# Macromolecules as Novel Transdermal Transport Enhancers for Skin Electroporation

**Rita Vanbever,[1] Mark R. Prausnitz,[2] and Véronique Préat[1,3]**

*Received January 7, 1997; accepted February 19, 1997*

***Purpose.*** Macromolecules were investigated as chemical enhancers of transdermal transport by skin electroporation. Although unable to enhance passive or iontophoretic transport, macromolecules are proposed to enhance electroporation-assisted delivery by stabilizing the increased permeability caused by high-voltage pulses.

***Methods.*** To test this hypothesis, we examined the timescale of transport, the influence of electrical protocol and the influence of macromolecule size, structure, and charge on enhancement of transdermal mannitol transport *in vitro* by heparin, dextran-sulfate, neutral dextran, and poly-lysine.

***Results.*** Skin electroporation increased transdermal mannitol delivery by approximately two orders of magnitude. The addition of macromolecules further increased transport up to five-fold, in support of the proposed hypothesis. Macromolecules present during pulsing enhanced mannitol transport after pulsing for hours, apparently by a macromolecule-skin interaction. No enhancement was observed during passive diffusion or low-voltage iontophoresis, suggesting that macromolecules interact specifically with transport pathways created at high voltage. Although all macromolecules studied enhanced transport, those with greater charge and size were more effective.

***Conclusions.*** This study demonstrates that macromolecules can be used as trandermal transport enhancers uniquely suited to skin electroporation.

**KEY WORDS:** macromolecule; electroporation; iontophoresis; skin; permeation enhancer; transdermal transport.

## INTRODUCTION

Drug delivery across skin offers a noninvasive, user-friendly alternative to conventional oral or parenteral administration (1–2). However, the skin's outer layer, the stratum corneum, is an extremely effective barrier which prevents transport of most drugs at therapeutic rates. Chemical, electrical, mechanical, and ultrasonic approaches to increasing transdermal transport have been explored. Recently, the intermittent application of short (e.g. msec), high-voltage (e.g., 100 V) pulses has also been shown to increase transport across skin by many orders of magnitude, probably by a mechanism involving electroporation (3–4).

Electroporation (or electropermeabilization) involves the creation of transient aqueous pathways across lipid bilayer membranes by applying a short, high-voltage pulse (5–7). This phenomenon occurs in the lipid bilayers of nonliving systems, such as liposomes and red blood cell ghosts, as well as the plasma membranes of living cells, either isolated or part of a tissue (5). Most recently, electroporation of the multilamellar, intercellular lipid bilayers of stratum corneum has also been demonstrated (3–4). During an electric pulse which causes electroporation, membrane permeability temporarily increases by orders of magnitude, thereby increasing transmembrane transport of compounds as large as macromolecules (5–7). After the pulse, membranes remain permeable for milliseconds to hours, depending on conditions such as temperature, but can eventually reseal to their original structure. This transient elevation of membrane permeability has found widespread use as a means for introducing DNA into viable cells (8) and, more recently, as a clinical tool for targeting and enhancing uptake of chemotherapeutic agents by tumors (9–10).

Electroporation of skin can increase transdermal transport for compounds ranging in size from small ions (e.g., $Na^+$, $Cl^-$; ref. 11) to moderate-sized molecules (e.g. calcein, metoprolol; refs. 3, 12), to macromolecules (e.g. LHRH, heparin, oligonucleotides; refs. 13–15), to latex microspheres (16). The effects of skin electroporation are largely or completely reversible, where the skin's barrier properties are restored within approximately one hour. Unlike iontophoresis, which acts primarily by electrophoretically driving compounds through existing pathways in skin (1–2), electroporation transiently creates new pathways for transport (17). Although most work has been performed *in vitro*, animal studies *in vivo* show similar results (3,18).

Chemical enhancers which increase transdermal transport by passive diffusion and those which increase transport by skin electroporation are not necessarily the same. Traditional chemical enhancers of passive transdermal delivery (e.g. DMSO, Azone) act largely by disrupting stratum corneum lipid structure (1–2). In contrast, an effective chemical enhancer for electroporation-assisted delivery does not need to disrupt lipids, but should stabilize the transient disruptions already created by skin electroporation.

We hypothesized that macromolecules, although not expected to enhance passive transport, could enhance electroporation-assisted delivery by stabilizing the increased permeability caused by high-voltage pulses. A study of cell electroporation showed that DNA could stabilize the elevated membrane permeability of electroporated simian Cos-1 cells, possibly by being inserted into "electropores" and hinder their closure (19). In skin, an initial study concerning transdermal delivery of heparin by skin electroporation indirectly suggested that heparin molecules could prolong increased skin permeability (14). A subsequent study supported this conclusion with direct evidence of enhanced and prolonged skin permeability when heparin was present during skin electroporation (20).

In this study, we investigated the possibility that macromolecules could enhance transdermal transport by skin electroporation. We first confirmed the enhancing effect of heparin, and then explored the effects of dextran-sulfate, neutral dextran, and poly-lysine on transdermal transport of mannitol during *in vitro* skin electroporation. A broad range of electrical protocols were employed to help elucidate the mechanisms by which

[1] Unité de Pharmacie galénique, Ecole de Pharmacie, Université catholique de Louvain, Brussels, Belgium.

[2] School of Chemical Engineering, Georgia Institute of Technology, Atlanta, Georgia 30332.

[3] To whom correspondence should be addressed. (e-mail: preat@farg.ucl.ac.be)

0724-8741/97/0500-0638$12.50/0 

macromolecules could enhance electroporation-mediated transport across skin.

## MATERIALS AND METHODS

The experimental approach has been described previously (12,21) and is summarized and expanded upon below.

### Chemicals

D-mannitol (crystalline), dextran (average molecular weight, $MW_{ave}$ = 10 kDa), dextran-sulfate ($MW_{ave}$ = 5 and 10 kDa), heparin ($MW_{ave}$ = 20 kDa; sodium salt from porcine intestinal mucosa), poly-L-lysine (MW = 4 − 15 kDa; hydrobromide salt) and NaCl were obtained from Sigma Chemical Co. (St. Louis, MO). Heparin ($MW_{ave}$ = 12 kDa; sodium salt from porcine intestinal mucosa) was kindly donated by Pharmacia Hepar Inc. (Franklin, OH). D-1-$^{14}$C-mannitol was obtained from New England Nuclear Research (Du Pont de Nemours, Brussels). $NaH_2PO_4$ and $Na_2HPO_4$, and n-butanol were supplied by Union Chimique Belge (UCB, Drogenbos, Belgium). Buffer A contained 0.06 M sodium phosphate at pH 7.4. Buffer B contained 0.048 M sodium phosphate at pH 7.4 supplemented with 0.15 M glucose (Merck, Darmstadt, Germany) to provide physiological osmolarity. All solutions were prepared in ultrapure water (Sation 900, Vel, Leuven, Belgium).

### Transdermal Transport Measurements

Skin electroporation was performed *in vitro* using upright diffusion chambers. Freshly excised abdominal hairless rat skin (mutant rat Iops hairless from Iffa Credo, Saint Germain les Arbreles, France) separated the donor (or upper) and receiver (or lower) compartments with an exposed skin area of 3 cm$^2$. The donor reservoir was filled with 1.5 ml of donor solution: mannitol (1 mg/ml) and $^{14}$C-mannitol (0.5 μCi/ml) in buffer A either with or without an "enhancer" compound. The receiver compartment (7.5 ml) was filled with buffer B, continuously stirred with a magnetic stir bar and maintained at 37°C. Platinum electrodes were immersed in the donor and receiver solutions and connected either to an electroporation device for pulse application or to a current source for iontophoresis (see below). Unless otherwise noted, the cathode was in the donor compartment and the anode in the receiver compartment.

"Enhancer" compounds were supplied in the donor solution at the following comcentrations: $10^{-2}$ M heparin (12 or 20 kDa), $10^{-2}$ M dextran-sulfate (5 or 10 kDa), $10^{-2}$ M neutral dextran (10 kDa), $2 \times 10^{-3}$ M poly-L-lysine or 0.16 M NaCl. The NaCl concentration was chosen to give the same electrical conductivity as $10^{-2}$ M dextran-sulfate (10 kDa).

After application of an electrical protocol (i.e., electroporation and/or iontophoresis), mannitol permeation was measured for 6 h. Mannitol solution was present in the donor compartment both during and after pulsing, unless otherwise noted. In those cases when the mannitol solution was present only during pulsing and then removed afterwards, the donor compartment was emptied, rinsed three times, and filled with buffer B within 1 min after the last pulse. When mannitol solution was added only after the electrical protocol, pulsing was carried out with buffer A either with or without dextran-sulfate (10 kDa), and replaced with mannitol solution within 1 min after the last pulse.

Measurement of transdermal mannitol transport was carried out by removing samples (0.4 ml) from the receiver compartment at regular intervals. Samples were replaced with an equal volume of buffer B. The amount of $^{14}$C-mannitol in each sample was determined by scintillation counting (liquid scintillation cocktail Ready Safe, Beckman, Belgium; liquid scintillation counter Wallac 1410, LKB, Pharmacia, Uppsala, Sweden).

The stability of mannitol and its $^{14}$C radiolabel during high-voltage pulsing was verified by thin layer chromatography using silica gel 60 sheets (Merck, Darmstadt, Germany). The mobile phase contained 85% n-butanol and 15% water by volume. Chemical detection of mannitol was performed with iodine vapor (UCB, Drogenbos, Belgium) (22). Radioactivity was detected by scintillation counting (Bioscan Type AO 3000 A, System 200 Auto Changer and Imaging Scanner, Canberra—Packard, Washington, DC). Both mannitol and $^{14}$C-labeled mannitol were shown to remain intact under the conditions of this study.

### Electroporation and Iontophoresis

The electroporation device used was an Easyject Plus (Equibio Ltd., Kent, England), which delivers exponential-decay capacitive discharge pulses. The pulse time constant ($\tau_{pulse}$) is defined as the length of time between the beginning of the pulse (maximum voltage) and the time when the voltage reaches 37% of its initial value. Voltages are expressed as voltages applied across the electrodes ($U_{electrodes}$). However, a pulse, apparent skin resistance can drop well below 100 Ω (23). As a result, significant voltage drops occur within donor and receiver solutions making the voltage across the skin ($U_{skin}$) approximately three-fold lower than the total voltage applied, $U_{electrodes}$ (23). For example, pulses applied with $U_{electrodes}$ = 150 V and $\tau_{pulse}$ = 180 ms yielded $U_{skin} \approx$ 40 V, whether 10 kDa dextran-sulfate was present or not. The total charge transfered across the skin by a pulsing protocol was determined by the product of $U_{electrodes}$, $\tau_{pulse}$ and the number of pulses applied divided by the apparent chamber resistance during the pulse (12).

Iontophoresis, alone or within 1 min subsequent to electroporation (started within 1 min after pulsing), was carried out at constant current using a custom-built device. The total charge transfered across the skin during an iontophoresis protocol was calculated by multiplying current times the duration of application.

Abbreviated descriptions of electrical protocols are given in the tables and figures and can be interpreted according to the following examples: "5 × (150 V − 180 ms) 1/6 min" indicates that 5 pulses were applied with $U_{electrodes}$ = 150 V, $\tau_{pulse}$ = 180 ms and at a rate of 1 pulse every 6 min; "5 × (750 V − 1.2 ms; 0.1 s; 74 V − 350 ms) 1/6 min" indicates that 5 twin pulses were applied at a rate of 1 pulse every 6 min, where each twin pulse was composed of a first pulse with $U_{electrodes}$ = 750 V and $\tau_{pulse}$ = 1.2 ms, followed after 0.1 s by a second pulse with $U_{electrodes}$ = 74 V and $\tau_{pulse}$ = 350 ms; "1 × (150 V − 180 ms) + ionto 0.2 mA/cm$^2$ − 24 min" indicates that one pulse was applied with $U_{electrodes}$ = 150 V, $\tau_{pulse}$ = 180 ms and was followed by iontophoresis at 0.2 mA/cm$^2$ for 24 min.

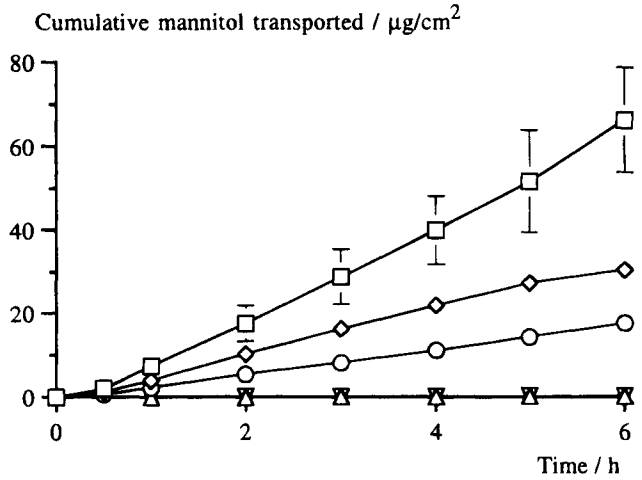


**Fig. 1.** Macromolecules enhance transdermal transport by skin electroporation. Application of high-voltage pulses increased transdermal permeation of mannitol. The addition of macromolecules (heparin or dextran sulfate) further increased mannitol permeation: (▽) passive diffusion, no macromolecule present. (△) passive diffusion, 10 kDa dextran-sulfate in the donor solution. (○) 5 × (150 V − 180 ms) 1/min, no macromolecule present. (◇) 5 × (150 V − 180 ms) 1/min, 12 kDa heparin in the donor. (□) 5 × (150 V − 180 ms) 1/min, 10 kDa dextran-sulfate in the donor. Explanation of electrical protocol abbreviations is given in the Methods section of the text.

### Statistical Analysis

Mannitol transport data represent averages of measurements from 3–11 skin samples (Figures 1–2, Table I). Standard errors of the mean (sem) are given. An enhancement ratio is the ratio of the average cumulative mannitol transported 6 h after pulsing and/or iontophoresis with enhancer present divided by the average cumulative mannitol transported 6 h after pulsing and/or iontophoresis without enhancer present (Figures 3–5). Overall cumulative transport data at different time points were compared by a two-way analysis of variance (Anova type III, $p < 0.05$). The enhancement ratios were compared by Student's t-test of their logarithm ($p < 0.05$).

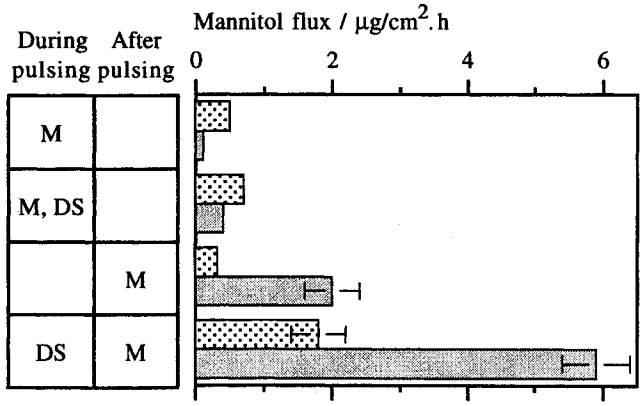


**Fig. 2.** Timescale of macromolecule-enhanced transport. Average mannitol flux (mean ± sem) is shown during 0–2 h (▨) and 4–6 h (▩) after an electroporation protocol using 5 × (150 V − 180 ms) 1/min (see Methods section). Mannitol (M) was present either during or after pulsing. Pulsing was carried out either with or without 10 kDa dextran-sulfate (DS) in the donor solution. The greatest flux was achieved when dextran-sulfate was present during pulsing (and thereby potentially introduced into skin) and mannitol was present after pulsing (for post-pulse transdermal diffusion; see text).

## RESULTS

### Macromolecules Enhance Transdermal Transport

In this study, we wanted to determine if macromolecules might represent a novel class of transdermal transport enhancers for skin electroporation. Although they do not enhance passive or iontophoretic transdermal transport, macromolecules could enhance electroporation-assisted transport by stabilizing the increased permeability created by high-voltage pulses. We were motivated by previous experiments with cell suspensions which show that the presence of DNA macromolecules can enhance transmembrane transport due to cell electroporation (19), as well as recent experiments with skin which suggested that the presence of heparin macromolecules may increase transdermal transport following skin electroporation (14,20). A possible mechanism for this enhancement suggests that highly charged macromolecules such as DNA or heparin can be electrophoretically driven into and trapped within electropores. This could widen pores and hinder their closing by electrical repulsion and steric effects (14,19,20). In this way, transport pathways created by electroporation might be larger and kept open longer, thereby increasing transmembrane or transdermal transport.

To test this hypothesis, we measured transdermal mannitol permeation after electroporation protocols applied either with or without macromolecules present in the donor solution. Figure 1 shows that the presence of heparin or dextran-sulfate macromolecules during skin electroporation enhanced transdermal transport of mannitol. Under passive conditions (no electric fields), rates of transport were slow and did not increase when macromolecules were added (Anova, $p > 0.05$). Application of an electroporation protocol increased mannitol transport by almost two orders of magnitude (Anova, $p < 0.05$). The addition of heparin or dextran-sulfate further increased transport by a factor of up to 3.5 in Figure 1 (Anova, $p < 0.05$). This supports the hypothesis that macromolecules can be effective enhancers of transdermal transport by skin electroporation.

### Timescale of Enhanced Transport

Because previous studies suggested that macromolecules interact with electropores in part by preventing their resealing (14,19,20), we characterized the timescale over which macromolecules enhanced transdermal mannitol transport. In a first set of experiments, mannitol solution, either with or without dextran-sulfate, was put in the donor compartment when electroporation pulses were applied. After pulsing, the mannitol solution was replaced with buffer (containing no mannitol or dextran-sulfate). Mannitol transport out of the skin was then measured for 6 h. Figure 2 shows that the presence of dextran-sulfate during pulsing increased transdermal transport by approximately 2.5 times (Anova, $p < 0.05$). This demonstrates that macromolecules increase mannitol transport into and across the skin at the time of pulsing.

In a second set of experiments, electroporation pulses were applied using a donor solution containing buffer either with or without dextran-sulfate (and no mannitol). Then, the donor solution was replaced with mannitol solution (containing no dextran-sulfate) and transdermal mannitol permeation was mea-

**Table I.** Influence of Electrical Parameters on Macromolecule-Enhanced Transport

| Electrical Protocol[a] | Total application time (min) | Total charge transfered (C) | Cumulative mannitol transported after 6 h (μg/cm²)[b] | |
|---|---|---|---|---|
| | | | Without dextran-sulfate | With dextran-sulfate[c] |
| 5 × (150 V − 180 ms) 1/6 min | 30 | 1.1 | 16 ± 4 | 40 ± 6 |
| 5 × (150 V − 180 ms) 1/1 min | 5 | 1.1 | 16 ± 2 | 51 ± 10 |
| 5 × (150 V − 180 ms) 1/15 s | 1 | 1.1 | 10.5 ± 0.3 | 52 ± 12 |
| 60 × (500 V − 1.2 ms) 1/10–15 s | 12 | 0.4 | 8 ± 1 | 17 ± 3 |
| 120 × (750 V − 1.2 ms) 1/15 s | 30 | 1.1 | 36 ± 4 | 46 ± 8 |
| 5 × (750 V − 1.2 ms; 0.1 s; 74 V − 350 ms) 1/6 min | 30 | 1.0 | 21 ± 6 | 28 ± 2 |
| ionto 0.2 mA/cm² − 30 min | 30 | 1.1 | 2.2 ± 0.7 | 2.7 ± 0.4 |
| 1 × (150 V − 180 ms) + ionto 0.2 mA/cm² − 24 min | 24 | 1.1 | 4 ± 0.5 | 7 ± 2 |
| 5 × (750 V − 1.2 ms) 1/15 s + ionto 0.2 mA/cm² − 29 min | 30 | 1.1 | 3.6 ± 0.4 | 3.6 ± 0.6 |
| passive diffusion | — | — | 0.32 ± 0.08 | 0.27 ± 0.01 |

[a] Explanation of electrical protocol abbreviations is given in the Methods section of the text.
[b] Expressed as mean ± sem.
[c] Dextran-sulfate (10 kDa).

sured for 6 h. Figure 2 shows that the presence of dextran-sulfate during pulsing significantly increased post-pulse mannitol transport (Anova, $p < 0.05$). This demonstrates that the effects of macromolecules added at the time of pulsing persist for at least several hours after pulsing. Comparison of the mannitol flux with and without dextran-sulfate in Fig. 2 shows that the enhancement due to dextran-sulfate was twice as great during the first two hours after pulsing as during the last two hours, indicating that the prolonged permeabilization diminishes over time.

Comparison of these two sets of experiments shows that much greater mannitol transport occurred when mannitol was present after pulsing than when it was present only during and between pulses (Figure 2). This suggests that transport of mannitol occurred primarily by post-pulse diffusion through long-lived changes in skin permeability, as expected for a small, uncharged molecule (24). These experiments also show that dextran-sulfate introduced at the time of pulsing more effectively enhanced transport after pulsing than transport at the time of pulsing (Figure 2).

## Electrical Parameters

The preceeding experiments suggest that dextran-sulfate can be introduced into skin during pulsing, perhaps involving electrophoresis into electropores, and thereby enhance transder-

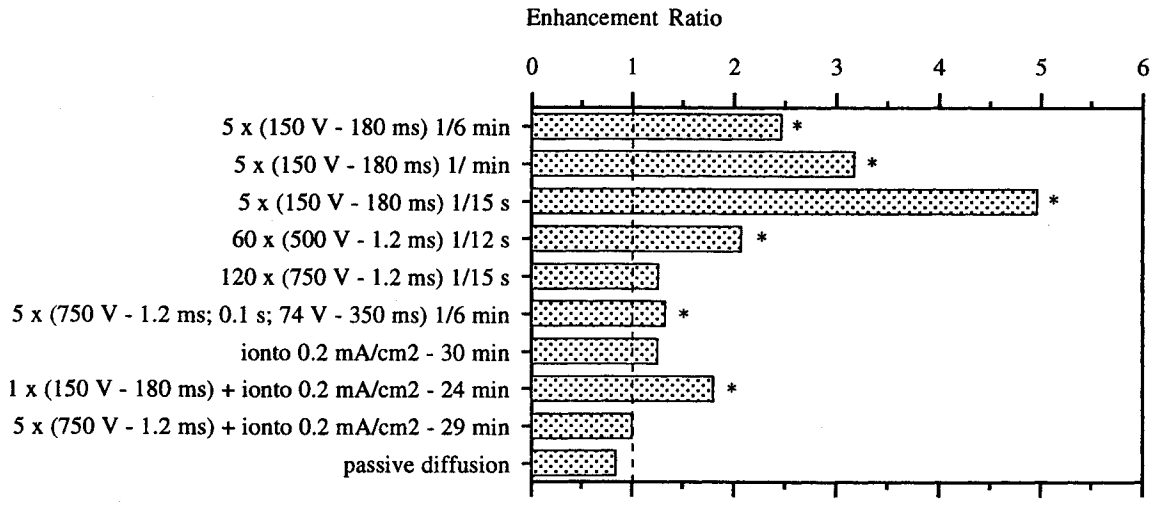


**Fig. 3.** Influence of electrical parameters on macromolecule-enhanced transport. For different electrical protocols, the enhancement of transdermal mannitol transport provided by the addition of 10 kDa dextran-sulfate is expressed as an enhancement ratio (see Methods section for definition of enhancement ratio and electrical protocol abbreviations). A star symbol (*) indicates significant enhancement of mannitol transport by dextran-sulfate (Anova, $p < 0.05$). The greatest enhancement was seen when dextran-sulfate was added during long, medium-voltage pulse protocols.

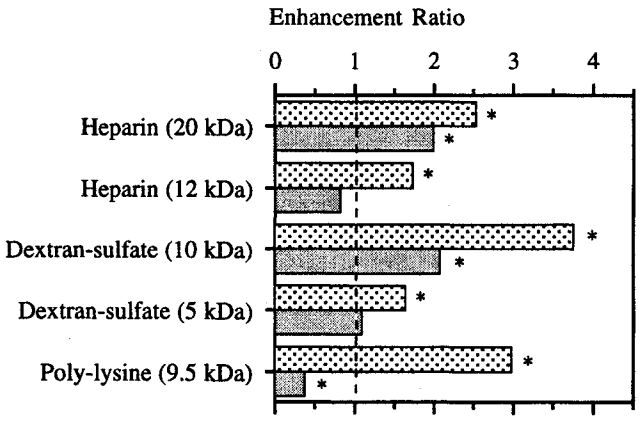


**Fig. 4.** Influence of macromolecule properties on enhancement. Enhancement ratios provided by macromolecules with different physicochemical properties are given for two different pulse protocols : 5 × (150 V − 180 ms) 1/min (▨) and 60 × (500 V − 1.2 ms) 1/10–15 s (▩). A star symbol (*) indicates significant enhancement or reduction of mannitol transport by the macromolecule (Anova, $p < 0.05$). Within each chemical class, more enhancement resulted from compounds of greater molecular weight, and therefore also greater charge. In addition, dextran-sulfates were more effective than heparins.

mal diffusion of mannitol. In the following experiments, we studied the effect of the electrical protocol on dextran-sulfate's ability to enhance mannitol transport (Table I). For each protocol, the total electrical charge delivered to the skin was kept approximately constant, so that each protocol caused the same total amount of ionic electrophoresis (Table I) (25).

First the effect of pulse spacing was examined. As shown in Figure 3, pulses spaced more closely together resulted in greater enhancement by dextran-sulfate (t-test, $p < 0.05$). Similarly, previous studies have shown that more closely spaced pulses cause greater increases in transdermal transport (12). Combined, these results suggest that protocols which cause greater dextran-sulfate penetration into skin can result in greater enhancement of post-pulse mannitol transport.

The effects of pulse length and pulse voltage were also studied. In the experiments described so far, pulses of "medium" voltage and "long" time constant (e.g., 150 V, 180 ms) were used. While this type of protocol has received attention in the literature (12,21), pulses of higher voltage and shorter time constant (e.g., 500 V, 1 ms) have also been studied (3,23). Figure 3 shows that the addition of dextran-sulfate during either type of protocol can enhance mannitol transport. However, enhancement is considerably less when higher voltages and shorter pulses are used (Figure 3, Table I; t-test, $p < 0.05$). This could be explained by previous studies which compared these two protocols and indicate that pulses of longer time constant and lower voltage may create larger transport pathways (25). Larger pathways would favor introduction of macromolecules such as dextran-sulfate into the skin.

Unlike skin electroporation, iontophoresis enhances transport by electrically driving molecules across the skin and generally does not create new pathways for transport (1–2). Based on the hypothesis that dextran-sulfate's mechanism of enhancement involves interaction with electropores (14,19,20), addition of dextran-sulfate during iontophoresis should not affect mannitol transport. Consistent with the proposed hypothesis, iontophoretic mannitol transport was not enhanced by dextran-sulfate as shown in Figure 3.

The application of a single electroporation pulse prior to iontophoresis has been shown to yield up to 10 times greater transdermal peptides fluxes than iontophoresis alone (13). When a long, medium-voltage pulse was applied and followed by iontophoresis, the addition of dextran-sulfate further enhanced mannitol transport by a factor of approximately 2. However, when shorter, higher-voltage pulses were followed by iontophoresis, no dextran-sulfate enhancement was observed (Figure 3, Table I). "Twin-pulse" protocols have also been explored previously (12,19,21,26). The presence of dextran-sulfate during such a protocol provided a modest enhancement in transport (Figure 3, Table I).

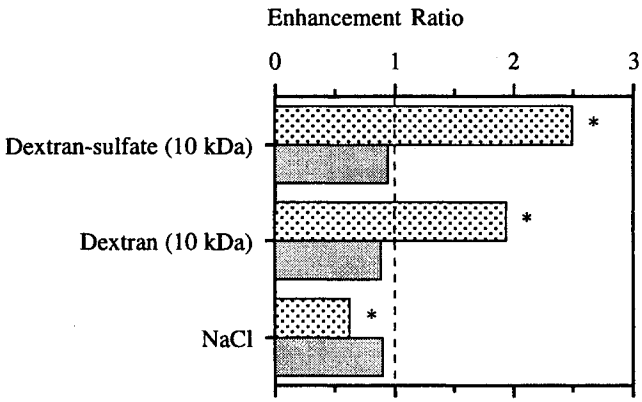


**Fig. 5.** Influence of pulse polarity and macromolecule size and charge on enhancement. Enhancement ratios provided by compounds of different size and charge are given for a pulse protocol of 5 × (150 V − 180 ms) 1/min (see Methods section) with either the cathode (▨) or anode (▩) in the donor compartment. A star symbol (*) indicates significant enhancement or reduction of mannitol transport by the added compound (Anova, $p < 0.05$) Dextran-sulfate's size, rather than its charge, appears to be more important to enhance mannitol transport.

## Macromolecular Size, Structure, and Charge

The different levels of enhancement produced by dextran-sulfate and heparin suggest that the size, structure, and charge of a macromolecular enhancer can influence its ability to modify skin permeability (Figure 1). We therefore evaluated the enhancement provided by a number of different compounds with different physicochemical properties: dextran-sulfate (5 and 10 kDa), neutral dextran (10 kDa), heparin (12 and 20 kDa), poly-L-lysine (9.5 kDa) and NaCl. In this context, the effect of pulse voltage, length, and polarity were also studied (Figures 4 and 5).

All of the macromolecular compounds provided enhanced transport, but to different extents. In each case, macromolecules provided greater enhancement with long, medium-voltage protocols than short, high-voltage protocols (Figure 4, t-test $p < 0.05$), in agreement with previous results for dextran-sulfate (Figure 3, Table I). Within each chemical class (e.g., dextran-sulfates or heparins), compounds of greater molecular weight, and therefore also greater charge, were better enhancers (Figure 4, t-test $p < 0.05$). Comparison between chemical classes shows that dextran-sulfates were more effective than heparins in

enhancing mannitol permeation, even though the dextran-sulfates tested had less charge and lower molecular weight (27). When using long, medium-voltage pulses, the positively-charged macromolecule poly-lysine increased mannitol transport almost as well as negatively-charged dextran-sulfate. In contrast, poly-lysine significantly decreased mannitol transport when short, high-voltage pulses were applied. In these experiments with poly-lysine, the electrode polarity was reversed (i.e., anode in the donor compartment) to promote electrophoresis of poly-lysine into the skin. It should be noted that under these conditions (i.e., anode in the donor compartment) the absolute value of mannitol transport in the absence of enhancer was significantly lower for long, medium-voltage pulses (data not shown).

To further evaluate the importance of molecular size and charge, the enhancement provided by dextran-sulfate was compared to that of neutral dextran (having the same molecular weight) and NaCl (present at a concentration which provided the same amount of charge) using both anodal and cathodal polarity (Figure 5). With the cathode in the donor compartment, neutral dextran enhanced mannitol permeation while NaCl caused a significant decrease in transport. This suggests that enhancement caused by dextran-sulfate comes more from its molecular size than its charge. When the electrode polarity was reversed (i.e., anode in the donor compartment), dextran-sulfate provided no enhancement, which is consistent with a mechanism involving introduction of macromolecules into skin by electrophoresis. However, the effects of neutral dextran and NaCl also disappeared when the "reverse" electrode polarity was used, which cannot easily be explained by an electrophoretic or electroosmotic mechanism.

## DISCUSSION

We wanted to determine if macromolecules could be effective enhancers of transdermal transport by skin electroporation. In this study we directly showed that macromolecules increase transdermal transport apparently by stabilizing the effects of skin electroporation. We also investigated the mechanism of macromolecular enhancement by examining the timescale of transport, the effects of electrical parameters, and the effects of macromolecule size, structure and charge. The results indicate that a number of different macromolecules can interact with skin during electric pulsing and thereby increase transdermal transport by promoting and/or prolonging the effects of skin electroporation.

Transdermal mannitol transport was increased up to five-fold by a number of different macromolecules present during skin electroporation (Table 1, Figures 3–5). Levels of enhancement were similar to those seen in previous electroporation studies involving cells (19). Significantly, if dextran-sulfate was present at the time of pulsing and then removed, transdermal transport of mannitol added after pulsing was enhanced (Figure 2). This indicates that enhancement was provided by an interaction between dextran-sulfate and skin rather than an interaction between dextran-sulfate and mannitol. This also suggests that dextran-sulfate was introduced into skin at the time of pulsing and increased skin permeability for hours after pulsing.

The effects of electrical parameters on enhancement suggest that protocols which introduce more macromolecules into skin provide greater enhancement. The use of (a) reduced inter-pulse spacing, (b) long, medium-voltage (as opposed to short, high-voltage) pulses and (c) pulse polarity which promoted electrophoresis of macromolecules into skin each resulted in greater enhancement by macromolecules (Figures 3 and 5). Previous studies showed that (a) reduced inter-pulse spacing is associated with greater transdermal transport (12), (b) long, medium-voltage pulses may be associated with larger transdermal transport pathways (25) and (c) electrophoresis contributes significantly to transdermal transport of charged, hydrophilic compounds during skin electroporation (3,12). Therefore, the greater enhancement of mannitol transport observed after pulsing could be caused by greater introduction of macromolecules into skin during pulsing with these protocols. Direct measurement of the amount of dextran-sulfate in skin was not performed in this study, but would provide additional useful information.

Enhancement by macromolecules was observed for electroporation protocols, but not for passive diffusion or iontophoresis (Table 1, Figures 1 and 3). This shows that interactions between macromolecules and the transport pathways used during non-electroporation protocols do not lead to enhancement, while interactions with transport pathways created at high voltage do enhance transport. This is consistent with the hypothesis that macromolecules stabilize electropores. These results also provide additional evidence that the high-voltage protocols used here are not just "high-voltage iontophoresis," but have distinctly different effects, probably due to the creation of new transport pathways by skin electroporation (17).

While the physicochemical properties of different macromolecules affected the degree of enhancement, all macromolecules examined in this study significantly enhanced mannitol transport (Figures 4 and 5). Although charged compounds (e.g., dextran-sulfate) were somewhat better than uncharged ones (e.g., neutral dextran), both negatively- (e.g., dextran-sulfate, heparin) and positively- (e.g., poly-lysine) charged macromolecules were good enhancers (Figures 4 and 5). Within each chemical class, macromolecules of greater molecular weight, and therefore greater charge, were more effective than smaller, less-charged macromolecules (e.g., 10 kDa vs. 5 kDa dextran-sulfate; Figure 4). Small ions provided no enhancement at all (e.g., NaCl; Figure 5). Differences between chemical classes were also observed (e.g., dextran-sulfates vs. heparins; Figure 4). Together, this provides a preliminary guide for optimizing macromolecule properties for macromolecule-enhanced transdermal transport by electroporation.

For applications in transdermal drug delivery, this study demonstrates ways in which macromolecules can be used and suggests other approaches not yet tested. The data show that macromolecules prolong the lifetime of skin permeabilization (Figure 2), suggesting that fewer and/or "weaker" electroporation pulses may be required when macromolecules are present. The possibility that macromolecules may also enlarge transport pathways, as seen in cell membranes (19), has not been clearly demonstrated in skin. Pathways enlarged by macromolecule enhancers could provide a valuable approach to increasing transdermal delivery of macromolecular drugs, such as insulin. There is also the possibility for "self-enhancement" by macromolecular drugs, as may have occurred in studies with heparin (14). Both charged and uncharged macromolecules enhanced transport of a neutral permeant (i.e., mannitol, Figures 4 and 5). In contrast, we previously observed that charged macromolecules may impede transdermal transport of permeants having the

same charge (20), presumably by competition in electrophoretic transport and charge repulsion. It is not yet known if neutral macromolecules or macromolecules having the opposite charge could enhance delivery of charged drugs. Finally, the macromolecules studied here all had flexible, linear structures (28). Structurally—different macromolecules (e.g., globular) might behave differently.

## CONCLUSIONS

Previous studies suggested that macromolecules could enlarge and stabilize transport pathways created by electroporation. To further test this hypothesis, we examined the timescale of transport and the effects of electrical protocol and macromolecule size, structure, and charge on enhancement of transdermal mannitol transport by a number of different macromolecules. These studies support the hypothesis that macromolecules are introduced into skin during electroporation and thereby stabilize the increased skin permeability caused by high-voltage pulses. This demonstrates that macromolecules can be used as transdermal transport enhancers uniquely suited to skin electroporation.

## ACKNOWLEDGMENTS

We are grateful for the technical assistance of N. Lecouturier and G. Langers. We thank Equibio for lending the electroporation device and Pharmacia Hepar Inc. for donating the heparin. This work was supported in part by the Fonds National de la Recherche Scientifique (FNRS, Belgium) and a National Science Foundation CAREER Young Investigator Award to Prof. M. Prausnitz (BES-9624832). Prof. V. Préat is a Senior Research Associate of the FNRS (Belgium).

## REFERENCES

1. J. Hadgraft and R. H. Guy, eds. *Transdermal Drug Delivery: Developmental Issues and Research Initiatives*, Marcel Dekker, New York, 1989.
2. E. W. Smith and H. I. Maibach, eds. *Percutaneous Penetration Enhancers*, CRC Press, Boca Raton, FL, 1995.
3. M. R. Prausnitz, V. G. Bose, R. Langer, and J. C. Weaver. *Proc. Natl. Acad. Sci. USA* **90**:10504–10508 (1993).
4. M. R. Prausnitz. In Berner, B. and S. M. Dinh (eds), *Electronically Controlled Drug Delivery*, CRC Press, Boca Raton, FL, (in press).
5. E. Neumann, A. E. Sowers, and C. A. Jordan. In *Cell Biology*, Plenum Press, New York, 1989.
6. S. Orlowski and L. M. Mir. *Biochim. Biophys. Acta* **1154**:51–63 (1993).
7. J. C. Weaver. *J. Cell. Biochem.* **51**:426–435 (1993).
8. J. A. Nickoloff, eds. *Animal cell electroporation and electrofusion protocols*, Humana Press, Totowa, N. J., 1995.
9. R. Heller, M. J. Jaroszeski, L. F. Glass, J. L. Messina, D. P. Rapaport, R. C. DeConti, N. A. Fenske, R. A. Gilbert, L. M. Mir, and D. S. Reintgen. *Cancer* **77**:964–971 (1996).
10. C. Domenge, S. Orlowski, B. Luboinski, T. De Baere, G. Schwaab, J. Belehradek, and L. M. Mir. *Cancer* **77**:956–963 (1996).
11. U. Pliquett, T. E. Zewert, T. Chen, R. Langer, and J. C. Weaver. *Biophys. Chem.*, **58**:185–204 (1996).
12. R. Vanbever, N. Lecouturier, and V. Préat. *Pharm. Res.* **11**:1657–1662 (1994).
13. D. Bommannan, J. Tamada, L. Leung, and R. O. Potts. *Pharm. Res.* **11**:1809–1814 (1994).
14. M. R. Prausnitz, E. R. Edelman, J. A. Gimm, R. Langer, and J. C. Weaver. *Bio/Technology* **13**:1205–1209 (1995).
15. T. E. Zewert, U. F. Pliquett, R. Langer, and J. C. Weaver. *Biochem. Biophys. Res. Com.* **212**:286–292 (1995).
16. M. R. Prausnitz, J. A. Gimm, R. H. Guy, R. Langer, J. C. Weaver, and C. Cullander. *J. Pharm. Sci.* **85**:1363–1370 (1996).
17. M. R. Prausnitz. *J. Control. Rel.* **40**:321–326 (1996).
18. R. Vanbever, G. Langers, S. Montmayeur, and V. Préat (submitted).
19. S. I. Sukharev, V. A. Klenchin, S. M. Serov, L. V. Chernomordik, and Y. A. Chizmadzhev. *Biophys. J.* **63**:1320–1327 (1992).
20. J. C. Weaver , R. Vanbever, T. Vaughan, and M. R. Prausnitz (submitted).
21. R. Vanbever, E. Le Boulengé, and V. Préat. *Pharm. Res.* **13**:559–565 (1996).
22. K. Randerath. *Chromatographie sur couches minces.*, Gauthier-Villars (eds), Paris, 1964.
23. U. Pliquett and J. C. Weaver. *Bioelectrochem. Bioenerget.* **39**:1–12 (1996).
24. R. Vanbever, M.-A. Leroy, and V. Préat (submitted)
25. R. Vanbever, U. F. Pliquett, V. Préat, and J. C. Weaver (in preparation).
26. R. Vanbever and V. Préat. *Bioelectrochem. Bioenerget.* **38**:223–228 (1995).
27. S. Budavari. *The Merck Index.*, Merck and Co., Rahway, NJ, 1996.
28. H. F. Mark, N. M. Bikales, C. G. Overberger, G. Menges, and J. I. Kroschwitz. *Encyclopedia of Polymer Science and Engineering*, Wiley-Interscience, 1992.