In Vitro Percutaneous Penetration: Evaluation of the Utility of Hairless Mouse Skin

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The permeability barrier of hairless mouse skin has been determined in vitro after exposure of the epidermal surface to volumes of acetone typically used in human in vivo skin penetration studies. It has been shown that the transport of tritiated water (when applied for limited 5-h periods) across hairless mouse skin is not affected by acetone treatments of approximately 15 $\mu l/cm^2$. Submersion of the membranes between aqueous donor and receptor phases for periods greater than 24 h, however, leads to significant and catastrophic barrier impairment. The acetone dose in the experiments reported is greater than that employed in vivo when the solvent is used to deposit a penetrant on human skin. We suggest, therefore, that acetone-mediated facilitation of percutaneous absorption in humans is unlikely. A further conclusion of this work is that in vitro solvent-deposition penetration experiments using hairless mouse skin should provide reliable transport information for at least 48 h postadministration. Although hairless mouse skin is more permeable than its human counterpart, in vitro measurements using the murine barrier should, therefore, provide useful and relevant guidelines for risk assessment calculations and bioavailability determinations. *J Invest Dermatol 93:87–91, 1989*

The use of animal skin in the study of percutaneous absorption has provided fundamental knowledge toward our understanding of barrier function. There are important differences, however, in the permeabilities of skin taken from different species and these inconsistencies have been highlighted in a number of publications [1–4]. Currently, there is considerable activity in the area of in vitro skin permeation measurement. At least three major driving forces for this effort can be identified: 1) The U.S. Food and Drug Administration recently sponsored a workshop on in vitro methods for the purpose of establishing guidelines that could be followed when new topical drug formulations are under development [5]. 2) There is a continuing need for reliable, and meaningful, procedures that can be used to predict the health risk resulting from dermal exposure to toxic substances [6]. 3) The emergence of transdermal drug delivery to provide systemic pharmacologic effect has introduced percutaneous penetration measurement as a key component of the pharmaceutic research and development effort [7].

The heightened interest in assessing percutaneous transport has led several investigators to use substitutes for human skin. It is sometimes difficult to obtain human tissue in a regular or timely fashion; in addition, the high level of variability associated with cadaver skin [8] has frustrated researchers and has directed them to consider alternatives. Of the various models that have been studied the skin of the hairless mouse is probably the most popular. There is no doubt that this tissue has enabled a number of key studies that have greatly increased our understanding of the skin permeation process. For example, it has allowed fundamental research into structure–penetration relationships [9–12], concurrent transport and metabolism [13–16], and the effects of skin damage on barrier function [17–19]. The advantages of hairless mouse skin include its availability and reproducibility. It is more permeable than human skin, too, and this is an asset for both bioavailability and risk assessment, as a result obtained with hairless mouse skin will err on the conservative side. In risk assessment, for instance, a permeation measurement through hairless mouse skin will not lead to an underestimate of dermal exposure in humans. This higher permeability, however, is also considered by some to be a major disadvantage of the tissue, although there is little evidence to document this concern. A more serious question, though, pertains to the response of hairless mouse skin, relative to that of human skin, to situations or circumstances often encountered in percutaneous penetration work, e.g., the effect of hydration and of organic solvents. The hydration issue was recently addressed by Bond and Barry [20], who showed that prolonged exposure of hairless mouse skin to aqueous donor and receptor phases in simple diffusion cells caused considerable derangement of barrier function. The interests of our laboratory have centered on in vivo evaluation of percutaneous absorption [21–25]. Typically, topical application of chemicals has involved deposition from an organic solvent, usually acetone. The question posed by the research presented here, therefore, was: "Does the amount of acetone used as the vehicle in human skin penetration studies cause significant changes to the barrier function of hairless mouse skin in vitro?" A negative response would imply that 1) human skin in vivo is not damaged by the acetone deposition and delivery process and 2) in vitro hairless mouse skin experiments involving chemical application in acetone may provide information relevant to percutaneous absorption in humans.

MATERIALS AND METHODS

To assess barrier function status of hairless mouse skin, the permeability of tritiated water (^3H_2O, 0.05 μCi/ml, New England Nuclear, Boston, MA) was determined at designated times after various acetone treatments. Permeation experiments were performed in conventional flow-through diffusion cells (Laboratory Glass Apparatus, Berkeley, CA) [26]. The area of skin exposed was 3.14 cm^2; the volume of the receptor phase was approximately 5 cm^3. The flow-rate was adjusted by a cassette pump (Manostat, New York,

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NY) so that the receptor solution was completely exchanged in 1 h. The receptor phase was normal saline in phosphate buffer at pH 7.4. Perfusate was collected in test tubes mounted on a fraction collector (Gilson FC-220, Middleton, WI) and the samples were then analyzed by liquid scintillation counting (Searle Mark III Model 6880, Elk Grove, IL). The diffusion cells were thermostatted at 35°C throughout the experiments; under these conditions, with the skin open to the laboratory atomosphere, the surface temperature of the membrane was 32°C ± 1°C.

In all experiments, full-thickness skin from hairless mice (HRS/hr hr, 6–16 wk, Simonsen Laboratories, Gilroy, CA) was used. The skin was removed from the animal immediately after killing, any small fatty deposits were carefully removed, and the membrane was then mounted in the diffusion cell. Typically, eight diffusion cells were used in each experiment, requiring skin from four mice. When comparisons were made within a run (acetone treatment vs. no treatment, for example), the four skins were halved so that each animal contributed to both the "control" and "test" set of cells. Because of the time involved in setting up eight diffusion cells and adjusting the receptor solution flow-rate appropriately, an experiment was typically started within 2 h after killing of the animals.

The experiments performed are summarized in Table I. The design was selected to test the effects of an acetone dose on barrier function and tissue constancy. The water treatments involved application of 1 cm^3 of ^3H_2O to the skin surface. To prevent evaporation, the upper half of the diffusion cell was then covered until the water was removed. Whenever water was *not* in contact with the skin, the surface was open to ambient conditions. Acetone treatments involved application of 50 μl of the solvent by a capillary pipet. As peformed in vivo, the acetone was distributed evenly over the skin surface, which, again, was open to the laboratory atmosphere. When water was administered subsequent to acetone treatment (experiments IIIa, IVa, V) there was a 2- to 3-min time lapse between solvent applications. No liquid acetone remained at this point.

Experiments I and II simply determined water permeation over 24- and 48-h periods, in the absence of acetone treatment. Experiments III and IV used short 5-h exposures of the tissue to water and assessed the long-term consequences of an acetone dose at $t = 0$. Experiment V involved a greater potential insult to the tissue and included three volatile solvent treatments. Experiment VI considered the effect of a time delay postacetone application followed by prolonged water contact.

RESULTS

In experiments I and II, the skin remained sandwiched between aqueous solutions throughout the measurement periods (24 and 48 h, respectively). Figure 1 shows that in experiment I, ^3H_2O flux is essentially constant over the 3- to 20-h postapplication period, corresponding to a permeability coefficient of about 2.95×10^{-3} cm/h (in good agreement with recently published data [20]). Increased permeation, however, is suggested by the later time points, an inference confirmed by experiment II. Figure 2 indicates that prolonged and complete hydration leads to barrier breakdown after 24 h of

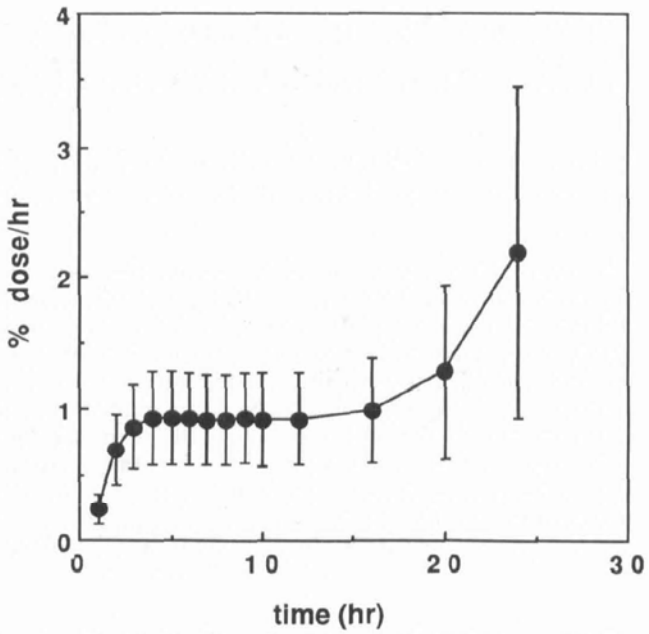

Figure 1. Permeation (mean flux ± SD, n = 8) of ^3H_2O through hairless mouse skin when the membrane is sandwiched between aqueous solutions for 24 h (experiment I).

contact. Indeed, in six out of eight cells, the membrane has been so damaged that ^3H_2O flux decreases at later times due to the substantial depletion of radiolabel in the donor phase.

The results of experiments III and IV are summarized in Figures 3 and 4, respectively. It is apparent that when water is dosed intermittently to the skin surface for 5-h periods, pretreatment with acetone does not cause any significant difference to the permeability behavior. This conclusion was substantiated by statistical comparisons (paired Wilcoxon and Student's *t*-tests) of the cumulative amounts of water transported across the control and acetone-treated membranes, following the 5-h applications. Acetone elicited an insignificant effect ($\alpha > 0.2, p > 0.1$) on water permeation. Figure 5, which contains the data from experiment V, demonstrates that repeated acetone administration before dosing with water also elicits no significant derangement of barrier function. Although Figures 3, 4, and 5 appear to suggest that, regardless of acetone treatment or not, the permeability of ^3H_2O increases with time, the trend is not statistically significant. It is perhaps reasonable, however, to conclude that hydration and the detrimental effects thereof, can continue during the water "off" periods.

Finally, Figure 6 illustrates the results of experiment VI, in which the permeation of ^3H_2O was followed 24 h after acetone treatment. Again, no difference from the control studies was observed al-

Table I. Experimental Design Summary (n = number of replicates)

Experiment	n	Treatments
I	8	↓ ↑
II	8	↓ ↑
IIIa	8	*↓ ↑ ↓ ↑
IIIb	8	↓ ↑ ↓ ↑
IVa	12	*↓ ↑ ↓ ↑ ↓ ↑
IVb	16	↓ ↑ ↓ ↑ ↓ ↑
V	4	*↓ ↑ *↓ ↑ *↓ ↑
VIa	12	* ↓ ↑
VIb	12	↓ ↑

0 4 8 12 16 20 24 28 32 36 40 44 48 52 56
Time (hours)

* = 50 μl acetone; ↓ = water on; ↑ = water off

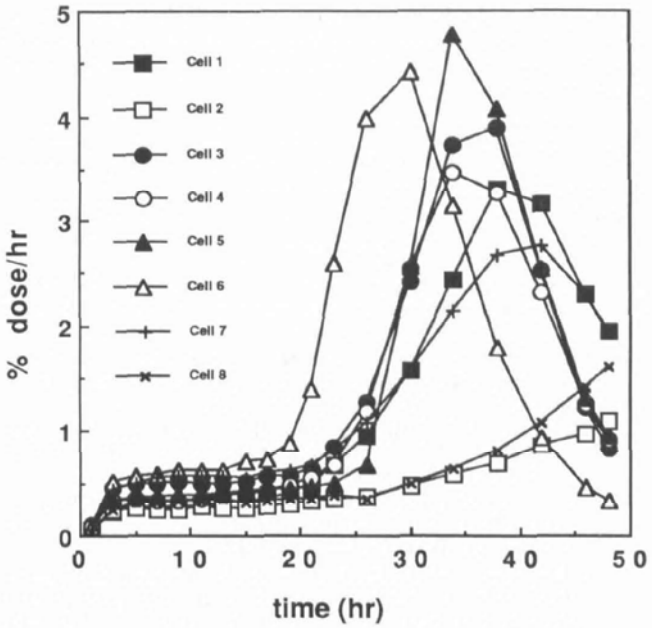

Figure 2. Permeation of ^3H_2O through hairless mouse skin when the membrane is sandwiched between aqueous solutions for 48 h (experiment II). The results from eight separate experiments are shown. The average ^3H_2O permeability coefficient during the 5–15-h postdosing period is 1.27×10^{-3} cm/h.

though, as before, prolonged exposure to aqueous solutions did begin to compromise the skin.

DISCUSSION

The experiments performed in this investigation lead to two important conclusions. First, as recently reported by Bond and Barry [20], the barrier function of hairless mouse skin does not withstand prolonged submersion in aqueous solution. The data in Figures 1 and 2 clearly reveal that exposure of the tissue to aqueous solutions, in both donor and receiver compartments, for periods in excess of 24 h, leads to substantial degeneration of the stratum corneum. The second key finding revealed by this study is that treatment of hairless mouse skin with acetone, in a fashion that mimics a typical "solvent-deposition" application procedure [22–25] does not appear to alter permanently the barrier to water to any significant degree. The results of experiments III, IV, and V indicate that acetone administration per se does not contribute to derangement of the stratum corneum. The data also suggest that if a penetrant were delivered in

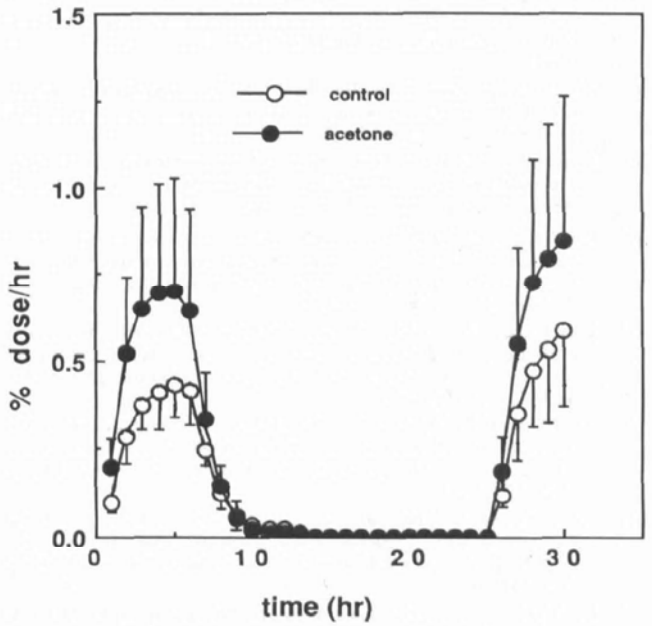

Figure 3. Effect of acetone pretreatment (15 μl/cm²) on tritiated water flux (mean ± SD, n = 8) across hairless mouse skin after applications from t = 0–5 h and t = 24–29 h (experiment III).

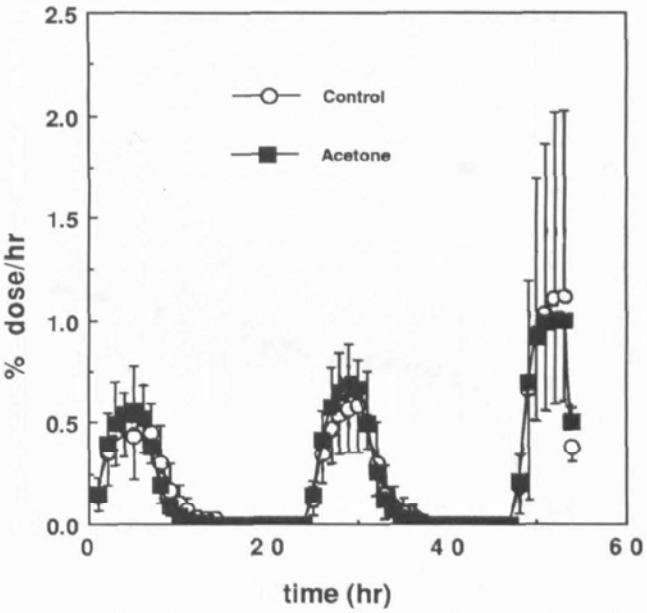

Figure 4. Effect of acetone pretreatment (15 μl/cm²) on tritiated water flux (mean ± SD, n ≥ 12) across hairless mouse skin after applications from t = 0–5 h, t = 24–29 h, and t = 48–53 h (experiment IV).

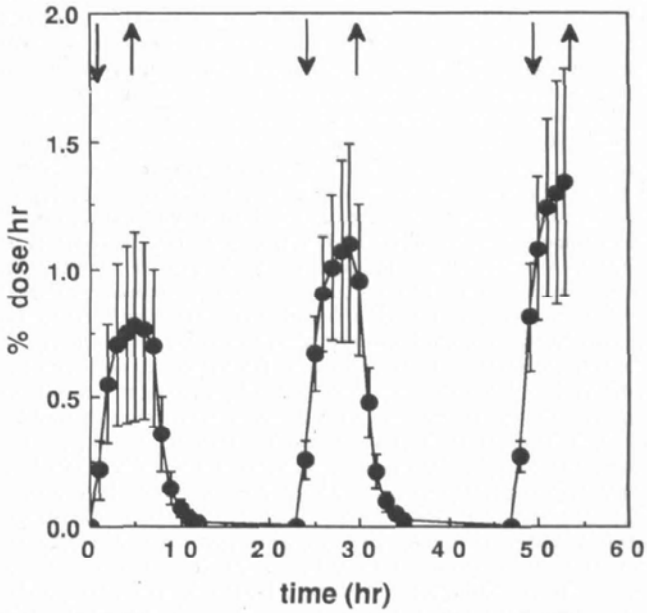

Figure 5. Tritiated water flux (mean ± SD, n = 4) across hairless mouse skin after applications from t = 0–5 h, t = 24–29 h, and t = 48–53 h. Before *each* administration of water, the skin was pretreated with acetone at a dose of 15 μl/cm².

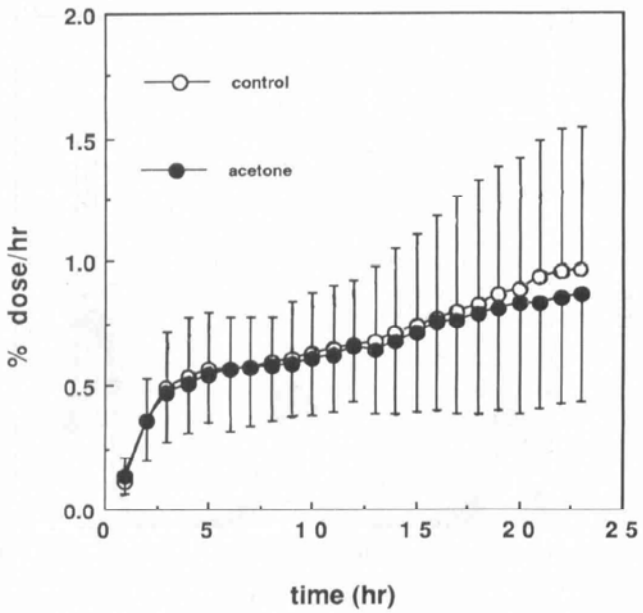

Figure 6. Permeation (mean flux ± SD, n = 12) of ^3H_2O through hairless mouse skin 24 h after mounting the membrane in the diffusion cell, and after treating the "acetone" specimens with a solvent dose of 15 $\mu l/cm^2$. For the subsequent 24-h period of observation presented here, the skin is sandwiched between aqueous donor and receptor phases (experiment VI). Based on the essentially constant ^3H_2O fluxes between 5 and 10 h, permeability coefficients for the control and acetone-treated membranes are calculated to be 1.88×10^{-3} cm/h and 1.83×10^{-3} cm/h, respectively.

an acetone vehicle under similar circumstances, one may expect the barrier function of hairless mouse skin to remain reasonably constant for at least 48 h. The short 5-h ^3H_2O applications were designed to test the skin permeability while minimizing hydration effects. In this respect, they would appear to have fulfilled their function adequately. Experiment V challenged the tissue further by considering multiple acetone treatments. Again, however, no significant effect of the solvent, over that observed in the controls (experiment IVb), was apparent. Furthermore, experiment VI showed that air-exposure of the epidermal surface for 24 h (acetone pretreated or not) did not significantly alter subsequent ^3H_2O permeation compared with the "control", i.e., experiment I.

One ramification of our research is that the warning of Bond and Barry [20], that " . . . hairless mouse skin should not be used . . . in . . . permeation studies incorporating long-term hydration, as erroneous results can be expected after . . . 3 days," appears somewhat conservative. On the basis of our data, we would be reluctant to draw conclusions from any flux measurements made after 24 h submersion. More important, though, we have shown that administration of acetone, at a dose of approximately 15 $\mu l/cm^2$, does not appear to alter significantly the stratum corneum barrier function of hairless mouse skin. Given that a typical topical dose of acetone in a solvent-deposition, human in vivo skin penetration study is less than 10 $\mu l/cm^2$ [22–25], it seems reasonable to suggest that no acetone-mediated facilitation of transport will be evident. In addition, one may also deduce that an in vitro penetration study using hairless mouse skin and solvent-deposition of penetrant from acetone (at a dose of 15 $\mu l/cm^2$ or less), should provide a reasonable model experiment for the in vivo situation. We recognize, however, that the latter two conclusions are based on observations that use water as the model permeant. It remains to be seen whether these deductions are sustained when the penetating molecule is lipophilic in character. Finally, although hairless mouse skin is generally more permeable than its human counterpart [20], the application of small volatile solvent volumes does not appear to place the murine barrier under measurable stress. The effects of larger volumes of solvent or of more structurally destructive chemicals (e.g., penetration enhancers) [27] will, no doubt, be greater and may be amplified in the less substantial stratum corneum of the hairless mouse.

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