

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 $15 \mu\text{l}/\text{cm}^2$. 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 pharmaceutical 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 ($^3\text{H}_2\text{O}$, $0.05 \mu\text{Ci}/\text{ml}$, 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 atmosphere, 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 cm³ of ³H₂O 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 performed 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, ³H₂O 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

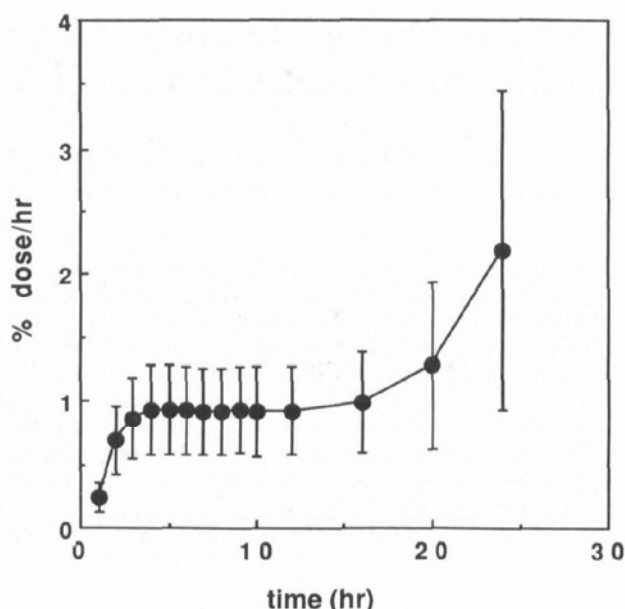


Figure 1. Permeation (mean flux ± SD, n = 8) of ³H₂O through hairless mouse skin when the membrane is sandwiched between aqueous solutions for 24 h (experiment I).

contact. Indeed, in six out of eight cells, the membrane has been so damaged that ³H₂O 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 ³H₂O 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 ³H₂O 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			

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

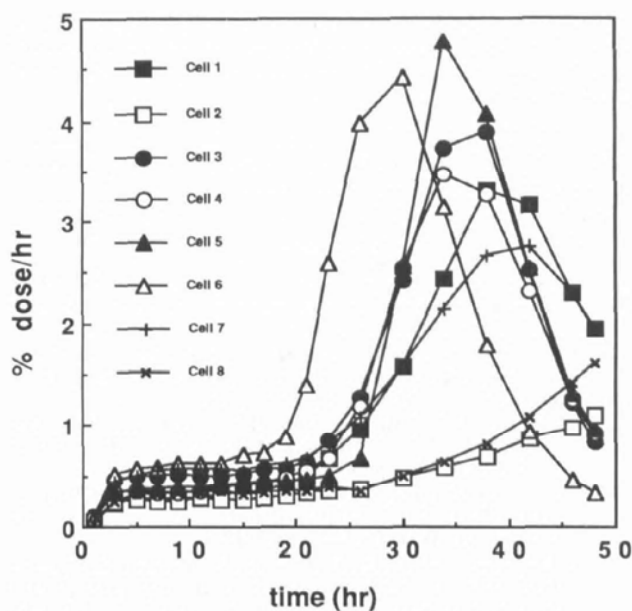


Figure 2. Permeation of $^3\text{H}_2\text{O}$ through hairless mouse skin when the membrane is sandwiched between aqueous solutions for 48 h (experiment II). The results from eight separate experiments are shown. The average $^3\text{H}_2\text{O}$ permeability coefficient during the 5–15-h postdosing 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

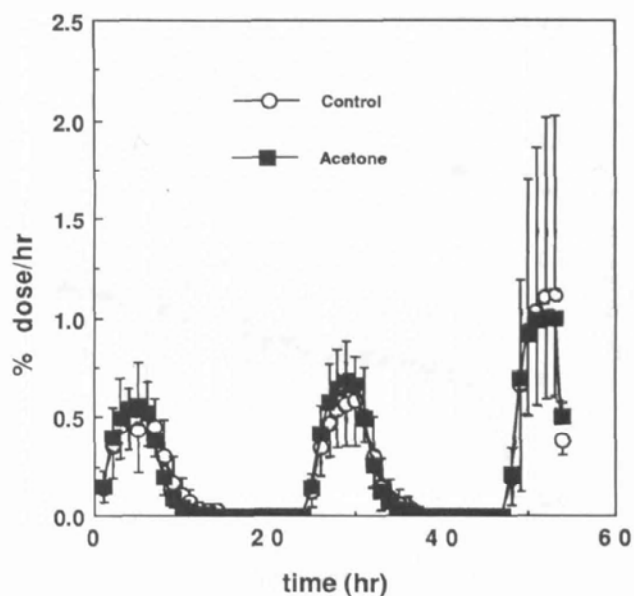


Figure 4. Effect of acetone pretreatment ($15 \mu\text{l}/\text{cm}^2$) on tritiated water flux (mean \pm SD, $n \geq 12$) across hairless mouse skin after applications from $t = 0-5$ h, $t = 24-29$ h, and $t = 48-53$ h (experiment IV).

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

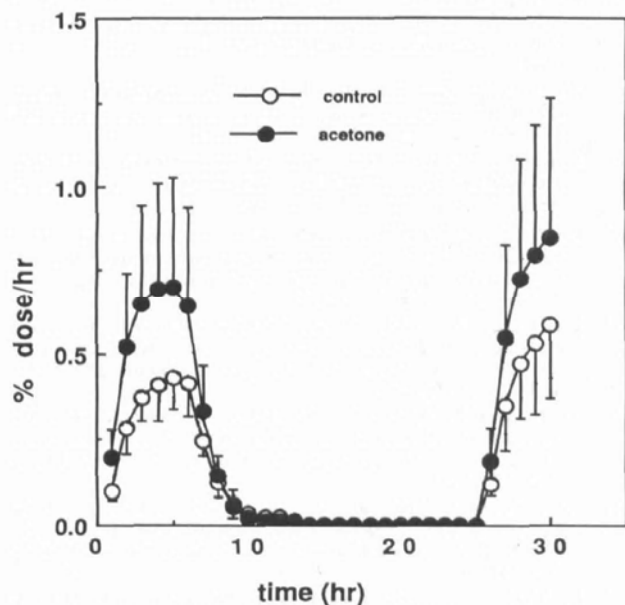


Figure 3. Effect of acetone pretreatment ($15 \mu\text{l}/\text{cm}^2$) on tritiated water flux (mean \pm SD, $n = 8$) across hairless mouse skin after applications from $t = 0-5$ h and $t = 24-29$ h (experiment III).

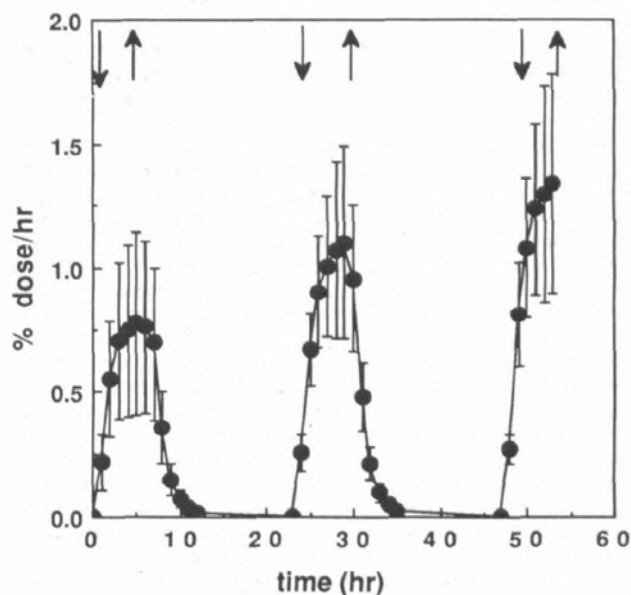


Figure 5. Tritiated water flux (mean \pm SD, $n = 4$) across hairless mouse skin after applications from $t = 0-5$ h, $t = 24-29$ h, and $t = 48-53$ h. Before each administration of water, the skin was pretreated with acetone at a dose of $15 \mu\text{l}/\text{cm}^2$.

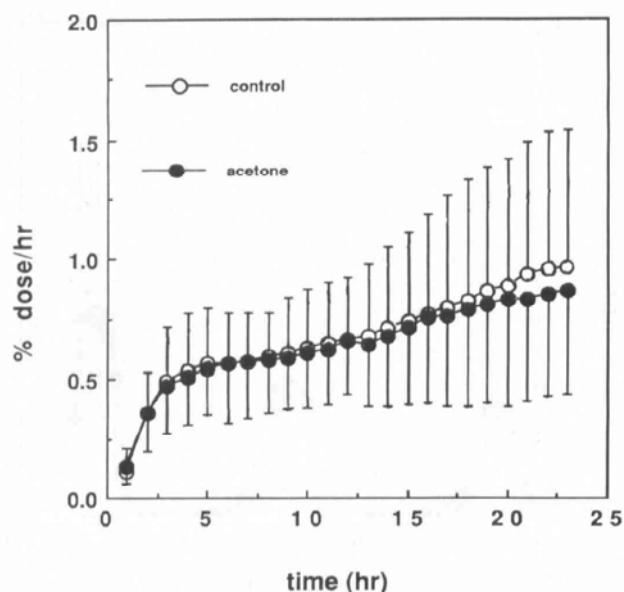


Figure 6. Permeation (mean flux \pm SD, $n = 12$) of $^3\text{H}_2\text{O}$ through hairless mouse skin 24 h after mounting the membrane in the diffusion cell, and after treating the "acetone" specimens with a solvent dose of $15 \mu\text{l}/\text{cm}^2$. For the subsequent 24-h period of observation presented here, the skin is sandwiched between aqueous donor and receptor phases (experiment VI). Based on the essentially constant $^3\text{H}_2\text{O}$ fluxes between 5 and 10 h, permeability coefficients for the control and acetone-treated membranes are calculated to be $1.88 \times 10^{-3} \text{ cm/h}$ and $1.83 \times 10^{-3} \text{ cm/h}$, 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 $^3\text{H}_2\text{O}$ 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 $^3\text{H}_2\text{O}$ 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 $15 \mu\text{l}/\text{cm}^2$, 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 $10 \mu\text{l}/\text{cm}^2$ [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 $15 \mu\text{l}/\text{cm}^2$ 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 penetrating 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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REFERENCES

- Bartek MJ, LaBadde JA: Percutaneous absorption *in vitro*. In: Maibach HI (ed.). *Animal Models in Dermatology*. New York, Churchill Livingstone, 1975, pp 103–120
- Bronaugh RL, Stewart RF: Methods for *in vitro* percutaneous absorption studies. II. Animal models for human skin. *Toxicol Appl Pharmacol* 62:481–488, 1982
- Wester RC, Maibach HI: Animal models for percutaneous absorption. In: Maibach HI, Lowe NJ (eds.). *Models in Dermatology*, vol. 2. Basel, Karger, 1985, pp 159–169
- Reifenrath WG, Chellquist EM, Shipwash EA, Jederberg WW: Evaluation of animal models for predicting skin penetration in man. *Fundam Appl Toxicol* 4:S224–S230, 1984
- Skelly JP, Shah VP, Maibach HI, Guy RH, Wester RC, Flynn G, Yacobi A: FDA and AAPS report of the workshop on principles and practices of *in vitro* percutaneous penetration studies: Relevance to bioavailability and bioequivalence. *Pharmacol Res Commun* 4:265–267, 1987
- Mathias CGT, Hinz RS, Guy RH, Maibach HI: Percutaneous absorption: interpretation of *in vitro* data and risk assessment. In: Honeycutt RC, Zweig G, Ragsdale NN (eds.). *Dermal Exposure Related to Pesticide Use*. Washington, DC, American Chemical Society, 1985, pp 3–17
- Guy RH, Hadgraft J: Selection of drug candidates for transdermal drug delivery. In: Hadgraft J, Guy RH (eds.). *Transdermal Drug Delivery: Developmental issues and research initiatives*. New York, Marcel Dekker, 1989, pp 59–81
- Barry BW: *Dermatological formulations: percutaneous absorption*. New York, Marcel Dekker, 1983, pp 235–236
- Behl CR, Flynn GL, Kurihara T, Harper N, Smith W, Higuchi WI, Ho NFH, Pierson CL: Hydration and percutaneous absorption. I. Influence of hydration on alkanol permeation through hairless mouse skin. *J Invest Dermatol* 25:346–352, 1980
- Durrheim HH, Flynn GL, Higuchi WI, Behl CR: Permeation of hairless mouse skin. I. Experimental methods and comparison with human epidermal permeation by alkanols. *J Pharm Sci* 69:781–786, 1980
- Flynn GL, Durrheim HH, Higuchi WI: Permeation of hairless mouse skin. II. Membrane sectioning techniques and influences on alkanol permeabilities. *J Pharm Sci* 70:52–56, 1981
- Flynn GL: Mechanism of percutaneous absorption from physicochemical evidence. In: Bronaugh RL, Maibach HI (eds.). *Percutaneous Absorption*. New York, Marcel Dekker, 1985, pp 17–42
- Yu CD, Fox JL, Ho NFH, Higuchi WI: Physical model evaluation of topical prodrug delivery—Simultaneous transport and bioconversion of vidarabine-5'-valerate. I. Physical model development. *J Pharm Sci* 68:1341–1346, 1979
- Yu CD, Fox JL, Ho NFH, Higuchi WI: Physical model evaluation of topical prodrug delivery—Simultaneous transport and bioconversion of vidarabine-5'-valerate. II. Parameter definitions. *J Pharm Sci* 68:1347–1357, 1979
- Fox JL, Yu CD, Higuchi WI, Ho NFH: General physical model for simultaneous diffusion and metabolism in biological membranes. The computational approach for the steady-state case. *Int J Pharm* 2:41–57, 1979
- Santus G, Watari N, Hinz RS, Benet LZ, Guy RH: Cutaneous metabolism of transdermally delivered nitroglycerin *in vitro*. In: Shroet B, Schaefer H (eds.). *Skin Pharmacokinetics*. Basel, Karger, 1987, pp 240–244
- Behl CR, Flynn GL, Kurihara T, Smith W, Gatmaitan O, Higuchi WI, Ho NFH, Pierson CL: Permeability of thermally damaged skin. I. Immediate influences of 60°C scalding on hairless mouse skin. *J Invest Dermatol* 25:340–345, 1980

18. Behl CR, Flynn GL, Barrett M, Linn EE, Higuchi WI, Ho NFH, Pierson CL: Permeability of thermally damaged skin. IV. Influence of branding iron temperature on the mass transfer of water and n-alkanols across hairless mouse skin. *Burns* 8:86-98, 1981
19. Flynn GL, Behl CR, Linn EE, Higuchi WI, Ho NFH, Pierson CL: Permeability of thermally damaged skin. V Permeability over the course of maturation of a deep partial thickness burn. *Burns* 8:196-202, 1981
20. Bond JR, Barry BW: Limitations of hairless mouse skin as a model for in vitro permeation studies through human skin: hydration damage. *J Invest Dermatol* 90:486-489, 1988
21. Guy RH, Hadgraft J, Hinz RS, Roskos KV, Bucks DAW: In vivo evaluations of transdermal drug delivery. In: Chien YW (ed.). *Transdermal controlled systemic medications*. New York, Marcel Dekker, 1987, pp 179-224
22. Bucks DAW, Maibach HI, Guy RH: Percutaneous absorption of steroids: effect of repeated application. *J Pharm Sci* 74:1337-1339, 1985
23. Guy RH, Carlstrom EM, Bucks DAW, Hinz RS, Maibach HI: Percutaneous penetration of nicotines: in vivo and in vitro measurements. *J Pharm Sci* 75:968-972, 1986
24. Roskos KV, Maibach HI, Guy RH: Percutaneous absorption in the aged. In: Gilchrist B (ed.). *Dermatologic Clinics*, vol 6: The aging skin. Philadelphia, W.B. Saunders, 1986, pp 455-465
25. Bucks DAW, McMaster JR, Maibach HI, Guy RH: Bioavailability of topically administered steroids: a "mass balance" technique. *J Invest Dermatol* 90:29-33, 1988
26. Gummer CL, Hinz RS, Maibach HI: The skin penetration cell: A design update. *Int J Pharm* 40:101-104, 1987
27. Mirejovsky D, Takturi H: Dermal penetration enhancement profile of hexamethylenelauramide and its homologues: in vitro versus in vivo behavior of enhancers in the penetration of hydrocortisone. *J Pharm Sci* 75:1089-1093, 1986