J. Pharm. Pharmacol. 1993, 45: 882–886
Received April 8, 1992

Percutaneous Permeation of Basic Compounds Through Shed Snake Skin as a Model Membrane

KOICHI TAKAHASHI, SATOKO TAMAGAWA, TOYOSHI KATAGI, J. HOWARD RYTTING*, TOSHIAKI NISHIHATA* AND NOBUYASU MIZUNO

*Faculty of Pharmaceutical Sciences, Mukogawa Women's University, 11–68 Koshien Kyuban-cho, Nishinomiya, Hyogo, Japan, and *Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, Kansas 66045, USA*

Abstract—Relationships between the in-vitro permeability of basic compounds through shed snake skin as a suitable model membrane for human stratum corneum and their physicochemical properties were investigated. Compounds with low pK_a values were selected to compare the permeabilities of non-ionized forms of the compounds. Steady-state penetration was achieved immediately without a lag time for all compounds. Flux rate and permeability coefficient were calculated from the steady-state penetration data and relationships between these parameters and the physicochemical properties were investigated. The results showed that permeability may be controlled by the lipophilicity and the molecular size of the compounds. Equations were developed to predict the permeability from the molecular weights and the partition coefficients of basic compounds.

The stratum corneum, the skin's outermost layer, typically provides the major barrier to transdermal drug absorption. To investigate the skin permeability of drugs, a variety of model membranes have been used (Bond & Barry 1988; Houk & Guy 1988; Hinz et al 1989; Hatanaka et al 1990). However, these model membranes are usually more permeable than human skin (Bartek et al 1972; Campbell et al 1976; Walker et al 1983). Although the human skin is the best model membrane, the use of human skin is limited. The stratum corneum is a heterogeneous membrane consisting of a mosaic of cornified cells containing cross-linked keratin filaments and intercellular lipid-containing regions (Elias 1981). Shed snake skin consists of three distinctive layers of a β-keratin-rich outermost layer, an α-keratin- and lipid-rich intermediate layer, and an α-keratin-rich innermost layer (Landmann 1979; Landmann et al 1981; Wong et al 1989). Itoh et al (1990a) suggested that shed snake skins of *Elaphe obsoleta* (black rat snake) are similar to human stratum corneum in terms of composition. The permeability of compounds through shed snake skin was found to be similar to, but often slightly less than, that through human skin (Rigg & Barry 1990; Itoh et al 1990a). These results may indicate that shed snake skin is a better model membrane than other animal skins.

In this study, several basic compounds with low pK_a values were selected to compare the permeabilities of non-ionized forms of the compounds.

Materials and Methods

Materials

Aniline, *p*-toluidine, acetanilide, antipyrine and aminopyrine were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Diazepam was kindly supplied by Takeda Chemical Ind. Ltd (Osaka, Japan). Other reagents used were of analytical grade and were used without further purification.

Correspondence: K. Takahashi, Faculty of Pharmaceutical Sciences, Mukogawa Women's University, 11–68 Koshien Kyuban-cho, Nishinomiya, Hyogo, Japan.

In-vitro penetration study

Shed snake skins of *Elaphe obsoleta* (black rat snake) were used as a model membrane for in-vitro penetration studies. Skin was hydrated by immersing it in water at 40°C for 30 min before experiments. The skin was mounted in a Franz-type diffusion cell. In this study, 10 mL 0·1 M sodium phosphate buffer (pH 7·2) was used as the receptor solution, and a suspension of compound in 0·1 M sodium phosphate buffer (pH 7·0) was placed on the donor side. The surface exposed for diffusion was 1·77 cm^2 (diam. = 1·5 cm). The receptor solution was kept at 32°C and stirred with a magnetic stirrer.

Aliquots (0·1 mL) of the receptor solution were withdrawn periodically. Immediately after each collection of the solution, 0·1 mL of the fresh buffer was added. The concentration of the compound in the sample was determined by high performance liquid chromatography (HPLC).

Determination of partition coefficient

Octanol and pH 7·0 phosphate buffer (0·1 M) were saturated with each other before use. Five millilitres octanol was mixed with 5 mL pH 7·0 buffer solution and model drug (0·12 mM) for 8 h at 32°C. The mixture was then centrifuged and the compound concentration in each phase was determined spectrophotometrically.

Solubility measurements

Model drug was stirred in 10 mL of pH 7·0 phosphate buffer at 32°C for 24 h. The solution was centrifuged and the supernatant filtered with a cellulose acetate-membrane filter (0·45 μm pore size, Toyo Roshi, Tokyo, Japan). The concentration of the compound in the filtrate was determined spectrophotometrically.

Analysis

The HPLC system consisted of a pump (880-PU, Jasco, Tokyo, Japan), and a detector (875-UV, Jasco), a 4·6 × 250 mm column packed with Fine SIL C18T-5 (Jasco), and an integrator (C-R3A, Shimazu, Kyoto, Japan). The flow rate

was 1·0 mL min^{-1} and the separation was performed at ambient temperature. The mobile phase composition and the UV detection for each compound were as follows: 8·7 mM H_3PO_4 at 254 nm for aniline; 8·7 mM H_3PO_4:CH_3OH, 95:5 at 254 nm for *p*-toluidine; 8·7 mM H_3PO_4:CH_3OH, 55:45 at 254 nm for acetanilide; 8·7 mM H_3PO_4:CH_3OH, 50:50 at 250 nm for antipyrine; 8·7 mM H_3PO_4:CH_3OH, 80:20 at 250 nm for aminopyrine; 8·7 mM H_3PO_4:CH_3OH, 30:70 at 254 nm for diazepam.

Results and Discussion

Fig. 1 and Table 1 show the structures and the physicochemical properties of compounds used in this study. To compare the permeabilities of non-ionized forms of compounds, compounds with low pK_a values were selected in this study. Further, compounds having mol. wt between 100 and 300 Da were selected to investigate the effect of mol. wt of compounds on the permeation.

Fig. 2 shows the cumulative amount of compounds that penetrated through shed snake skin from their aqueous suspensions. A steady-state penetration was achieved immediately without a lag time for all compounds. Permeability coefficients (P_e) were calculated according to equations from the initial linear portion of the penetration curve.

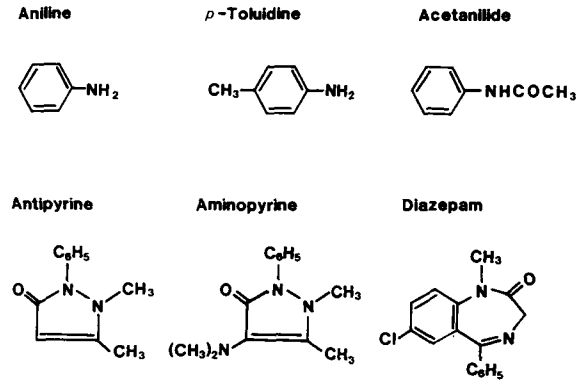

FIG. 1. Structures of cationic drugs.

$$P_e = \frac{dQ/dt}{A\,C_d} \quad (1)$$

where dQ/dt is the slope of the straight portion of the penetration curve, A is the surface area (1·77 cm^2 for the diffusion cells used in the present study), and C_d is the drug solubility in the donor phase. The flux, dQ/dt, and permeability coefficients thus calculated are listed in Table 1.

To compare the physicochemical properties listed in Table 1 with the experimental permeability coefficients, relationships and possible correlations of permeability coefficients of basic compounds against the melting points, partition coefficients and mol. wts are given in Figs 3, 4, and 5, respectively. It is possible that the melting points may reflect hydrophobicities, because that attribute may be associated with a low level of crystallinity. In this study, as shown in Fig. 3, the permeability coefficients of compounds having low melting points exhibited higher values, but a significant relationship between the permeability coefficients and melting points was not observed.

Compounds with high partition coefficients are likely to be the best permeants of the skin. Since there are difficulties in determining the actual skin/water partition coefficients of drugs, octanol/water partition coefficients have been used for ranking the lipophilicities of compounds for skin permeation predictions (Scheuplein 1965; Flynn & Yalkowsky 1972; Roy & Flynn 1989). The relationship between partition coefficient and permeability coefficient shows a distinct biphasic character (Fig. 4), an observation also reported with human skin (Scheuplein & Bronaugh 1983; Roy & Flynn 1989).

Mol. wt dependencies for the diffusion of organic solutes through membranes are well-documented phenomena (Li et al 1987). Since the diffusion of molecules through liquids is inversely proportional to the square root or cubic root of their molecular weights, one might expect higher permeability coefficients to be associated with the lower mol. compounds. In this study, the permeability coefficient decreased with increased mol. wt. up to about 200 Da (Fig. 5) for in the case of compounds with mol. wts greater than 200, the permeability coefficient increased proportionally with an increase in mol. wt, reflecting an increase in partition coefficient. It is possible that the permeabilities of basic compounds may be controlled by the lipophilicity and by the molecular size of the drugs. Itoh et al (1990b) suggested that

Table 1. Physicochemical properties and transport parameters of basic drugs.

	Aniline	*p*-Toluidine	Acetanilide	Antipyrine	Aminopyrine	Diazepam
Mol. wt	93·13	107·15	135·16	188·23	231·30	284·74
pK_a	4·6	5·3	0·6	1·4	5·0	3·4
mp (°C)	(−6)	44·5	114	112	108	125·5
P_{oct}[a]	8·5	25·3	18·5	2·2	8·6	637·2
Solubility[b] (μmol mL^{-1})	547·6	87·0	43·7	3187·6	289·3	0·158
dP/dt[c] (μmol cm^{-2} h^{-1})	8·961 ±0·309	2·895 ±0·154	$0·427 \times 10^{-1}$ $\pm 0·033 \times 10^{-1}$	$0·352 \times 10^{-1}$ $\pm 0·030 \times 10^{-1}$	$0·342 \times 10^{-1}$ $\pm 0·013 \times 10^{-1}$	$0·485 \times 10^{-3}$ $\pm 0·047 \times 10^{-3}$
P_e[d] ($\times 10^{-3}$ cm h^{-1})	16·36 ±0·56	33·3 ±1·77	0·977 ±0·076	0·011 ±0·001	0·118 ±0·043	3·07 ±0·30

[a] Octanol/phosphate buffer (pH 7·0) partition coefficient (P_{oct}) was measured at 32°C. [b] Solubility of basic compounds in the phosphate buffer (pH 7·0) was measured at 32°C. [c] The values of steady-state flux (J) were calculated from the straight lines in Fig. 2. Each value represents the mean ± s.d. (n = 4–6). [d] Permeability coefficient (P_e) was calculated from the steady-state flux and the initial concentration of compound in the donor compartment. Each value represents the mean ± s.d. (n = 4–6).

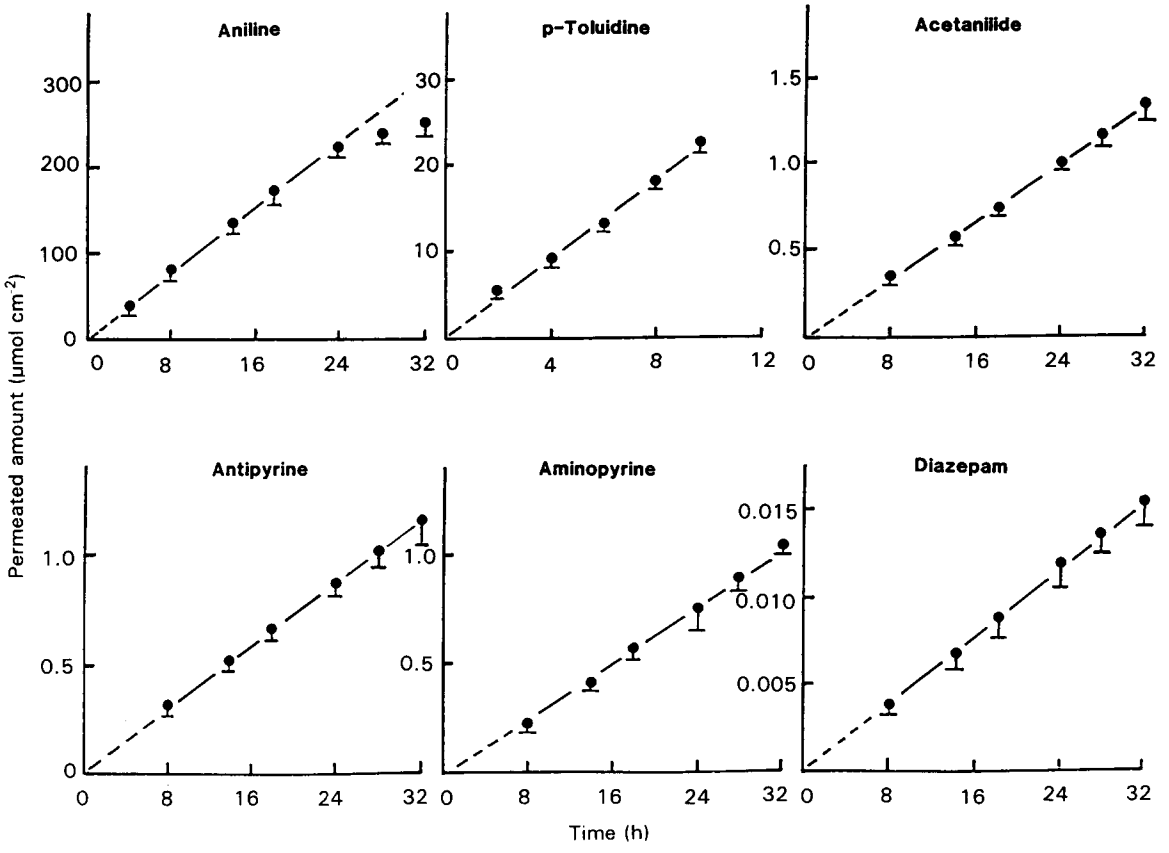

FIG. 2. Time-course penetration profiles of cationic drugs through shed snake skin. One millilitre of aqueous suspension was applied to donor cell at 32°C. Each bar represents s.d. of 4–6 trials.

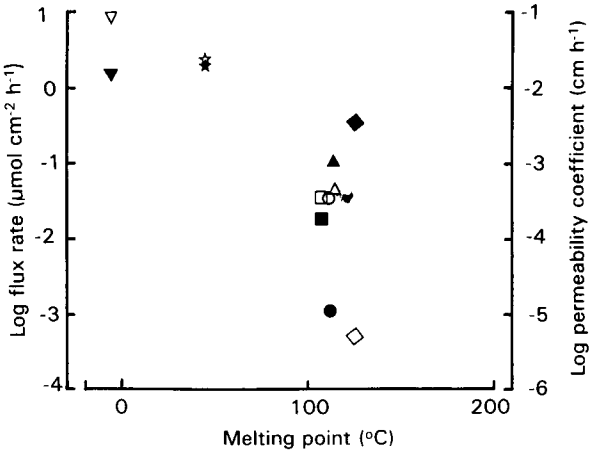

FIG. 3. Relationship between melting point and flux rate (open symbol) or permeability coefficient (closed symbol). Aniline (▽, ▼), *p*-toluidine (☆, ★), acetanilide (△, ▲), antipyrine (○, ●), aminopyrine (□, ■), diazepam (◇, ◆).

the following equations may predict the permeability coefficient from the mol. wt and the partition coefficient of neutral and acidic compounds.

$$\ln(P_e) = a \ln(P_{oct}) + b(\text{mol. wt}) + c \quad (2)$$

$$\ln(P_e) = a' \ln(P_{oct}) + b' \ln(\text{mol. wt}) + c' \quad (3)$$

where P_e is the permeability coefficient (cm h^{-1}), P_{oct} is the octanol/water partition coefficient, and a, a′, b, b′, c, and c′ are regression constants. The present data were also fitted using the above equations. By a least-squares fit of the data, the following values were obtained; a = 1·481, b = −0·040, c = −3·93 with a correlation coefficient of 0·938 for equation 2, a′ = 1·361, b′ = −6·691, c′ = 23·25 with a correlation coefficient of 0·960 for equation 3. The calculated ln(P_e) according to equation 2 was plotted against the observed ln(P_e), which was very similar to the plot between the observed ln(P_e) and the calculated ln(P_e) from equation 3. There is a good agreement between the calculated ln(P_e) and observed ln(P_e) (Fig. 6).

To determine whether these equations are able to predict the permeabilities of basic drugs through human skin, the calculated permeabilities were compared with the reported permeabilities (Roy & Flynn 1989). The same order of permeabilities was observed for morphine, hydromorphone, codeine and pethidine (Table 2). However, one or two higher orders of magnitude of calculated values of the permeabilities compared with the reported values were observed for fentanyl (eqns 2,3) and sufentanil (eqn 3). Fentanyl and sufentanil, which are highly lipophilic compounds (log P > 4), may be underestimated in studies using saline or buffer solution as the receptor fluid because of a low tendency to partition into the receptor medium beneath the skin. Thus, Pozzo et al (1991) suggested that a receptor

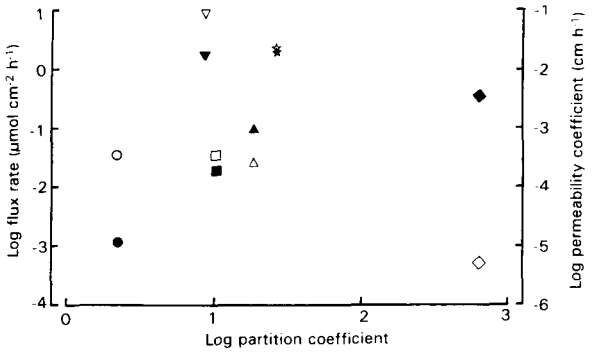

FIG. 4. Relationship between partition coefficient and flux (open symbol) or permeability coefficient (closed symbol). Aniline (▽, ▼), *p*-toluidine (☆, ★), acetanilide (△, ▲), antipyrine (○, ●), aminopyrine (□, ■), diazepam (◇, ◆).

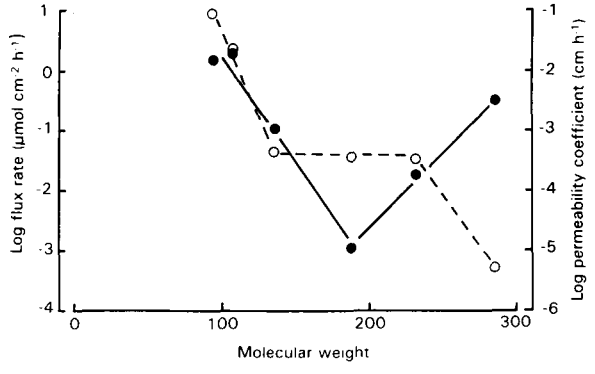

FIG. 5. Relationship between mol. wt and flux (○) or permeability coefficient (●).

medium containing bovine serum albumin is useful in obtaining sink conditions for compounds with log P > 3.

Similar values for a and a′ were reported for neutral and acidic compounds (a = 1·07, a′ = 1·11) (Itoh et al 1990a, b). This indicates that the effect of P_{oct} on the permeation is the same for basic, acidic and neutral compounds. However, somewhat greater values of b and b′ were observed in this study compared with the reported values for neutral and acidic compounds (b = −0·015 and b′ = −3·721). This may

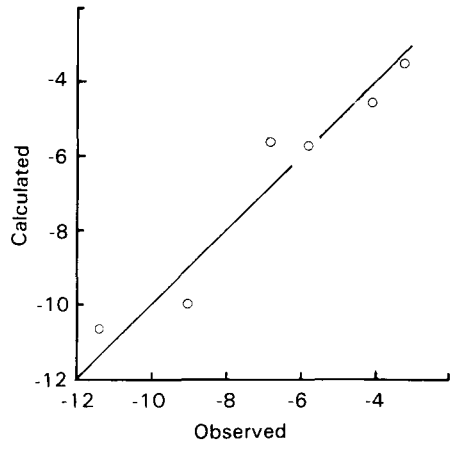

FIG. 6. The observed ln(P_e) vs calculated ln(P_e) according to equation 2. The straight line is for the perfect correlation between the observed and the calculated ln(P_e).

indicate a greater sensitivity to size or may be an artifact due to the relatively small number of compounds tested.

For neutral and acidic compounds (Itoh et al 1990a, b), lag times were not observed with compounds of mol. wt below about 200. However, a lag was observed for compounds of mol. wt above 200. From these results, it is suggested that lag times are affected by molecular size. Percutaneous penetration may occur via an aqueous pathway or a lipoidal pathway (Wester & Maibach 1985). Compounds of small molecular size may penetrate through the aqueous pathway more easily than larger molecules, which more readily penetrate through the lipoidal pathway. In this study, lag times were not observed for any of the compounds tested. The basic compounds appear to penetrate via the same pathways as neutral and acidic compounds. As basic compounds interact with phospholipids in the membrane, it is possible that these basic compounds of large molecular size may rapidly partition into the stratum corneum, eliminating the lag times observed with large acidic and neutral compounds. Further investigations are needed to clarify the detailed mechanism of these phenomena. However, from the results of this study, the prediction of penetration of basic compounds may be possible from the mol. wt and partition coefficient as has been observed earlier for acidic and neutral compounds.

Table 2. Prediction of permeability (P_e) of narcotic analgesics in human skin.

	Mol. wt	P_{oct}[a]	P_e[a] (cm h^{-1})	Calculated P_e	
				Equation 2	Equation 3
Morphine	285·3	5·7	$9·3 \times 10^{-6}$	$2·9 \times 10^{-6}$	$5·0 \times 10^{-6}$
Hydromorphone	285·3	7·7	$1·5 \times 10^{-5}$	$0·4 \times 10^{-5}$	$0·8 \times 10^{-5}$
Codeine	299·3	17·7	$4·9 \times 10^{-5}$	$0·9 \times 10^{-5}$	$1·7 \times 10^{-5}$
Fentanyl	336·5	23390	$5·6 \times 10^{-3}$	$8·3 \times 10^{-2}$	$1·4 \times 10^{-1}$
Sufentanil	387·5	38621	$1·2 \times 10^{-2}$	$2·3 \times 10^{-2}$	$1·1 \times 10^{-1}$
Pethidine	247·0	529	$3·7 \times 10^{-3}$	$1·1 \times 10^{-2}$	$6·2 \times 10^{-3}$

[a] From Roy & Flynn (1989).

References

Bartek, M. J., LaBudde, J. A., Maibach, H. I. (1972) Skin permeability in vivo. Comparison in rat, rabbit, pig and man. J. Invest. Dermatol. 58: 114–123

Bond, J. R., Barry, B. W. (1988) Limitations of hairless mouse skin as a model for in vitro permeation studies through human skin: hydration damage. J. Invest. Dermatol. 90: 486–489

Campbell, P., Watanabe, T., Chandrasekaran, S. K. (1976) Comparison of in vitro skin permeability of scopolamine in rat, rabbit, and man. Fed. Proc. 35: 639

Elias, P. M. (1981) Lipids and the epidermal permeability barrier. Arch. Dermatol. 270: 95–117

Flynn, G. L., Yalkowsky, S. H. (1972) Correlation and prediction of mass transport across membrane I: influence of alkyl chain length on flux-determining properties of barrier and diffusant. J. Pharm. Sci. 61: 838–852

Hatanaka, T., Inuma, M., Sugibayashi, K., Morimoto, Y. (1990) Prediction of skin permeability of drugs. I. Comparison with artificial membrane. Chem. Pharm. Bull. 38: 3452–3459

Hinz, R. S., Hodson, C. D., Lorence, C. R., Guy, R. H. (1989) In vitro penetration: evaluation of the utility of hairless mouse skin. J. Invest. Dermatol. 93: 87–91

Houk, J., Guy, R. H. (1988) Membrane model for skin penetration study. J. Am. Chem. Soc. 88: 455–471

Itoh, T., Xia, J., Magavi, R., Nishihata, T., Rytting, J. H. (1990a) Use of shed snake skin as a model membrane for in vitro percutaneous penetration studies: comparison with human skin. Pharm. Res. 7: 1042–1047

Itoh, T., Magavi, R., Casady, R. L., Nishihata, T., Rytting, J. H. (1990b) A method to predict the percutaneous permeability of various compounds: shed snake skin as a model membrane. Pharm. Res. 7: 1302–1306

Landmann, L. (1979) Keratin formation and barrier mechanisms in the epidermis of *Natrix natrix* (Reptilia: Serpentes): an ultrastructural study. J. Morphol. 162: 93–126

Landmann, L., Stolinski, C., Martin, B. (1981) The permeability barrier in the epidermis of the grass snake during the resting stage of the sloughing cycle. Cell Tissue Res. 215: 369–382

Li, V. H. K., Robinson, J. R., Lee, V. H. L. (1987) Influence of drug properties and routes of drug administration on the design of sustained and controlled release systems. In: Robinson, J. R., Lee, V. H. L. (eds) Controlled Drug Delivery, Fundamentals and Applications. 2nd edn. Marcel Dekker Inc., New York, pp 3–94

Pozzo, A. D., Liggeri, E., Delucca, C., Calabrese, G. (1991) Prediction of skin permeation of highly lipophilic compounds: in vitro model with a modified receptor phase. Int. J. Pharm. 70: 219–223

Rigg, P. C., Barry, B. W. (1990) Shed snake skin and hairless mouse skin as model membranes for human skin during permeation studies. J. Invest. Dermatol. 94: 235–240

Roy, S. D., Flynn, G. L. (1989) Transdermal delivery of narcotic analgesics: comparative permeabilities of narcotic analgesics through human cadaver skin. Pharm. Res. 6: 825–832

Scheuplein, R. J. (1965) Mechanism of percutaneous absorption I. Routes of penetration and the influence of solubility. J. Invest. Dermatol. 47: 344–346

Scheuplein, R. J., Bronaugh, R. L. (1983) Percutaneous absorption. In: Goldsmith, L. A. (ed.) Biochemistry and Physiology of the Skin. Oxford University Press, New York and Oxford, pp 1271–1273

Walker, M., Dugard, P. H., Scott, R. C. (1983) In vitro percutaneous absorption studies: a comparison of human and laboratory species. Hum. Toxicol. 2: 561–565

Wester, R. C., Maibach, H. I. (1985) Structure-activity correlations in percutaneous absorption. In: Bronaugh, R. L., Maibach, H. I. (eds) Percutaneous Absorption. Marcel Dekker Inc., New York and Basel, pp 107–123

Wong, O., Huntington, J., Nishihata, T., Rytting, J. H. (1989) New alkyl *N,N*-dialkyl-substituted amino acetates as transdermal penetration enhancers. Pharm. Res. 6: 286–295