Transepidermal Water Loss Does Not Correlate with Skin Barrier Function *In Vitro*

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The purpose of this study was to investigate the relationship between transepidermal water loss and skin permeability to tritiated water (^3H_2O) and the lipophilic penetrant sulfur mustard *in vitro*. No correlation was found between basal transepidermal water loss rates and the permeability of human epidermal membranes to ^3H_2O ($p = 0.72$) or sulfur mustard ($p = 0.74$). Similarly, there was no correlation between transepidermal water loss rates and the ^3H_2O permeability of full-thickness pig skin ($p = 0.68$). There was no correlation between transepidermal water loss rate and ^3H_2O permeability following up to 15 tape strips ($p = 0.64$) or up to four needle-stick punctures ($p = 0.13$). These data indicate that transepidermal water loss cannot be unconditionally ascribed to be a measure of skin barrier function. It is clear that further work should be conducted to interpret the significance of measuring transepidermal water loss by evaporimetry. ***Key words: skin punctures/sulfur mustard/tape stripping/tritiated water permeability. J Invest Dermatol 118:871–875, 2002***

Transepidermal water loss (TEWL) is the normal, constitutive loss of water vapour from the skin in the absence of sweat gland activity (Pinson, 1942). The development of robust instrumentation for measuring TEWL rates has provided an invaluable tool for the noninvasive evaluation of skin function in diseased and damaged skin (Lamke *et al*, 1977; Nilsson, 1977; Idson, 1978). As such, TEWL has become a ubiquitous parameter of *in vivo* skin studies.

Transepidermal water loss is commonly ascribed to be a measure of skin barrier function, either at baseline (Lotte *et al*, 1987; Nangia *et al*, 1999) or following topical treatments (Freeman and Maibach, 1988; Nicander *et al*, 1996). More precisely, TEWL is thought to represent the water barrier function of the skin (Pinnagoda and Tupker, 1995). Previous studies have demonstrated that exposure of the skin to certain chemical, physical, or radiologic insults will lead to perturbations in skin barrier function, as indicated by an elevation in TEWL rates (Lamaud and Schalla, 1984; Van der Valk and Maibach, 1990; Lévêque *et al*, 1993).

The purpose of this study was to investigate the relationship between TEWL and skin permeability *in vitro* to validate the assumption that TEWL is an appropriate measure of skin barrier function. Excised skin is free of many factors that may potentially affect TEWL and/or skin absorption rates, such as variations in blood perfusion (Benowitz *et al*, 1992), temperature fluctuations (Blank and Scheuplein, 1969), sweat gland activity (Pinnagoda *et al*, 1990), metabolism (Kao *et al*, 1984), and other diurnal variations (Yosipovitch *et al*, 1998; Chilcott and Farrar, 2000). In addition, the permeability of excised skin can be measured directly using validated, standard techniques (Howes *et al*, 1996). Thus, an *in vitro* investigation of the relationship between TEWL and skin permeability may arguably be considered superior to *in vivo* studies. In this study, sulfur mustard (bis-[2-chloroethyl]sulfide, SM) was used as a model lipophilic penetrant to complement the use of tritiated water.

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MATERIALS AND METHODS

Chemicals Dulbecco's phosphate-buffered saline (DPBS), gentamycin, sodium lauryl sulfate (> 99.9%), and ethanol (100%) were purchased from the Sigma-Aldrich (Poole, U.K.). Liquid scintillation counting fluid (Emulsifier-safe) and Soluene-350 were purchased from Canberra-Packard (Michigan). Tritiated water (^3H_2O) was purchased from NEN Life Science Products (Hounslow, U.K.). Radiolabeled SM (^{35}SM) was synthesized at CBD Porton Down (purity > 98%). Tape stripping was performed with 14 mm diameter D-Squame stripping discs (CuDerm, Texas). The majority of experiments were conducted in an environmentally controlled chamber (40% ± 2% relative humidity, 20°C ± 0.5°C). Skin absorption of ^{35}SM was measured in a normal laboratory fume cupboard (35%–55% relative humidity, 20°C ± 3°C).

Skin Full-thickness pig-back skin was freshly obtained from large-white pigs (Sus scrofa domestica) bred at CBD Porton Down (six animals total, weight range 30–40 kg). Subcutaneous fat was dissected from each piece of skin and the (epidermal) surface was carefully clipped to remove excess hair prior to storage at –25°C for a maximum of 14 d. Human epidermal membranes were prepared and stored as previously described (Chilcott *et al*, 2000) by heat separation of full-thickness breast skin (Kligman and Christophers, 1963).

Diffusion cells Full-thickness pig skin or human epidermal membranes were placed into Franz-type (Franz, 1975) glass diffusion cells with an available skin surface area of 2.54 cm^2 and 5 ml receptor chamber containing DPBS (containing 86.6 μg per ml gentamycin) or 50% aqueous ethanol (depending on the skin penetrant being measured). Groups of six diffusion cells were mounted on purpose-built aluminum heating blocks (CBD Porton Down) heated to 35°C to attain a skin surface temperature of 31°C ± 1.5°C. Each heating block was placed on a six-place magnetic stirrer bed (Whatman, Kent, U.K.) that stirred the receptor fluid of each diffusion cell via a magnetic follower. All diffusion cells were left to equilibrate in the environmental chamber for at least 24 h prior to measurements of TEWL or skin permeability.

Measurement of TEWL Transepidermal water loss was measured using a calibrated, ServoMed EP-3 Evaporimeter (ServoMed, Varberg, Sweden) in accordance with current guidelines (Pinnagoda *et al*, 1990). A 1.5 cm stainless steel collar was attached to the TEWL probe in order to ensure contact with the pig skin or human epidermal membranes in each diffusion cell. The probe collar was placed on the skin surface for 60 s prior to an average rate being taken over a 15 s measurement period. The probe was immediately serviced after experiments using 50% ethanol water receptor chamber fluid, as ethanol has been reported to have adverse effects on the TEWL probe (Abrams *et al*, 1993).

Measurement of skin permeability Skin water barrier function was measured directly using ^3H_2O (1 ml, 10 μCi per ml) placed into the donor chamber of cells containing pig skin or human epidermal membranes. Samples (20 μl) of receptor chamber fluid were removed at 0, 1, 2, 4, and 6 h intervals, placed into 5 ml liquid scintillation counting fluid and analyzed using a Wallac 1409DSA liquid scintillation counter. The amount of radioactivity in each sample was related to the amount of ^3H_2O by reference to standards prepared and measured simultaneously. Skin absorption rates were calculated by linear regression analysis of the amount of ^3H_2O penetrated against time between 2 and 6 h (steady state, where the amount penetrating per unit time was constant). Skin absorption of ^{35}SM through human epidermal membranes was measured under unoccluded conditions as previously described (Chilcott *et al*, 2000).

Tape-stripping damage Stripping discs were applied to pig skin for 10 s using a purpose-built applicator that applied a constant pressure of approximately 0.5 N per m^2 (Emanuel, 1999). The number of tape strips ranged from 1 to 20. For comparison, epidermis-free "full-thickness" skin was obtained by heat separation.

Skin puncture damage Skin in each diffusion cell was punctured (up to four times) using a lancet needle delivered from an auto-injector (Monojector Lancet Device) set to a maximum penetration depth of 2 mm. A new needle was used for each puncture.

Skin damage protocol Full-thickness pig skin was used in all skin damage experiments. Diffusion cells were assembled within the environmentally controlled chamber on day 1. On day 2, baseline (–24 h) TEWL and ^3H_2O measurements were taken. Skin damage (tape stripping or punctures) were conducted on the third day and were accompanied, where appropriate, with TEWL measurements. Day 4 comprised a 24 h postdamage measure of TEWL and ^3H_2O permeability, which was followed on subsequent days (relating to 48, 72, and 96 h postdamage) with TEWL measurements only.

Human epidermal membrane study Diffusion cells containing heat-separated human epidermal membranes were assembled in the environmentally controlled chamber on day 1. On day 2, baseline measurements of TEWL were taken prior to measurement of ^3H_2O skin permeability (conducted *in situ*) or ^{35}SM (conducted within a fume cupboard in a laboratory that was not equipped with a rigorous environmental control system).

Statistical analysis Comparison of ^3H_2O flux predamage and postdamage was analyzed using a paired, nonparametric test (Wilcoxon signed rank) subject to adequate pairing as indicated by the Spearman correlation coefficient. Ineffectively paired data were analyzed using the Mann–Whitney test. Rates of TEWL were analyzed using a multivariate analysis of variation followed by Dunnet's adjustment for multiple comparisons post-test (one-sided). Correlation coefficient (r^2) values were used to evaluate the linear regression analysis of skin absorption rates. For $r^2 < 0.90$, the data were deemed to be nonlinear (i.e., steady-state conditions were not attained).

RESULTS

Penetration of ^3H_2O through human epidermal membranes A total of 48 epidermal membranes were placed into diffusion cells and subjected to ^3H_2O permeability measurements. Following linear regression analysis (of the amount of ^3H_2O penetrated against time), six of these membranes were rejected on the basis that the correlation coefficient (r^2) was less than 0.90. A further two were subsequently rejected, as nearly all (>93%) the applied dose had penetrated within 1 h, and whilst the linear correlation coefficients were high ($r^2 > 0.94$) the apparently low calculated fluxes (measured between 2 and 6 h) were meaningless. The remaining flux values were plotted against initial TEWL rates (**Fig 1**), which indicated that there was no correlation between TEWL rates and ^3H_2O permeability ($r^2 = 0.26$). Similarly, a plot of the total amount of ^3H_2O penetrated (at 3 h) against TEWL rates for all 48 epidermal membranes did not indicate any correlation (data not shown).

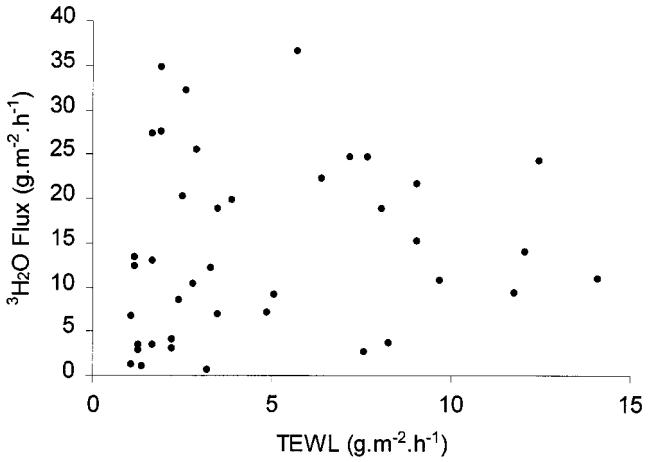

Figure 1. Human skin permeability to ^3H_2O as a function of TEWL. Skin absorption rates of ^3H_2O through heat-separated human epidermal membranes plotted as a function of TEWL rate measured under controlled environmental conditions (40% relative humidity, 20°C). Data collated from n = 40 diffusion cells containing skin derived from seven individuals.

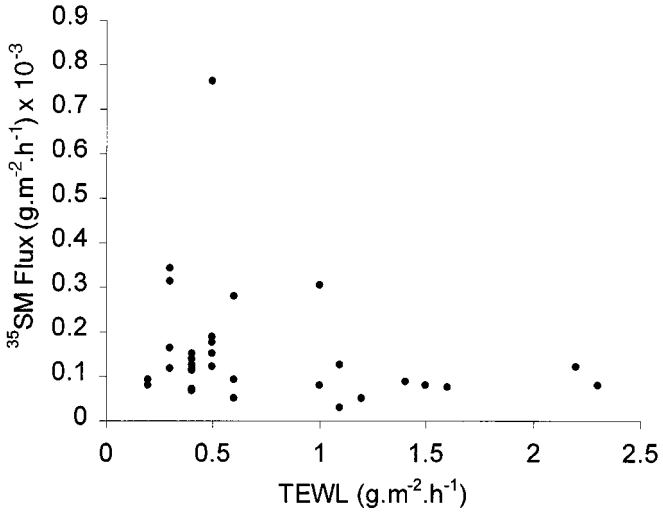

Figure 2. Human skin permeability to ^{35}SM as a function of TEWL. Skin absorption rates of ^{35}SM through heat-separated human epidermal membranes plotted as a function of TEWL rate measured under controlled environmental conditions (40% relative humidity, 20°C). Data collated from $n = 32$ diffusion cells containing skin derived from six individuals.

Penetration of ^{35}SM through human epidermal membranes Skin absorption rates of ^{35}SM were subject to the same criteria as above and resulted in 15 membranes being rejected from 48 on the basis of allowing a high percentage of applied dose to penetrate (> 70%), with one additional membrane being rejected on the basis of a poor linear correlation coefficient ($r^2 < 0.90$). A plot of ^{35}SM skin absorption rates against TEWL ($n = 32$) indicated no apparent correlation ($r^2 = 0.24$; **Fig 2**) as did a plot of

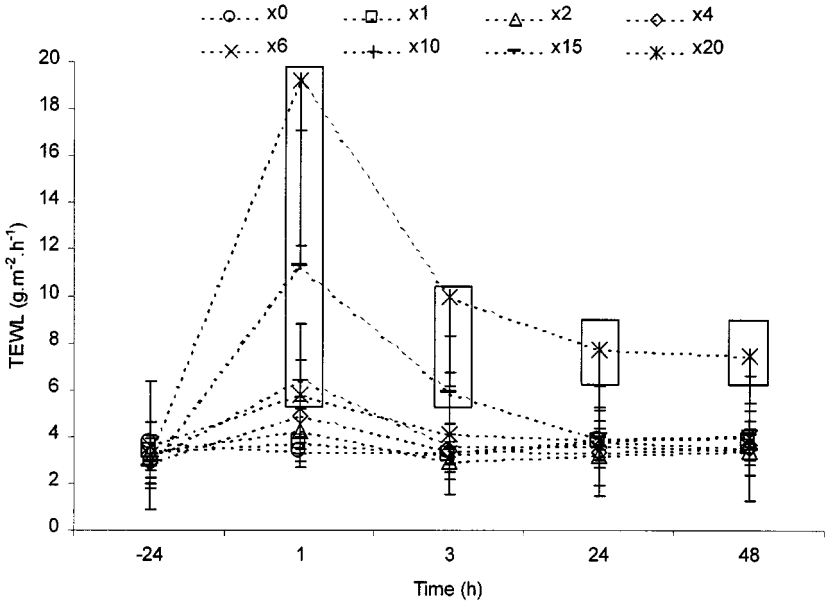

Figure 3. Effect of tape stripping on pig skin TEWL. Variation in TEWL rates measured from full-thickness pig-back skin before (–24 h) and up to 48 h after tape stripping (1× to 20×) conducted under controlled environmental conditions (40% relative humidity, 20°C). All values are mean ± SD of $n = 6$ diffusion cells containing skin from two animals. Boxed values are significantly different ($p < 0.05$) from control (0×) at 1 h (4×, 6×, 10×, 15×, and 20×), 3 h (15× and 20×), 24 h (20×), and 48 h (20×).

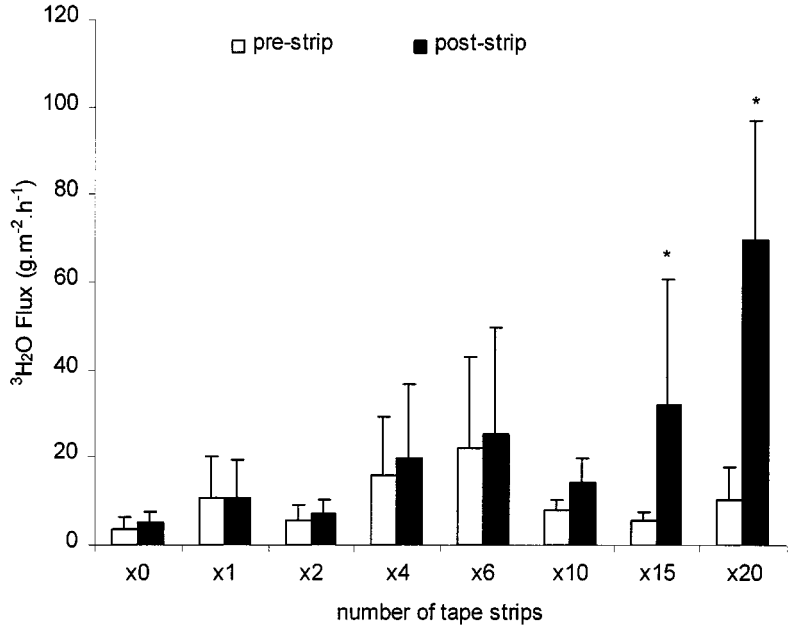

Figure 4. Effect of tape stripping on permeability of pig skin ^3H_2O. Comparison of ^3H_2O fluxes through full-thickness pig-back skin 24 h before (pre) and 24 h following (post) tape stripping (1× to 20×; control, 0×). All values are mean ± SD of $n = 6$ diffusion cells containing skin from two animals. Asterisks indicate significant ($p < 0.05$) differences between pre- and post-exposure fluxes.

percentage of applied dose penetrated at 3 h against TEWL for all epidermal membranes (data not shown).

Effects of tape-stripping on full-thickness pig skin Changes in TEWL rates were dependent on exceeding a certain number of tape strips (**Fig 3**). Up to six strips had no significant effect. After 10 strips, there was a small (1.9-fold) but significant ($p < 0.05$) increase in TEWL 1 h after stripping. Following 15 tape strips, there was a significant (3.4-fold) increase in TEWL for up to 3 h, whereas 20 strips caused a significant (2–6-fold) elevation in TEWL for the duration of the experiment. Skin permeability to ^3H_2O 24 h post-stripping did not significantly alter until 15 or 20 tape strips, which resulted in a 6- or 7-fold increase in ^3H_2O permeability, respectively (**Fig 4**), relating to skin absorption rates of 3.18 ± 2.89 and 6.98 ± 2.73 g per m^2 per h, respectively, in comparison with controls (0.36 ± 0.25 g per m^2 per h). The skin absorption rate of ^3H_2O through epidermis-free (heat-separated) skin was 13.43 ± 5.25 g per m^2 per h.

There was no significant correlation between TEWL rates and ^3H_2O permeability following tape stripping.

Effects of single or multiple skin punctures on full-thickness pig skin There was a significant ($p < 0.05$) increase in TEWL rates 1 h after puncturing the skin, the magnitude of which was proportional to the number of punctures (**Fig 5**). After 24 h, TEWL rates were not significantly different from controls (nonpunctured). Two or more punctures caused a significant increase in ^3H_2O permeability at 24 h (**Fig 6**). There was no significant correlation between the number of punctures and ^3H_2O permeability ($p = 0.13$).

DISCUSSION

It is logical to assume that changes in TEWL represent alterations to the skin's "barrier function" (permeability), as it is consistent with the fact that the stratum corneum is the main barrier to the egress of

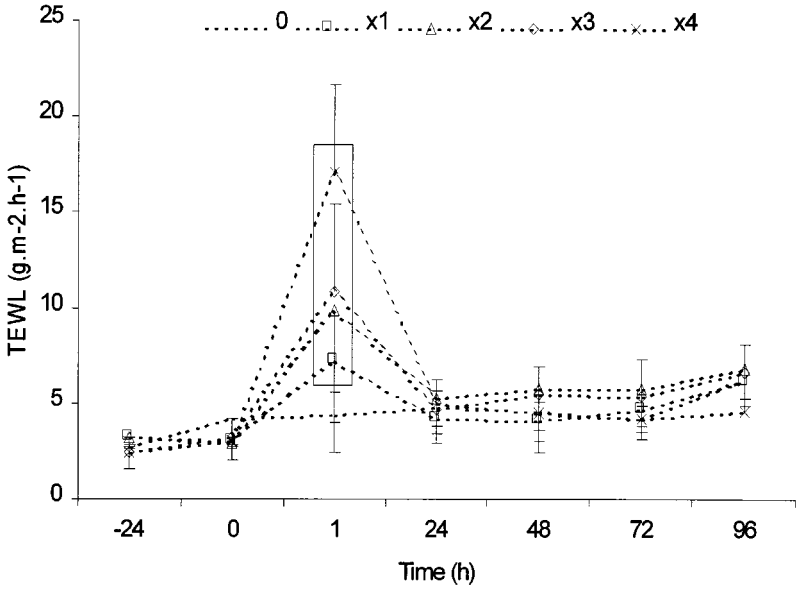

Figure 5. Effect of punctures on pig skin TEWL. Variation in TEWL rates measured from full-thickness pig-back skin before (= 24 h) and up to 48 h after skin punctures (1× to 4×). All values are mean ± SD of $n = 6$ diffusion cells containing skin from one animal. Boxed values are significantly different ($p < 0.05$) from control (0×).

water and ingress of most xenobiotics (Scheuplein and Blank, 1971). Thus, TEWL rates are frequently interpreted as damage to skin barrier function following exposure to various chemical or physical insults. The results of this *in vitro* study clearly do not support the supposition that baseline TEWL rates are a reflection of intrinsic skin barrier function. Furthermore, there was no correlation between the change in TEWL rates and skin permeability following damage by tape stripping or frank skin punctures.

Basal TEWL and skin permeability This study has demonstrated no correlation between TEWL rates and skin permeability to ^3H_2O or ^{35}SM in structurally viable skin. Furthermore, TEWL failed to identify damaged membranes (where an excessive percentage of the applied dose had penetrated in a short time). In addition, collation of all full-thickness pig skin data for baseline ^3H_2O fluxes measured in this study and previous (unpublished) studies in our laboratory also indicated no correlation ($r^2 = 0.11$) with baseline TEWL values (**Fig 7**). Thus, it is apparent that baseline TEWL rates cannot be used as a tool to evaluate epidermal membrane integrity prior to *in vitro* percutaneous absorption experiments using epidermal membranes or full-thickness skin.

Postdamage measurements of TEWL and skin permeability Previous studies have demonstrated that sequential removal of the stratum corneum by tape stripping results in a transient increase in TEWL rates, particularly when the lowest cornified layers are removed (Blank, 1953). The mechanisms of barrier restoration observed *in vivo* for normal skin (Matoltsty *et al*, 1962; Spruit and Malten, 1965; Frödin and Skogh, 1984) would not be expected to be reproducible in our *in vitro* system, as the skin had been frozen and subject to nutrient-free media for at least 24 h prior to tape stripping (Holland *et al*, 1984; Collier *et al*, 1989). Consequently, any energy-requiring repair processes such as parakeratosis would almost certainly be absent in our *in vitro* model. Therefore, damage caused by tape-stripping in this *in vitro* study would be expected to be irreversible. That is, the elevation in TEWL rates would be persistent. However, the results of this study were similar to the early repair phase observed *in vivo* (Matoltsty *et al*, 1962). An immediate increase in TEWL was followed by a rapid decrease to basal (up to 15 strips) or supra-basal TEWL rates (20 strips). There are three obvious interpretations of these data: the apparent restoration of barrier function is a nonenergy-dependent mechanism, or the transient increase in TEWL is due to the temporary exposure of deeper, hydrated tissue that subsequently re-equilibrates, or TEWL rates are simply not indicative of "barrier function". It is possible that the transient increase in TEWL for moderate (up to 10×) strippings is due to the exposure of more

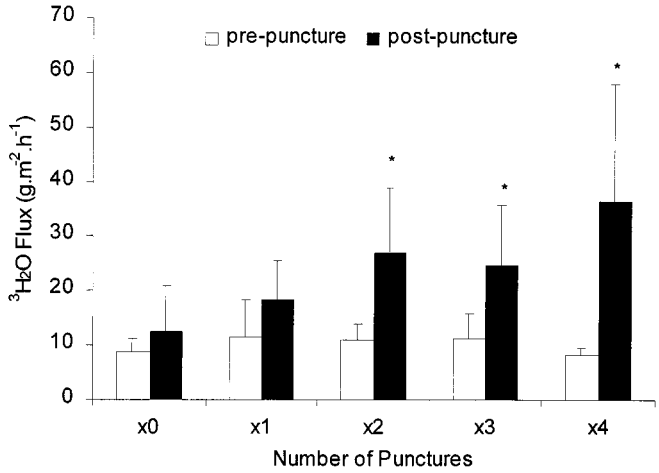

Figure 6. Effect of punctures on permeability of pig skin to ^3H_2O. Comparison of ^3H_2O fluxes measured from full-thickness pig-back skin 24 h before (pre) and 24 h after (post) puncture(s). All values are mean ± SD of $n = 6$ diffusion cells containing skin from two animals. Asterisks indicate that the post-value is significantly different from the pre-value ($p < 0.05$).

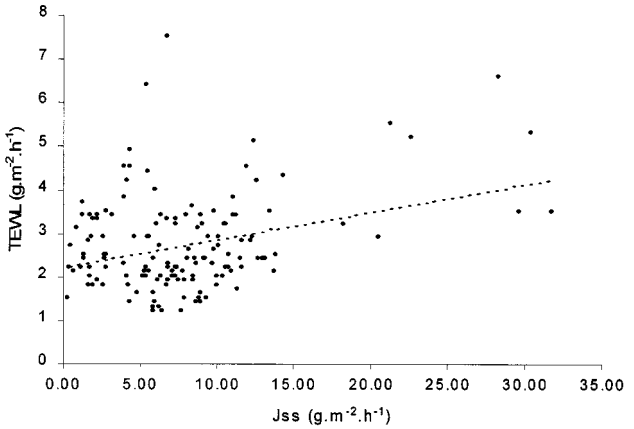

Figure 7. Basal pig skin TEWL rates and permeability to ^3H_2O. Baseline ^3H_2O permeation and baseline TEWL rate data from all experiments using full-thickness pig skin conducted during this study ($n = 144$ pieces of skin from $n = 6$ animals). Dotted line indicates the result of linear regression analysis ($r^2 = 0.1075$).

hydrated tissue that subsequently undergoes a re-establishment of the water gradient leading to an apparently normal TEWL value (Emanuel, 1999). It is conceivable that the rate at which the water gradient is re-established is proportional to the diffusivity of water within the remaining stratum corneum – this would explain why some individuals appear to be resistant to an increase in TEWL following tape stripping (Bashir *et al*, 2001), as those with a high water diffusivity in the stratum corneum will re-establish the gradient more rapidly, leading to apparently normal TEWL values. This may also explain why certain pathologic skin appears to have a faster early repair phase after stripping (Tanaka *et al*, 1997), as water within the stratum corneum is more mobile and may equilibrate faster with the surrounding air. The fact that skin permeability to ^3H_2O was elevated 6-fold at 24 h after 15 strips, yet there was no significant elevation of TEWL at that time, would imply that TEWL is, at best, a poor indicator of skin (water) barrier function. The 2-fold increase in TEWL (24 h) after 20 strips was also an insensitive measure of the actual 7-fold increase in ^3H_2O permeability. The permeability of heat-separated (epidermis-free) skin was approximately 2-fold higher than skin stripped 20×, 4-fold higher than skin stripped 15×, and 37-fold higher than controls, confirming that substantial damage was inflicted by tape stripping.

The results of this study are in agreement with previous work, which demonstrated that damage caused by scratching the skin surface with a needle results in a significant increase in permeability to ^3H_2O (Nangia *et al*, 1999) and a range of other compounds (Bronaugh and Stewart, 1985). Whilst there was a transient increase in TEWL that was related to the number of punctures administered, TEWL rates at 24 h were not significantly different from controls, despite a 2–5-fold increase in ^3H_2O permeability. Thus, TEWL did not identify frank, physical damage to the water barrier layer. It is possible that the transient alteration in TEWL may be attributable to exposure and re-equilibration of deeper, hydrated tissue, as discussed above.

GENERAL CONCLUSIONS

The results of this study clearly demonstrate that basal TEWL rates do not correlate with baseline skin permeability to water or SM and that, following physical damage, there is no interrelationship between TEWL and skin permeability. It can be inferred that elevated TEWL rates should not be unconditionally ascribed to an alteration of skin barrier function, as other factors may be largely responsible for variation in TEWL rates *in vivo*. It is evident that further work is required to elucidate the meaning of elevated TEWL rates, given its increasing utility in clinical studies. With regard to measuring skin barrier function by evaporimetry, perhaps the fundamental principle that the gradient of water vapour on the skin surface represents skin barrier function should be questioned.

REFERENCES

Abrams K, Harvell JD, Shriner S, Wertz P, Maibaich H, Maibach HI, Rehfeld SJ: Effect of organic solvents on *in vitro* human skin water barrier. *J Invest Dermatol* 101:609–613, 1993

Bashir SJ, Chew A-L, Anigbogu A, Dreher F, Maibach HI: Physical and physiological effects of stratum corneum tape stripping. *Skin Res Technol* 7:40–48, 2001

Benowitz NL, Jacob III, Olsson P Johansson CJ: Intravenous nicotine retards transdermal absorption of nicotine: evidence of blood-flow limited percutaneous absorption. *Clin Pharmacol Ther* 52:223–230, 1992

Blank IH: Further investigations on factors which influence the water content of the stratum corneum. *J Invest Dermatol* 12:259–271, 1953

Blank IH Scheuplein RJ: Transport into and within the skin. *Br J Dermatol* 81:4–10, 1969

Bronaugh RL Stewart RF: Methods for *in vitro* percutaneous absorption studies V. Permeation through damaged skin. *J Pharm Sci* 74:1062–1066, 1985

Chilcott RP Farrar R: Biophysical measurements of human forearm skin *in vivo*. Effects of site, gender, chirality and time. *Skin Res Technol* 6:64–69, 2000

Chilcott RP Jenner J Carrick W Hotchkiss SAM Rice P: Human skin absorption of bis-2-(chloroethyl) sulphide (sulphur mustard) *in vitro*. *J Appl Toxicol* 20:349–355, 2000

Collier SW Sheikh NM Sakr A Lichtin JL Stewart RF Bronaugh RL: Maintenance of skin viability during *in vitro* percutaneous absorption/metabolism studies. *Toxicol Appl Pharmacol* 99:522–533, 1989

Emanuel A: Effects of controlled physical damage on transepidermal water loss and permeability of human and pig skin. MSc Thesis. University of Birmingham, U.K., 1999

Franz TJ: Percutaneous absorption. On the relevance of *in vitro* data. *J Invest Dermatol* 64:190–195, 1975

Freeman S Maibach HI: Study of irritant contact dermatitis produced by repeat patch test with sodium lauryl sulfate and assessed by visual methods, transepidermal water loss, and laser Doppler velocimetry. *J Am Acad Dermatol* 19:496–502, 1988

Frödin T Skogh M: Measurement of transepidermal water loss using an evaporimeter to follow the restitution of the barrier layer of human epidermis after stripping the stratum corneum. *Acta Derm Venereol* 64:537–540, 1984

Holland JM Kao JY Whitaker MJ: A multisample apparatus for kinetic evaluation of skin penetration *in vitro*: the influence of viability and metabolic status of the skin. *Toxicol Appl Pharmacol* 72:272–280, 1984

Howes D Guy R Hadgraft J, *et al*: Methods for assessing percutaneous absorption. *ATLA* 24:81–106, 1996

Idson B: *In vivo* measurement of transepidermal water loss. *J Soc Cosmet Chem* 29:573–580, 1978

Kao J, Hall J, Shugert LR, Holland JM: An *in vitro* approach to studying cutaneous metabolism and disposition of topically applied xenobiotics. *Toxicol Appl Pharmacol* 75:289–298, 1984

Kligman AM, Christophers E: Preparation of isolated sheets of human stratum corneum. *Arch Dermatol* 88:70–73, 1963

Lamaud E, Schalla W: Influence of UV irradiation on penetration of hydrocortisone. *In vivo* study in hairless rat skin. *Br J Dermatol* 111:152–157, 1984

Lamke L-O, Nilsson GE, Reithner HL: Insensible perspiration from the skin under standardized environmental conditions. *Scand J Clin Laboratory Invest* 37:325–331, 1977

Lévêque JL, de Rigal J, Saint-Léger D, Billy D: How does sodium lauryl sulfate alter the skin barrier function in man? A multiparametric approach. *Skin Pharmacol* 6:111–115, 1993

Lotte C, Rougier A, Wilson DR, Maibach HI: *In vivo* relationship between transepidermal water loss and percutaneous penetration of some organic compounds in man: effect of anatomic site. *Dermatol Res* 279:351–356, 1987

Matoltsty AG, Schragger A, Matolstdy MN: Observation on regeneration of the skin barrier. *J Invest Dermatol* 38:251–253, 1962

Nangia A, Berner B, Maibach HI: Transepidermal water loss measurements for assessing skin barrier function during *in vitro* percutaneous absorption experiments. In: Bronaugh RL, Maibach HI: eds. *Percutaneous Absorption: Drugs, Cosmetics, Mechanisms, Methodology*, 3rd edn, Chapter 34. New York: Marcel Dekker, 1999

Nicander I, Ollmar S, Eek A, Rozell BL, Emtestam L: Correlation of impedance response patterns to histological findings in irritant skin reactions induced by various surfactants. *Br J Dermatol* 134:221–218, 1996

Nilsson GE: Measurement of water exchange through skin. *Med Biol Eng Comput* 15:209–218, 1977

Pinnagoda J, Tupker RA: Measurement of transepidermal water loss. In: Serup J, Jemec GBE: eds. Handbook of Non-invasive Methods, the Skin. Chapter 9: Epidermal Barrier Functions. London: CRC Press, 1995

Pinnagoda J, Tupker RA, Agner T, Serup J: Guidelines for transepidermal water loss (TEWL) measurement. *Contact Dermatitis* 22:164–178, 1990

Pinson EA: Evaporation from human skin with sweat glands inactivated. *Am J Physiol* 187:492–503, 1942

Scheuplein RJ, Blank IH: Permeability of the skin. *Physiol Rev* 51:702–745, 1971

Spruit D, Malten KE: Epidermal water barrier formation after stripping of normal skin. *J Invest Dermatol* 45:6–14, 1965

Tanaka M, Zhen YX, Tagami H: Normal recovery of the stratum corneum barrier function following damage induced by tape stripping in patients with atopic dermatitis. *Br J Dermatol* 136:966–967, 1997

Van der Valk PGM, Maibach HI: A functional study of the skin barrier to evaporative water loss by means of repeated cellophane-tape stripping. *Clin Exp Dermatol* 15:180–182, 1990

Yosipovitch G, Xiong GL, Haus E, Sackett-Lundeen L, Ashkenazi HI: Time dependant variations of the skin barrier function in humans: transepidermal water loss, stratum corneum hydration, skin surface skin temperature. *J Invest Dermatol* 110:20–23, 1998