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International Journal of Pharmaceutics 193 (1999) 27–35

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

The back diffusion of glucose across human skin in vitro

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Received 19 April 1999; received in revised form 24 August 1999; accepted 2 September 1999

Abstract

For diabetic patients, blood glucose monitoring is an important part in the management of their disease, however the acquisition of blood requires the use of invasive and often painful methods, and the development of a technique that removes these problems would represent a major advance. The uppermost membrane of the skin, the stratum corneum, has been shown to be the main barrier to percutaneous absorption, but there have been claims that polar water-soluble compounds diffuse across it via aqueous pathways. In this study, skin diffusion cells were used to investigate the back diffusion of tritiated water and the convective transport of ^{3}H-glucose across full thickness human skin after the application of a number of different materials to the stratum corneum. Significant amounts of ^{3}H-glucose back diffused only after complete removal of the stratum corneum by tape stripping, and it is likely that any future attempts to monitor blood glucose levels using non-physical techniques will require a certain degree of damage to the stratum corneum. The extraction through the skin of tritiated water and ^{3}H-glucose after the application of solutions with different osmotic pressures were consistent with the theory that solutions with high osmotic pressures dehydrate the stratum corneum which suggests that passive transport of these radiolabelled molecules through porous pathways was insignificant.

Keywords: Diabetes; Back diffusion; Blood glucose monitoring; Routes of diffusion; Human skin

1. Introduction

Currently, patients with Type I diabetes routinely check their blood glucose levels using invasive and often painful methods. Normally, blood is obtained by pricking a finger and then glucose levels are assessed using meters or test strips. The nature of diabetes is such that regular puncturing of a finger to obtain blood for these tests may infrequently lead to sepsis or gangrene (Knezevic and Mastaglia, 1983; Ryan et al., 1983). The use of less invasive techniques would be beneficial to diabetics in avoiding the potential morbidity and pain associated with the current methods. Many diabetics consider the sampling of blood for assessment of glucose levels to be more painful than injecting insulin. One of the methods to minimise such pain is to assess glucose levels from the same

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0378-5173/99/$ - see front matter
PII: S0378-5173(99)00314-2

insertion point as the insulin injection (Suzuki et al., 1993).

Noninvasive methods for measuring glucose have been attempted by a number of workers with variable success rates. Morenkova and Gussev (1995) stimulated the production of sweat using pilocarpine in both healthy and diabetic subjects, and showed that levels in sweat were similar to blood levels, however our own unpublished work suggests that bacterial breakdown of glucose collected through sweating can lead to anomalous results. The measurement of glucose in urine, urinalysis, has also been used as an indication of blood levels, but due to its storage in the bladder, there is a often a lag period between the levels excreted in the urine and those found in the blood. Similarly, glucose levels in other biological fluids, such as saliva, lag behind blood levels (Ginsberg, 1992), and in order to obtain a more immediate measure through non-invasive techniques, investigators have attempted to extract fluid through skin. The determination of glucose levels in this skin derived fluid can be used to provide a more immediate prediction of blood levels (subcutaneous levels lag behind blood levels by only 5–15 min; Ginsberg, 1992). However, the main drawback of this technique is the impermeability of the stratum corneum to glucose. Ito et al. (1994) attempted to overcome this problem by tape stripping the stratum corneum and applying a vacuum to suck fluid through the skin into a reservoir where it was analysed for glucose using a sensitive biosensor. The system was first tested on rabbits followed by human males and under the experimental conditions used showed very short lag times between glucose levels in the blood and those in the effusion fluid (5–10 min). Jensen et al. (1995) raised suction blisters in Type I diabetic patients and showed that the glucose levels in the blisters correlated closely with levels in the venous circulation ($R = 0.998$).

Reverse iontophoresis has also been used to facilitate glucose transport across human skin using in vitro diffusion cells (Rao et al., 1993). Glucose dissolved in aqueous solutions will be transported with the convective solvent flow (electroosmosis) through the skin during iontophoresis. Glucose concentrations at the anode, as assessed using 5 mM solutions of glucose spiked with radiolabelled material on the dermal side, were approximately ten fold that at the cathode after iontophoresis for 2 h. It was suggested that metabolism of glucose occurred during its passage through the skin leading to the accumulation of negatively charged metabolites at the anode. The amount of glucose that accumulated at the cathode correlated with the concentrations of glucose in the sub-dermal phase. Passive diffusion of glucose was reported to be negligible. The use of such a technique in a practical situation was limited by the time required for iontophoresis, but in an in vivo study conducted by the same authors (Rao et al., 1995), the period of time required for iontophoresis was reduced to less than an hour. Limitations of the technique were reported to include contamination by concentrations of glucose resident in the skin at early collection times and a large inter-subject variation. Futhermore, the long term effects of short term exposures of electric currents have not been fully resolved (Ledger, 1992).

In this study, we examined the potential of facilitating passive glucose transport across excised human skin, with particular attention to the effects of changing osmotic pressure. We quantified the passive flow of tritiated water and ^{3}H-glucose across human skin from a subdermal phase by applying to the stratum corneum different preparations traditionally used to enhance the penetration of pharmaceuticals in the opposite direction.

In addition, we attempted to deduce the likely route of transport of glucose through the stratum corneum. The main route of diffusion of permeants across this biological membrane is thought primarily to occur around the cells and through the intercellular lipids (Albery and Hadgraft, 1979; Boddé et al., 1989) but there have been claims that pores may be involved in the diffusion of polar water soluble compounds (Sznitowska et al., 1995). Essentially, there are two basic mechanisms by which drug permeation through the stratum corneum may occur, namely partitioning, or porous transport. Guy and Hadgraft (1988) doubted the existence of porous pathways by showing that permeability coefficients for a range

of compounds could be accounted for by correlation with their ether: water partition coefficients and molecular weights. Siddiqui et al. (1989) used the high correlation in the transport of a range of solutes through a silicone membrane (which lacks pores) and human skin to deduce that pore transport for steroids was minimal relative to transepidermal penetration.

The choice of a suitable subdermal phase for maintaining skin viability during in vitro experiments is an important consideration for glucose in that it may undergo metabolism in the skin. Indeed, glucose utilisation has been used as a measure of tissue viability (Collier et al., 1989) in the assessment of the effect of different receptor media on the cutaneous metabolism of glucose in fuzzy rat skin over a 24 h period. Tissue viability was maintained in receptor media consisting of minimum essential medium, Dulbecco modified phosphate buffered saline (DMPBS) or HEPES buffered Hanks' balanced salt solution. In a phosphate buffered saline with 0.1% glucose media, glucose utilisation decreased after 3 h. In the present study, DMPBS containing 22.2 mM of glucose was chosen as a subdermal phase, and was maintained as an iso-osmotic solution by altering the concentration of sodium chloride.

2. Materials and methods

2.1. Materials

^{3}H-glucose, 1 mCi/ml, was purchased from Dupont (NEN) Australia Ltd, Sydney; tritiated water, 100 mCi/ml, from Amersham Australia; Magnaplasm from Faulding Pharmaceuticals, Salisbury, South Australia; eucalytpus oil from Seldon Drug Co. Pty Ltd., Sydney; dimethyl sulphoxide (DMSO) from Sigma Chemical Co., Sydney; Span 85 from Sigma Chemical Co, St. Louis, USA; Aquacare (10% urea cream) from Allergen Australia Pty Ltd., Sydney. 3M Blenderm tape was used for tape stripping. The scintillation cocktails, Optima Gold and Ultima Gold, were purchased from Packard, Canberra, Australia. Dulbecco's modified phosphate buffered saline pH 7.4 was used as the subdermal medium, and distilled water was used throughout this study. All chemicals were of at least reagent grade and used as supplied unless indicated. Human skin was obtained from abdomenoplasty after cosmetic surgery at a local hospital and stored frozen until required. Silicone membranes of 300 μm thickness were purchased from Samco, St. Albans, Herts, UK.

2.2. Preparation of diffusion cells

Skin samples were thawed overnight, and the subcutaneous fat was removed by blunt dissection. Diffusion cells providing a diffusional surface area of approximately 1 cm^2 and receptor volumes of about 3 ml were used. In the first experiment, side-by-side diffusion cells were used. In the remaining experiments, Franz-type diffusion cells were used. Iso-osmotic Dulbecco modified phosphate buffered saline (DMPBS), adjusted to pH 7.4 with sodium hydroxide, was used as the subdermal phase for both types of diffusion cell. It contained 22.2 mM glucose which was spiked with either ^{3}H-glucose (0.4 or 4 μCi/ml) or tritiated water (1 μCi/ml) and was vacuum filtered through a 0.45 μm filter membrane prior to use. In the upright diffusion cells, rubber O-rings (1.2 cm diameter) were placed on the surface of the skin to provide a lip over which a silicone membrane could be placed providing a volume of about 0.14cm^3 for the test materials. The silicone membranes had a dual role in that they prevented evaporation of the volatile components of the test materials, and minimised uptake of atmospheric water by hygroscopic materials. The diffusion cells were allowed to equilibrate in a water bath at 37°C for 1 h prior to starting an experiment.

2.3. Experimental

2.3.1. Side-by-side diffusion cells

A DMPBS subdermal solution containing 22.2 mM of glucose with 0.4 μCi/ml of ^{3}H-glucose was used. Either an iso-osmotic DMPBS solution, or a DMPBS solution with a 20-fold higher osmotic pressure than the iso-osmotic solution was applied to the epidermal side of the skin. Glucose was not present in either of these solutions. The entire

contents of the chamber adjacent to the stratum corneum was removed after a 48 h period and analysed for ^{3}H-glucose.

2.3.2. Franz-type diffusion cells

Subdermal phases containing either 4 μCi/ml of ^{3}H-glucose or 1 μCi/ml of tritiated water were used. After an appropriate period of time, the material adjacent to the stratum corneum was removed by successive washings with iso-osmotic DMPBS, and the silicone membranes and O-rings were also washed. All washings and test materials were added together for each cell and analysed for ^{3}H by scintillation counting. The following test materials were applied to the stratum corneum:

(a) 140 μl of iso-osmotic DMPBS (without glucose)

(b) 140 μl of a DMPBS solution (without glucose) with a 20-fold increase in the osmotic pressure

(c) 140 μl of water

(d) 100 mg of Magnaplasm

(e) 140 μl of a 10% aqueous solution of DMSO

(f) 140 μl of an aqueous dispersion of 1% Span 85

(g) 100 mg of Aquacare (10% urea cream)

(h) Aquacare massaged into skin (12.5 mg/cm^2) and allowed to equilibrate for 1 h prior to application of (i) 140 μl of water or, (ii) a DMPBS solution with a 20-fold increase in the osmotic pressure

(i) pre-treatment for 1 h with 70 μl of a 2% ethanolic solution of eucalyptus oil and then application of 140 μl of water

(j) removal of the stratum corneum with 15 strips of Blenderm tape prior to mounting in diffusion cells, and then application of 140 μl of water or a DMPBS solution with a 20-fold increase in the osmotic pressure.

2.4. Analysis

The scintillation cocktail, Ultima Gold, was mixed with the aqueous samples, which were subsequently analysed for ^{3}H on a 1600TR Liquid Scintillation Analyser, Packard, Canberra, Australia.

3. Results and discussion

Osmosis is the movement of solvents such as water from hypotonic solutions to hypertonic solutions through a semi-permeable membrane. In an aqueous system, an increase in the osmotic potential across a membrane would result in water flowing towards the hypertonic solution. Ultimately, if the system is left to equilibrate, the osmotic pressure on both sides of the membrane would be identical and there would be no net flow of water. In a physiological environment, iso-osmotic solutions play an important role in homeostasis, and consequently many pharmaceutical preparations are often maintained iso-osmotic with physiological fluids using saline solutions (0.9%). Other constituents of a formulation may contribute to the osmotic pressure, and different amounts of sodium chloride are generally required to maintain iso-osmolarity. Four methods can be used to calculate the amount of sodium chloride required (The Pharmaceutical Handbook, 1980): sodium chloride equivalents, depression of freezing point, amount of substance concentration, and serum osmolarity. Calculations based on sodium chloride equivalents were used in this study to adjust solutions so that they were iso-osmotic with an aqueous solution of 0.9% sodium chloride.

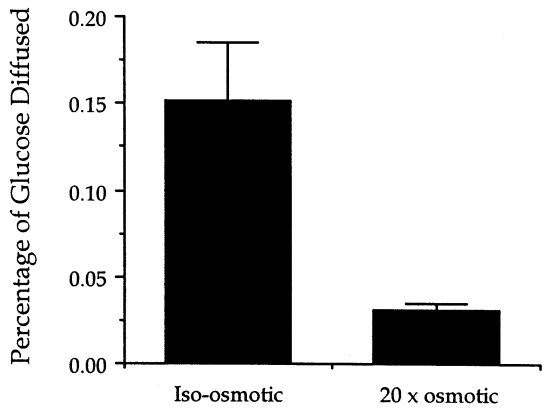

Fig. 1. Total amount of ^{3}H-glucose that has back diffused across full thickness human skin after 48 h (mean values plotted ± standard error, $n = 6$).

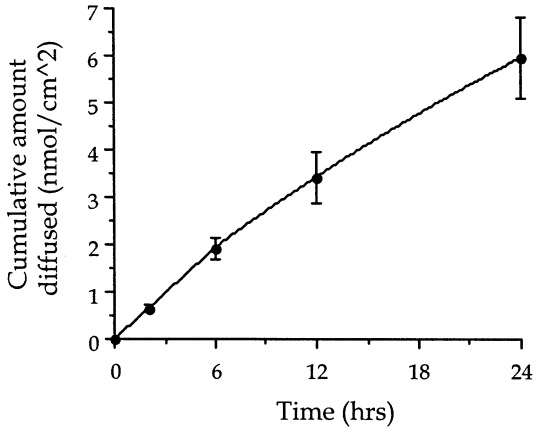

Fig. 2. Amount of glucose that back diffused across human skin when an iso-osmotic solution is placed on the surface of the stratum corneum (mean values plotted ± standard error, $n = 4$).

Fig. 1 shows, unexpectedly, no increase in glucose diffusion with increasing osmotic pressure when using the side-by-side diffusion cells. Indeed, the glucose transport from the subdermal compartment for 20-fold osmotic pressure solution was one fifth that for the iso-osmotic solution. One explanation for this result is that the solution of increased osmotic pressure extracted water from the stratum corneum faster than it could be replaced by the aqueous subdermal phase. The overall effect of this imbalance in water transport is dehydration of the stratum corneum.

Fig. 2 shows the cumulative amount of glucose back transported through full thickness excised human skin, with time, using conventional upright diffusion cells. The subdermal phase contained a ten-fold increase in the activity of glucose (4 μCi/ml) with an iso-osmotic solution on the stratum corneum side of the skin. Under these conditions, glucose levels on the stratum corneum side could be detected after 2 h. No attempt was made to ascertain the degree of glucose metabolism that occurred in the skin during these experiments. Although glucose metabolites may have accounted for much of the ^{3}H glucose counted, it was deemed that the amounts of glucose transported did not warrant investigation of the significance of glucose metabolism. Furthermore, it was recognised that, whilst glucose metabolism may be significant in the low concentrations studied, the process would probably be saturated when physiologically more realistic amounts of glucose (Rao et al., 1993) were permeating. The extent of this metabolic process may be further hindered through using skin that has been previously frozen, and any future attempts to quantitate this effect would require the use of fresh, unfrozen human tissue.

Fig. 2 also shows that the cumulative amount of diffused glucose increased with time and that after 24 h, less than 0.01% of the subdermal glucose concentration diffused. The lag time also appears to be negligible suggesting that, provided sufficient quantities of glucose diffuse across the skin, the penetrating flux of glucose may be a predictor of glucose blood levels.

Fig. 3 compares the percentages of the subdermal glucose concentrations that have back diffused after applying other enhancers to the stratum corneum for 2 h with the results from the osmotic studies. It was thought that the application to the stratum corneum of Magnaplasm, a commercial formulation containing magnesium sulphate and glycerol, both of which are known humectants (Barry, 1983), could result in the increased transport of water and subsequent co-

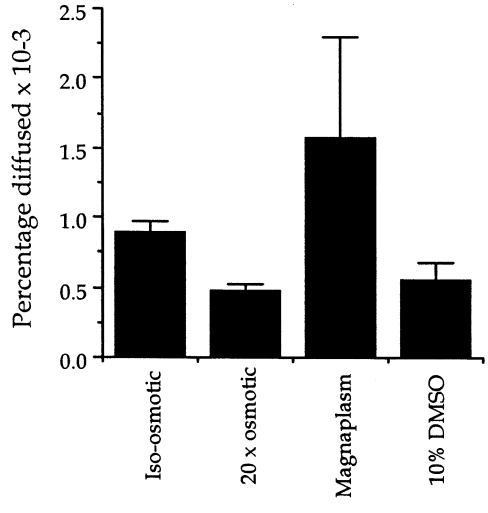

Fig. 3. Back diffusion of ^{3}H-glucose across human skin after 2 h (mean values plotted ± standard error, $n = 3$).

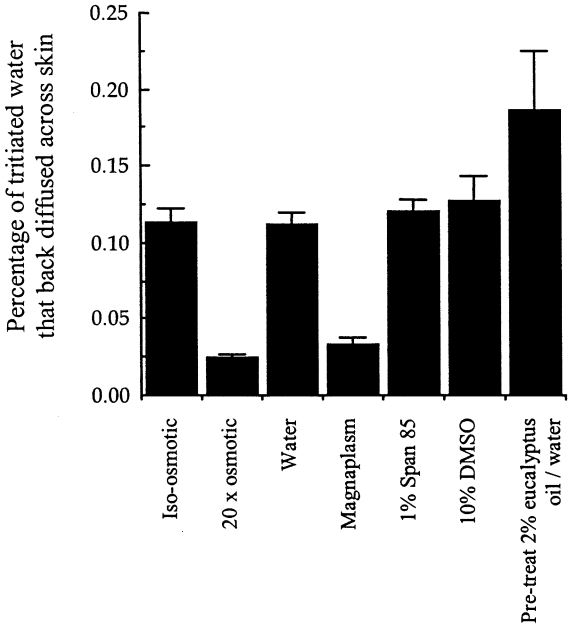

Fig. 4. Back diffusion of tritiated water across human skin after 2 h using different treatments (mean values plotted ± standard error, $n = 3$).

transport of glucose across skin. The enhancer, DMSO, in a 10% aqueous solution was also used in the comparison. It appears that there are no statistical differences in the back diffusion of glucose with the materials used (iso-osmotic versus Magnaplasm: $P = 0.37$; iso-osmotic versus 10% DMSO: $P = 0.15$).

As a result of the apparent dehydration effect by a hypertonic solution, an attempt was made to increase glucose transport using a hypotonic solution consisting only of water. This was tested by examining the back diffusion of ^{3}H-water (see below). Fig. 4 shows the results obtained for the formulations shown in Fig. 3 under these conditions. It also shows the results obtained with a range of other known penetration enhancers and pre-treatment methods. The effect of a 1% aqueous dispersion of Span 85 was studied because, in a study on a range of non-ionic surfactants on the absorption of salicylic acid and sodium salicylate in rabbits, it was demonstrated that Span 85 (sorbitan trioleate) significantly enhanced the permeation of salicylic acid (Shen et al., 1976). Eucalyptus oil, of which the main constituent is 1,8-cineole, was used as a 2% ethanolic solution as it is used as a penetration enhancer. A 70 μl volume of this solution was applied to the stratum corneum for a 1 h period before adding 140 μl of water. Williams and Barry (1991), in a study investigating the effects of a series of terpenes on the enhancement of oestradiol, a model lipophilic compound, and 5-fluorouracil, a model hydrophilic compound, showed enhancement ratios of up to 94 with 1,8-cineole for 5-fluorouracil after pretreatment of the stratum corneum. The data in Fig. 4 show that hypertonic solutions and Magnaplasm result in a lower diffusion than the other treatments, and none of the other treatments caused any greater diffusion of tritiated water than the iso-osmotic solution.

Water molecules in the stratum corneum are thought to associate with the polar head groups of intercellular lipids. Data exhibiting reductions in the penetration of water soluble polar compounds, such as glucose, and indeed tritiated water, can be explained by the removal of these resident water molecules, simply as a result of dehydration of the membrane. If aqueous pores were present, then the back diffusion of tritiated water across skin would be expected to increase when a hypertonic solution was applied to the stratum corneum which is contrary to the data obtained in this study. Furthermore, direct visual evidence of pores in the stratum corneum has yet to be observed. However, there are reports of pores after the application of an electric current (Cullander and Guy, 1991). Therefore, the stratum corneum does not act as a simple semi-permeable membrane, which reinforces the theory that partitioning of compounds into the intercellular lipids provides the main route for percutaneous diffusion.

Fig. 5 shows the ratio of water: glucose molecules that diffused across the skin after four different treatments. If aqueous pores were present in the stratum corneum, then it is likely that the ratios for these treatments would be the same, because the different treatments would be expected to have the same effect on the back diffusion of water and the highly soluble glucose molecules. Since the ratios are different, it suggests that there are no pores in the membrane.

Claims that urea moisturises the skin and its use in the management of ichthyosis as a mild keratolytic (Barry, 1983) lead to its use in this study to increase the water content of the stratum corneum and the back diffusion of tritiated water. A series of different pre-treatment procedures and applications were assessed (Fig. 6). There appears to be little difference between the different urea applications and the back diffusion of ^{3}H-water. Reasons for the difference between the test solution with high osmotic pressure, and the combination of the high osmotic pressure and Aquacare pre-treatment are unclear. There may have been excipients in the commercial product which were not declared on the labelling which resulted in this effect, but were not seen between equivalent treatments with water.

When the stratum corneum has been removed by tape stripping, the back diffusion of water increased more than ten-fold, whereas the amount of glucose that back diffused increased by over 400-fold (see Table 1). This demonstrates that the stratum corneum is the main barrier to the back diffusion of glucose. Furthermore, any future attempts to monitor blood glucose levels using non-physical techniques such as those used in this study will probably require removal of the stratum corneum barrier.

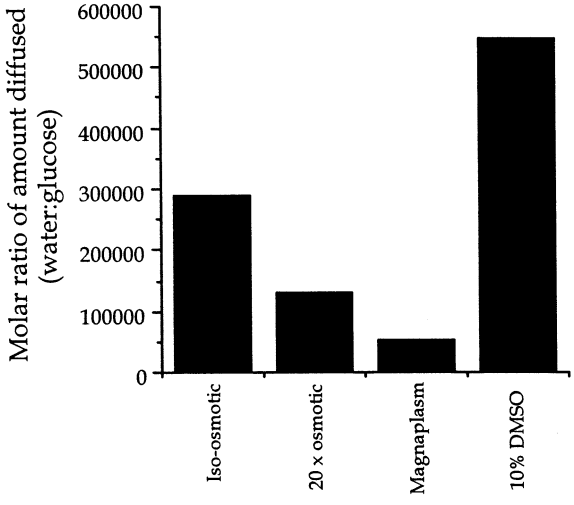

Fig. 5. Ratio of amount of tritiated water: ^{3}H-glucose that back diffused across human skin using four different treatments (and data presented in Figs. 3 and 4).

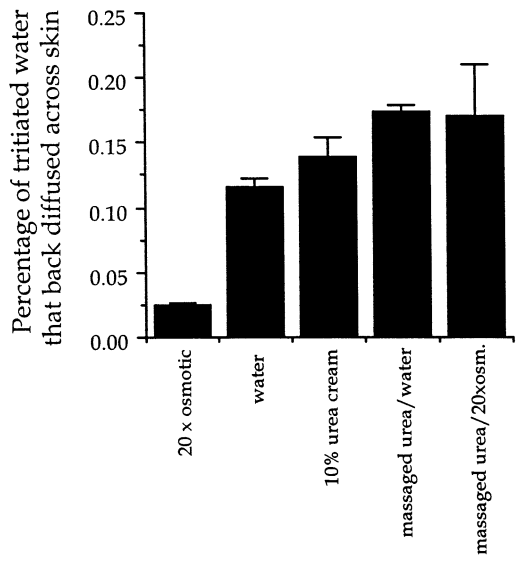

Fig. 6. Back diffusion of tritiated water across human skin using solutions of different osmotic potential after the application of 10% urea cream (mean values plotted $\pm$ standard error, $n = 3$).

4. Conclusions

This study has attempted to use osmotic pressure as a means of facilitating transport of hydrophilic solutes such as glucose through skin. We are not aware of other studies attempting this approach. Previous studies have used chemical, electrical, ultrasound, physical damage (e.g. laser ablation) and invasive techniques, e.g. high pressure 'injections'. Glucose is a polar, water soluble compound, and the application of different materials to the stratum corneum failed to increase its back diffusion across human skin in vitro. Furthermore, the use of solutions with high osmotic pressures decreased diffusion compared with iso-osmotic solutions which is consistent with the theory that the stratum corneum became dehydrated by the hypertonic solutions. These observations were reinforced by monitoring the back diffusion of tritiated water which also suggested that the stratum corneum does not act as a semi-permeable membrane and the contribution of passive transport to the back diffusion of glucose or tritiated water through porous pathways was insignificant. Comparatively high concentrations of

Table 1
Effects of tape stripping on the back diffusion of tritiated water and ^{3}H-glucose after application of water (except *, where the values quoted are those obtained with an iso-osmotic solution) to the stripped surface of the skin for 2 h

	^{3}H-glucose		^{3}H-water	
	Stripped	*Not stripped	Stripped	Not stripped
Total amount back diffused (μmol)	0.269 ± 0.007	$0.653 \pm 0.059 \times 10^{-3}$	2739 ± 181	212 ± 13
Percentage of subdermal concentration diffused	0.37 ± 0.01	$0.89 \pm 0.08 \times 10^{-3}$	1.48 ± 0.10	0.12 ± 0.01

glucose back diffused only after the removal of the stratum corneum, and consequently, it is likely that any future attempts to back diffuse this non-electrolyte across skin using passive techniques will involve the complete removal of the stratum corneum by suction blisters or tape stripping.

Acknowledgements

The authors would like to thank the Queensland Cancer Fund, the Lions Kidney and Medical Research Foundation of Northern NSW, the National Health and Medical Research Council of Australia, SmithKline Beecham at Weybridge, UK and the EPSRC of UK for providing financial support for this project.

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