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Report

Iontophoretic Transport of a Homologous Series of Ionized and Nonionized Model Compounds: Influence of Hydrophobicity and Mechanistic Interpretation

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An *in vitro* study was carried out to elucidate the mechanisms controlling iontophoretic transport. The investigation focused on three areas, including the nature of the permeant (state of ionization and hydrophobicity), skin structures (hair follicle distribution and stratum corneum), and various parameters influencing iontophoresis (current, permeant concentration, and competitive ion effects). The data indicate that iontophoretic-facilitated transport is essentially pore mediated and that the transport of ionized and nonionized molecules may be enhanced through the pore-type pathway. The data presented show that iontophoresis has a detrimental effect on the lipoidal transport pathway and that the transport of more hydrophobic nonionized molecules is decreased compared with passive diffusion. The iontophoretic enhancement values decreased linearly with increasing alkyl chain length of *n*-alkanols. The iontophoretic permeability coefficients of ionized *n*-alkanoic acids was shown to decrease with increasing permeant hydrophobicity.

KEY WORDS: iontophoresis; facilitated transport; ion transfer; iontophoretic mechanisms; electroosmosis.

INTRODUCTION

Iontophoretic devices for the delivery of drugs through the skin were extensively reviewed by Tyle (1). Until recently, few investigators have attempted to quantify the mechanisms controlling transdermal iontophoresis. Iontophoresis (or ion transfer) is defined as the transdermal migration of ions when an electric current is passed through a conducting medium containing ionized species. Abramson and Gorin (2) observed the development of a "dot pattern" on human skin following iontophoresis of various dyes. Closer examination showed that the pattern occurred at the orifices of sweat glands. Thus, iontophoretic transport appears to be via sweat pores. Burnette and Marrero (3) and Grimnes (4) confirmed in separate studies that iontophoretic flow is negligible through the stratum corneum and that the dominant pathway for ionic migration is through the "pore pathway." Bellantone *et al.* (5) recently studied the *in vitro* iontophoretic diffusion of benzoate anion, phenethylamine cation, and benzyl alcohol as model compounds in diffusion cells. They reported that iontophoresis did not enhance the flux of benzyl alcohol, a nonionized species. Gangarosa *et al.* (6), however, showed increased diffusion of nonelectrolytes into the skin during *in vivo* iontophoresis, which they postulated was the result of a water transport mechanism.

The purpose of the present investigation was to assess systematically the effects of various experimental parameters on iontophoretic transport. The *in vitro* permeability of a homologous series of ionizable *n*-alkanoic acids and a corresponding series of nonionized *n*-alkanols was quantified using passive and iontophoretic-assisted transport. These studies provided insight into the influence of ionic state and hydrophobicity on iontophoretic transport. Other experimental parameters studied included the influence of current, permeant concentration, and competitive ion effects, with respect to both concentration and type. Finally, the effects of stratum corneum removal and hair follicle distribution on iontophoretic transport were assessed. It was expected that the data from these studies could be used to delineate the mechanisms associated with transdermal iontophoretic transport.

MATERIALS AND METHODS

Chemicals. Radiolabeled ^{14}C-alkanoic acid sodium salts, ^{14}C-alkanols, and ^{3}H-water were obtained from New England Nuclear Corp., Boston, Mass., or ICN Radiochemicals Inc., Irvine, Calif. Stock solutions (100 μCi/mL) were prepared for each compound in deionized distilled water.

Membranes. Excised abdominal skin from nude rats (strain RNU, Harlen Sprague Dawley, Inc., Indianapolis, Ind.) was used unless otherwise specified. The skin was stored at −20°C and thawed prior to use (7). In one subset of

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experiments, the nude rat stratum corneum was removed by stripping the skin 20 times with Scotch brand tape. In another subset of experiments furry rat skin was used (Sprague Dawley rats, strain CD, Sprague Dawley Inc., Kingston, N.Y.). When furry rat skin was used, the abdominal fur was closely clipped using scissors.

Power Source and Electrodes. A DC constant-current power source was designed and built at Hoffmann-La Roche, Inc., Nutley, N.J. The unit can power 12 individual diffusion cells, supplying each cell with a specified current ranging between 0 and 1 mA. Platinum wires were used as electrodes (99.95% purity, 0.3 mm, Allied Fisher Scientific), having an effective working length of 10 mm.

Permeation Procedure. In vitro iontophoretic and passive skin permeation studies were carried out using Valia-Chien cells (Crown Glass Co., Sommerville, N.Y.). The excised skin was sandwiched between the half-cells with the stratum corneum facing the donor side. The exposed skin area was 0.64 cm^2. The half-cell volume was 3.5 ml and the contents were magnetically stirred at 600 rpm. The temperature was controlled at 30 ± 0.2°C. The diffusion cell medium was 0.01 *M* potassium chloride solution (pH 6.5). The radiolabeled test permeants, in the concentration range of 25–50 μM, were added to the donor compartment. For iontophoretic diffusion studies, the cathodic (−) electrode was always placed in the donor compartment and the anodic electrode (+) was placed in the receiver compartment. The electrodes were energized with 0.1 mA of constant current unless otherwise specified. During the iontophoresis experiments, the sodium salts of the alkanoic acids were essentially fully ionized, whereas the alkanols served as model permeants for nonionized compounds. The diffusion of these compounds was monitored by periodically sampling and assaying the receiver compartment.

Data Analyses. Permeability coefficients were determined for each compound by plotting the cumulative amounts detected in the receiver compartment as a function of time. The steady-state flux was calculated from the slope of the linear portion of each curve, using the following equations (8):

$$J = P \cdot A \cdot \Delta C = V \cdot dc/dt$$

and

$$P = \frac{V \cdot dc/dt}{A \cdot \Delta C}$$

where

J = the total pseudo-steady-state flux across the skin (dpm/hr);
A = the diffusion area (cm^2);
ΔC = the concentration differential across the membrane, which was taken to be equal to the donor phase concentration (dpm/cm^3);
P = the permeability coefficient (cm/hr);
V = the half-cell volume of the receptor compartment (cm^3); and
dc/dt = the steady-state slope in terms of dpm/cm^3, hr.

RESULTS AND DISCUSSION

Physical Parameters Affecting Iontophoretic Skin Transport

Current. A series of experiments was performed in which the influence of applied current on *in vitro* iontophoretic transport of sodium butyrate was assessed. The current was varied between 0.05 and 1.0 mA. The data, which indicate a linear increase in butyrate diffusion with applied current, are depicted in Fig. 1. These data are consistent with those previously reported and discussed by Bellantone *et al.* for the *in vitro* flux of benzoate ions across hairless mouse skin (5).

The observed average voltage profiles as a function of time which were necessary to maintain constant currents are shown in Fig. 2. These data represent the net voltage drop between the diffusion cell electrodes. At constant current, the observed voltages reflect the sum of the resistances in the diffusion cell, including voltage drops across the skin, solution, and skin/solution/electrode interfaces. Higuchi (9), using a four-electrode system, has more precisely defined the voltage drop across the skin and its interfaces. A direct comparison between the four- and the two-electrode systems remains to be investigated and is beyond the scope of this paper. The voltage data demonstrate the basis for the selection of 0.1 mA for the remaining studies in the present work. The relatively constant voltage indicates that steady-state conditions were maintained in the diffusion cell.

Permeant Concentration. The influence of permeant concentration on iontophoretic transport was investigated by varying the donor compartment concentration of sodium butyrate over an approximately eightfold range. Based upon the amounts of butyrate detected in the receiver compart-

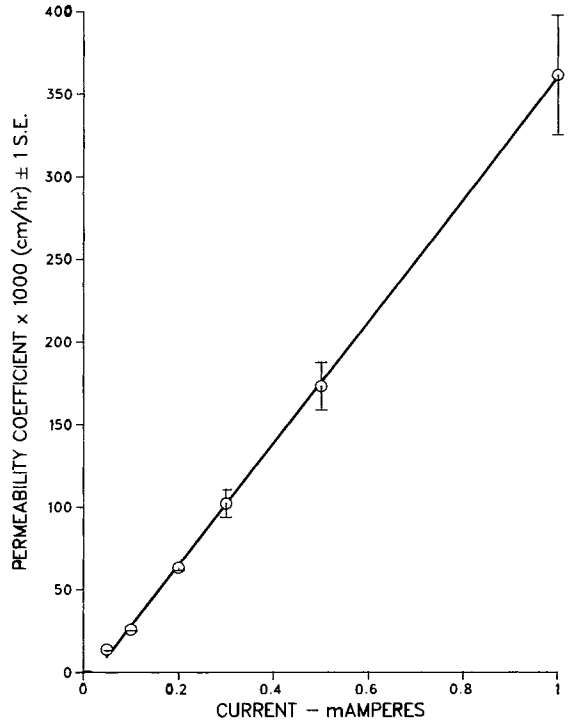

Fig. 1. Iontophoretic diffusion of sodium butyrate as a function of applied current to the cell.

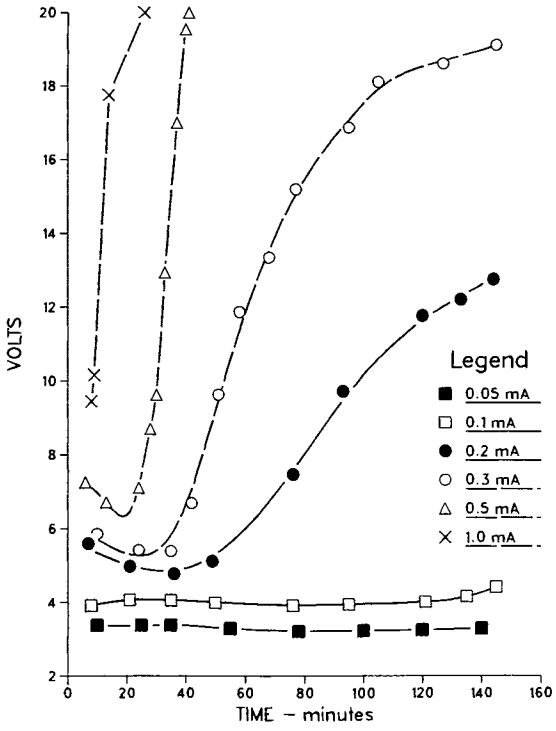

Fig. 2. Cell voltage profile as a function of time. Voltage necessary to maintain constant current.

ment, the steady-state fluxes were calculated and these data are plotted as a function of butyrate concentration in Fig. 3. As in passive diffusion experiments, a linear and proportional relationship was obtained, indicating that, under the present experimental conditions of low permeant and electrolyte concentrations, the permeability coefficient is independent of concentration.

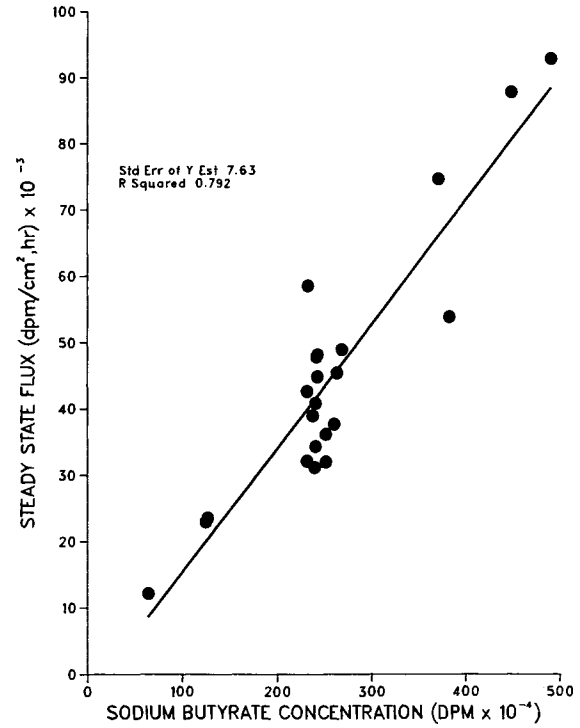

Fig. 3. Iontophoretic diffusion of sodium butyrate as a function of applied current.

Competitive Ions: Influence of Type and Concentration. The iontophoretic transport of butyrate ions was studied in the presence of various 0.01 *M* electrolyte solutions, while holding all other diffusion variables constant. The permeability coefficients (*P* values) as a function of electrolyte type, for the iontophoretic diffusion of butyrate, are summarized in Table I. There appears to be a complex interaction between electrolyte dissociation (number of ions generated, ion changes, ionic mobilities, etc.) and other iontophoretic transport parameters (permeant, vehicle and membrane factors, etc.) which precludes any in-depth analysis of the data. The data, however, provide some insight with respect to selection of electrolytes for use in iontophoretic delivery systems. Dissociation of mixed valence salts results in greater numbers of competitive ions in solution and the use of these salts in iontophoretic studies should generally be avoided. Also, electrolytes having multivalent cations appear to be superior to those with univalent cations. It has been suggested that multivalent cations could neutralize the negative charge of the skin (6), which may partially explain this observation.

The influence of the competitive ion concentration on the iontophoretic transport of butyrate ions was investigated and these data are presented in Fig. 4. The curves obtained by plotting the butyrate permeability against the square root of the salt concentration appear identical to the conductivity curves predicted by Kohlrausch's law (10) for weak electrolytes. The significance of Kohlrausch's law is that at infinite dilution the conductance of each ion is independent of the oppositely charged ion with which it is associated. It postulates that with increased dilution, ionization increases for weak electrolytes, whereas for strong electrolytes that are completely ionized, the interionic interference diminishes. In our experimental system, the butyrate concentration is in a micromolar range and thus is essentially fully ionized. Applying the above concepts to the butyrate iontophoretic data, competitive ions in solution impede diffusion through electrostatic interactions between butyrate anions and electrolyte cations. Extrapolating this conclusion to a system containing high concentrations of sodium butyrate, with no other competitive ions, one would not expect a direct relationship between increased donor concentration and increased flux, due to incomplete dissociation of molecules in

Table I. Iontophoretic Diffusion of Sodium Butyrate at 0.1 mA as a Function of Electrolyte Type in Diffusion Medium

0.01 *M* Electrolyte	*P* value × 1000 (cm/hr)		
	Mean	SD	N^a
$MgSo_4$	59.7	2.5	3
NaCl	39.1	3.8	3
$MgCl_2$	34.5	3.6	3
KCl	25.9	4.7	26
K_2HPO_4	16.9	1.3	2
K_2SO_4	10.5	3.8	3

[a] Number of replicates.

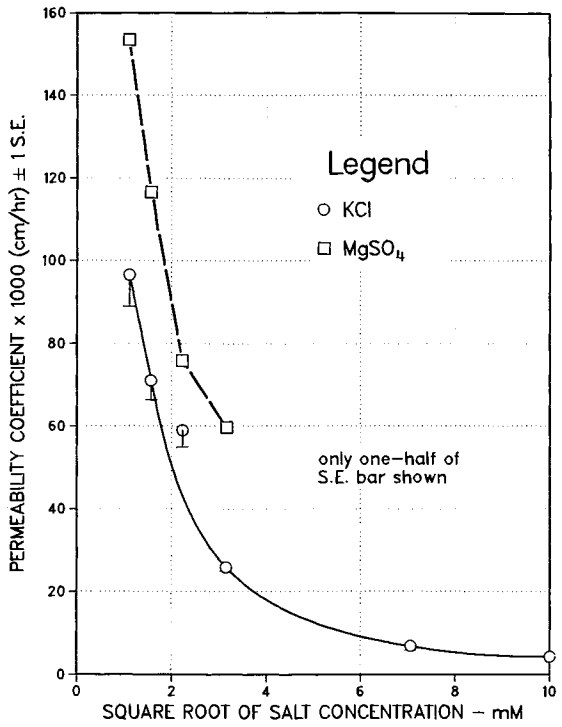

Fig. 4. Influence of the competitive ion concentration on the iontophoretic diffusion of sodium butyrate.

solution. This may partially explain why Bellantone *et al.* (5) observed only a 1.7-fold increase in benzoate flux with a 20-fold increase in donor concentration.

Influence of Skin Structures on Iontophoresis

Furry Skin vs Nude Skin. In these experiments the effect of hair follicle distribution on iontophoretic transport was studied. Comparisons were made between the iontophoretic transport of various alkanoic acids across furry rat skin and that across nude rat skin, which has only a sparse hair distribution. The purpose of this particular study was to assess the influence of iontophoresis on the pore-type transport pathway. The term "pore-type transport" is used in a broad sense and is not limited just to the sweat pores in the skin. It includes all skin structures or imperfections that act as channels through which molecules can migrate. One such structure which is easily evaluated is hair follicle distribution.

Iontophoretic transport, using furry skin, increased 61% for acetate, 44% for valerate, and 12% for octanoate over that obtained with nude rat skin. These compounds were selected in order to delineate between pore-mediated and lipoidal transport pathways, i.e., acetate generally favoring the first and octanoate the latter pathway. These data indicate that all three compounds are transported through pores, although with decreasing efficiency, during iontophoresis. The increased transport could not be explained by increased lipoidal transport in the furry rat. Passive diffusion data indicate that the permeability coefficient for octanoate was 38% lower with furry rat skin compared to nude rat skin. Therefore, increased transport may be attributed to the increased number of shunts in furry rat skin and this observation appears to support the pore transport model.

Stripped Skin vs Whole Skin. The purpose of this study was to assess the effect the stratum corneum has on iontophoretic transport. Iontophoretic transport of acetate, valerate, and octanoate increased by 4.1-, 3.0-, and 2.9-fold, respectively, after removal of the stratum corneum from nude rat skin. These data indicate that the stratum corneum retards the iontophoretic transport of all three molecules including the more lipophilic octanoate.

Permeant Ionization State and Hydrophobic Character

Nonionized Molecules. A homologous series of *n*-alkanols and water were used as model nonionized compounds to assess their transport by iontophoresis and to gain insight into the operative diffusion pathways in iontophoresis. These permeants have been used previously to define the mechanisms of passive skin transport and to study the effect of variables such as hydration and skin aging on transport kinetics (11–13). The iontophoretic and passive diffusion profiles of *n*-alkanols and water are presented in Fig. 5 (water data provided for informational purposes only). Compared to passive diffusion, there is enhanced iontophoretic transport of methanol, ethanol and butanol; equivalent transport of hexanol; and decreased transport of octanol. This last finding was further investigated by testing decanol (Fig. 5), whose transport was even further decreased. Enhancement ratios were calculated for each compound, comparing iontophoretic diffusion with passive diffusion, and these data

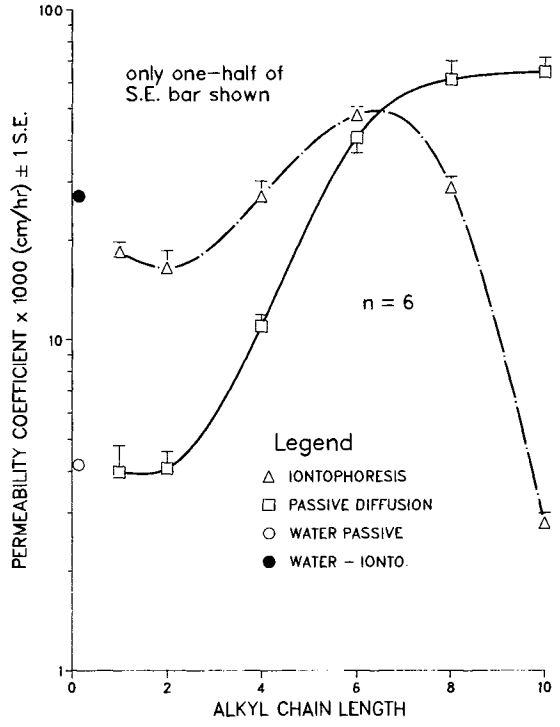

Fig. 5. Comparison between iontophoretic and passive diffusion of *n*-alkanols and water. *n* represents the number of replicates.

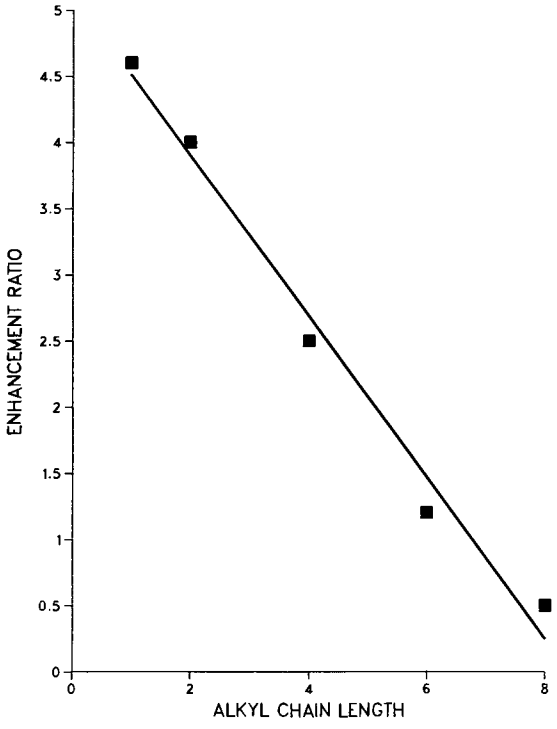

Fig. 6. Iontophoretic enhancement of alkanol diffusion compared with passive diffusion.

are plotted as a function of alkyl chain length (ACL) in Fig. 6. The curve shows a linear decrease in enhancement ratio with increasing ACL. This is the first time that an observation has been made relating decreased iontophoretic enhancement to increases in permeant hydrophobicity.

Gangarosa *et al.* (6) have previously reported increased transport of thymidine, D-arabinofuranosyladenine, and water, all nonconductive compounds, during anodal iontophoresis in dilute sodium chloride solutions. These investigators postulated that increased transport results from water movement (iontohydrokinesis) associated with sodium ion transfer using anodic iontophoresis. This concept may explain the increased iontophoretic diffusion of alkanols in the present study. Nonionized compounds may be passively carried in association with the hydration shells of ions in solution during iontophoresis.

The detrimental effect of iontophoresis on the transport of more hydrophobic alkanols was not expected. However, in retrospect, such data are compatible with the iontohydrokinesis diffusion model presented previously by Gangarosa et al. (6). Increasingly more hydrophobic molecules would have greater difficulty in associating with water molecules which form the hydration shells of ions in solution. Therefore, the iontophoretic transport of alkanols with increasing chain lengths should decrease. The rise seen in the iontophoretic curve for the shorter-chain alkanols (Fig. 5) may be explained by the fact that partitioning into, and saturation of, the hydration shells becomes a limiting factor only for alkanols of alkyl chain length six and above in the present experimental design. The significant point of this study is that iontophoresis can facilitate the transport of some nonionized molecules. Our data suggest a modest fourfold to fivefold increase over that of passive diffusion (Fig. 6). However, iontophoresis may have a detrimental effect on the transport of more hydrophobic molecules, and therefore, such molecules may not be good candidates for iontophoretic delivery.

Ionized Molecules. A homologous series of *n*-alkanoic acids was used to study the influence of increasing permeant hydrophobicity on iontophoretic transport of ionized compounds. These data are presented in Fig. 7, along with passive diffusion data for the same compounds. The iontophoretic data show a linear decrease in permeability with increasing alkyl chain length over the entire range of compounds studied (ACL 1–8). These data extend the finding that the efficiency of iontophoretic transport decreases with increasing permeant hydrophobicity to include ionized species. Enhanced iontophoretic transport of ions ranging in size from sodium to insulin has been reported (1), therefore, limitations based on molecular size have not yet been delineated. The present study indicates that iontophoretic transport of relatively small hydrophobic molecules is retarded, indicating that permeant hydrophobicity may be a more critical parameter influencing transport than is molecular size. Of course, the issue of hydrophobicity vs size is complex and needs further studies for a more complete understanding.

Transport Mechanisms

Skin diffusion pathways are well defined as a result of using homologous series of compounds with increasing hy-

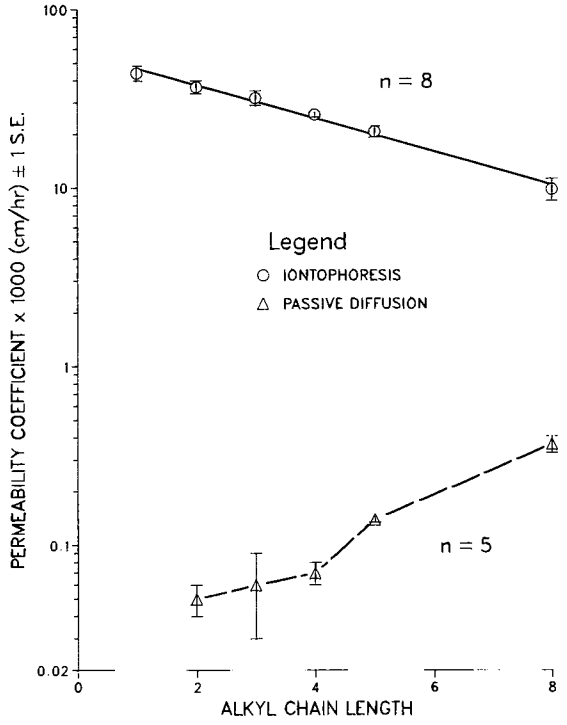

Fig. 7. Iontophoretic and passive diffusion profiles of ionized alkanoic acids. *n* represents the number of replicates.

drophobicity to delineate transport through the skin's pores, lipids, and aqueous tissues (7,11–13). This approach has been found useful in assessing the influence of iontophoresis on these transport pathways during the present study. Total flux during iontophoresis experiments is composed of the normal passive diffusion component plus the facilitated iontophoretic transport component. Since charged permeants are usually selected to study iontophoretic transport, the passive diffusion component is generally insignificant (Fig. 7). In the present study, the iontophoretic transport data obtained for the nonionized alkanols, which are known to be significantly transported by passive diffusion (Fig. 5), indicate inhibition of passive transport. Since the observed inhibition becomes apparent for the more hydrophobic alkanols, which are normally transported through the lipoidal pathway, we postulate that iontophoresis has a detrimental effect on this pathway. The increased iontophoretic transport of the lower alkanols has been discussed above and probably represents facilitated transport through the pore-type pathway. It is suggested that the pore environment, with its close packing of ionic species, represents a relatively more hostile environment for hydrophobic molecules, charged or uncharged, than does the vehicle. Therefore, the efficiency with which these molecules enter the pore-type pathway decreased with increasing hydrophobicity.

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