Chapter 8

Transdermal Delivery of Macromolecules: Recent Advances by Modification of Skin's Barrier Properties

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Great advances in transdermal delivery of macromolecules have been made within the last few years using ultradeformable liposomes, electroporation, and low-frequency ultrasound, each of which has been shown to deliver macromolecules at clinically-useful rates. Transdermal drug delivery is a potentially useful method by which macromolecules, such as proteins, could be administered for local or systemic therapy. Until recently, transdermal delivery was not a realistic option, since skin's great barrier properties had prevented transport of macromolecules across human skin at therapeutically-relevant rates. In this chapter, chemical, electrical, and ultrasonic delivery methods are reviewed. Mechanistic perspective, a summary of key experimental findings, and an assessment of potential for impact on medicine are provided for each enhancement technique.

Biotechnology has produced a generation of novel macromolecular compounds with great therapeutic promise. While a number of challenges sometimes slow progress of these new drugs to clinical application, difficulties in meeting their special drug delivery requirements can be a significant impediment. This is because biologically-active macromolecules, such as proteins, generally have low oral bioavailability, making oral administration difficult, and often have short biological half-lives, making parenteral delivery impractical outside a hospital setting (*1-3*). Delivery of drugs across the skin addresses these problems by offering a number of potential advantages compared to conventional methods, such as pills and injections: (1) no degradation due to stomach, intestine, or first pass of the liver, (2) likely improved patient compliance because of a user-friendly method, and (3) potential for steady or time-varying controlled delivery (*4-9*). However, delivery of therapeutic quantities of macromolecules across human skin is extremely difficult. This chapter describes the current status of transdermal drug delivery, focusing on recent advances involving the modification of skin's barrier properties, which indicate that transdermal delivery of macromolecules (> 1 kDa) may now be possible.

Transdermal drug delivery. The advantages of delivery across skin have led to the clinical success of a number of transdermal products, indicated by annual sales in excess of one billion dollars. Transdermal drugs approved by the United States Food and Drug Administration (FDA) include clonidine, estradiol, fentanyl, lidocaine, nicotine, nitroglycerin, scopolamine, and testosterone (*10*).

Applications of transdermal drug delivery are limited largely by skin's great barrier properties, which prevent transdermal diffusion of most compounds at therapeutic rates (*4-9*). Drugs which have been successfully delivered (i.e., the FDA-approved drugs listed above) each share three common traits: effectiveness at relatively low doses, molecular mass less than 400 Da, and lipid solubility. While proteins and other macromolecular drugs are often effective at low doses, they generally are much larger than 400 Da and have very poor lipid solubility, which explains their extremely slow percutaneous absorption.

Pathways for transport across the skin's stratum corneum. The outer 10 - 15 μm of human skin is the stratum corneum (*11*), a dead layer of tissue which provides the primary and extremely effective barrier to transdermal transport (Figure 1) (*12-14*). Below is the viable epidermis, which consists of living cells, but is devoid of nerves and blood vessels. Deeper still is the dermis, which also contains living cells, in addition to blood vessels and nerves. While most drugs traverse the stratum corneum very slowly, they diffuse with great ease through deeper tissues to the capillary bed in the dermis (*4-9*).

The stratum corneum's barrier properties are generally attributed to multilamellar lipid bilayers which fill the extracellular spaces (*15, 16*). The bulk of stratum corneum is composed of flattened cells called keratinocytes, which are filled with cross-linked keratin. Their relatively permeable cell interiors are not normally accessible for transport, since they are surrounded by the relatively impermeable intercellular lipids. Unlike the phospholipid bilayers of cell membranes, these intercellular bilayers contain very few phospholipids, being composed primarily of ceramides, cholesterol, and fatty acids (*17*).

There are three transport pathways across the skin which molecules are likely to follow (Figure 2). One involves transport directly across the bulk of stratum corneum, where a molecule must sequentially cross keratinocytes and intercellular lipid bilayers. Normally, this route is not available to most molecules, because it involves crossing on the order of 100 intercellular bilayers, which is energetically unfavorable (*18*), and is therefore extremely slow. Another pathway reduces the number of bilayer crossings by following a tortuous path exclusively within the intercellular lipids, where drugs travel predominantly along the multilamellar bilayers, rather than across them. This route is probably taken by small drugs which diffuse across the skin (*19-22*). The third pathway, often termed the "shunt" route, avoids the intercellular lipid bilayers altogether by following a path within sweat ducts and hair follicles. Although the shunts make up only a small fraction of the skin (~ 0.1 % (*12*)), this route is important for transport of charged compounds, especially when electrophoretically driven by an imposed electric field (see below) (*23-27*).

Because stratum corneum lipids limit transport of most compounds, efforts to increase transdermal delivery have often focused on altering lipid bilayer structure to increase permeability. Modification of skin's barrier properties in this way has been achieved by chemical and physical approaches. Recent advances in this field, most of which have been published since 1995, suggest that the tools needed for transdermal delivery of macromolecules are now available.

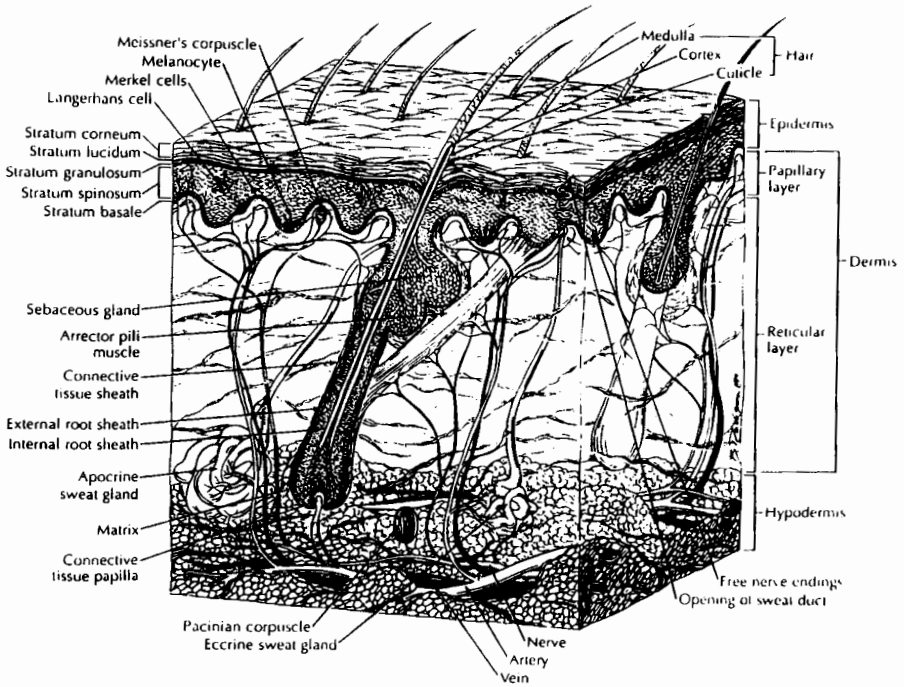


Figure 1. A composite representation of the anatomical structures found in mammalian skin. The outermost layer, stratum corneum, provides the primary resistance to transdermal transport of most compounds. Because the epidermis is avascular, drugs must reach the capillaries (or lymphatic vessels) in the dermis for systemic administration. Reproduced with permission from reference (*13*). Copyright 1991 CRC Press, Inc.

Modification of Skin's Barrier Properties.

Chemical enhancers can alter the skin's lipid environment. Because transport across skin by passive diffusion is too slow for most applications, the effects of a broad variety of chemicals on transdermal drug delivery have been investigated. Although extensively studied, their potential for significant impact on macromolecule delivery has not been demonstrated.

Mechanistic perspective. Transdermal transport by the tortuous intercellular route followed by most drugs can be viewed as a two-step process: (1) drug must first partition from an external donor solution into the skin's lipids and then (2) diffuse across the stratum corneum within the lipid domain. Models based on this approach have successfully described transdermal transport (*19, 22, 28-32*). Chemical enhancers should therefore be effective if they alter the skin's lipid environment in ways which (1) increase drug solubility in skin and/or (2) increase drug diffusivity in skin.

Experimental transdermal permeability values for some hydrophilic compounds are inconsistent with transport via an intercellular lipid route and have led to the hypothesis that there are additional hydrophilic pathways, sometimes called "aqueous pores" (*33-37*). The physical nature of these pathways remains controversial, but may represent hydrophilic domains within the bulk of stratum corneum. Others suggest that diffusion through hair follicles and sweat ducts may be significant (*33, 38, 39*).

Experimental findings. Chemical approaches to increasing transport have received extensive attention from the transdermal community (*4-9, 40, 41*). Most effective chemical enhancers act by disrupting or fluidizing lipid bilayer structures within the stratum corneum, thereby increasing drug diffusivity within the skin. Examples include dimethyl sulfoxide (DMSO), Azone (1-dodecylazacycloheptan-2-one), unsaturated fatty acids (e.g., oleic acid, linoleic acid), and surfactants (e.g. sodium dodecyl sulfate). Chemical enhancers have been shown to increase transdermal transport of small compounds by as much as orders of magnitude, but also frequency cause signifcant skin irritation and may affect drug stability (*4-9, 40, 41*). However, studies addressing chemical enhancement of macromolecules report only modest or no enhancement under clinically-relevant conditions (*42-50*).

Potential for impact. Despite extensive research, chemical enhancers have so far had little practical impact on transdermal delivery beyond preclinical studies. While ethanol is used in FDA-approved formulations (*10*), other enhancers with much greater effects on skin permeability have not yet found clinical acceptance due largely to safety concerns and the costly FDA approval process. Moreover, although transdermal delivery of small molecules is significantly increased by a number of different chemical additives, delivery of proteins and other macromolecules generally is not.

Liposomes facilitate transdermal transport by a poorly understood mechanism. Encapsulation of drugs within liposomes has been studied for many drug delivery applications, including transdermal delivery. Liposomes are spherical lipid bilayer membranes which surround an aqueous interior. In addition to being found in hundreds of cosmetic formulations, liposomes are currently used to enhance transdermal transport of low molecular weight drugs in some pharmaceutical products (*51-54*). Recent laboratory studies indicate that liposomes may also play a useful role in transdermal delivery of macromolecules.

Mechanistic perspective. It is again useful to consider transdermal transport as a two-step process, involving partitioning and diffusion. The ability of liposomes to facilitate drug partitioning into skin is generally accepted (*51-54*). Liposomes can be

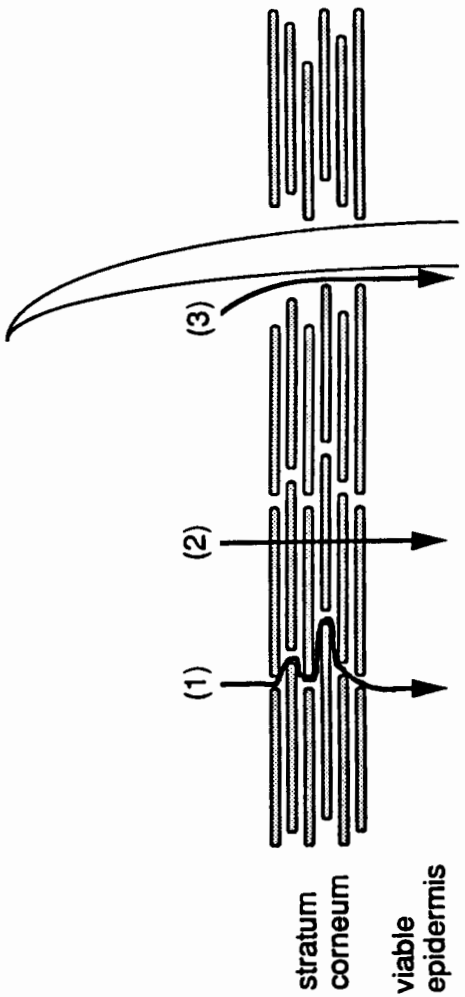


Figure 2. Three primary routes across the stratum corneum are available for transport. (1) A tortuous intercellular route solely within the stratum corneum lipids is usually followed by small compounds diffusing through normal or chemically-modified skin. (2) Transcellular pathways, which cross both the cells and intercellular lipids of stratum corneum, may only be accessible following skin permeabilization by electroporation or ultrasonic cavitation. (3) "Shunt" pathways through the hair follicles and/or sweat ducts may be important during iontophoretic and liposomal transport.

used with lipophilic drugs, which localize within the liposome's lipid bilayer shell, and with hydrophilic drugs, which localize within the aqueous interior. In either case, an interaction of liposomes with the lipids of the stratum corneum could increase drug entry into the skin. Partitioning could be enhanced by liposomes which provide a high local drug concentration at the skin surface. Adsorption or fusion of liposomes onto stratum corneum lipid bilayers could also promote drug partitioning into skin.

The role of liposomes in enhancing the second step in the transport process — diffusion across the stratum corneum — remains controversial. Most researchers agree that conventional liposomes do not serve as drug carriers which cross the bulk of stratum corneum as intact vesicles (*54-56*). Enhanced drug diffusion within stratum corneum could result from liposomal lipids becoming incorporated into stratum corneum bilayers, thereby acting as chemical enhancers which fluidize or otherwise change lipid properties to facilitate transport (*57*).

Under special circumstances, some suggest that liposomes cross the stratum corneum as intact vesicles. The hair follicles may provide a shunt route through which liposomes could cross the stratum corneum and deposit drug deep within the skin, primarily within or near hair follicles (*58, 59*). Moreover, liposome formulations designed to make vesicle shape very deformable might penetrate intact skin more readily (*60, 61*).

Experimental findings. Liposomes have been shown to increase topical and transdermal delivery of a variety of low molecular weight compounds (*51-54*). Moreover, it has been shown that liposomes can increase drug localization within the skin while decreasing systemic distribution (*62-64*). This has made the use of liposomes a popular enhancement technique for local drug delivery in dermatological applications.

Delivery of macromolecules can also be enhanced by liposomes, where transport is usually localized within hair follicles. Increased macromolecule penetration into skin has been shown following topical administration with liposomes for cyclosporin (1.2 kDa) (*62, 65*), a DNA repair enzyme (16 kDa) (*66*), γ-interferon (16 - 25 kDa, in monomeric form) (*67*), α-interferon (18 - 20 kDa) (*65*), melanin (*68*), superoxide dismutase (33 kDa) (*69*), a monoclonal antibody (~150 kDa) (*70*), and DNA (1 kb) (*71*). These studies generally used animal skin in vivo, in vitro, or from histoculture. Some studies have directly shown localization of these compounds within follicles, while others have inferred it. It has not been clearly shown that intact liposomes penetrate deep into follicles without breaking up. Moreover, some studies indicate that molecules need not be encapsulated within liposomes for increased follicular penetration, but can be co-administered in solution (*72*). This suggests that intact liposomes may not carry drug across the skin, but enhance transport by a different mechanism.

To facilitate liposome penetration into skin, ultradeformable liposomes (termed "transfersomes") have been developed through the addition of bile salts to liposome bilayers (*60, 61*). Enhanced transport of a number of small drugs has been shown using this approach, including the clinical delivery of lidocaine to increase local anesthesia (*73*). Studies which demonstrate systemic delivery of macromolecules across the skin have used insulin (5.8 kDa, in monomeric form) (*74*), bovine serum albumin (69 kDa) (*75*), and gap junction protein (> 178 kDa) (*75*) (Figure 3). These liposomes must be applied non-occlusively so that the formulation dries, thereby potentially enhancing an osmotic driving force for transport (*60*). It has been proposed that ultradeformable liposomes cross the skin as intact vesicles, following a non-follicular pathway and being taken up by the lymphatic system before entering systemic circulation (*61*).

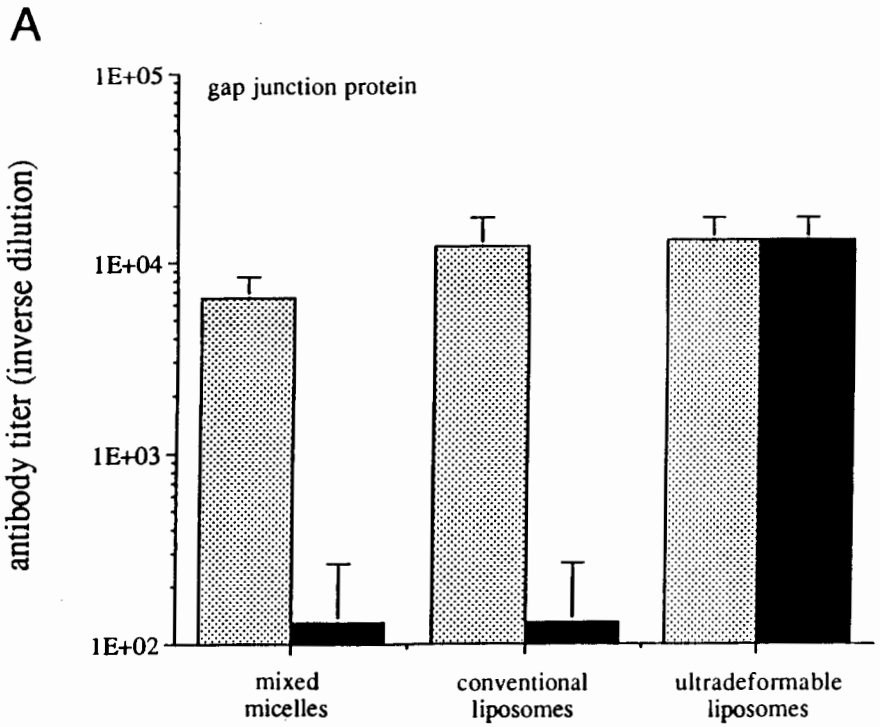


Figure 3. Immune response to (A) gap junction protein or (B) bovine serum albumin given to mice either by injection (▨) or topical application (■). Immunogenic protein was administered with mixed micelles (soybean phosphatidylcholine (SPC) and bile salt at 1:1 mole ratio) or encapsulated within liposomes, either "conventional" (SPC only) or ultradeformable (SPC and bile salt at 9:2 mole ratio). Antibody titers were determined by the serum-dependent, complement-mediated lysis of antigen-sensitized liposomes. Topically applied protein elicited an immune response equal to that caused by injection only when administered with ultradeformable liposomes. Standard deviation bars are shown. Reproduced with permission from reference (*75*).

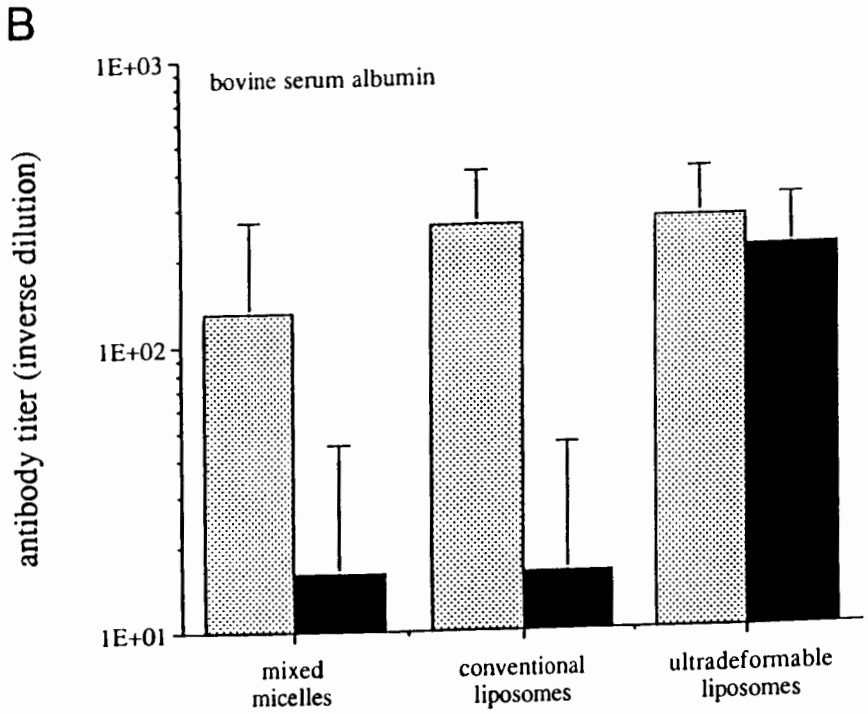


Figure 3B. *Continued*

Potential for impact. Although only a limited number of studies address liposomal delivery of macromolecules, they demonstrate penetration of large compounds into or across skin. Topical delivery of macromolecules to hair follicles has a number of potential clinical applications. Reports on systemic macromolecule delivery using ultradeformable liposomes offer still more possibilities.

Iontophoresis provides an electrical driving force for transdermal transport. As an alternative to enhancing transport by modifying the chemical environment in skin, the possibility of driving drugs across skin by the application of an electric field has received considerable attention, especially since the mid 1980s. Extensive in vitro, in vivo, and clinical study, coupled with a few commercial products, suggest that iontophoresis is a viable means of enhancement. Nevertheless, therapeutic delivery of macromolecules across human skin has been difficult to achieve.

Mechanistic perspective. Application of an electric field across the skin can enhance transdermal transport of both charged and uncharged drugs by electrophoresis and/or electroosmosis. A charged compound in an electric field moves by electrophoresis at a rate determined by the product of the electric field strength and the compound's mobility (a function of molecular size and charge) in the surrounding medium (i.e., the skin) (*76*). Electrophoretic enhancement is often possible, since many drugs have a net charge, including most macromolecules of therapeutic interest.

Because the skin carries a net fixed negative charge, transdermal transport of positively-charged ions is favored. As a result, during iontophoresis there is a net flux of ions from the anode to the cathode, which provides a convective driving force for transport across the skin, termed electroosmosis (*77*). With the proper electric field orientation, this effect can be used to enhance transport of uncharged compounds. Moreover, positively-charged drugs delivered across the skin by electrophoresis will be further enhanced by electroosmosis. However, electroosmosis will oppose electrophoretic transport of negatively-charged drugs. Theoretical models have been developed which describe transdermal electrophoresis and electroosmosis (*22, 77-79*). While some studies suggest that during iontophoresis ions cross the stratum corneum via the tortuous intercellular routes followed during passive diffusion (*80, 81*), others identify appendageal shunts as the primary pathways (*23-27*).

Electrical studies show that exposure of skin to transdermal voltages of approximately one volt or more reduces skin resistance. At typical iontophoretic voltages (< 10 V), human skin resistivity drops one to two orders of magnitude (from 100 $k\Omega$-cm^2) over a timescale of seconds to tens of minutes (*24, 27, 82-88*). Lowered skin resistance and increased skin permeability can persist after the electric field is removed, demonstrating either partial or full reversibility over a timescale of minutes to hours. Mechanistically, this has been explained by voltage-dependent rearrangements in skin microstructure (*79, 87*) and by an electroosmotic mechanism (*85*). These electrical measurements suggest that electric fields are capable of not only driving molecules across skin, but directly changing skin barrier properties. The possibility of utilizing electric fields in this way has received some attention (*24, 77, 87*), with recent efforts directed outside the context of traditional iontophoresis, as described below (see Electroporation).

Experimental findings. Transdermal iontophoresis of small compounds has been the subject of extensive in vitro and in vivo studies, some of which have led to clinical success (*89-92*). Today, commercial products exist for iontophoresis of: pilocarpine to induce sweating as part of a cystic fibrosis screening test (e.g., CF Indicator, Medtronic, Inc., Minneapolis, MN) (*93*), tap water as a treatment for hyperhidrosis (e.g., Drionic, General Medical Co., Los Angeles, CA) (*94*), and

lidocaine and other therapeutic agents from an "all-purpose" device (e.g., Iontocaine and Phoresor, IOMED, Inc., Salt Lake City, UT) (*95*). Iontophoresis is generally well tolerated, although mild skin irritation, erythema, and non-painful sensation are sometimes reported.

Iontophoresis of macromolecules has been more difficult than electrically-assisted delivery of small compounds (*6, 8, 96*). Those reporting success have generally not used human skin, but have demonstrated transport across animal skin, which is often more permeable than the human integument. Macromolecules delivered across animal skin include: arginine-vasopressin (1.1 kDa) (*97*), leuprolide (1.2 kDa) (*98, 99*), calcitonin (3.5 kDa) (*100*), growth hormone releasing factor (3.9 kDa) (*101*), carboxy-inulin (5.2 kDa) (*84*), insulin (*102-105*), and bovine serum albumin (*84*) (Figure 4). Notable exceptions include the clinical delivery of leuprolide to human subjects (*106, 107*) and delivery of cytochrome c (12 kDa) across human skin in vitro (*182, 183*). Delivery of leuprolide, a cholecystokinin-8 analogue (1.2 kDa), and insulin were also achieved across human skin in vitro, where detectable fluxes were measured only when iontophoresis was preceded by a two hour exposure to absolute ethanol (*108, 109*), a process unlikely to find clinical acceptance.

Potential for Impact. Overall, the iontophoresis literature shows that despite success with animal skin, clinically-relevant iontophoretic protocols have been capable of transporting macromolecules across human skin in very few cases. Perhaps an iontophoretic driving force coupled with a means of reversibly altering the skin's barrier properties (e.g., chemical, electrical, or ultrasonic) will be more broadly useful.

Electroporation creates new transdermal pathways by disrupting lipid bilayer structure. Short, high-voltage pulses which cause electroporation are known to transport large numbers of macromolecules across cell membranes without killing cells in vitro and in vivo. Recently, electroporation of the stratum corneum's lipid bilayers was shown to occur and to increase rates of transdermal transport by orders of magnitude (*110, 111*). Subsequent studies indicate that electroporation can also enhance transdermal macromolecule delivery to clinically-relevant levels.

Mechanistic perspective. Electroporation (also called electropermeabilization) is believed to involve the creation of transient aqueous pathways in lipid bilayers by the application of a short (μs to ms) electric field pulse (*112-114*). Permeability and electrical conductance of lipid bilayers are rapidly increased by many orders of magnitude, where membrane changes can be reversible or irreversible, depending mainly on pulse magnitude and duration. This phenomenon is known to occur when the transmembrane voltage reaches approximately 1 V for electric field pulses typically of 10 μs to 100 ms duration when applied to bilayers in either living cells or metabolically-inactive systems (e.g., liposomes). During electroporation the following sequence of events is believed to take place: (1) new aqueous pathways ("pores") are created on a timescale of microseconds or less, (2) molecules are moved through these pathways by diffusion and local electrophoresis and/or electroosmosis, and (3) after the pulse, pores close over characteristic times ranging from milliseconds to hours.

Because the rate-limiting barrier to transdermal transport is the lipid bilayers of the stratum corneum, electroporation of these bilayers could significantly increase drug delivery across skin. A simple theoretical estimate indicates that millisecond electric field pulses of approximately 100 V could electroporate the approximately 100 multilamellar bilayers crossed in a path directly across the stratum corneum (*111, 115, 116*). Voltages typically applied during iontophoresis (< 10 V) are considerably lower, but might be sufficient to electroporate a few bilayers, perhaps affecting the lining of appendages (*79, 87*). Experiments investigating skin electroporation usually apply a

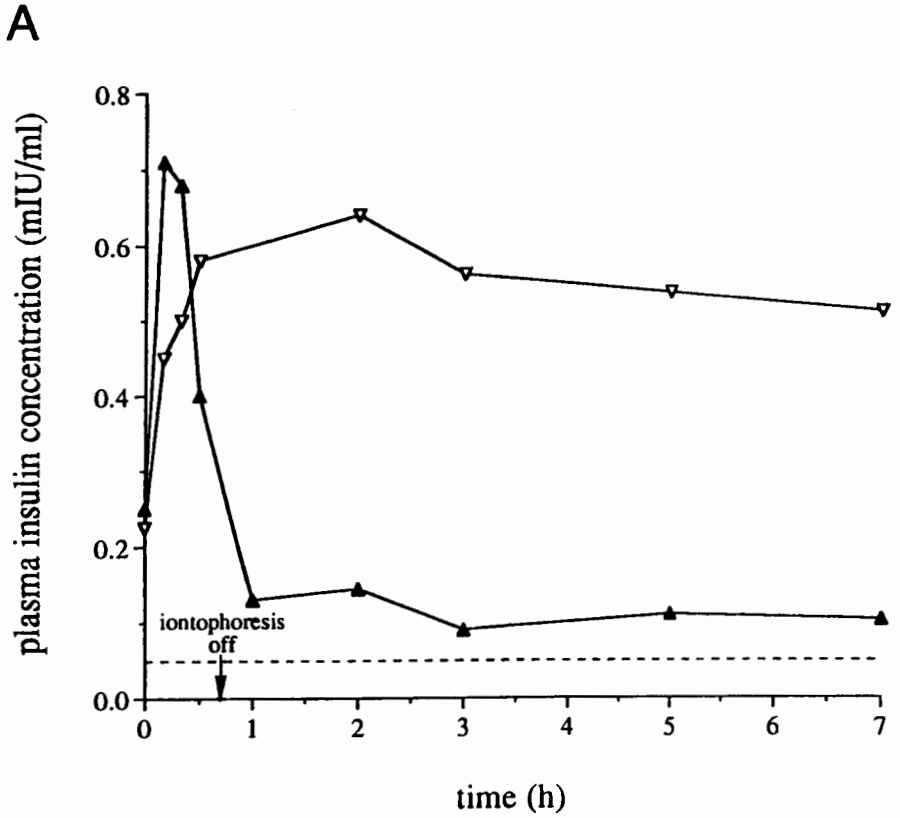
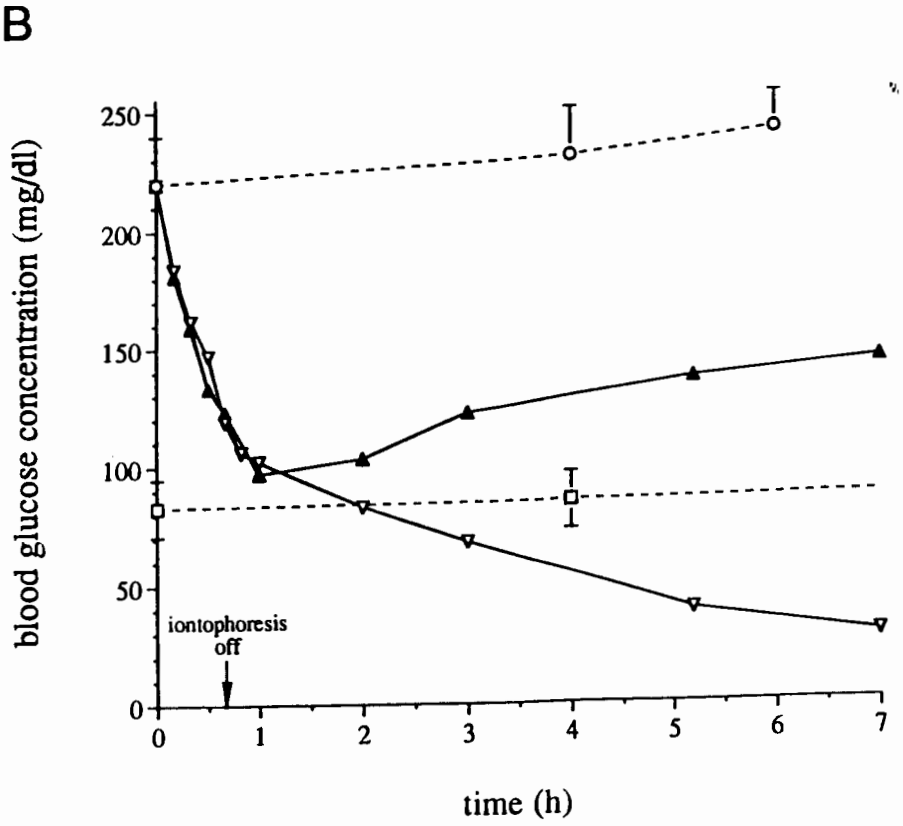


Figure 4. (A) Plasma insulin and (B) blood glucose concentrations following insulin administration to rabbits. Diabetic rabbits were given insulin either by subcutaneous injection (▽) or transdermal administration using iontophoresis (▲). As controls, additional diabetic (○) and normal (□) rabbits received no treatment. Iontophoresis was applied at 1 mA for 40 min using a pulsed waveform. Insulin concentration was determined by radioimmunoassay. Standard deviation bars are shown. Reproduced with permission from reference (*96*).



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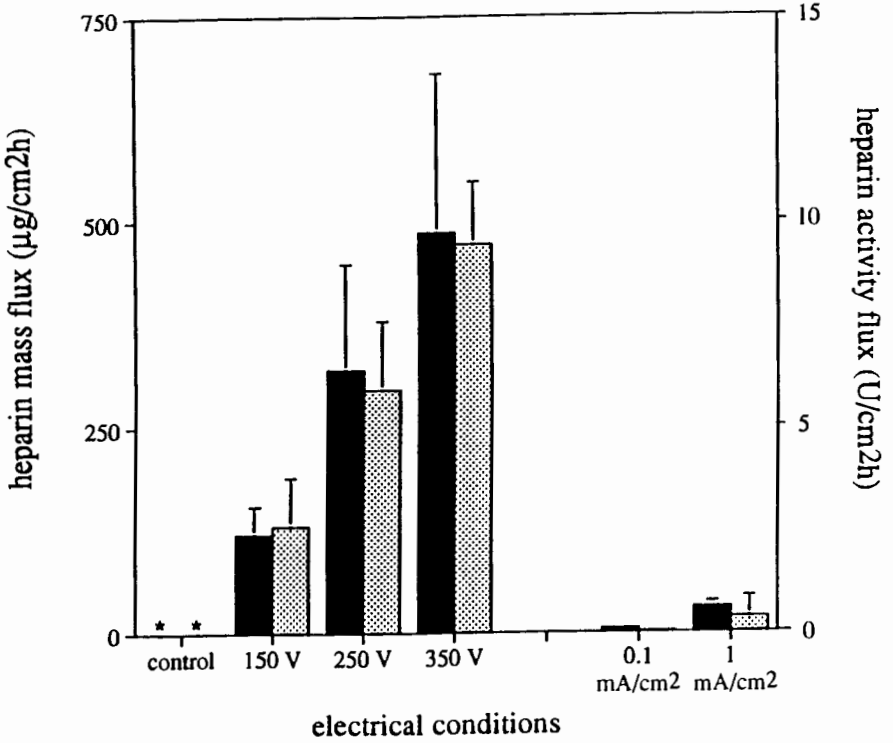

Figure 5. Transdermal heparin fluxes caused by electroporation or iontophoresis of human skin in vitro. Heparin mass flux (■) was determined by scintillation counting measurements of radioactively-labeled heparin. Biological activity flux (▨) was determined by a blood clotting time assay. Heparin fluxes caused by electroporation may be sufficient for clinical applications (*126*). Electrical exposures were each 1 h of either continuous iontophoresis (0.1 or 1 mA/cm^2) or intermittent electroporation pulses (150, 250, or 350 V) each lasting 1.9 ms and applied at a rate of 12 pulses per minute. Standard deviation bars are shown. Asterisk indicates a flux below the detection limit (of order 1 $\mu g/cm^2h$ for radioactivity measurements and 0.1 U/cm^2h for biological activity measurements). Reproduced with permission from reference (*126*).

series of pulses at rates of one pulse every five seconds to one pulse every five minutes, where each pulse causes a transdermal voltage of 30 to 300 V and lasts for 1 to 300 ms. Theoretical studies suggest that the described experimental findings could be accounted for by electroporation of stratum corneum lipids (*115, 116*).

Experimental findings. Electrical studies have shown that short, high-voltage pulses can have dramatic and reversible effects on skin electrical properties. During a pulse, skin resistance drops as much as three orders of magnitude within microseconds (*117, 118*). Skin resistance then generally recovers by a factor of ten within milliseconds, and exhibits either complete or partial reversibility within minutes. Skin capacitance has been observed to increase by up to an order of magnitude and later reverse to pre-pulse values (*117, 119*). Increased capacitance may indicate changes in skin lipids, since skin's capacitance is generally attributed to stratum corneum lipid bilayers (*80, 86*). In contrast, these electrical effects are not observed during low-voltage iontophoresis (*82, 85-87, 120, 121*).

High-voltage pulses also change skin transport properties such that up to 10,000-fold increases in transdermal delivery occur for compounds ranging in size from small ions to microspheres (*111, 118, 122-136*). Steady-state transport can be achieved in a matter of minutes (*130, 137*). Complete or partial reversibility is generally observed within an hour (*111, 130*). Microscopic imaging suggests that transport occurs through the bulk of stratum corneum via transcellular and intercellular pathways estimated to occupy up to 0.1% of skin area (*138, 139*). This observed transcellular transport contrasts with the tortuous intercellular pathways of passive diffusion and the shunt pathways of iontophoresis and liposomes. Limited work performed on hairless rats indicates that large transdermal fluxes are also achieved in vivo, where no effects beyond transient erythema and edema were observed (*111, 123, 140*). Additional safety studeis are required.

The effects of high-voltage pulses have been attributed to short-lived changes in skin structure (e.g., electroporation of stratum corneum lipid bilayers) followed by electrophoretically-driven transport across the skin. A number of studies indicate that electrophoresis alone cannot explain the large flux increases observed during high-voltage pulsing, supporting the hypothesis that skin structure is transiently disrupted (*111, 134, 136, 141*). Other studies more specifically suggest that high-voltage pulses can create enlarged transport pathways, the size of which is controlled by a voltage-dependent mechanism (*118, 126*).

Skin electroporation can increase delivery of macromolecules to therapeutically-useful rates across human skin. Transdermal transport of heparin (5 - 30 kDa) was increased by electroporation in vitro to rates sufficient for clinical anticoagulation therapy (*126*) (Figure 5). Other studies have demonstrated electroporation-enhanced delivery of arginine-vasopressin (*142*), luteinizing hormone releasing hormone (1.2 kDa) (*122, 140, 142*), neurotensin (1.7 kDa) (*142*), and oligonucleotides (4.8 and 7 kDa) (*129*). Increased penetration into skin has also been shown for latex microspheres of up to micron dimensions (*125, 139*). These studies almost all employed human skin in vitro.

Potential for impact. Electroporation's ability to both create new transport pathways and drive molecules through them has proved capable of delivering macromolecules across skin, especially for highly-charged compounds which are effectively moved by electrophoresis (e.g., heparin, oligonucleotides). This has the potential to lead to a variety of clinical applications. Electroporation-mediated delivery of macromolecules which carry a weak net charge, such as proteins, has not yet received attention. Issues of safety and drug stability also need further study.

Ultrasound creates new transdermal pathways by cavitation. Ultrasound is used extensively in clinical practice for applications ranging from diagnostic imaging to therapeutic heating to lithotripsy procedures. Transdermal delivery enhanced by ultrasound (sometimes called sonophoresis or phonophoresis) has received sporadic attention for half a century, but has recently sparked renewed interest by studies which demonstrate delivery of macromolecules at therapeutically-relevant rates (*143*).

Mechanistic perspective. Ultrasound is a pressure wave having a frequency too high to be heard by the human ear (> 16 kHz) (*144, 145*). When introduced into the body, ultrasound echoes off internal structures, thereby allowing diagnostic imaging. Ultrasound conditions used by diagnostic instruments are typically very high frequency (» 1 MHz) and low intensity (« 1 W/cm^2), selected in part to prevent damaging imaged tissue (*144, 146-150*). Ultrasound under these conditions should have no effect on skin properties.

Ultrasound applied at "therapeutic" conditions heats tissue. High frequencies (~ 1 MHz) and moderate intensities (~ 1 W/cm^2) are typically employed in physical therapy and cancer chemotherapy using ultrasonic hyperthermia (*144-150*). These conditions are favorable because they provide sufficient energy to heat tissue, even deep within the body, without causing other effects associated with ultrasound at greater intensity or lower frequency. Most studies using ultrasound to enhance transdermal drug delivery have used therapeutic conditions (*151-156*), in part because those intensities and frequencies are already FDA-approved for clinical use. Ultrasonic heating of skin could increase transdermal transport by fluidizing stratum corneum lipids and/or increasing convective flow.

Ultrasound can also cause non-thermal effects such as cavitation. If applied at lower frequencies (« 1 MHz) or greater intensities (» 1 W/cm^2) than used in therapeutic applications, ultrasound can cause extensive generation of gas bubbles, called cavitation (*144-150, 157, 158*). Stable cavitation creates bubbles which oscillate in size at the frequency of the applied ultrasound. Transient cavitation bubbles are short lived, imploding violently upon their collapse. Both forms of cavitation can have severe effects on biological tissue, as demonstrated by the shattering of kidney stones during ultrasonic lithotripsy procedures (*150, 159, 160*) and ultrasonic cell disruption techniques commonly employed in research laboratories (*150, 161*). If applied to the skin, significant changes in skin structure and permeability could result.

Experimental findings. Ultrasound has been used since the 1950's to enhance transport of small drugs into and across skin for local delivery. Early clinical studies showed increased absorption of hydrocortisone when accompanied by ultrasound at therapeutic intensity and frequency (*162, 163*). Other clinical studies have described local delivery of anesthetics, non-steroidal anti-inflammatories, antibiotics, and antivirals (*151-156*). In addition to local delivery, systemic transdermal administration of small drugs has also been enhanced by ultrasound. While some work has been performed clinically (*164*), most compounds have been examined through in vitro and in vivo laboratory investigation (*165-170*). These studies, in addition to others performed outside the context of drug delivery, suggest that application of therapeutic ultrasound to the skin is safe (*146, 149, 150*).

The mechanism(s) of ultrasonic enhancement remains controversial. While most conclude that thermal contributions are small, evidence for increased convection (e.g., acoustic streaming or mixing) (*165, 171*) and for cavitation-mediated effects (*165, 170, 172*) offer compelling explanations for increased transport. Although many studies have shown that therapeutic ultrasound can significantly enhance transdermal transport (*151-156*), some studies report that ultrasound has no effect (*173-175*). A recent analysis may reconcile these findings by identifying that studies which report no

enhancement used compounds which are small (< 250 Da), while those which observed enhanced transport used larger compounds (*176*). Because small molecules diffuse easily through stratum corneum lipids, their transport may not be significantly increased by increases in lipid fluidity caused by ultrasound. Concerning the route of transport during therapeutic ultrasound, studies suggest that compounds follow an intercellular path (*168*).

Deviating from conventional therapeutic ultrasound, a few studies have employed lower frequencies (e.g., 20 - 100 kHz), which cause increased cavitation (*145, 177*). Enhanced delivery of lidocaine (*178*) and insulin (*172, 179*) has been shown in animal models. Recently, transdermal delivery of insulin, γ-interferon, and erythropoeitin (48 kDa) was demonstrated using human skin in vitro, supported by data obtained with hairless rats in vivo (*143*) (Figure 6). Delivery of these large macromolecules was at rates sufficient for clinical applications. Preliminary histological examination showed no adverse effects (*143, 180*). In contrast to therapeutic ultrasound, the effects of low-frequency ultrasound are believed make transcellular pathways accessible by a mechanism involving cavitation (*180*).

Potential for impact. Although useful for local and systemic delivery of small compounds, therapeutic ultrasound has not significantly increased transdermal transport of macromolecules. When applied at lower frequencies, ultrasound has been shown to deliver large macromolecules across skin at therapeutically-relevant rates. Although only limited work has been done so far, the dramatic effects of low-frequency ultrasound suggest that it is a promising new approach which warrants close attention. Safety and drug stability concerns will require further study.

Discussion.

Methods of delivering macromolecules across skin which have been successful each provide a driving force for transport as well as modify skin's barrier properties. Methods which do not alter skin's properties, such as passive diffusion and iontophoretic enhancement by electrophoresis and/or electroosmosis, have weaker ability to transport large compounds. In contrast, barrier modification by means of electroporation or ultrasonic cavitation increases macromolecule delivery to levels of clinical interest. Disruption of the skin with chemicals can sometimes increase macromolecule delivery, but generally only under conditions which raise safety concerns. Liposomes are an exception, since they deliver macromolecules by a mechanism which is not yet completely understood, but appears not to involve modifying the skin barrier.

Introduction of electrical or ultrasonic energy into skin alters skin properties as a complex function of the energy input. As shown in Table I, iontophoresis, electroporation, therapeutic ultrasound and low-frequency ultrasound all introduce energy into the skin, but have very different effects on the skin barrier. Increases in macromolecule transport do not relate in a simple manner to the energy provided to the skin. For example, therapeutic ultrasound supplies the greatest average power, but has the weakest effect. While the instantaneous power associated with electroporation is 100-times greater than ultrasound, low-frequency ultrasound appears to deliver large macromolecules more effectively. Enhancement also does not relate simply to changes in skin resistance. The effects of low-frequency ultrasound on skin resistance are similar to those caused by iontophoresis, yet the abilities of these two methods to enhance macromolecule transport are very different. Clearly, the form of energy provided, and its microscopic distribution within the skin, are important. Perhaps it is the delivery of short doses of highly localized energy that is most effective. This is achieved during electroporation, where millisecond-pulses of energy are concentrated a

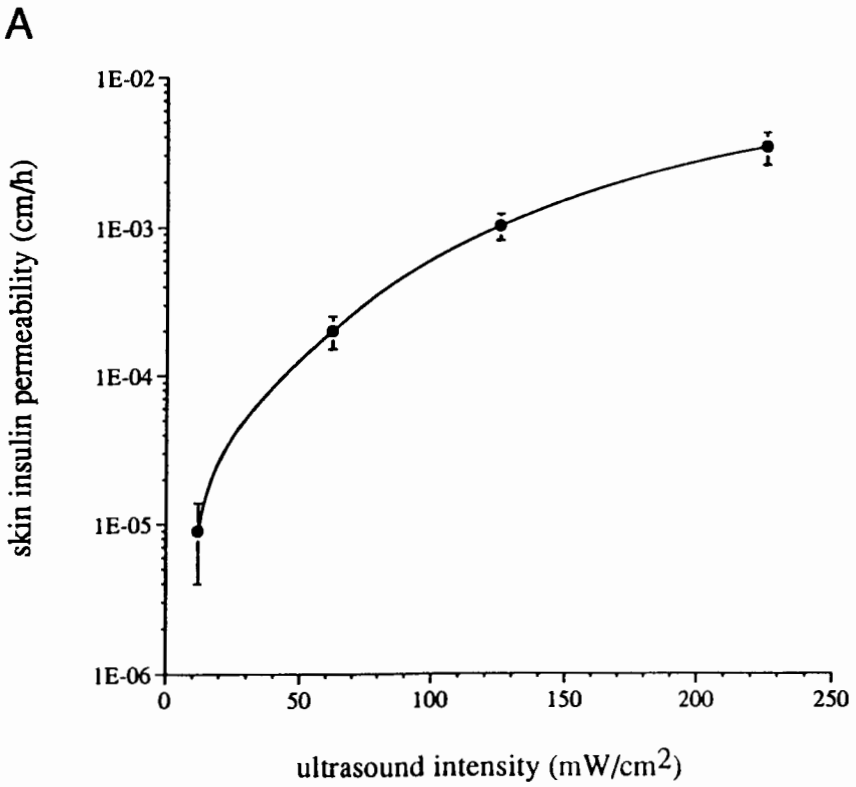

Figure 6. Transdermal delivery of insulin in the presence of ultrasound. (A) The permeability of human skin in vitro to insulin as a function of ultrasound intensity (at 20 kHz and 10% duty cycle). A permeability on the order of 10^{-3} cm/h may be sufficient to deliver insulin at clinically-useful rates (*143*). (B) Blood glucose concentration in hairless rats. The blood glucose level in diabetic rats was reduced to normal levels by application of ultrasound (225 mW/cm^2, 20 kHz, 10% duty cycle, 30 min) to an insulin solution on the rat's skin (▲). The blood glucose levels of diabetic (○) and normal (□) rats which received neither insulin nor ultrasound remained constant. Standard deviation bars are shown. Reproduced with permission from reference (*143*).

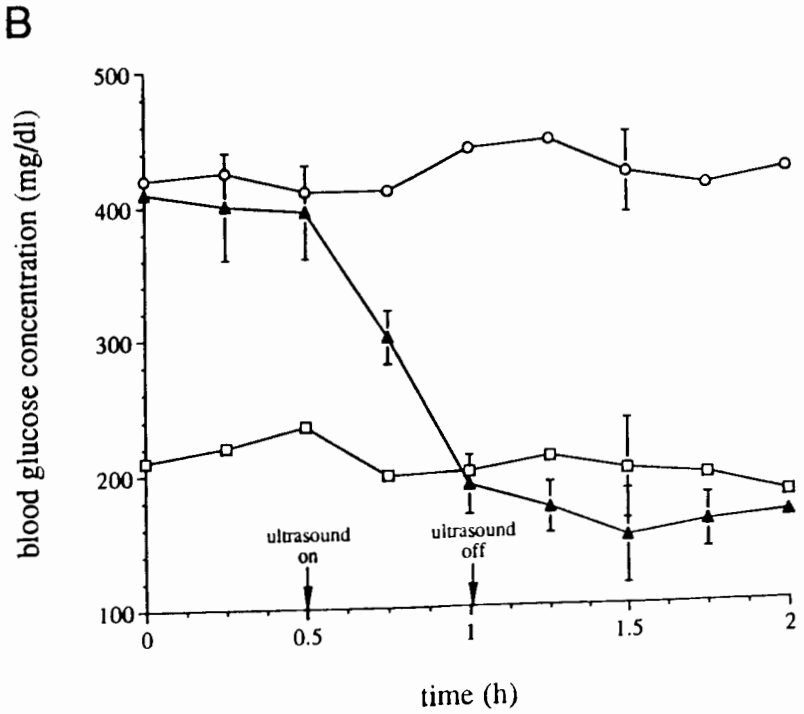

Figure 6B. *Continued*

Table I. Characteristics of ultrasonic and electrical methods of transdermal transport enhancement.[a]

characteristic properties	*ultrasound (therapeutic)*	*iontophoresis*	*electroporation*	*ultrasound (low-frequency)*
instantaneous power[b] (W/cm^2)	1	0.001	100	1
average power[b] (W/cm^2)	1	0.001	0.001 - 0.1	0.1
skin resistance[c] ($k\Omega$–cm^2)	100	1 - 10	0.1	1 - 10
timescale of resistance change[c]	1 h	1 - 10 min	1 μs	1 h
macromolecule transport[d]	not likely	possible	possible	likely

[a] Numerical values provided in this table are based on data from references (*88, 118, 180*).

[b] Iontophoresis is typically applied continuously, while pulsed fields are often used in ultrasound (e.g., 10% duty cycle) and electroporation (0.001 - 0.1% duty cycle). A typical iontophoresis exposure could apply up to a few volts and ≤ 0.5 mA/cm^2 across the skin. Electroporation typically applies ~100 V and ≤ 1 A/cm^2 instantaneously during pulses.

[c] Exposure to ultrasound and electric fields can lower skin resistance. Representative steady state resistance values and the timescales over which those values are reached are shown for human skin.

[d] The possibility of delivering macromolecules across human skin at clinically-relevant rates is assessed, based on information presently available in the literature.

the lipid bilayers, and during low-frequency ultrasound, where cavitation releases localized bursts of energy within the skin.

Recognizing that successful macromolecule delivery involves two important components — (1) modifying the skin barrier and (2) providing a driving force for transport — combinations of enhancement methods may provide new opportunities. Used alone, electroporation and ultrasound inherently affect both of these components, where electroporation simultaneously disrupts the skin and transports molecules by electrophoresis and/or electroosmosis, and ultrasound disrupts the skin via cavitation and enhances transport by acoustic streaming and other convective effects. An example where two different methods have been combined, iontophoresis has been used to provide a driving force for transport across skin exposed to chemical enhancers which alter the skin barrier (*108, 109*). Similarly, electrophoretic transport by iontophoresis has been preceded by electroporation, used to permeabilize the skin (*140, 141*). Therapeutic ultrasound has also been applied during electroporation to enhance the ability of electroporation to disrupt the skin barrier (*181*).

Conclusion.

Research on transdermal transport has led to a number of successful clinical applications involving delivery of low molecular weight drugs. However, the special delivery requirements of proteins and other therapeutic products of biotechnology present the need to expand the scope of transdermal administration to include delivery of macromolecules. Just a few years ago, this need could not be met, since transdermal delivery of macromolecules across human skin at clinically-relevant rates had not been performed. However, successful macromolecule delivery has now been demonstrated, in which transient modification of skin's barrier properties is an important component. Techniques involving ultradeformable liposomes, electroporation, and low-frequency ultrasound, perhaps supplemented with chemical or iontophoretic enhancement, show real promise as tools for delivering biologically-active macromolecules across skin.

Despite the great importance of developing drug delivery technologies for macromolecules, most transdermal delivery research continues to focus on traditional methods of enhancement useful primarily for small compounds. With few exceptions, the research groups which first reported the effects of ultradeformable liposomes, electroporation, and low-frequency ultrasound remain the only ones who have published on the subject. Contributions by other researchers would confirm their exciting results and provide new perspectives on the rapidly evolving field of transdermal macromolecule delivery.

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183 In reference (*182*), this compound is referred to as protein X, but has been identified as cytochrome c in a personal communication from Ron Haak.