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Drug delivery routes in skin: a novel approach

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Abstract

The role of hair follicles in transdermal delivery remains difficult to elucidate due partly to animal model complications. This paper explores a novel technique employing two human skin membranes to differentiate shunt route delivery from bulk transepidermal input. The method monitors penetration through epidermal membranes and compares this with delivery through a sandwich of stratum corneum and epidermis, with the corneum forming a top membrane. As orifices of shunts occupy only 0.1% of the area, there is negligible chance that shunts in the membranes will superimpose. The top layer blocks shunts available in the bottom layer. If shunts are important, delivery through sandwiches will be much reduced compared with that through epidermis, allowing for increased double membrane thickness. Experiments with penetrants under passive, iontophoretic and electroporation conditions illustrated the value of the method. A Monte Carlo simulation suggested that any failure of membrane adherence would not affect conclusions drawn.

Keywords: Skin sandwich; Diffusion; Iontophoresis; Electroporation; Shunt route; Hair follicle

Contents

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1. Importance of shunt route penetration

Almost since the introduction of the modern scientific study of percutaneous absorption (transdermal delivery), workers have debated the relative importance of three potential routes of entry from the surface of the skin into the subepidermal tissue—through the hair follicles with their associated sebaceous glands, via the sweat ducts, or across the continuous stratum corneum between these appendages (Fig. 1). Nearly 40 years ago in pioneering work, Scheuplein and co-workers [1,2] proposed that the rapid transport of large polar molecules and ions arose from a follicular shunt route; the investigations of Feldmann and Maibach [3,4] also suggested the possibility of follicular delivery. Early experiments on this concept were summarised by Barry [5], and more modern techniques have included fluorescence microscopy and confocal laser scanning microscopy applied to a range of drugs in a variety of vehicles [6]. Even 'naked' DNA, a large molecule, can immunise by topical application, suggesting follicular transport, and the hair follicle promises to be a target for gene therapy (e.g. Refs. [7,8]). Liposomes and their analogues, microspheres and small crystals have all been claimed to target the hair follicle [9–25]. Recently, Grams and Bowstra [26] determined the distribution of a fluorophore in scalp skin hair follicles and provided other relevant references.

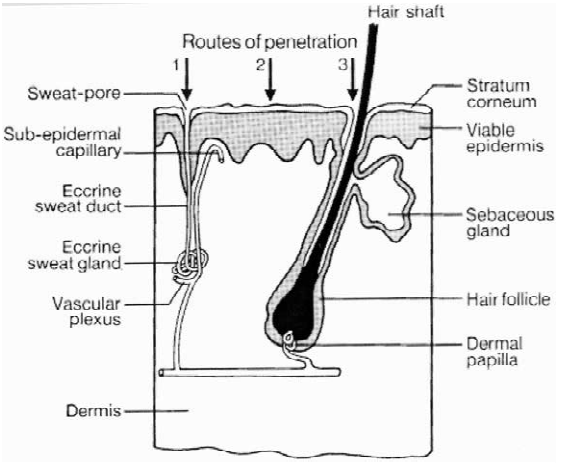

Fig. 1. Macroroutes for penetration through human skin (1 = sweat gland, 2 = transepidermal, 3 = hair follicle).

A major problem when we attempt to assess the relative importance of shunt route flux to the overall skin flux (as distinct from targeting to the follicle) is the absence of an entirely satisfactory experimental model with which to separate out the three parallel routes. Mathematical analysis of human flux data (to disentangle the contributions of serial and parallel routes) is fraught with difficulties, mainly arising from the complexity, variability and occasional instability of skin membranes [5]. Workers have therefore often used animal models, such as hairless rodents (mice, rats, guinea pigs) and newborn rats, including a follicle-free tissue developed by scarring hairless rat skin. Other preparations include the Syrian hamster ear, the follicle-free area of guinea pig skin, the Macaque monkey and histocultures with hair follicles (for a review, see Ref. [27]). However, all such approaches have their individual problems. This presentation describes a new technique (the skin sandwich or composite) that holds promise for assessing the importance or otherwise of the shunt route in specific skin penetration scenarios. The paper considers a simple theoretical background for the skin composite approach, together with some examples of the use of the method that illustrate fundamental principles. The selections all use flux data, but similar conclusions were obtained as to the value of the sandwich technique when lag time was the assessed variable. The paper closes with a theoretical validation of the robustness of the system, based on a Monte Carlo approach.

2. Theoretical background for the skin sandwich technique

This novel method for assessing shunt route and transepidermal passage compares the delivery of a

test penetrant through a composite or sandwich made from human stratum corneum and epidermis (consisting of its own stratum corneum plus nucleated epidermis), with that through the epidermis alone [28]. The membranes are formed from the same sample of human skin and we assume that the top stratum corneum blocks essentially all the shunts in the bottom epidermis (Fig. 2). Thus, as the orifices of the shunts only occupy approximately 0.1% of the total skin area [29,30], the chance is negligible that shunts in the two-membrane composite will co-locate or superimpose. A further advantage of the technique is that the blocking stratum corneum layer has the same physicochemical and biological properties as the epidermal stratum corneum, since it is derived from the same skin sample, i.e. a foreign material is not used to block the shunts. This avoids further complications with respect to the interpretation of flux data.

2.1. Simple diffusional relationships [5]

Flux relationships for a simple membrane without shunts may be written as follows.

2.1.1. An infinite dose at steady state

$$J = (DPC)/h \quad (1)$$

where J is the steady state flux, D the diffusion coefficient through the membrane, P the partition coefficient, C the donor concentration and h is the membrane thickness.

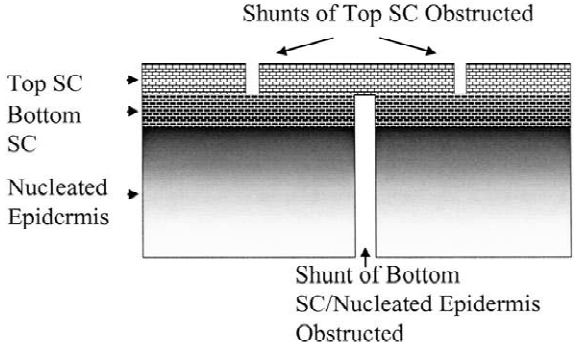

Fig. 2. Diagrammatic representation of the skin sandwich.

2.1.2. A finite dose deposited on the membrane

The maximum flux, J_{max}, is given approximately by:

$$J_{max} = (1.85DPC\delta)/h^2 \quad (2)$$

where δ is the thickness of a thin film deposited on the surface of the skin.

2.1.3. Additional definitions

h_{sc} = stratum corneum thickness; stratum corneum shunt distance
h_{ne} = nucleated epidermis thickness; nucleated epidermis shunt distance
J_e = flux through epidermis
J_{maxe} = maximum flux through epidermis, finite dose
J_{es} = flux through epidermal shunt
J_s = flux through sandwich
J_{maxs} = maximum flux through sandwich, finite dose
J_{ss} = flux through sandwich shunts
J_{ssn} = flux through sandwich shunts, membranes nonadhering

3. Examples illustrating the technique

3.1. Lipophilic permeant

An easy way to assess the validity of the composite or sandwich technique is to select as our model permeant one for which we would expect that the shunt route through epidermis would contribute negligibly to the overall flux at relatively long times. Then we could confirm whether or not theoretical predictions for the transepidermal flux through the composite, compared with that of the simple epidermis, agreed with experimental data. A suitable molecule is estradiol, a poorly soluble neutral compound with a log octanol–water partition coefficient of 2.29 [31] and a water solubility of 0.0003–0.00036% [32,33].

3.1.1. Lipophilic permeant at steady state (infinite dose)

For an epidermal membrane, according to Eq. (1), the steady state flux should be inversely proportional

to the thickness of the stratum corneum and the nucleated epidermis i.e.:

$$J_e \propto 1/h_{sc}, \quad 1/h_{ne}$$

Neglecting the small resistance of the nucleated epidermal barrier to molecules other than very lipophilic entities [34]:

$$J_e \propto 1/h_{sc}$$

For the sandwich membrane, the drug would have to permeate through two layers of stratum corneum so that the flux should obey the relationship:

$$J_s \propto 1/2h_{sc}$$

Thus the flux ratio (sandwich:epidermal membrane) is given by:

$$J_s/J_e = 0.5$$

Fig. 3a is a histogram of this flux ratio derived from the steady state permeation profiles of saturated aqueous estradiol penetrating epidermal membranes and composites [28]. For comparison, the theoretical value is shown when shunt penetration is negligible

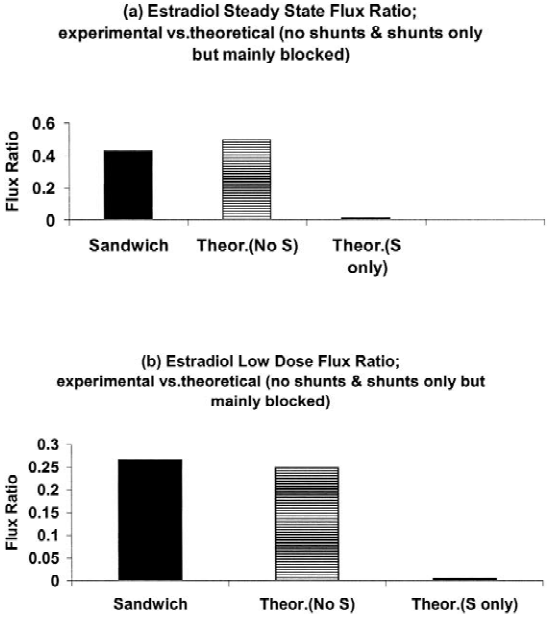

Fig. 3. Flux ratios for estradiol aqueous solution permeating through epidermis and sandwich—experimental and theoretical: (a) steady state, (b) low dose.

(0.5) and the result to be expected in the unlikely event that all the flux through the epidermis was via the shunts and these are blocked in the sandwich (0.0). The experimental flux ratio is indeed close to 0.5, in excellent agreement with theory. We can thus conclude that the skin composite technique is valid in respect to the concept of doubling the membrane thickness, and hence its resistance, compared with the simple epidermis.

3.1.2. Lipophilic permeant (low dose)

With a low (finite) dose applied to an epidermal membrane, we expect that the maximum flux attained would be a function of the square of the membrane thickness (Eq. (2)), i.e.:

$$J_{max} \propto 1/h_{sc}^2, \quad 1/h_{ne}^2$$

or, as before, neglecting the nucleated epidermis:

$$J_{max} \propto 1/h_{sc}^2$$

For the composite membrane with its double thickness in the rate limiting tissue:

$$J_{max} \propto 1/(2h_{sc})^2$$

Thus, the relevant low dose flux ratio, sandwich to epidermis, is:

$$J_{maxs}/J_{maxe} = 0.25$$

Fig. 3b provides data on a low dose study in which estradiol saturated solution was allowed to dry out on epidermal membranes and composites [28]. For comparison, the theoretical ratios are also provided for no shunt contribution and for the unrealistic situation of the main route of penetration of a lipophilic molecule being the shunt route, with all shunts being blocked.

The flux ratio is close to the theoretical value for no shunt contribution (0.25), again confirming the accuracy of the sandwich technique.

3.2. Hydrophilic (polar) permeant

If we select as our next permeant a very polar molecule, then we have the possibility of isolating a shunt route component of the flux and examining the validity of the sandwich technique as applied to this pathway. Then we would expect that, at short

diffusional times, the major contribution to the flux that comes down the follicles would essentially be completely blocked in the double membrane. A suitable test molecule here is mannitol, a highly water-soluble compound (approximately 18–22% at 25–37 °C [35]) with a log partition coefficient of −2.47 [36].

3.2.1. Hydrophilic permeant: shunt diffusion only

When there is no bulk (epidermal) diffusion and only shunt passage, which is blocked in the skin composite, then for the epidermal membrane the flux through the shunts is related by:

$$J_{es} \propto 1/(h_{sc} + h_{ne})$$

which assumes that the diffusional and partitioning properties of shunts h_{sc} and h_{ne} are similar.

For the sandwich composite in which the shunts are completely blocked:

$$J_{ss} = 0$$

Then the flux ratio (composite/epidermal membrane) is also zero:

$$J_{ss}/J_{es} = 0$$

Fig. 4 illustrates the 9-h permeation profiles for the diffusion of mannitol from an aqueous solution through epidermis and sandwich [37]. The epidermal plot is one that would be expected for a polar penetrant entering the membrane mainly by a low-resistance, but small fractional area, route. However, the effect of the sandwich is dramatic. The top stratum corneum does indeed block the shunts in the lower epidermis—so efficiently that negligible mannitol passes through the composite during 9 h permeation. Such data are strong evidence for the validity of the skin composite technique.

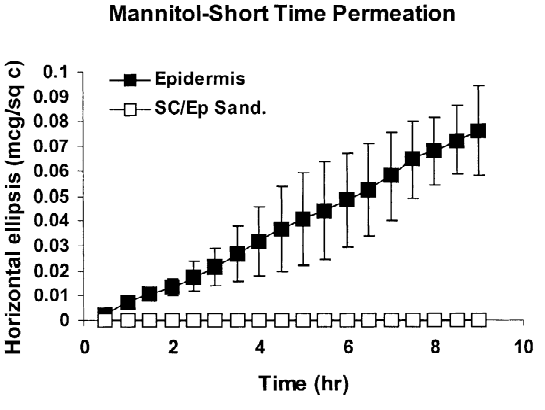

Fig. 4. Permeation profiles for mannitol aqueous solution through epidermis and sandwich: short times.

3.3. Liposomes (low dose)

In recent years, there has been much interest in delivering drugs to and through the skin using specific types of liposomes, variously described as deformable, ultradeformable, or flexible. For example, Cevc and co-workers [38–41] reported that transfersomes penetrate well through intact skin, but only under nonoccluded conditions. We have compared such vesicles with 'traditional' liposomes [42–46] and taken the opportunity to assess the sandwich technique with these nano particles. For example, we applied the sandwich approach to four types of liposomes each containing estradiol as a model drug and formulated as:

Ultradeformable (Ch.)—phosphatidylcholine plus sodium cholate
Ultradeformable (Sp.)—phosphatidylcholine plus Span 80
Non-rigid—phosphatidylcholine
Rigid—dipalmitoylphosphatidylcholine plus cholesterol

The experiment applied low doses of unilammellar vesicles, diameters 127–146 nm, to epidermal membranes and their sandwiches. As in Section 3.1.2, we expect that if the main route of penetration is via the bulk epidermis, for a low dose study the flux ratio (sandwich: epidermis) should be 0.25. However, if the shunt route is the major pathway and this continuous route is blocked in the composite, then the ratio should be 0.

Fig. 5 compares these ratios with theoretical values with either no shunt permeation or with 100% shunt passage in the epidermis but fully blocked in the sandwich. All liposomes produced ratios much closer to 0.25 than to 0; ultradeformable and traditional liposomes behaved similarly. We conclude that under these experimental conditions, the shunt route is not important for liposome delivery *through* the

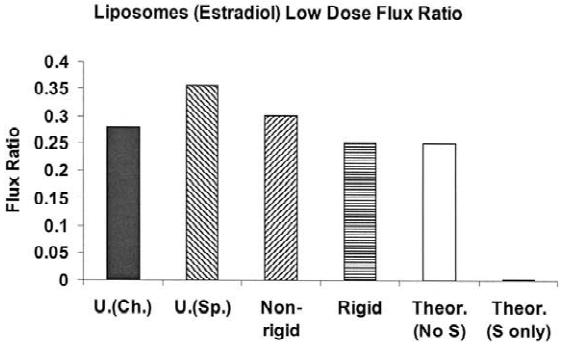

Fig. 5. Flux ratios for liposomes containing estradiol (low dose) penetrating through epidermis and sandwich—experimental and theoretical (no shunt delivery and shunt only delivery but blocked in sandwich). See text for definitions.

skin. These results correlate with work that shows images of intact elastic vesicles (in channel-like regions of the horny layer) in electron micrographs developed from tape strips of stratum corneum up to the depth represented by the ninth strip [47]. Few vesicular structures were found in the 15th tape-strip. However, in our work we were not concerned with whether or not liposomes *localize* in hair follicles, i.e. do they target the follicle? There is good evidence that solid particles greater than 10 μm tend to remain on the skin surface, those in a band between 3 and 10 μm concentrate within the follicle and that when the size is decreased to below 3 μm, they can penetrate stratum corneum and follicles alike [48]. Our work only assessed if liposomes would delivery their contents *through* the skin via shunt passage.

3.4. Electrical delivery

As an example of the sandwich technique used to assess aspects of iontophoresis and electroporation, we can consider some investigations with a buffered aqueous solution of a simple model compound, the negatively charged amino acid, L-glutamic acid.

3.4.1. Iontophoresis

The electrical driving of charged species into tissue (iontophoresis) passes a low DC current via an electrode in contact with a drug and the skin; a grounding electrode completes the circuit [49–51]. Three main mechanisms promote drug delivery: (i) the driving electrode repels ions; (ii) the flow of the current may decrease the resistance of the skin so as to increase its permeability; and (iii) electroosmosis may affect uncharged molecules and large polar peptides. How well drugs penetrate depends mainly on the polarity, valence and mobility of the ions, as well as formulation components and electrical duty cycles [52]; for a review, see Ref. [53]. During iontophoresis, most ions are expected to follow the path of least resistance and to penetrate through damaged regions of the skin and down the shunts of the hair follicles and sweat glands. The current, if high enough, may form artificial shunts as the lipids of the stratum corneum temporarily disrupt to form pores [54–57].

Our example iontophoretic study [58] used a constant voltage (5 V) applied for 2 h to an aqueous solution of L-glutamic acid and compared the steady state flux through the sandwich with that through the epidermis (Fig. 6). The flux ratio was close to 0, confirming that the shunt route dominates iontophoretic passage through the epidermis. It is also noteworthy that, under conditions of low constant voltage rather than constant current, few, if any, new shunts formed in the sandwich. This is not the situation with constant current conditions, where pores may develop in the stratum corneum (data not shown). A similar condition may apply with iontophoretic delivery of liposomes [59].

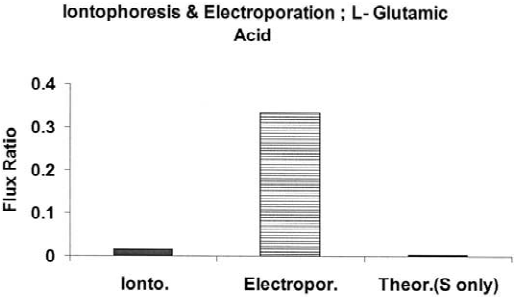

Fig. 6. Flux ratios for L-glutamic acid under iontophoresis and electroporation penetrating through epidermis and sandwich—experimental and theoretical (shunt only delivery but blocked in sandwich).

3.4.2. Electroporation

Skin electroporation ('electropermeabilization' [60]) applies micro- to millisecond electrical pulses of about 100–1000 V to create transient aqueous pores in the intercellular lipid bilayers. Such pores provide drug penetration routes that travel straight through the stratum corneum [61–66]. During the pulse, molecules move mainly because of iontophoresis and/or electroosmosis. Between pulses, drugs can also penetrate well by simple diffusion because of the (relatively) large changes in the horny layer resistance [66]; for drug examples, see Ref. [53]).

In a companion study to the iontophoresis regime with L-glutamic acid, we applied an electroporation cycle of two rounds of five pulses (each of 100 V and 100 ms width) at 1-min intervals at the start, and at 1 h into, a 2-h study. Fig. 6 shows that the flux ratio did *not* fall to zero. The significant flux ratio provides compelling evidence that the voltage pulses formed new shunts through the sandwich by the process of electropermeabilization.

4. Theoretical system validation: Monte Carlo approach

These examples of the use of the skin sandwich (composite) technique, together with others to be published, provide strong evidence for our basic assumption that the top stratum corneum essentially closes shunts in the underlying epidermis (Fig. 2). However, a question naturally arises—what are the consequences if in practice the two membranes of the composite do not adhere tightly together? There would then be the possibility of lateral drug transport between the layers of the sandwich, so that molecules could theoretically penetrate through the top pores of the sandwich and find an exit into the lower layers of the skin. We could, of course, attempt to stick the layers together, using a tissue adhesive. But this process would introduce further complications arising from the presence of a new layer and its (unknown) effects on permeation. Fortunately, a simple model analysis reveals that the complication of possible adherence failure is not a practical problem.

If for argument sake we allow for the laminates of the sandwich not sticking together, we can imagine molecules diffusing down the top shunts, then between the layers and finally exiting through the bottom shunts (Fig. 7).

If we consider flux relationships at steady state, for shunt penetration only (very hydrophilic or polar penetrant) and the shunts *not* blocked, we have the following:

For flux through the epidermal shunt, J_{es}, we write as before:

$$J_{es} \propto 1/(h_{sc} + h_{ne})$$

Flux through the sandwich shunts for nonadhering membranes, J_{ssn}, obeys:

$$J_{ssn} \propto 1/(2h_{sc} + h_{ne} + h_l)$$

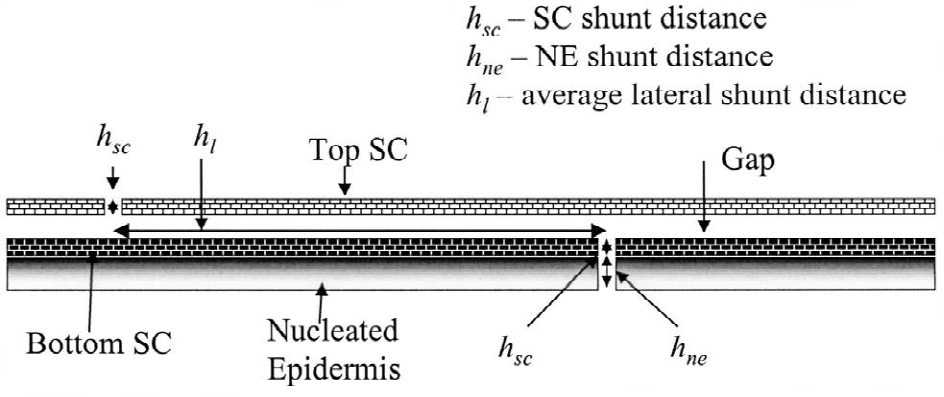

Fig. 7. Theoretical pathway for hydrophilic molecules diffusing down a top shunts, then between the layers and exiting through a bottom shunts (skin sandwich layers not adhering). See text for abbreviations and definitions.

Where h_l is the average lateral distance between a top shunt and the lower, epidermal shunts. The treatment assumes for simplicity that the transport properties of h_{sc}, h_{ne} and h_l are similar.

The flux ratio (sandwich:epidermis) is:

$$J_{ssn}/J_{es} = (h_{sc} + h_{ne})/(2h_{sc} + h_{ne} + h_l)$$

If h_l is very long compared with the other distances then:

$$J_{ssn}/J_{es} \rightarrow 0$$

Thus, to ensure that nonadherence of the membranes will not obviate the basic assumption of shunt route blockage, we need only show that h_l *is* very long compared with the thicknesses of the stratum corneum and nucleated epidermis. This approach is valid because of the inherent variability and accuracy of experimental data obtained from human skin.

Fig. 8 is a plan view illustrating diffusion down a top shunt in the upper stratum corneum and how molecules could reach possible open shunts in the bottom (epidermal) layer. Radial diffusion profiles show molecules reaching, or usually bypassing, bottom shunts as time increases. We used a Monte Carlo approach to assess average lateral shunt distances h_l for a variety of simulations using reasonable anatomical dimensions (data to be published). A typical simulation yielded:

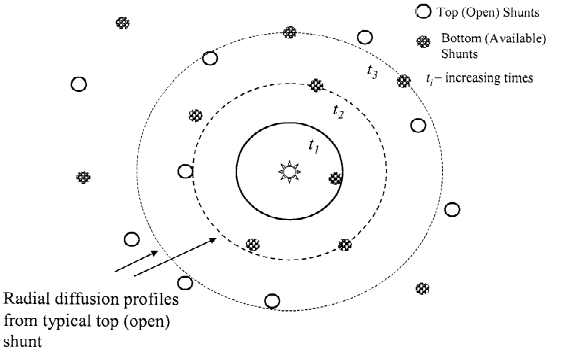

Fig. 8. A plan view illustrating diffusion down a top shunt in the upper stratum corneum and how molecules could reach possible open shunts in the bottom (epidermal) layer. Radial diffusion profiles show molecules reaching or bypassing bottom shunts as time increases.

$J_{ssn}/J_{es} = 0.015$ (theoretical value for blocked shunts in an adhering sandwich = 0.0)

Thus the simulation suggested a ratio within 1.5% of the assumed value at full shunt blockage. This is well within the accuracy of skin penetration experiments.

The situation is even more advantageous to our basic premise of shunt blockage when we realise that the simulations did not allow for the fact that most of the diffusant would miss a lower open shunt on the first pass, i.e. at steady state h_l would be even longer than the calculated value. Also, the simple model permitted unhindered diffusion between the skin layers, whereas in reality we would expect at least some points of adherence between the layers. These areas of obstruction would further increase the lateral diffusional path length by a tortuosity factor. Both features would decrease further the ratio J_{ssn}/J_{es}.

The relevant conclusion is that even if the adherence in the sandwich is not perfect in a particular experiment, the test results may safely be interpreted on the basis of ideal contact behaviour.

5. Overall conclusion

The epidermal sandwich technique is valuable for probing the relative importance of the various routes of delivery of chemicals and colloidal particles into and through skin. The method is useful for passive diffusion and electrical methods (iontophoresis and electroporation). It should apply equally well to ultrasound and magnetophoresis studies [53]. The approach is particularly valuable for shunt route analysis.

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