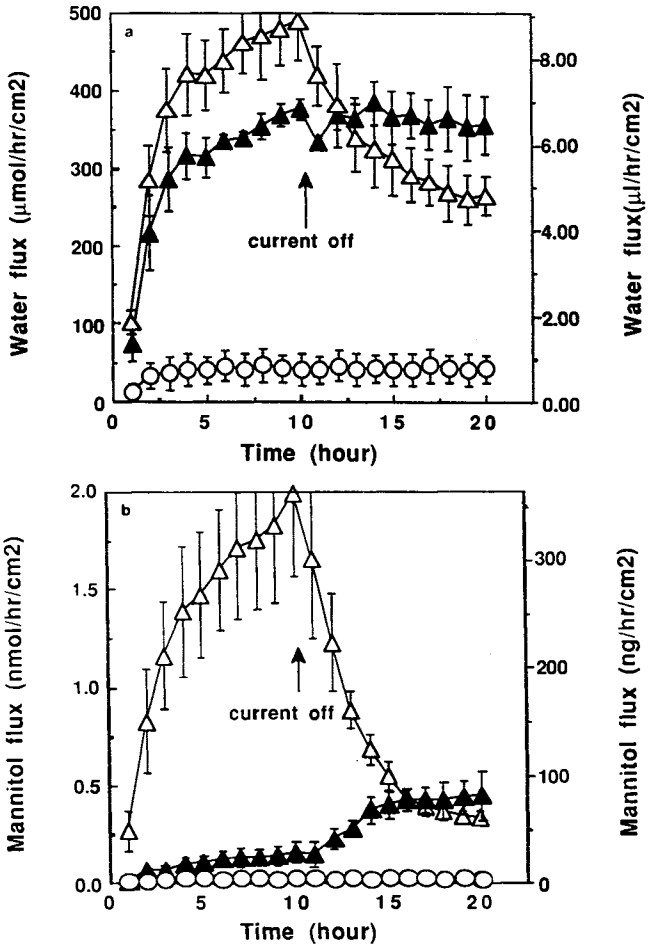

Fig. 2. Molar fluxes of (a) water and (b) mannitol across HMS during, and after, 10 hr of iontophoresis at 0.36 mA/cm^2. Anodal (△), cathodal (▲), and passive (○) transport profiles are shown. Each data point represents the mean (±SD) of three determinations.

and the anodal flux increased to the same final level (which was similar to that attained after iontophoresis at pH 7.4). The electroosmotic behavior in this experiment is opposite to that observed at pH 7.4 (Fig. 2b), i.e., there is net volume flow from cathode to anode, indicating that the permselectivity of the membrane has been changed.

Imposition of a pH gradient across the membrane (donor solution at pH 3.8, receptor at pH 7.4) resulted in the mannitol flux profiles shown in Fig. 3b. During the first hours of iontophoresis, anodal and cathodal fluxes were not significantly different. After ~5 hr, however, cathodal flux plateaued, whereas the anodal flux continued to increase until the current was terminated. At this point, the cathodal delivery continued at the same steady value, while the anodal transport declined. Once again, the postiontophoresis fluxes were similar (anode vs cathode) and were not significantly different from those observed when symmetrical pH conditions were maintained. The donor solution pH did not change measurably during this experiment and the voltage applied across the system was comparable to the experiments in which no pH gradient existed.

The results for iontophoresis of mannitol across tape-stripped skin (pH 7.4 donor and receiver solutions) are

Table I. Iontophoretic Fluxes of Water and Mannitol under Various Conditions

	pH (D/R)[a]	Anodal[b]	Cathodal[c]	Passive-a[d]	Passive-c[e]	Δa[f]	Δc[g]
Water (μmol/hr cm^2)	7.4/7.4	384	303	237	299	148	4.6
SD (n = 11)		(124)	(119)	(70)	(118)	(71)	(33)
Mannitol (nmol/hr cm^2)	7.4/7.4	1.65	0.17	0.42	0.37	1.23	−0.2
SD (n = 11)		(0.74)	(0.14)	(0.22)	(0.22)	(0.59)	(0.18)
Mannitol (nmol/hr cm^2)	3.8/3.9	0.14	0.54	0.25	0.25	−0.11	0.29
SD (n = 4)		(0.05)	(0.07)	(0.06)	(0.10)	(0.03)	(0.04)
Mannitol (nmol/hr cm^2)	3.8/7.4	0.52	0.24	1.30	0.21	0.23	0.007
SD (n = 7)		(0.18)	(0.04)	(0.26)	(0.05)	(0.10)	(0.10)
Mannitol (nmol/hr cm^2)[h]	7.4/7.4	13.2	10.5	9.4	10.5	3.73	−0.04
SD (n = 12)		(8.52)	(8.30)	(6.15)	(7.56)	(3.05)	(1.17)

[a] pH values of the donor (D) and receptor (R) solutions.
[b] Anodal flux at 10 hr of 0.2 mA (0.36 mA/cm^2) iontophoresis.
[c] Cathodal flux at 10 hr of 0.2 mA (0.36 mA/cm^2) iontophoresis.
[d] Passive flux 10 hr after termination of anodal iontophoresis.
[e] Passive flux 10 hr after termination of cathodal iontophoresis.
[f] Anodal iontophoretic flux at 10 hr − passive flux 10 hr after termination of current.
[g] Cathodal iontophoretic flux at 10 hr − passive flux 10 hr after termination of current.
[h] Data from tape-stripped skin.

shown in Fig. 4 and Table I. With the high variability in the data obtained, no significant difference between the anodal and the cathodal transport of mannitol could be detected. However, there was a statistically significant decrease in anode flux after current termination (whereas the cathodal flux remained at the iontophoretically elevated level). These findings suggest that skin stripped of its stratum corneum retains, nevertheless, a certain permselectivity, which continues to favor anions over cations.

Transport Numbers

The value of t_{Na^+} in aqueous solution was 0.38, in agreement with previous determinations (7). In hairless mouse skin, t_{Na^+} at pH 7.3 was 0.46 (±0.08; n = 7) by the Hittorf method and 0.43 (±0.02; n = 4) from measurement of membrane potential. Pikal and Shah (3) reported that, at pH 7 (38 mM NaCl), t_{Na^+} = 0.44, while at pH 6 (50 mM NaCl, t_{Na^+} = 0.33. Relative to human skin at pH 7.4 (t_{Na^+} = 0.6) (2,8), the cation permselectivity of HMS is smaller. Through stripped skin, we found (by the Hittorf method) t_{Na^+} = 0.47 (±0.07; n = 3), suggesting that the permselectivity was maintained even after removal of the stratum corneum.

DISCUSSION

Initiation of current flow across the skin caused the resistance of the membrane to decrease rapidly and to achieve a new lower value (Fig. 1). While this change has been ascribed to an (deleterious) alteration in the skin's barrier properties, it has been shown that the effect can be induced simply by increasing the number of ions in the membrane (which results, inevitably, in a decrease in resistance). Indeed, it can be shown that, if skin iontophoresed in the presence of 0.1 M electrolyte is subsequently immersed in a solution of much lower ionic strength (0.005 M, for example), then the membrane's resistance will gradually increase and, with sufficient time, ultimately exceed the preiontophoresis value. Thus, although it is clear from the re-

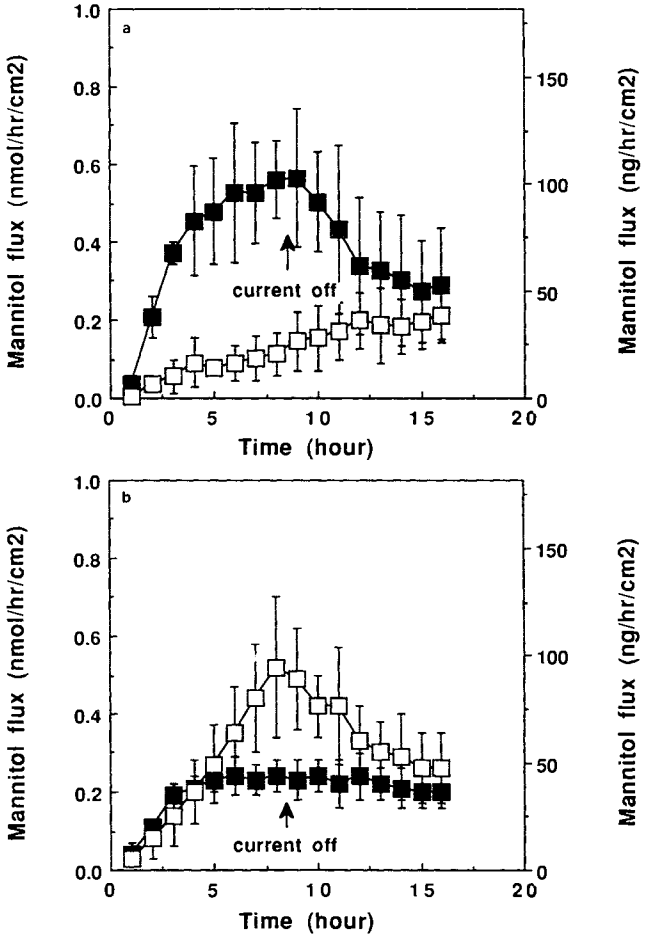

Fig. 3. Mannitol flux during, and subsequent to, 10 hr of iontophoresis at 0.36 mA/cm^2 (a) from donor solution of pH 3.8 into a receptor solution at the same pH and (b) from a donor solution at pH 3.8 into a receptor solution at pH 7.4. Anodal (□) and cathodal (■) transport profiles are shown. Each data point represents the (±SD) of (a) two and (b) seven determinations.

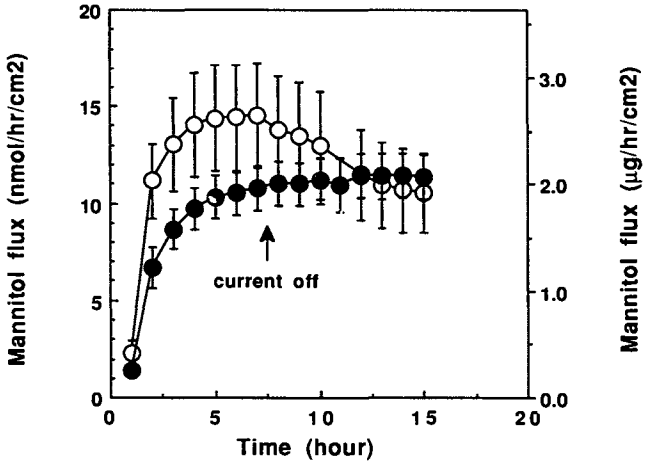

Fig. 4. Mannitol flux across tape-stripped HMS during, and after, 7 hr of iontophoresis at 0.36 mA/cm^2. Anodal (○) and cathodal (●) transport profiles are shown. Each data point represents the mean (±SE) of 11 determinations.

sults described below that iontophoresis does alter the inherent barrier characteristics of the skin, the observation that the membrane resistance decreases is not, in and of itself, sufficient evidence to diagnose that a detrimental change has occurred.

We present here, for the first time, the kinetic profile of water flux during, and subsequent to, constant-current iontophoresis. A large enhancement of water permeation is apparent following the period of iontophoresis. Furthermore, as reported by Burnette and Ongpipattanakul (2), considerable augmentation of mannitol transport across hairless mouse skin is achieved by iontophoresis. Similar findings with human skin have been observed too (9). The anodal iontophoretic and passive permeability coefficients (i.e., steady-state flux divided by donor concentration) of mannitol across HMS were found to be 2.6×10^{-5} and 1.2×10^{-6} cm/sec, respectively. These values are about an order of magnitude higher than the corresponding results in human epidermis (9).

Figures 2a and b show that the passive fluxes of water and mannitol were significantly increased by the period of iontophoresis (regardless of the polarity of the electrode with which the permeant was associated). It follows that the electroosmotic enhancement determined at the end of iontophoresis includes a significant contribution from the elevated passive transport. In assessing the electroosmotic component, therefore, the passive flux following iontophoresis was subtracted from the iontophoretic flux measured just prior to the termination of current flow. These corrected anodal and cathodal fluxes are summarized in Table I for the different experiments performed. The data clearly show that net volume flow takes place in the anode-to-cathode direction at pH 7.4. The corrected fluxes of water and mannitol in the opposite direction (cathode-to-anode) were not significantly different from zero. The permselectivity of a charged membrane can be assessed by the degree to which the transport number of small cations (e.g., t_{Na^+}) exceeds its corresponding value in water. The measurements of t_{Na^+} here indicate that HMS is permselective to positive ions, but much less so than human skin. Direct comparison of the extent of net electroosmotic flow in HMS with human skin, however, is precluded because previous studies using human tissue have not reported corrected electroosmotic flow as presented in Table I.

The molar fluxes in Table I were converted to equivalent volume fluxes to normalize for the difference in the applied concentrations of water and mannitol (55.5 *M* versus 1 m*M*) (see Table II). While the cathodal fluxes are not significantly different from zero, the anodal flux of water was approximately twice that of mannitol ($P < 0.005$, paired t test). It follows that the electroosmotic transport of mannitol is only ~50% coupled to that of water flux. Interestingly, on the other hand, iontophoresis leads to changes in the passive permeation properties of the skin that have a much greater impact upon mannitol flux than upon that of water (see Table III). Iontophoresis for 10 hr leads to a 6-fold augmentation of water permeability compared to a 30-fold increase for mannitol. By comparison, tape-stripping the skin increases the K_p values of water and mannitol by factors of 270 and nearly 700, respectively (Table III).

When iontophoresis was performed with the pH of the donor and receptor solutions at slightly less than 4, net electroosmotic volume flow was from cathode to anode (i.e., the reverse of the situation at pH 7.4). For mannitol, the corrected cathodal iontophoretic flux at pH 4 was, however, about fourfold smaller than the corrected anodal flux at pH 7.4 (Table I). It follows that decreasing the pH by 3 units changes dramatically the charge characteristics of HMS, such that the membrane now appears to have anion permselectivity. Phipps and Gyory (10) also described a decrease in the anodic electroosmotic flow of L-glucose when the pH was lowered from 8 to 4. Other results describing a fall in cation flux at pH 4 (11) and an increase in anion flux at pH 4.2 (12) are also consistent with anion permselectivity at pH 4.

When a pH gradient was maintained across the membrane (donor pH 3.8, receptor pH 7.4), the electrotransport behavior was more complex but, ultimately, resulted in retention of the skin's inherent cation permselectivity. Thus, although neutralization of negative charge (or even charge reversal) at the skin surface almost certainly took place, the deeper parts of the barrier remained preferentially permeable to positively charged species. Interestingly, skin stripped of its stratum corneum is also cation-permselective (see Table I and Fig. 4), although it is, of course, very much more permeable than the intact barrier. We conclude that in an *in vivo* experiment (in which the "receptor-phase" pH cannot be changed from 7.4), it is unlikely that the skin will lose its cation selectivity; modulation of the donor-phase pH may

Table II. Electroosmotic Volume Flux of Water and Mannitol Calculated from the Corrected Anodal Molar Flux Summarized in Table I

	Anodal	Cathodal
Water (μL/hr cm^2)	2.7 (±1.3)*	0.08 (±0.6)**
Mannitol (μL/hr cm^2)	1.23 (±0.59)*	−0.2 (±0.18)**

* These values are significantly different ($P < 0.005$).
** Not significantly different ($P > 0.1$).

Table III. Passive Permeability Coefficients[a] of Water and Mannitol Across HMS Under Various Conditions

	Water (cm/hr)	Mannitol (cm/hr)
Intact HMS (n = 3)	1.0×10^{-3} ($\pm 4.4 \times 10^{-4}$)	2.6×10^{-5} ($\pm 2.3 \times 10^{-5}$)
Post-iontophoresis (n = 3)[b]	6.0×10^{-3} ($\pm 1.7 \times 10^{-3}$)	8.0×10^{-4} ($\pm 6.4 \times 10^{-4}$)
Stripped HMS	2.7×10^{-1} ($\pm 4.1 \times 10^{-1}$)	1.8×10^{-2} ($\pm 3.5 \times 10^{-3}$)

[a] Permeability coefficient (K_p) is the ratio of the steady-state flux (J; mol/hr cm^2) to the donor concentration (C_o), $K_p = J/C_o$.
[b] Ten hours after termination of current flow (0.2 mA) for a 10-hr period.

mitigate the degree of preferential cation passage but will probably not obliterate the phenomena.

Comparison of the results discussed above with previously published findings is complicated by inconsistencies in the experimental conditions employed by different laboratories. Pikal and Shah (3), for example, reported volume flow from anode to cathode at pH 3.8, even though this finding seemed at odds with the t_{Na^+} measured under these conditions. A heterogeneous pore model was invoked to account for aspects of these data. Our flux results at pH 4 are in agreement of those of Pikal and Shah (3) when volume flow is observed from cathode to anode. In these circumstances, Pikal and Shah report a value for the Na^+ transport number of ~0.35. Because our measurement of t_{Na^+} at pH 7.4 agrees with that of Pikal and Shah at the same pH, we believe that the Na^+ transport number in our pH 4 experiments is very close to that reported previously. Parenthetically, we note that Kasting and Bowman (13) found that t_{Na^+} was 0.24 through human skin at pH 4.

Burnette and Marrero (1) showed that iontophoresis of thyrotropin releasing hormone (TRH) was greater at pH 8 than at pH 4 even though the peptide had a net positive charge at pH 4 (but was effectively neutral at pH 8). In contrast, Green *et al.* (4,14) found that histidine and a tripeptide, Ac-Ala-His-Ala-NH(Bu^t), were more efficiently iontophoresed at pH 4 (net charge = +1) than at pH 7.4 (where the solutes were predominantly nonionized). Differences in buffering electrolyte and transport numbers probably contribute to the divergence between these two investigations. Green *et al.* (4) did show, using lysine (which has a net charge of +1 at both pH 7.4 and pH 4), that lowering the pH reduced the level of iontophoretic enhancement, an observation consistent with the perceived charge neutralization of the skin reported in this paper. Clearly, there are complicated, interacting factors that are not yet fully understood. Further, as pointed out by Burnette and Marrero (1), (a) the pH profile across the skin is not known, and (b) whether the pK_a values of these ionizable solutes are the same inside the skin as they are in aqueous solution is not established. What is apparent is that electroosmotic flow can provide an important mechanism of enhanced delivery under iontophoretic conditions. Additional, systematic research is required to understand fully the complex interplay of forces occurring during electrotransport and to maximize the application of iontophoresis for optimal drug delivery.

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