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Report

Electrical Analysis of Fresh, Excised Human Skin: A Comparison with Frozen Skin[1]

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Samples of human allograft skin prepared without freezing ("fresh skin") were found to have electrical and sodium ion transport properties which differed only slightly from those of skin which had been similarly treated but stored frozen ("frozen skin"). The fresh skin samples were less permeable to sodium ions during passive diffusion and less conductive than frozen skin at low current levels. They were more permselective for sodium versus chloride during constant-current iontophoresis and showed slightly more asymmetry in their current–voltage properties. Overall, the electrical behavior of the two tissues was similar enough to support the use of frozen tissue in iontophoresis studies. However, caution should be exercised when considering the use of frozen skin for applications, such as those based on electroosmosis, where the observed differences could have a major impact on the results.

KEY WORDS: iontophoresis; human skin; current–voltage characteristic; sodium ion transport; fresh skin; frozen skin.

INTRODUCTION

Drug ions passing from an iontophoretic patch into skin under the influence of an electric field must compete as charge carriers with one of the two major extracellular ions of the body, sodium or chloride. In order to make a priori estimates of the electrical current and voltage required to deliver a given amount of drug, the permeability of the membrane to sodium and chloride as a function of applied potential (i.e., the skin's current–voltage characteristic) must be known.

In an earlier study (1) we described the electrical and sodium ion transport properties of human allograft skin which had been slowly frozen in 10% glycerol to −150°C, stored frozen for up to 2 months, then thawed prior to use (herein called "frozen skin"). The DC resistance of frozen skin was found to be lower than that reported for human skin *in vivo*, yet the sodium ion permeabilities were similar to *in vivo* values. In this study we examined samples of allograft skin which had never been frozen ("fresh skin") in order more accurately to infer the effects of freezing on the tissue. The objective of the work was to validate the use of the more readily available frozen skin for iontophoresis studies.

MATERIALS AND METHODS

The apparatus and methods have been previously described (1,2). An outline of the procedures is given below.

Back skin from a male Caucasian donor was obtained from the Ohio Valley Skin and Tissue Center, Cincinnati, OH. The skin was procured with a dermatome set to 0.25 μm after the hair had been clipped and the skin washed. The skin was bathed in a solution of antibiotics (penicillin G, streptomycin) for 24 hr, then transported to the laboratory, where it was used immediately.

The skin was cut into small squares and mounted in either side-by-side iontophoresis cells (n = 12) or horizontally oriented passive diffusion cells (n = 24). The diffusional cross section for both types of cells was 0.7 cm^2. Both sides of the tissue were bathed in an isotonic saline buffer, which was Dulbecco's phosphate-buffered saline, pH 7.4, to which 0.02% (w/v) sodium azide had been added. For sodium ion transport measurements, the donor solution (epidermal side of skin) was spiked with 2.0 μCi/ml of $^{22}NaCl$, 99% radiochemical purity.

Protocol for Passive Diffusion Cells

After an overnight equilibration period with both sides of the skin immersed in buffer, the receptor solutions (5 ml) were replaced with fresh buffer and the donor solutions were removed and replaced with 0.5 ml of buffer containing $^{22}NaCl$. The receptor solutions were removed for radioactivity analysis at 2, 4, 6.5, 23, 47, and 71 hr postdose. Sodium ion permeability coefficients and diffusional time lags were determined from the cumulative penetration versus time curves by fitting straight lines through the data between 6.5 and 71 hr. Visual inspection and linear regression (mean r^2 = 0.988) showed the data to be highly linear over this range. The ratio of the slope of these lines to the sodium ion concentration in the donor solution yielded the permeability co-

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efficient, and their intercept with the time axis provided the time lag.

Protocol for Iontophoresis Cells

The following sequence of measurements was conducted: initial resistance determination (Day 1), current–voltage measurements (Days 2 and 3), $^{22}Na^+$ passive diffusion (Days 3–5), followed by a second resistance determination and $^{22}Na^+$ iontophoresis (Day 5).

The procedures were those of Reference 1 (Study 1), except that the passive diffusion stage was conducted for 45 hr. Skin resistance was determined by passing a direct current, I, of 10 μa (or current density, j, of 14 μA/cm^2) through each tissue for 5–10 sec, using a constant-current source. Additional current–voltage measurements were made using short bursts of direct current having alternating polarity and an intensity which first increased and then decreased in a stepwise manner. Three consecutive current regimens were imposed: 0 to ±10, 0 to ±50, and 0 to ±250 μA. A positive potential was defined as epidermis positive with respect to dermis. Completion of one sequence (20 readings) required about 10 min per sample. The current–voltage (j–V) data were characterized by the following equation (1):

$$V = (4aRT/F)\ \sinh^{-1}(bj) \qquad (1)$$

where R is the gas constant, T the absolute temperature, F the Faraday constant, and a and b are parameters which are properties of the membrane. Due to the constant-current methodology, voltage was treated as the dependent variable and current as independent. Following the passive permeability and resistance determinations, $^{22}Na^+$ iontophoresis was conducted for 6 hr at a constant current of 50 μA. Permeation samples were obtained every 2 hr during the iontophoresis treatment and analyzed for radioactivity using a gamma counter.

RESULTS

Skin Resistance and Sodium Ion Permeability Coefficients

Skin resistance values and passive sodium ion permeabilities are given in Table I. Summary parameters are shown in Table II, along with corresponding results from frozen skin (1). The fresh skin samples had a slightly higher median resistance and a 45% lower median permeability to Na^+ than the frozen skin samples. However, a statistical comparison of the results using the Wilcoxon rank sum test failed to show a significant difference in either R or k_p between the two tissues ($P > 0.2$, two-sided test).

Table I also shows that for 10 of 12 samples in the present study, electrical resistance decreased over the 4-day interval between mounting the skin and completing the passive sodium ion permeability measurements. The inverse of the final resistance values (i.e., the final conductance) showed a strong linear correlation with sodium ion permeability ($r^2 = 0.96$, excluding the very high permeability sample). This relationship was considerably tighter than that between initial conductance and Na^+ permeability ($r^2 = 0.24$). This change in the tissue properties over time explains our failure to observe a strong relationship between conductance and permeability in Ref. 1. Note that the resistance of one sample actually increased by more than 100% during the course of the study. We have observed similar resistance changes in other skin samples in subsequent experiments. The operating mechanism here is unknown; however, we offer the possibility that conductive shunts in such samples that are initially open to ion flow may swell shut as the tissue hydrates.

Diffusional Lag Times

Typical penetration versus time curves for the passive diffusion of Na^+ through skin in the side-by-side iontophoresis cells are shown in Fig. 1a. The mean lag time for achieve-

Table I. Sodium Ion Permeability Coefficients and Electrical Resistance for Fresh Excised Human Skin *in Vitro*

$k_p \times 10^6$ (cm/min)	R, kΩ[a]		$k_p \times 10^6$ (cm/min)	R, kΩ		$k_p \times 10^6$ (cm/min)	R, kΩ	
	Init.[b]	Final[c]		Init.	Final		Init.	Final
0.04[d]			0.23			1.49		
0.06			0.27	185	146	1.56	77	39
0.07			0.33			1.82		
0.07			0.35	253	161	1.83		
0.08			0.41			1.84		
0.10	238	198	0.44	87	118	1.85		
0.11	75	182	0.48	135	95	6.07		
0.12			0.49	127	107	6.16		
0.13			0.49	149	112	6.42		
0.14			0.50	135	81	12.83		
0.21			0.80			28.28		
0.23	193	126	0.89			51.40	26	12

[a] Effective resistance at 10 μA of 0.7-cm^2 samples.
[b] 15–60 min after mounting skin.
[c] Following k_p determination.
[d] k_p values without an associated value of R were determined in passive diffusion cells; the others were determined in iontophoresis cells following the current–voltage measurements.

Table II. Comparison of the Electrical Resistance, Passive Sodium Ion Permeability, and Sodium Ion Diffusional Time Lag for Fresh and Frozen Human Skin

Parameter	Units	Summary statistic	Value	
			Fresh skin	Frozen skin[a]
R_{init}[b]	kΩ	Median ± MAD[c]	135 ± 54 (n = 12)	126 ± 41 (n = 34)
$k_p \times 10^6$	cm/min	Median ± MAD	0.46 ± 0.38 (n = 36)	0.84 ± 0.39 (n = 19)
t_L	hr			
Iontophoresis cells		Mean ± SD	1.2 ± 1.0 (n = 12)	ND[d]
Passive cells		Mean ± SD	3.1 ± 6.2 (n = 23)	ND

[a] Data from Ref. 1.
[b] Initial resistance at a current of 10 μA (j = 14 μA/cm^2).
[c] Median absolute deviation.
[d] Not determined.

ment of steady-state diffusion of Na^+ in these cells was about an hour (see Table II). This value is small compared to lag times typical of low permeability lipophilic solutes in skin (in our experience, usually 3–4 hr or more). Considering the form of the lag time for an ideal membrane, $t_L = h^2/6D$, this result suggests that Na^+ has either a larger diffusion coefficient, D, or a smaller effective path length, h, than the lipophilic solutes.

Sodium ion lag times determined from the passive diffusion cell data (Table II) were not as consistent as those from the iontophoresis cells. This could be a consequence of cell construction (horizontal rather than vertical) or of the additional handling they received—the passive cells were removed from their heating blocks and turned 120° during sampling. A third possibility is that the extra immersion time and mild electrical treatment undergone by the iontophoresis cell samples prior to the passive diffusion measurements actually led to greater stability for these tissues. No differences were evident in the Na^+ permeability coefficients determined in the two types of cells, however, based on a rank sum test on the data in Table I.

Figure 1b shows penetration versus time curves from a separate experiment in which the $^{22}NaCl$ solution was applied to dry skin which had not been pretreated with saline buffer. The Na^+ flux for two of the four samples was initially quite high, then gradually decreased to levels comparable to those in the present study. Similar behavior was observed for additional samples treated with different concentrations of NaCl (data not shown). We suspect that sodium ion (and presumably chloride as well) was carried into and through the skin as the tissue hydrated. It therefore appears to be important to prehydrate the skin in order to obtain steady state ionic permeability data.

Skin Current–Voltage Characteristic

The results of current–voltage determinations on fresh skin samples are shown in Fig. 2. The electrical behavior was, in most respects, similar to that of frozen skin (1). The j–V curves were nonlinear and slightly asymmetric with respect to the sign of the applied potential. Resistance decreased with increasing current; this effect was time dependent and largely reversible for the power levels and exposure times used in the study. Upon completion of each current–voltage cycle, the skin resistance increased to near its initial value over a period of tens of minutes.

Figure 3a shows the ascending portion of all three j–V curves plotted together, along with a line indicating the result for frozen skin. For both data sets the plot of V versus the logarithm of j (Fig. 3b) was nearly linear. The data were satisfactorily described by Eq. (1), so long as the positive and negative arcs of the j–V curve were treated independently; the values of the parameters determined by least-squares fitting are given in Table III. Compared to frozen

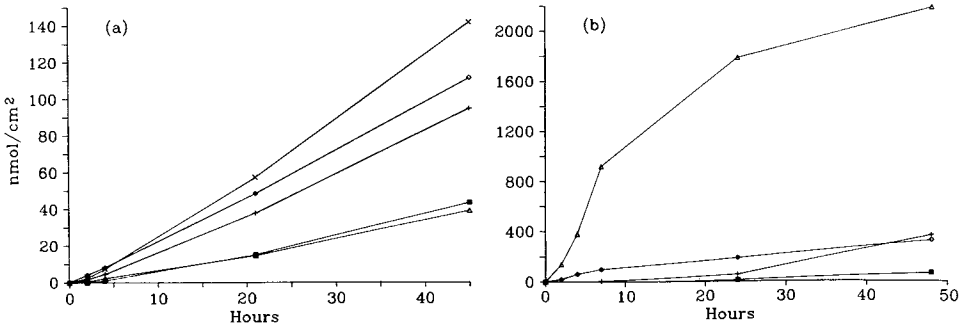

Fig. 1. Passive permeation of $^{22}Na^+$ from a normal saline buffer through excised human skin. (a) Iontophoresis cell data. Both sides of the skin were pretreated with buffer for 2 days prior to dosing the radiolabeled solution. (b) A study in which the radiolabeled solution was applied directly to the dry epidermal surface of the skin. Each curve represents an individual tissue sample. Note the scale difference between a and b.

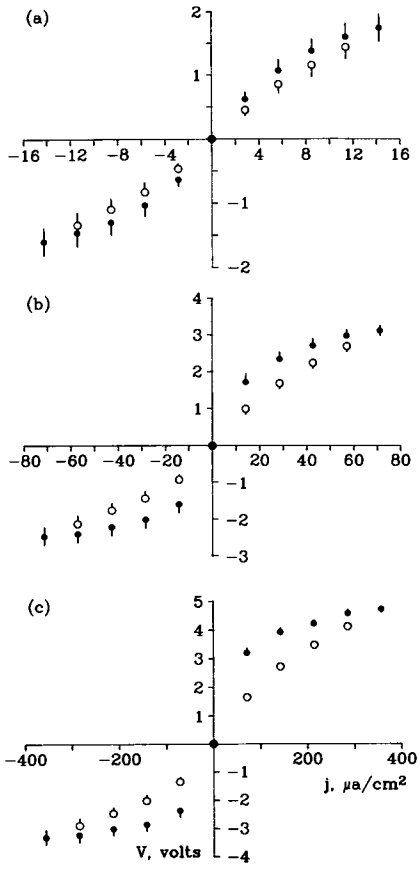

Fig. 2. Mean current–voltage characteristic (n = 10) for fresh human skin samples immersed in a normal saline buffer. Data obtained with increasing current magnitude are drawn as filled circles; those with decreasing current magnitude, as open circles. The error bars are 1 SE. (a) 0- to ±10-μA regimen; (b) 0- to ±50-μA regimen; (c) 0- to ±250- μA regimen.

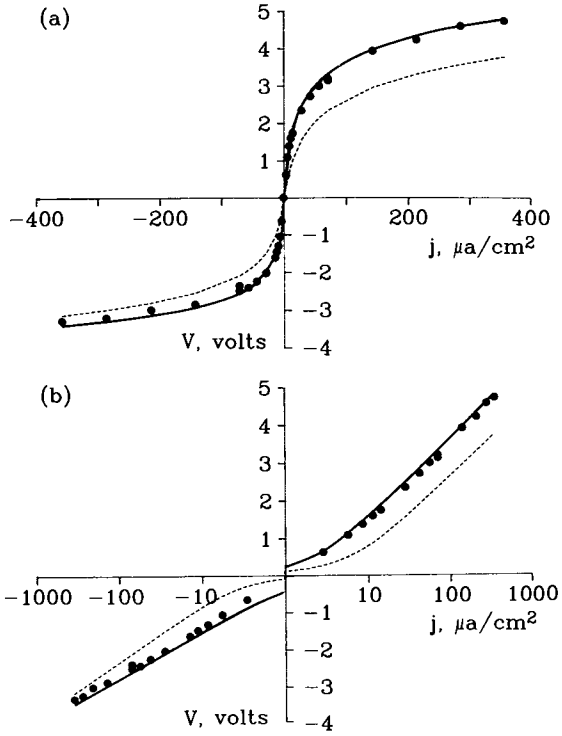

Fig. 3. Ascending current–voltage data from Fig. 2 plotted with (a) a linear current axis or (b) a logarithmic current axis. The smooth curves are calculated according to Eq. (1), using the values of a and b given in Table III. The dashed curves show the corresponding results for frozen skin from Ref. 1.

skin, the fresh skin samples showed more asymmetry with respect to the polarity of the applied potential and were appreciably less conductive at low voltages. These properties of fresh skin are reflected, respectively, by its slightly greater disparity between values of a for positive and negative currents ($P < 0.09$) and by its significantly higher values of b ($P < 0.01$), relative to frozen skin. The probabilities given here are the results of one-sided t tests on the individual cell parameters.

Sodium Ion Transference Number

Sodium ion transference numbers during constant current iontophoresis are shown in Table IV. The value for fresh skin, t_{Na} = 0.60 ± 0.02, was significantly higher (t test) than that for frozen skin, 0.51 ± 0.05, and agreed closely with the value of 0.62 determined for freshly excised human thigh skin immersed in a HEPES buffer by Burnette and Ongpipattanakul (3). This difference may well reflect changes induced by the freeze/thaw cycle, leading to lower cation permselectivity for frozen skin relative to fresh skin. Such a difference could be of importance when studying phenomena dependent on this property, e.g., electroosmosis and neutral molecule flux enhancement.

DISCUSSION

Based on j–V characteristics and a comparison with human *in vivo* results (4,5), two acceptance criteria were proposed in Ref. 1 for selecting excised human skin samples for iontophoresis studies: (1) specific resistance ($R \times$ area) ≥35 kΩ cm² at a current density of 14 μA/cm² or (2) $k_p(Na^+) \leq 2 \times 10^{-6}$ cm/min. The data in Table I support this recommendation. There was an apparent break in permeability values

Table III. Results of Fitting Equation (1) to the Current–Voltage Data for Excised Human Skin

Data set	No. of pts	Mean parameter value ± SD		MSR[b]
		a	log b[a]	
Fresh skin (n = 9)				
All data	33	7.0 ± 1.1	−0.34 ± 0.43	0.2107
$I \geq 0$	18	9.0 ± 1.1	−0.55 ± 0.41	0.0270
$I \leq 0$	18	5.3 ± 1.8	−0.05 ± 0.44	0.0431
Frozen skin (n = 7)[c]				
All data	33	7.6 ± 1.5	−0.91 ± 0.34	0.0521
$I \geq 0$	18	8.8 ± 1.0	−1.02 ± 0.29	0.0183
$I \leq 0$	18	6.6 ± 2.0	−0.76 ± 0.46	0.0086

[a] Units of b are cm²/μA.
[b] Mean squared residuals in fit (V²).
[c] Data from Ref. 1.

Table IV. Sodium Ion Transference Numbers During Constant-Current Iontophoresis (I = 50 μA) in Normal Saline Buffer

ID	Time (hr)	t_{Na} (mean ± SD)
Fresh skin (n = 12)	0–2	0.52 ± 0.04
	2–4	0.59 ± 0.02
	4–6	0.62 ± 0.03
2- to 6-hr average		0.60 ± 0.02
Frozen skin (n = 8)[a]	0–2	0.39 ± 0.05
	2–4	0.50 ± 0.06
	4–6	0.53 ± 0.04
2- to 6-hr average		0.51 ± 0.05

[a] Data from Ref. 1.

between about 2×10^{-6} and 6×10^{-6} cm/min, and all of the samples having $k_p < 2 \times 10^{-6}$ cm/min had an initial resistance of 75 kΩ (52 kΩ cm^2) or higher. [The reported human *in vivo* range of k_p (Na^+) is 0.6–1.0 $\times 10^{-6}$ cm/min (5) and that of $R \times$ area is 590–1170 kΩ cm^2 (4) for skin immersed in normal saline solutions.] More than 75% of the samples tested in the previous (1) and present studies met the above criteria. Since the resistance measurement can easily be carried out at the outset of an iontophoresis study, it appears to be the more useful of the two tests.

Although these criteria appear to be desirable targets, we have some reservations about being able to meet them routinely, at least in the case of frozen tissue. Only 4 of 12 frozen skin samples tested in an earlier study using this skin preparation (2) met the 35-kΩ cm^2 resistance criterion. In a very recent study in our laboratory (unpublished), 0 of 12 samples of frozen back skin met the resistance criterion. While the freezing and storage techniques discussed in Ref. 1 may be useful for preserving tissue integrity, they do not ensure that the skin will be highly resistive. It may be that variability between sites and individuals will ultimately preclude the establishment of absolute limits for resistance or permeability. Nevertheless, the limits given above may be used as benchmarks for evaluating the integrity of human skin samples.

The nonlinear current–voltage behavior of excised skin, combined with the relatively short lag times for steady-state sodium ion transport, offer some insight into the routes of ionic flow through skin. We propose here a model, portions of which have been discussed previously (6,7), which is consistent with these results. In this scenario most ion flow is through the skin appendages, specifically sweat ducts and, to a lesser extent, hair follicles. The primary barriers encountered by the ions are the lipid envelopes of the two or three epithelial cell layers lining these appendages. The permeability of these lipid layers is first reversibly increased by an applied electric field, then permanently altered after prolonged or intense exposures.

Support for this model comes from several sources. Burnette and Ongpipattanakul (8) have shown by means of a microelectrode technique that areas of high current flow through human skin immersed in saline solutions usually correspond with pores in the skin. Staining studies with ionic dyes (9,10) and etching studies with metal foil electrodes (11) have led to similar conclusions for other current carriers.

Although the pores provide a transport pathway through the stratum corneum, it is unlikely that the resistance to ionic flow through skin resides in the lumen of the sweat ducts. Were this the case, it would be difficult to explain the nonlinear current–voltage behavior observed here and in Ref. 1 and the permselectivity of skin for small ions versus larger ones (2). For intact skin it is clear from the anatomy of an eccrine gland (12) that materials which pass through the skin via the lumen of the duct must necessarily pass through several epithelial cell layers in order to enter the body. Based on the current–voltage and sodium ion permeability data presented here and in Ref. 1, it seems that this is also true for excised skin. Since the epithelial cell membranes lining the sweat ducts are thin relative to the lipid layers of the stratum corneum, this model also provides an explanation for the relatively short lag times for achievement of steady-state Na^+ flux.

How can one explain the nearly exponential dependence of current on voltage for excised human skin? This type of electrical behavior is common in semiconductors (13) and at electrode–solution interfaces (14,15). It has been observed in synthetic lipid bilayers (16,17), at the interface of two immiscible liquids (17,18), and in biological membranes capable of producing action potentials (14). In each of these situations, the kinetics of charge transfer govern the current–voltage properties, and conditions at the interface(s) are far from equilibrium. According to Bockris and Reddy, in order to provoke this type of electrical behavior, "there must be a potential-energy barrier (at least in one direction) which hinders the movement of charged particles across the interface and which can be modified in height by the interfacial potential difference" (14).

One mechanism by which such a potential-dependent barrier could be generated in skin is that of hindered charge transport at lipid–water interfaces (17,18). This mechanism was proposed earlier by one of us as a possible explanation for skin current–voltage properties (7). A second mechanism is reversible dielectric breakdown of the epithelial cell membranes lining the skin appendages. Such a phenomenon is known to occur in model lipid membranes subjected to an electric field and is believed to proceed via a pore-formation mechanism (16). For short exposure times and moderate fields, the pore formation process is reversible; under harsher conditions the membranes rupture (16). It seems quite possible that the latter mechanism could explain both the reversible and the irreversible aspects of resistance change in skin during the passage of electric current.

CONCLUSIONS

The strong similarities between the electrical and the sodium ion transport properties of fresh and frozen human skin show that freezing, when carried out as described in Ref. 1, does not grossly alter the tissue with respect to these properties. However, in a limited comparison between skin from different donors, we noted several differences between fresh and frozen tissue. Fresh skin was appreciably less conductive than frozen skin at low current levels and showed a trend (not statistically significant) toward lower sodium ion permeability during passive diffusion. It was more permselective for sodium versus chloride during constant current

iontophoresis and showed more asymmetry in its current–voltage properties. These differences suggest two areas for caution when using frozen cadaver skin to study iontophoretic drug delivery: (1) *in vivo* delivery devices may require a higher-than-anticipated driving voltage to deliver equivalent amounts of drug, and (2) drug delivery based on the electroosmotic effect (which requires cation permselectivity) may not be well predicted by *in vitro* studies with frozen skin.

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