Alkali Halide-Assisted Penetration of Neostigmine across Excised Human Skin: A Combination of Structured Water Disruption and a Donnan-like Effect

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Abstract □ The penetration of neostigmine across excised human skin mounted in flow-through diffusion cells, delivered from a 0.28 M aqueous solution, was below detection limits. The presence of either NaCl or LiCl in the donor solution caused significant fluxes of neostigmine, with permeability coefficients (K_p's) in the range of 10^{-6} cm min^{-1}. Paradoxically, low concentrations of NaCl or LiCl (0.25 and 0.5 M) were more effective in this respect than the 1 M solution, which was the least effective concentration in the range of 0.25–3 M. Thus, the dependence of the experimental K_p values on inorganic ion concentration followed a biphasic course, suggesting the participation of two distinctive mechanisms in the penetration-enhancement process. The early phase corresponding to 0.25 and 0.5 M NaCl or LiCl is being partly ascribed to a decrease in the viscosity of lamellar water caused by the influx of the respective hydrated ions, hydration of LiCl or NaCl being more extensive at low alkali halide concentration that at higher ones (reference cited). The late phase corresponding to 2 and 3 M LiCl and NaCl is partly ascribed to a Donnan-like effect whereby the presence of a large excess of poorly diffusible common ion (Na^+ or Li^+) enhances the partitioning into the skin of the more diffusible ion, in this case neostigmine cation. The presence of inorganic ions at different concentrations had no effect on the partial molal volume of neostigmine bromide (V_i^∞ = 223.5 cm^3 mol^{-1}), which was practically the same for all concentrations of either LiCl and NaCl. Enhancement of the penetration of neostigmine probably by a Donnan-like effect was far more prominent in the presence of benzalkonium cation, which is less likely to penetrate the skin barrier in comparison to Li^+ or Na^+. The K_p's observed were of the order of 10^{-5} cm min^{-1} and showed a clear dependence on benzalkonium chloride molarity in the range of 0.25 to 1 M.

The thermodynamics and kinetics of transdermal drug penetration have usually been approached from a consideration of the partitioning of the penetrant between donor and skin.[1-6] In this context, the use of a range of penetrant probes comprising at one end lipophilic molecules and at the other end amphiphilic molecules proved highly effective, generating various trajectory models of drug diffusion across the skin barrier.[1,2,4-6] Thus, one can invoke an intercellular trajectory for the lipid-soluble drugs and the more amphiphlic ones[1,6] and perhaps a polar route for the hydrophiles,[1,6-9] the distinction between these routes not being always clear and sharp. The distinction among the various hypothetical barrier regions of the skin can be overlooked by applying the extended solubility approach[10] to the transfer of penetrant from donor to skin. In this approach, the transfer is governed by the excess free energy of the solute in the donor over that in the skin, both being deducible from the cohesion parameters of solute, donor system, and a mean cohesion parameter assigned to the skin.[2,10,11] But this approach, which is a variant of partition theory, reaches its limitations with penetrants at the extreme end of the hydrophilic scale such as the inorganic and organic ions which are known to associate strongly with water molecules to the point of formation of a hydration shell.[12,13] Water itself is known to permeate through skin at an estimated permeability coefficient (K_p) of the order of 10^{-3} cm h^{-1},[3] so that there might exist a common denominator between water penetration on the one hand and hydrophile penetration on the other. This view is partly borne out by data reported in conjunction with the iontophoretic-assisted transdermal penetration of inorganic and organic ions and also mannitol and the unchanged forms of thyrotropin-releasing hormone (TRH),[14] lidocaine,[15] insulin,[16-18] and perhaps others.[19] As a rule, the iontophoretic flux of Na^+ or Li^+ was much higher than their passive flux, the former being proportional to the applied current density. This finding is anticipated from the fact that a charged species such as Na^+ is expected to migrate in an electric field from the anode (+) to the cathode (−). Less expected is the concurrent increase in the anodal transport of the highly hydrophillic mannitol, which bears no formal positive charge, and hence should not have been a candidate for iontophoretic migration. Burnette and Ongpipattanakul[9] ascribed this phenomenon to a convection flow arising from a net volume flow during iontophoresis from anode to cathode. In other words, hydrophiles and ions might be carried over the barrier region of the skin by a water current which is evoked, in this case, by iontophoresis. Would such a current arise under conditions other than iontophoresis? Conceivably, a convection flow across the skin barrier could arise from a Donnan-like effect, whereby the penetration of a diffusing ion is enhanced by the presence of a nondiffusing ion bearing a common charge. The Donnan exclusion of co-ions was addressed by Burnette and co-workers,[9] in a different context, as these authors were primarily concerned with the permselectivity of the skin toward the positively charged species.

We have now approached the problem by determining the free transdermal flux of a positively charged organic molecule, neostigmine, across samples of dermatomed human skin mounted in flow-through diffusion cells. The choice of neostigmine for this study was guided by the following considerations: the molecule embodies a quaternary nitrogen and hence is permanently charged irrespective of pH in the physiological range, it has a relatively large molar volume which is an impediment to free flow, and pertinent data on the free transfer of the structurally related pyridostigmine is available from the work of Phipps and co-workers.[19]

Theoretical Section

The flux at steady state, J_{ss}, of a penetrant across a diffusion barrier, in this case the skin, is given by Fick's first law of diffusion:

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$$J_{ss} = (K_m D \Delta C)/h \quad (1)$$

where D is the diffusion coefficient as expressed in the Stokes–Einstein equation (see eq 4), ΔC is the concentration difference of penetrant between the proximal and distal ends of the barrier, and h is its thickness. K_m is the theoretical partition coefficient between the proximal region of the barrier and the donor system, usually approximated as the partition coefficient between a pair of solvents such as octanol and water or others.[1,2] Implicit in this approach is the assumption that the mean solvent property of the skin with respect to penetrant resembles that of octanol or olive oil. This significance of K_m becomes irrelevant in the case of hydrated ions which can effectively penetrate the skin only through a more compatible matrix, such as the interlamellar water of the lipid bilayer. The passive transfer of an ion C^i from an aqueous donor solution to lamellar water in the presence of a non-diffusing ion R^i bearing a common charge $i(+/-)$ is assumed to be governed by the Donnan equilibrium, as follows:

$$[C^i]_{acceptor}/[C^i]_{donor} = \{1 + [R^i]_{donor}/[C^i]_{donor}\}^{1/2} \approx K_m' \quad (2)$$

The left-hand side of eq 2 represents the equilibrium partition coefficient, K_m', of the penetrant between the aqueous compartment of the barrier and the donor aqueous solution. In this model, the stratum corneum is assumed to fulfill the double role of a diffusion barrier on the one hand and the acceptor solution of the Donnan system on the other. It can be seen that K_m' is expected to increase nonlinearly with an increase in $[R^i]_{donor}$. K_p, the experimental permeability coefficient, must be directly proportional to K_m' hence

$$K_p = (K_m' D)/h \quad (3)$$

The Stokes–Einstein equation gives the value of the diffusion coefficient D, as follows:

$$D = RT/6\pi r\eta N_0 \quad (4)$$

where R is the gas constant, T is the absolute temperature, r is the radius of the diffusing molecule, N_0 is Avogrados number, and η is the viscosity of the diffusion medium, in this case lamellar water.

Substitution of K_m' in eq 3 by its value in eq 2 and D by its value in eq 4 gives

$$K_p = \{1 + [R^i]/[C^i]\}^{1/2}(RT/6\pi r\eta N_0)1/h \quad (5)$$

Eq 5 predicts that the transdermal penetration of a water-soluble molecule bearing a charge will be enhanced by the presence of a nonpenetrating or poorly penetrating ion of similar charge in the donor solution and also by a decrease in the viscosity of the diffusion medium, in this case lamellar water, concomitant with an increase in convection flow, provided other parameters in the equation remain constant. This hypothesis is now being put to test by determination of the flux of neostigmine, delivered as the bromide salt from aqueous solutions containing additional electrolytes.

Experimental Section

Samples of dermatomed strips of human skin ≈ 500 μm thick were removed from the face and back of the leg soon after amputation. They were wrapped in saline-soaked pads and aluminum foil shortly after removal and kept frozen at −20 °C until used. Upon thawing at room temperature, skin samples were mounted in stainless steel doubly-jacketed diffusion cells of the flow-through type, thermostated at 37 °C. The skin was interposed between a donor and an acceptor compartment, the area of diffusion being 1 cm². To minimize individual and regional variations in permeability, a given series of tests was usually run as follows: A single strip was divided into two samples which were mounted in each of two diffusion cells. Using these, donor solutions (0.45 mL each) containing neostigmine bromide 0.28 M (≈135 μmol) and two different concentrations of a given electrolyte (NaCl or LiCl or benzalkonium chloride) were then applied to determine the flux of neostigmine. The consecutive series of tests were carried out with one of the donor solutions used in the earlier run and a third donor solution containing a new concentration of the given electrolyte. Thus, each successive run of tests included a donor solution that had been used in the preceding one as a check for acceptable reproducibility. The steady state flux of neostigmine over the entire sampling time was also indicative that the barrier properties of the skin were not altered. In certain cases, an organic solvent was used as a solvent for neostigmine or a penetrant other than neostigmine was used to probe the efficacy of penetration from aqueous solutions. These additional experiments were included in order to broaden the data base pertaining to the penetration process.

Unless otherwise stated, the collecting solution consisted of 8 mM KH_2PO_4 and 150 mM NaCl, pH 4.6, perfusing through the acceptor compartment at a rate of 10 mL/h. Perfusate samples, 10 mL each, were collected automatically over periods of 24 h under conditions approaching "sink effect".

Neostigmine contained in the perfusate was analyzed by reverse-phase ion pair chromatography using a Varian Model 5000 HPLC equipped with a UV detector set at 214 nm. An aliquot, of the sample, was injected into a 100-μL loop followed by a reverse-phase column (250 × 4.6 mm internal diameter) containing octyl Spherisorb S5-0DS. The column temperature was held at 30 °C.

The mobile phase was prepared as follows. A solution of 10 mM octanesulfonic acid sodium salt monohydrate, 10 mM NaH_2PO_4, and 2.5 mM tetramethylammonium chloride was brought to pH 3 with sulfuric acid. This solution (70 volumes) was mixed with acetonitrile (30 volumes) to give the mobile phase. The solvent was pumped through the column at a flow rate of 1.1 mL/min. A calibration curve was prepared from solutions of neostigmine bromide in the range 2–20 μM contained in the collecting buffer. The standard solution and perfusate samples were stored at 4 °C until used. Reproducibility was ensured by running a standard neostigmine solution with every series of tests. From the concentrations of neostigmine found in the perfusates and the volumes of these, the transfer of mass over time was calculated and represented in the usual penetration curves.

When adenosine or theophylline were used as penetrants in the presence of benzalkonium chloride, similar conditions were applied except the following: for theophylline, the mobile phase consisted of 90 volumes of 0.1 M KH_2PO_4 and 10 volumes of acetonitrile, and the run operated at a flow rate of 1 mL/min and 40 °C, with the detector set at 272 nM. For adenosine, the mobile phase consisted of 87 volumes of 0.1 M KH_2PO_4 and 13 volumes of acetonitrile, and the run operated at a flow rate of 1 mL/min and 30 °C, with the detector set at 262 nm. A provision for the detection of inosine, which is deaminated adenosine, was also included,[18] but if this had formed, it was below detection limits.

Determination of Partial Molal Volumes—High-precision density measurements using an Anton Paar DMA 602 and DMA-60 system were used to determine the partial molal volume at infinite dilution (V_i^∞) of neostigmine bromide in various solvents, following the procedure of Liron and Cohen.[20] A U-shaped glass cell of constant volume was electromagnetically vibrated in an undamped harmonic system and the period of oscillation (T) read directly on a digital display to the sixth decimal point. Changes in the oscillation period of the cell at its natural frequency were recorded in relation to changes in its mass when filled with pure liquids and solutions selected for density measurements. T is related to the density, ρ, of solution x by the following relation:[23]

$$\rho_x = 1/A(T_x^2 - B) \quad (6)$$

A and B are constants that are determined by precalibration with air and distilled water for which the densities are known at the existing atmospheric pressure.

The temperature inside the cell was kept constant with an accuracy range of ±0.005 °C. The desired mass of solvent was added to each of six or seven 22-mL vials, each containing neostigmine bromide preweighed to the fifth decimal point of a gram. The mass fraction of the solute in the series ranged from 10^{-3} to 1.2×10^{-2}. The specific volume of each of these solutions was plotted against the mass fraction of solute to give

Table 1—Penetration of Neostigmine across Dermatomed Human Skin Delivered from a Solution of Neostigmine Bromide in Various Organic Solvents at the Indicated Saturation Concentrations

Solvent	δ,[a] $(cal/cm^3)^{1/2}$	concn, M	T_L, min	$J_{ss} \times 10^4$, $\mu mol\ cm^{-2}\ min^{-1}$	$K_p \times 10^6$, $cm\ min^{-1}$
Propylene glycol	15[b]	0.45		ND	
Propionic acid	10[b]	1.7	960	6.3	0.38
Hexanoic acid	9.5[b]	0.13	60	0.96	1.2
Ethanol	12.7[b]	0.8	375	6.16	2.2
Isopropyl myristate + hexanoic acid (54:46 v/v)	8.9[c]	1.5×10^{-3}	69	6.16	410

[a] Cohesion parameter of the solvent. [b] Reference 21. [c] Calculated from $\delta = \sum \delta_i \phi_i$ (ref 21), where δ is the effective cohesion parameter of a mixture, and ϕ_i is the volume fraction of constituent having cohesion parameter δ_i. The respective δ_i and ϕ_i are 8.5 (ref 22) and 0.54 for isopropyl myristate and 9.5 (ref 21) and 0.46 for hexanoic acid.

a linear plot with $r^2 \geq 0.999$ and SE $< 5 \times 10^{-5}$. The product of the specific volume of the solute, extrapolated to mass fraction of 1, and its molecular weight gave the partial molal volume of neostigmine bromide. A detailed working example is given elsewhere.[20]

Osmolality Measurements—These were determined with a Wescor 5500 vapor pressure osmometer. The osmolality of a given solution was determined using each of two standard calibration plots of neostigmine bromide and NaCl in molal units against osmolality (in millimole/kilogram).

All chemicals were of the highest grade commercially obtainable.

Results and Discussion

The fluxes of neostigmine delivered from pure water solution, if any, were below detection limits and may be regarded as approaching zero. The use of an organic solvent as delivery vehicle produced fluxes of the order of 10^{-5}–10^{-4} $\mu mol\ cm^{-2}\ min^{-1}$, depending on the concentration of neostigmine in the donor solution (Table 1) and corresponding to K_p's in the wide range of 10^{-7}–10^{-4} $cm\ min^{-1}$. These data, even if not immediately relevant to the model being currently considered, are useful in the sense that they convey estimates of the limits of the percutaneous penetration of neostigmine under conditions favoring enhancement following both an increase in driving force, as with isopropyl myristate,[11,22] and barrier modification, as with propionic acid[18] and possibly ethanol.[15] The lack of penetration from propylene glycol is remarkable and is consistent with the relatively high cohesion parameter of this solvent ($\delta = 15$).

In contradistinction to pure water, the presence of either NaCl or LiCl in water as the donor solvent produced appreciable fluxes of neostigmine, with K_p's well in the range found for the membrane-modifying donor solvents such as propionic acid or ethanol (Table 2). Obviously, some of the predictions of the current working hypothesis, as given in eq 5, are being fulfilled but without complete compliance with the proposed model. The divergence from the model is seen as a biphasic dependence of K_p on donor solution molarity for either NaCl and LiCl (Figures 1 and 2). Unexpectedly, the K_p values of 0.25 and 0.5 M are higher than the K_p values at 1 M, the differences being of statistical significance. From 1 M onward, a moderate rise in K_p can be perceived with increasing electrolyte concentration, partly satisfying the prediction of the model.

At this point it must be recalled that different activities correspond to different concentrations of inorganic electrolytes. Thus, the activity coefficient of NaCl at 25 °C is 0.93 at 0.005 M but 0.67 at 2 M,[24] implying that the actual concentration of Na^+ is not a linear function of molarity. However, the activity coefficient of NaCl in the narrower range of 0.50–3.0 M is fairly constant (0.68–0.75), meaning that a plot of K_p over activity will differ only slightly from one of K_p over molarity, not affecting the biphasic course of the plot. It is not known how the presence of 0.28 M neostigmine bromide will affect the activity of coefficient of NaCl in solution. An alternative procedure is a plot of K_p over total osmolaity of the donor solution (Table 3, Figure 3). Again, the biphasic course of K_p over osmolaity suggests that osmolality may not be a major driving force in the skin penetration of neostigmine. In further support of this contention, the K_p of neostigmine delivered from 3 M NaCl against a 150 mM NaCl and 8 mM KH_2PO_4 acceptor solution (Table 2) was not different from the K_p found when the concentration of NaCl in the acceptor solution was raised from 150 mM to 3 M.

A similar argument may be offered in the case of LiCl solutions

Table 2—Penetration of Neostigmine across Dermatomed Human Skin[a] Delivered from Aqueous Solutions of Neostigmine Bromide in the Presence of the Indicated Concentrations of Electrolytes

Solvent	T_L, min	$J_{ss} \times 10^4$,[b] $\mu mol\ cm^{-2}\ min^{-1}$	$K_p \times 10^6$,[b] $cm\ min^{-1}$
Water		*d*	*d*
Water[c]		*d*	*d*
0.25 M NaCl	50	3.83 ± 1.0	0.73 ± 0.09
0.25 M LiCl	210	8.33 ± 2.83	2.58 ± 0.62
0.50 M NaCl	156	16.0 ± 8.0	3.43 ± 1.10
0.50 M LiCl	204	5.3 ± 1.0	2.03 ± 0.35
1.00 M NaCl	54	2.83 ± 0.83	0.14 ± 0.08
1.00 M LiCl	72	6.33 ± 1.66	0.33 ± 0.08
2.0 M NaCl	282	17.3 ± 7.83	1.81 ± 0.23
2.0 M LiCl	30	7.0 ± 1.16	1.76 ± 0.23
3.0 M NaCl	222	10.1 ± 4.6	2.36 ± 0.66
3.0 M LiCl	186	13.66 ± 2.6	3.74 ± 0.63

[a] Five different skin samples tested with each solvent. [b] Mean ± SEM. [c] Following exposure of the skin to propionic acid for 35 min and then washout of the propionic acid with water. [d] Below detection levels.

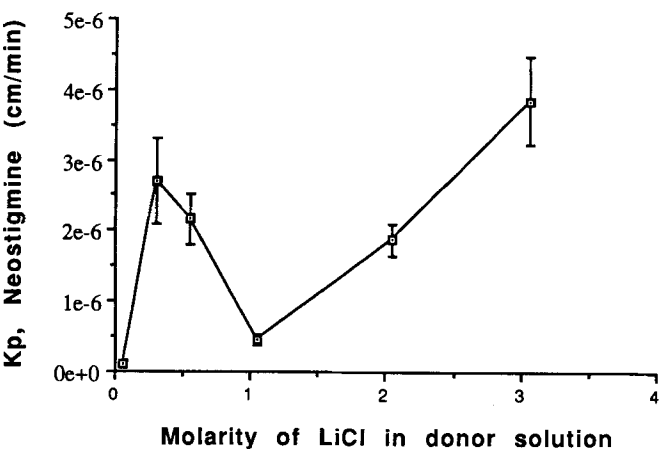

Figure 1—Relationship between K_p of neostigmine, delivered from solutions of LiCl across human skin samples, and LiCl concentration in the donor solution.

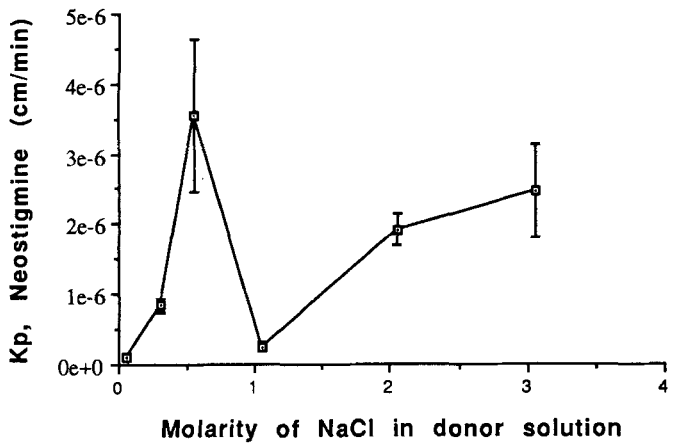

Figure 2—Relationship between K_p of neostigmine, delivered from solutions of NaCl across human skin samples, and NaCl concentration in the donor solution.

Table 3—Osmolality of Solutions Containing Neostigmine Bromide and NaCl at Different Concentrations

	Osmolality, mmol/kg			
NaCl, M	NaCl	Neostigmine	Total	$K_p \times 10^6$, cm/m
0.25	516	248	764	0.68
0.25	516	225	740	0.28
0.25	516	342	858	0.98
0.5	980	264	1244	4.12
0.5	980	264	1244	2.13
0.5	980	304	1283	2.12
1.0	1909	215	2124	0.16
1.0	1909	240	2149	0.07
1.0	1909	304	2213	0.30
2.0	3766	725	4492	1.91
2.0	3766	552	4318	2.37
2.0	3766	225	3991	1.30
3.0	5624	537	6161	2.40
3.0	5624	205	5829	1.20
3.0	5624	343	5966	3.04

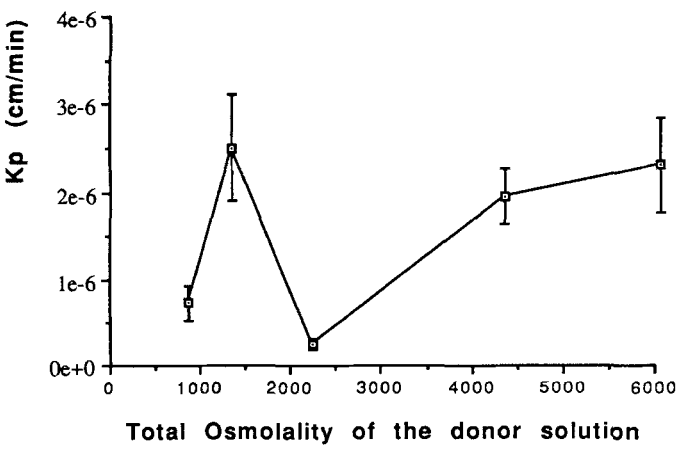

Figure 3—Relationship between K_p values of neostigmine bromide, delivered across human skin from NaCl solution, and the total osmolality of the donor solution (as given in Table 3) in millimoles/kilogram.

for which the activity coefficient is minimal at 0.5 M (0.739) but then rises to higher values at 1 M (0.774) and perhaps beyond.[25]

The extent of divergence of the experimental data from the theoretical model implied in eq 5 can be better perceived by comparing the proportionality of K_p values in each experimental

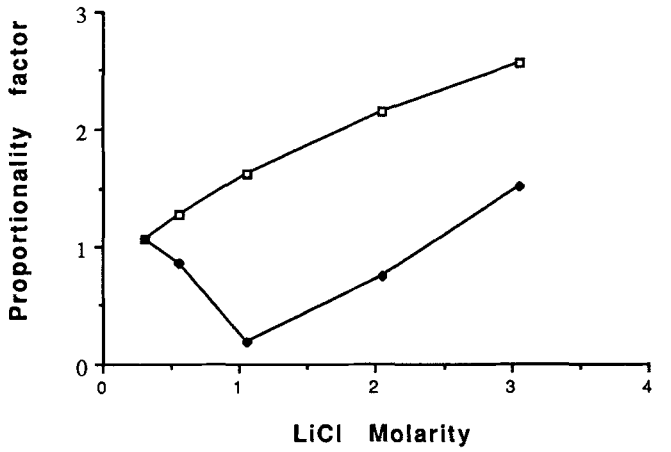

Figure 4—Proportionality of K_p values found for neostigmine delivered from LiCl solutions compared to the proportionality of the corresponding values calculated from eq 5, assuming constancy of the term $(RT/6\pi r\eta N_0)1/h$: □, expected values; ◆, observed values.

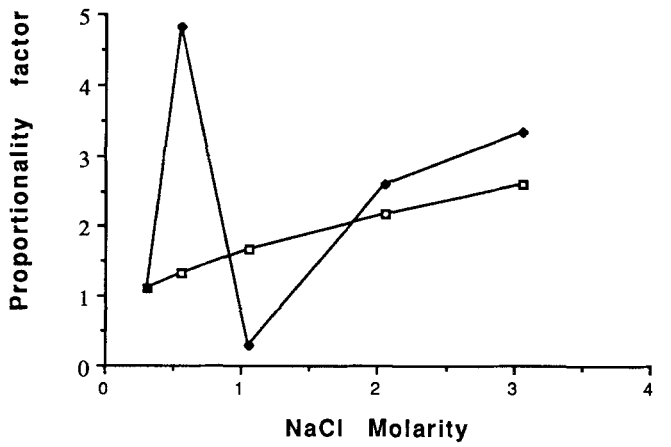

Figure 5—Proportionality of K_p values found for neostigmine delivered from NaCl solutions compared to the proportionality of the corresponding values calculated from eq 5, assuming constancy of the term $(RT/6\pi r\eta N_0)1/h$: □, expected values; ◆, observed values.

Table 4—Partial Molal Volume at Infinite Dilution (V_i^∞) of Neostigmine Bromide[b] in Various Solutions[c]

Solvent	V_i^∞,[a] cm^3 mol^{-1}	Solvent	V_i^∞,[a] cm^3 mol^{-1}
Water	222.25 ± 0.00	0.25 M LiCl	223.00 ± 0.07
0.25 M NaCl	223.46 ± 0.12	0.50 M LiCl	223.00 ± 0.36
0.50 M NaCl	223.57 ± 0.16	1.00 M LiCl	223.34 ± 0.37
1.00 M NaCl	223.53 ± 0.08	Methanol	192.53 ± 0.896

[a] Mean ± SEM. [b] Four samples tested for each solvent. [c] All experiments were carried out at 25 °C.

series and the proportionality of the corresponding K_p values calculated from eq 5 assuming constancy of the term $(RT/6\pi r\eta N_0)1/h$ (Figures 4 and 5). In either case, the proportionality factor at 0.25 M electrolyte is taken as 1. Noncompliance with eq 5 at electrolyte concentrations smaller than 1 M will now be considered for the hypothetical case where r and/or η could have changed under the conditions of the test. The partial molal volume at infinite dilution V_i^∞ of neostigmine bromide in aqueous solutions in the absence and presence of electrolytes showed little change with electrolyte concentrations over the entire range of 0.25–1 M NaCl or LiCl (Table 4). In view of this finding, the probability of a change in r in eq 5 is not a likely explanation of the paradoxical dependence of K_p on electrolyte molarity. Also, the finding that V_i^∞ in aqueous solutions is 223 cm^3 mol^{-1},

while that in methanol is about 193 cm^3 mol^{-1}, suggests that neostigmine bromide is predominantly hydrated in aqueous solutions. At this point, it is useful to record that V_i^∞ of the related pyridostigmine bromide in water is significantly smaller, being 178.59 cm^3 mol^{-1}, which may partly account for the skin penetrability of this molecule from water.[19]

There remains to consider the likelihood of the participation of η, the viscosity coefficient, in the effect produced. Lamellar water within the membrane is assumed to be structured and bound[26], therefore, owning poor mobility. However, the penetration of small inorganic ions into the lamellae causes disruption of the structured water molecules, resulting in increased mobility of the molecules and decreased viscosity of the medium. In the present case, a change in the viscosity of the diffusion barrier can only occur through the intervention of the ions Na^+, Li^+, and Cl^-, which are known to be strongly hydrated, and perhaps Br^-, which codiffuses to an unknown extent with neostigmine cation. This statement seems to invalidate the earlier assumption implicit in eq 5 whereby neostigmine is the permeable species while Na^+ or Li^+ are not. In fact, all three species are of such poor penetrability under the conditions of the assay that the inverse case may also be true. Namely, neostigmine cation is the impermeable species while Na^+ and Li^+ are the permeable ones, the total recovery of neostigmine in the acceptor solution after 24 h not exceeding 0.7% of the mass (135 μmol) placed in the donor compartment. Since the concentration of neostigmine bromide in all donor solutions was kept at 0.28 M, this inversion of roles between penetrant and nonpenetrant ions must be more important at low concentrations of NaCl or LiCl (0.25 and 0.5 M) than at higher molarities, leading to a lower viscosity of the aqueous barrier at these concentrations. The data of Pedersen, Dethlefsen, and Hvidt[23] on the dependence on concentration of the apparent molar volume V_i^{app} of alkali halides in water solution seem to support this view. They observed a biphasic dependence of V_i^{app} of lithium salts on concentration and suggested maximal interaction between the cation and the water molecules when the concentration of the former in the latter was low. The intrinsic volumes of LiCl and NaCl are 15.77 and 17.36 cm^3 mol^{-1} as compared to their 'liquid' volumes which are 24.10 and 29.58 cm^3 mol^{-1}, respectively. Progressive dilution, however, causes a significant decrease in V_i^{app}, which is ascribed to progressive hydration of the ions. "There is no tendency of a levelling off of V_i^{app} when the concentration approaches zero. On the contrary, the steepness of $(\partial V_i^{app}/\partial x_1)$ of the curves increases for $x_1 \rightarrow 0$", x_1 being the mole fraction of NaCl or LiCl.[23] Thus, the convection current proposed by Burnette and Ongpipattanakul[19] could be of consequence also under conditions of passive diffusion of NaCl or LiCl, being more important under conditions and concentrations which favor progressive hydration of the ions. At 1 M NaCl or LiCl and beyond, the effect of these salts on the viscosity of lamellar water becomes less pronounced, even counterproductive, and the Donnan-like effect predominates.

These findings invited the investigation of benzalkonium cation (as the chloride) as the potential nondiffusible partner R^i in eq 5. The benzalkonium molecule consists of a 12–18-membered hydrocarbon chain linked to a pyridinium moiety and is of such dimensions that any diffusion across the stratum corneum may be negligibly small compared to that of Na^+ or Li^+. The values of J_{ss} and K_p of neostigmine in the presence of three concentrations of benzalkonium chloride are given in Table 5 and Figure 6. These represent the best fit of penetration approaching a Donnan-like partitioning of the penetrant between donor and acceptor (Figure 7). Against this contention one could argue that the observed enhancement of penetration is due to the action of benzalkonium chloride as a surface-active agent, whereby the integrity of the stratum corneum is impaired. However, the nonionic surface-active agent polyethylene glycol lauryl ether (PEG-4-lauryl ether) failed to evoke penetration of neostigmine from water solution. On the other hand, 1 M benzalkonium chloride had no effect on the penetration of the nonionic theophylline (K_p from water = 1.02×10^{-5} cm min^{-1}; from benzalkonium chloride, 1.3×10^{-5} cm min^{-1}) or adenosine (J_{ss} from water or from benzalkonium chloride not detectable). Thus, even with due concession allowed for the membrane-modifying action of benzalkonium chloride, its role as a non-penetrating co-ion in a Donna-like system appears to be substantial.

Table 5—Penetration of Neostigmine across Dermatomed Human Skin[a] Delivered as Neostigmine Bromide from Aqueous Solutions of Benzalkonium Chloride at the Indicated Concentrations

Solvent	T_L, min	$J_{ss} \times 10^2$,[b] μmol cm^{-2} min^{-1}	$K_p \times 10^6$,[b] cm min^{-1}
Water		[c]	[c]
Benzalkonium chloride			
0.25 M	450	1.06 ± 0.3	21.0 ± 2.0
0.50 M	426	1.45 ± 0.23	45.0 ± 2.0
1.00 M	462	2.83 ± 0.7	57.9 ± 5.6

[a] Five different skin samples tested with each solvent. [b] Mean ± SEM. [c] Below detection levels.

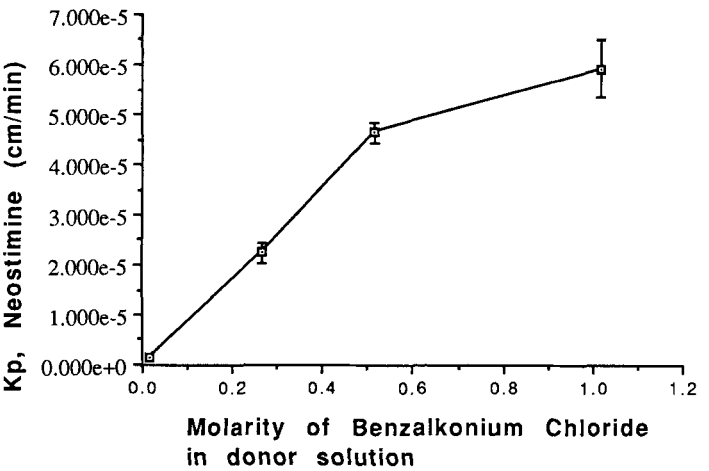

Figure 6—Relationship between K_p of neostigmine, delivered from solutions of benzalkonium chloride across human skin samples, and benzalkonium chloride concentration in the donor solution.

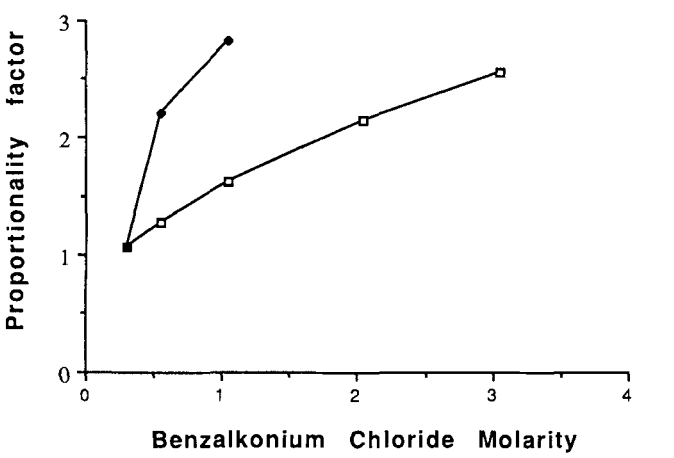

Figure 7—Proportionality of K_p values found for neostigmine delivered from benzalkonium chloride solutions and values calculated from eq 5 assuming constancy of the term $(RT/6\pi r\eta N_0)1/h$: □, expected values; ◆, observed values.

Conclusions

Hydrophilic molecules, especially those bearing a formal charge such as the quaternary ammonium compounds, can penetrate human skin from a water solution through a polar pathway consisting most probably of lamellar water. In this case, the stratum corneum fulfills the double role of a semipermeable diffusion barrier consisting of the lipid matrix of the stratum corneum and a hydrated acceptor compartment consisting of structured water molecules. Enhancement of hydrophile penetration can be caused by two distinct effects: a decrease in the viscosity of the hydrated compartment by an influx of hydrated ions and an increase in the partitioning of the hydrophile between the aqueous donor and the acceptor compartment. The first effect is caused by low concentrations of inorganic salts such as NaCl or LiCl, hydration of the corresponding ions being more extensive at low concentrations of these. The second effect is caused by the presence in the aqueous donor solution of a poorly diffusible or nondiffusible co-ion in a system approaching a Donnan-like effect. Both effects can occur jointly and to an extent depending on the relative concentrations in the donor of the penetrating hydrophile and of the inorganic halide used.

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