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In Vivo Evaluations of Transdermal Drug Delivery

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I. INTRODUCTION

In the development of a transdermal drug delivery (TDD) system, several crucial questions need to be answered. Most, if not all, of these demands are identified elsewhere in this book. Specifically, for the purposes of this chapter, though, only two basic questions must be addressed:

1. Does the drug proposed for transdermal delivery penetrate skin in vivo and, if so, to what extent?
2. How is a useful indicator of in vivo skin penetration obtained and, hence, how feasible is the transdermal route of drug administration?

Clearly, the answer to the first question may begin to resolve the objective of the second. However, the experimental system chosen to respond to question 2 may, if improperly selected, provide a

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misleading guide to the initial unknown. Thus, the fundamental questions pertaining to in vivo evaluations of TDD are inextricably linked.

What options are available, then, for the estimation of the potential of TDD in vivo? Two obvious, prime, candidates can be identified.

1. Measure skin penetration and subsequent biodisposition in an intact animal model.
2. Evaluate percutaneous absorption directly in human volunteers.

A third, simpler, but perhaps less readily acceptable alternative is:

3. Formulate a biophysical model for TDD and, after validation, use the simulation to predict the feasibility of drug administration via the skin.

Each of these three possibilities will be considered in this chapter. None of the approaches, needless to say, is problem-free, but all, when employed carefully in full knowledge of their likely limitations, can prove instructive and valuable.

As we will indicate, a definitive response to "What is the best method to evaluate TDD in vivo?" cannot, at this time, be made. Eventually, the development of a TDD system requires extensive pharmacokinetic and pharmacodynamic characterization in vivo in humans. These determinations can be performed and have already been documented (1–14) for the TDD devices currently on the market. Nevertheless, it cannot be stated too frequently that, although these systems are being safely and effectively used for therapeutic benefit, our knowledge of the process, by which the delivered drug gains systemic access (viz., percutaneous absorption) is incomplete. Among the unresolved issues, which should be borne in mind when one considers the results of an in vivo experiment, are the following:

1. How does the barrier function of human skin change with increasing age? Despite a number of studies (15–19), which have shown how the permeability of neonate skin differs from that of adults, almost no comparative information is available from the opposite end of the age spectrum. Since TDD is suited to chronic drug therapy, a requirement most often exhibited by elderly patients, the effect of aging on percutaneous absorption is a highly pertinent unknown factor for in vivo evaluation.

2. What is the significance of skin metabolism? An often-cited advantage (20,21) of TDD is that the cutaneous route of drug input avoids the hepatic first-pass effect. However, the ability of the skin to metabolize penetrating compounds is established (22); indeed,

the potential utility of this biotransformation capacity (via a prodrug approach) has attracted significant interest (23). The *extent* of metabolism is not yet quantified, nor is it known whether the skin handles transporting drugs in the same way as the liver; for example, it appears that the 1,2- and 1,3-dinitrate metabolites of nitroglycerin are formed in different ratios depending on the route (sublingual versus topical) of parent drug administration (24). In addition, skin surface microflora are capable of topical drug degradation (25, 26). The occlusive environment beneath a TDD device may be expected to increase the significance of this potential metabolic pathway.

3. Do we understand how penetration enhancers function in vivo? Although our comprehension of absorption promotion is improving and various classes of adjuvant can now be identified (27–29), it remains unclear whether drugs of different physicochemical properties require enhancers of different types. The breadth of applicability of TDD is presently determined by the barrier function of skin. If we can safely, reversibly, and controllably increase cutaneous permeability with suitable formulation components, the possibilities for the dermal route of administration will expand considerably.

4. What is the relationship between a drug's structure and properties and its potential to elicit an irritant or allergic reaction in human skin? The provocation of an inflammatory cutaneous response by an externally applied chemical requires both percutaneous penetration and intradermal pharmacological effect. A large reaction could be caused either by a poorly absorbed but highly potent molecule or by a well-absorbed, low-activity moiety; that is, the magnitude of response does not unequivocally categorize the antagonist. The literature in this field lacks a standardized reporting approach, which makes the anticipation of problems difficult. As discussed elsewhere, structure–activity relationships are required (30), and steps toward their establishment have been initiated.

We now discuss, in turn, three discrete approaches to the in vivo evaluation of TDD: animal models, human experimentation, and kinetic modeling and prediction. Problems considered above, and other difficulties, will be addressed within the context of each attack. The order of the discussion is as stated; at present, in preformulation studies, animal experiments precede human testing. Although, logically, one might consider the theoretical simulation approach to be the initial feasibility test, its relative infancy as a viable means of TDD prediction requires that it be debated last in the sequence as a promising but as yet "experimental" tool.

II. ANIMAL MODELS

Although the most relevant data pertaining to TDD are obtained in humans, this desirable approach is not always possible, since

considerable time and resources are required to conduct a safe and meaningful percutaneous absorption study in man. Consequently, one must be prepared to use an in vivo animal model. Although the range of species employed in previous work is very broad, there is no general agreement as to the "best," or most predictive, model for skin penetration work. Therefore, in addressing the suitability of an animal test system, the investigator should evaluate three basic considerations:

1. How does percutaneous absorption in different animals compare to or rank with that observed in man?
2. Which, if any, of the animal models provides the closest representation of penetration in humans for the widest range of tested compounds?
3. Are there new developments taking place that warrant careful monitoring in the immediate future?

The following discussion aims to respond to these questions. While it may not be possible, at present, to state exactly what should be done, it is suggested that the available literature both provides strong considerations as to those species whose use is questionable, and perhaps misleading, and offers a new approach that has demonstrated significant potential.

A. Rankings

The subject of in vivo animal models for percutaneous absorption has been reviewed recently (31,32). Only a brief discussion will be given here, therefore, with a few illustrative and representative examples. It is first appropriate to list the various species that have been used as experimental test systems either in vivo or in vitro. The following is a comprehensive, but not necessarily complete, compilation: mouse, rat, guinea pig, rabbit; hairless mouse, hairless rat, hairless dog; cat, dog, miniature pig, pig, horse, goat; squirrel monkey, rhesus monkey, chimpanzee (33–40). The diversity is striking and is manifested in the histological appearance of the skins from these different animals. It is clear that there have been studies in which an animal model has been chosen with no consideration whatsoever of the physiological/anatomical relevance of the "model" skin to that of man.

To rank the disparate animal types with respect to their ability to provide an acceptable prediction of in vivo skin absorption in man involves consideration of two groups of studies: (a) those in which percutaneous penetration of one or a few compounds is measured in several species (including human), and (b) those in which absorption of one or more chemicals is compared between the chosen animal model

and man. The former category is obviously labor-intensive and is customarily exemplified by the work of Bartek et al. (34) (Fig. 1). These experiments utilized a standard radiochemical approach (41–46) (see below) for the measurement of percutaneous absorption. The different chemicals were applied to the animals' shaved backs. Each site of administration was protected with a nonocclusive guard. While the results are not sufficiently comprehensive to draw unequivocal conclusions, it is clear that the rat and the rabbit do not offer reliable estimations of human penetration. The pig, on the other hand, appears to provide a reasonable approximation to absorption in man. As a general rule, it may be suggested that animals with high follicular density are less likely to provide good predictions of in vivo transdermal delivery in human subjects. This observation is also supported by in vitro measurements (47,48); furthermore, the preparation of hairy skin for study (e.g., by shaving or depilation) may, in itself, damage the barrier function, resulting in a membrane of unusually low (and nonrepresentative) resistance.

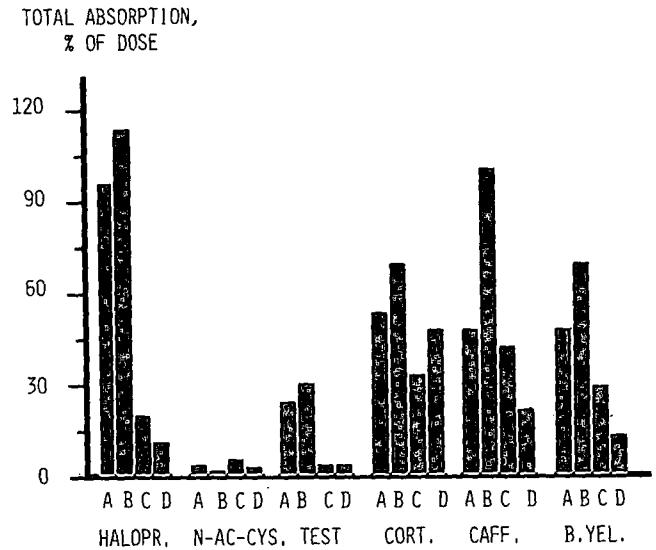


Figure 1 In vivo percutaneous absorption in various animals (A = rat, B = rabbit, C = pig) compared to that in man (D) for haloprogin, *N*-acetylcysteine, testosterone, cortisone, caffeine, and butter yellow. (Adapted from Ref. 34.)

Using the same procedures, Bartek and La Budde (35) later reported on the in vivo absorption of four pesticides in rabbit, pig, squirrel monkey, and man. Once again, penetration in the rabbit was very high (4–10 times greater than man). The squirrel monkey gave values for absorption that were as predictive as those from the pig, indicating that the lower primate may be an alternative representation for man. However, by far the most extensive in vivo comparison between a single animal and man (group b, in the preceding paragraph) has been performed with the rhesus monkey, details of which are now discussed.

B. The Rhesus Monkey

The laboratories of Maibach, Wester, et al. (49–59) have established the rhesus monkey as one of the most frequently cited "good" animal models for in vivo evaluation of TDD in man. As stated earlier, this area of research has been reviewed on a number of occasions (31,32) and only brief recapitulation is warranted here. Again, standard radiotracer methodology has been followed (41–46); the application site has generally been either the forearm or the abdomen. Compound is usually administered after light clipper shaving of the site to be treated. While the rhesus monkey is certainly "hairy," these typical sites of dosing are among the least well covered on the animal's body.

The compounds for which the rhesus–man comparison has been made include hydrocortisone, testosterone, benzoic acid, 2,4-dinitrochlorobenzene (DNCB), nitrobenzene, and a range of hair dyes (60–63). In some cases (hydrocortisone, testosterone, and benzoic acid), the comparisons have been performed following the administration of more than one topical dose (60) (Fig. 2). On the whole, as can be seen, the coincidence between rhesus penetration and percutaneous absorption in man is good. This agreement, as elsewhere, is based on "total" penetration (measured as % ^{14}C dose excreted) in a defined period of time, typically 5 or 7 days. True validation of the animal model requires the kinetic profiles of the chemical disposition to match; regrettably, this information is less often available in the published literature.

The nitroaromatic compounds also showed excellent correlation (61): for DNCB, human absorption was 53.1 ± 6.2% compared to 52.5 ± 4.3% in the rhesus; for nitrobenzene, the corresponding man and monkey values were 1.5 ± 0.3% and 4.2 ± 0.5%, respectively. In addition, impressive coincidence between penetration across human and monkey skin in vitro was demonstrated for the same two compounds and three additional nitroaromatic derivatives (*p*-nitroaniline, 4-amino-2-nitrophenol, and 2-nitro-*p*-phenylenediamine).

Wolfram and Maibach (62,63) measured the in vivo percutaneous absorption of various hair dyes in rhesus and man. Agreement was

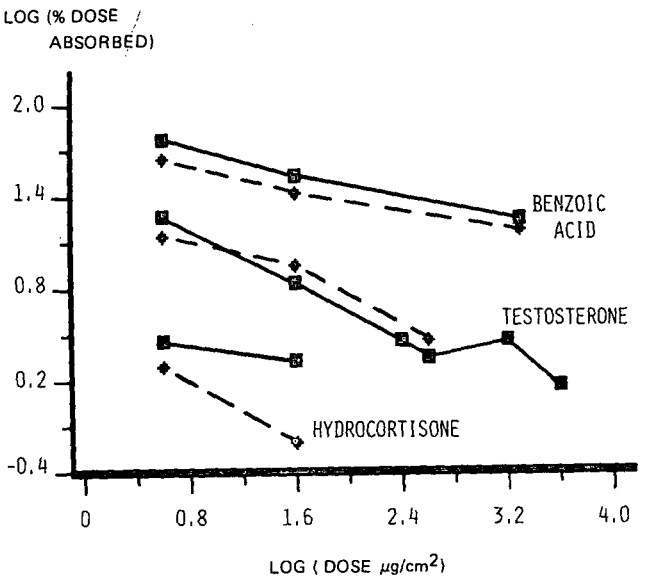


Figure 2 In vivo percutaneous absorption of hydrocortisone, testosterone, and benzoic acid in rhesus monkeys (solid curves) and in humans (dashed curves). (Adapted from Ref. 60.)

very close for resorcinol, *p*-phenylenediamine, and HC Blue No. 1 and was acceptable for 2,4-diaminoanisole and 2-nitro-*p*-phenylenediamine. Excretion half-lives of the topically administered radioactivity were also assessed in man and rhesus and were, on the whole, similar (Table 1). Although the assay procedures used in this work were identical to the studies described earlier in this section, the site of application (the scalp) and the method of chemical administration (working the hair dye lotion into the hair and scalp versus deposition of penetrant in acetone) were distinctly different. These investigations serve, therefore, as positive reinforcement of the potential utility and representative nature of the rhesus monkey as a model for human skin absorption (and, hence, TDD) in vivo.

C. Recent Developments

Despite the persuasive evidence to support the rhesus monkey as an excellent simulator for human percutaneous absorption, other

Table 1 Percutaneous Penetration of Hair Dyes in Rhesus Monkey and in Man[a]

Chemical	Species	% Dose absorbed (±SD)	Urinary excretion half-life (hr)
2,4-Diaminoanisole	Man	0.02 ± 0.01	18
	Rhesus	0.03	20
Resorcinol	Man	0.08 ± 0.03	31
	Rhesus	0.18 ± 0.03	31
4-Amino-2-hydroxytoluene	Man	0.20 ± 0.10	24
p-Phenylenediamine	Man	0.19 ± 0.06	16
	Rhesus	0.18 ± 0.06	22
2-Nitro-*p*-phenylenediamine	Man	0.14 ± 0.04	24
	Rhesus	0.55 ± 0.01	24
4-Amino-2-nitrophenol	Man	0.24 ± 0.08	10
HC Blue No. 1	Man	0.15 ± 0.12	18
	Rhesus	0.13 ± 0.03	40

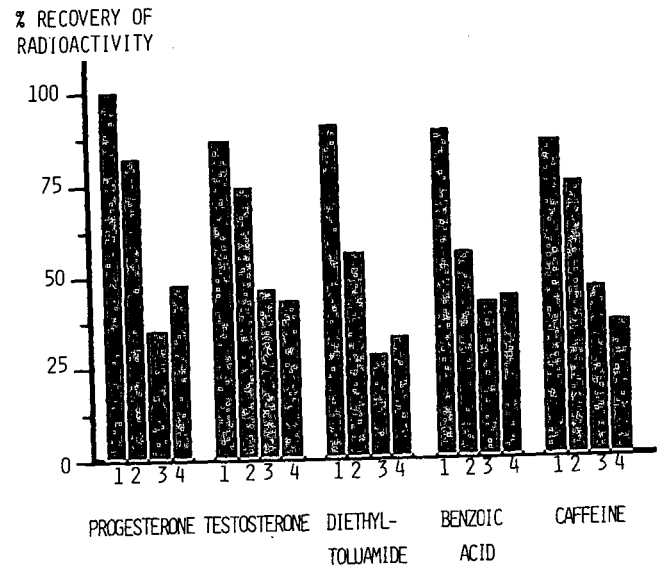
[a]The number of subjects was 2 or 3 for the monkey studies, 3 or 5 in man.
Source: Adapted from Refs. 62 and 63.

alternatives are being continually sought. The cost, relative accessibility, and handling capabilities required for these primate experiments precludes, at this time, the general use of rhesus monkeys. A more recent additional complication centers on the emotional issue of the use of animals in research, in particular the employment of primates and "domestic" species. A detailed and impressive example of the search and identification of other in vivo possibilities has been reported recently by Reifenrath et al. (64,65).

These authors have evaluated four possible models for predicting skin penetration in man: the human-skin-grafted congenitally (nude) mouse ("man–mouse"), the pig-skin-grafted nude mouse ("pig–mouse"), the hairless dog, and the weanling Yorkshire pig. To each model, at

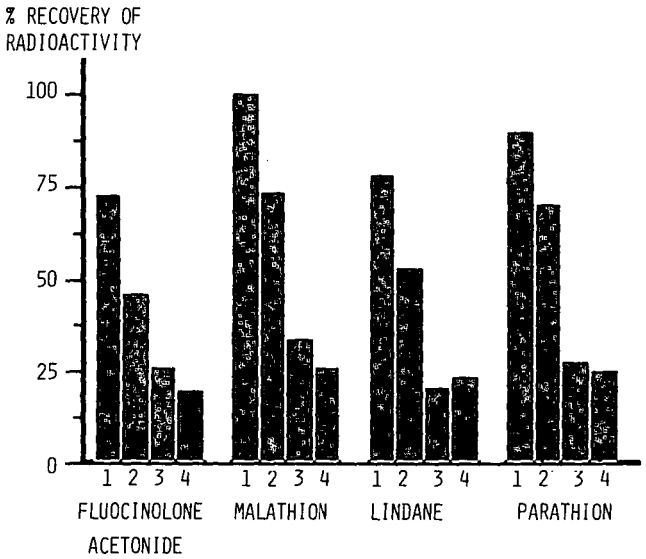
a single dose (4 μg/cm^2), were separately applied nine radiolabeled compounds, which had been previously tested in man. The chemicals were caffeine, benzoic acid, *N*,*N*-diethyl-*m*-toluamide; testosterone, progesterone, fluocinolone acetonide; lindane, parathion, and malathion. Percutaneous absorption was evaluated in the normal way by monitoring the excretion of radioactivity from the animal after dosing. To correct for incomplete excretion, a parenteral dose was also administered and the elimination monitored as before.

The results obtained from the nude mouse experiments are summarized in Figure 3. It can be seen that the mouse itself offers a negligible cutaneous barrier to chemical ingress; absorption for some compounds is almost as high as that seen following subcutaneous injection. The "man–mouse" and "pig–mouse," however, experience much lower penetration and are consistent. These observations can be highlighted by a consideration of Figure 4, which shows the marked



(a)

Figure 3 Absorption of nine chemicals following subcutaneous (1) and topical (2) administration to the nude mouse and topical application to the "man–mouse" (3) and "pig–mouse" (4). (Adapted from Ref. 65.)



(b)

Figure 3 (continued)

change in the stratum corneum of a human-skin-grafted mouse at the border of the xenograft. Whereas the nude mouse has only a minimal horny layer, the human skin demonstrates a particularly well-formed and appreciable keratinized layer.

Comparison between percutaneous absorption in the "man-mouse" with that previously reported in man (42,43,46) (Fig. 5) reveals a reasonably linear correlation, with the grafted animal generally overestimating the human results. The other significant correlation was that between human absorption and penetration in the weanling pig (Fig. 6). The hairless dog and the "pig-mouse" proved to be less representative models for human penetration.

When the percutaneous absorption ratio "man-mouse"/man is plotted as a function of penetrant octanol/water partition coefficient (Fig. 7), the deviation from unity increases with increasing hydrophobicity. It has been suggested that this effect occurs because the xenograft uses split-thickness skin approximately 0.7 mm thick (i.e., skin from which a significant portion of the dermis has been removed). It is conceivable that the graft procedure removes, therefore, part of the skin membrane that controls absorption for more lipophilic

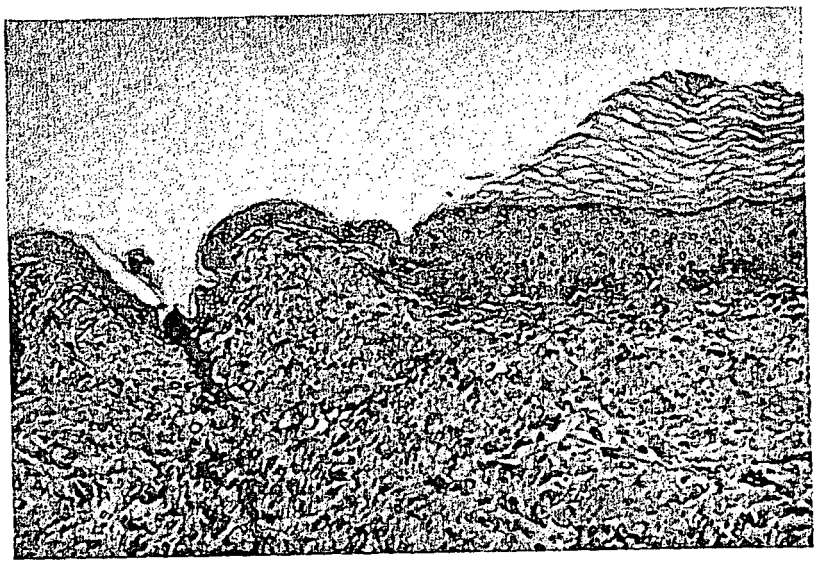


Figure 4 Micrograph of the border of a human skin xenograft onto the nude mouse. The marked difference between the stratum corneum layers is clear. Tissue processing has caused extensive swelling of the human horny layer.

substances. Unfortunately, "control" measurements (using full-thickness grafts) have not been reported because dermatoming of the tissue is necessary to ensure an acceptable surgical success rate (64,65).

D. Conclusions

The available information on in vivo animal models points to some very simple conclusions:

1. Small "hairy" animals (e.g., rat, rabbit) invariably yield penetration values much greater than those seen in man. Their usefulness as predictive models for human in vivo TDD is questionable.
2. The use of the rhesus monkey, if available, appears to be the most reliable and well-validated of the existing models for human skin penetration.

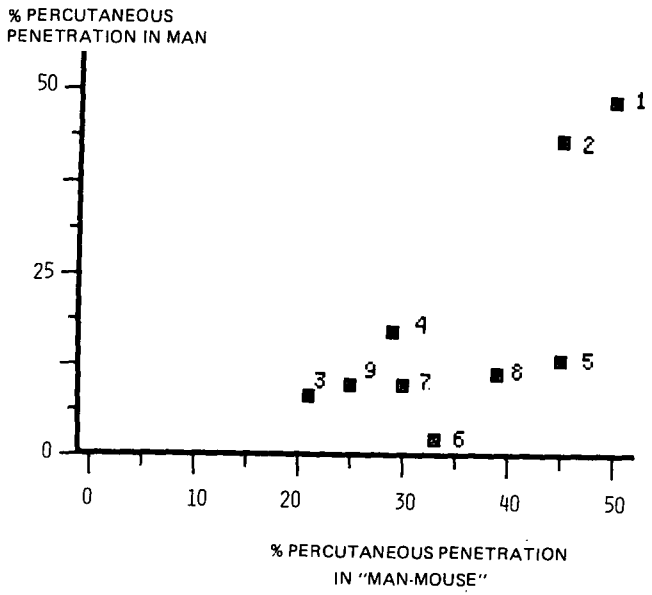


Figure 5 Percutaneous penetration in man (42,43) compared to that in the human-skin-grafted nude mouse ("man–mouse") for caffeine (1), benzoic acid (2), malathion (3), *N,N*-diethyl-*m*-toluamide (4), testosterone (5), fluocinolone acetonide (6), parathion (7), progesterone (8), and lindane (9). (Adapted from Ref. 65.)

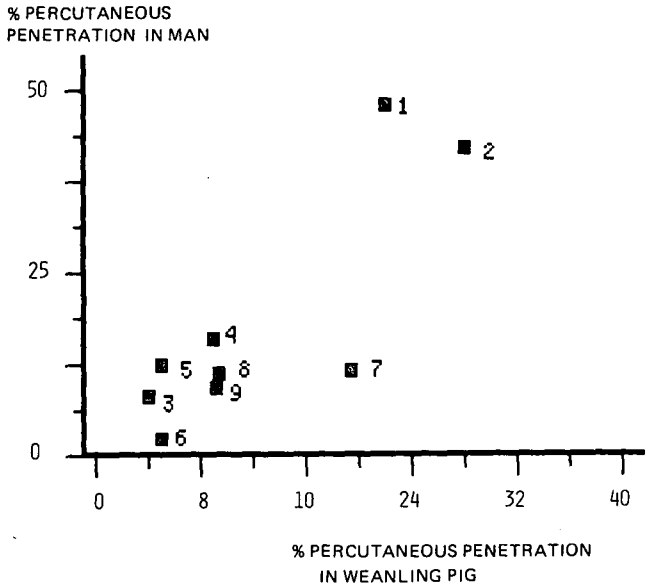


Figure 6 Percutaneous penetration in man (42,43) compared to that in the weanling pig for caffeine (1), benzoic acid (2), malathion (3), *N,N*-diethyl-*m*-toluamide (4), testosterone (5), fluocinolone acetonide (6), parathion (7), progesterone (8), and lindane (9). (Adapted from Ref. 65.)

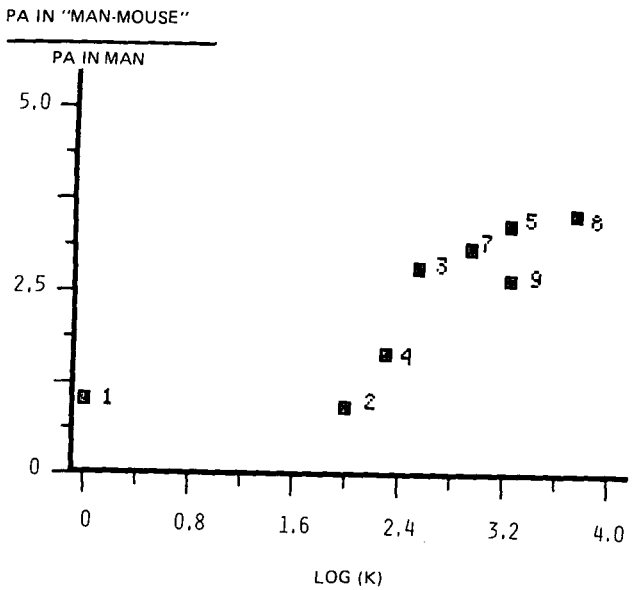


Figure 7 The ratio of percutaneous absorption (PA) in the "man-mouse" to that in man (42,43) plotted as a function of the octanol/water partition coefficient (K) (103) for caffeine (1), benzoic acid (2), malathion (3), *N,N*-diethyl-*m*-toluamide (4), testosterone (5), parathion (7), progesterone (8), and lindane (9). (Adapted from Ref. 64.)

3. The weanling pig (although perhaps not the most convenient and readily available species either) and the human-skin-grafted nude mouse (again, an experimental animal requiring surgical skill and clean laboratory space) offer new, alternative models of much promise and potential.

III. HUMAN EXPERIMENTATION

Eventually, in the development of a TDD system, the drug must be applied to human subjects and pharmacokinetic and pharmacodynamic data must be collected; specific, sensitive assays for the parent drug and any active metabolites will have been developed, and a suitable pharmacological response, which can be quantified, will have been identified.

But does the drug penetrate human skin in vivo? This question will first be asked. Even though in vitro measurements and animal model experiments may be supportive of the likely penetration behavior of the compound in human subjects, a direct demonstration allows the decision to proceed to detailed kinetic and dynamic studies to be made with considerably more confidence. Equally, if several possible agents are under consideration for TDD, a human in vivo evaluation may facilitate the selection of the most promising candidate(s) much more quickly and convincingly than other experimental options.

How, then, can a meaningful in vivo evaluation be performed such that: (a) there is minimal risk to the human volunteers, and (b) predictive results can be generated within a reasonable time? In this section we consider this question in three parts by:

1. Describing the advantages, drawbacks, and predictability of the standard methodology, which has been used for nearly 20 years.
2. Considering two refinements (to the basic approach) that have been reported recently.
3. Examining (again, through studies currently in progress) whether there are age-related changes in skin that alter the barrier function sufficiently and imply, thereby, a closer consideration of subject selection for in vivo studies.

A. Standard In Vivo Methodology

Most of the in vivo human skin penetration data in the literature have been obtained using the procedures first described by Feldmann and Maibach (41–46). As mentioned above, these techniques have also been used in many animal studies. In brief, percutaneous absorption is determined by an indirect method of measuring radioactivity in excreta following topical application of the labeled drug. Generally, ^{14}C has been the radiolabel of choice. Although plasma levels of administered compound are perhaps most desirable, the low levels typically found in the blood after transdermal input preclude the routine use of specific chemical assay procedures. The use of an isotope and quantification in the urine (and feces) allows penetration to be measured following a very small, nonpharmacologically active dose of the chemical. In addition, the approach is applicable to any molecule into which a radiolabel can be incorporated at sufficient specific activity. Hence many more compounds have been evaluated for their in vivo penetration than would have been possible if routine analytical procedures had been sought and used.

Determination of absorption following topical administration in the manner described requires the investigator to know the amount of radioactivity retained in the body, or excreted by routes not assayed.

This necessitates measurement of elimination following parenteral (ideally intravenous) administration of the compound. The percentage of dose absorbed transdermally is then calculated from Eq. (1).

$$\% \text{ dose absorbed} = \frac{\text{total radioactivity excreted after topical administration}}{\text{total radioactivity excreted after intravenous administration}} \times 100 \quad (1)$$

Obvious limitations are inherent in this simple methodology:

1. The radioactivity detected in the excreta is a mixture of parent chemical and metabolites.
2. The origin of the metabolites (e.g., that fraction generated in the skin during transdermal passage) is unknown.
3. Sophisticated kinetic analysis of the data on excretion rate versus time is therefore precluded.

From a practical standpoint, the procedure requires good subject compliance because excretion measurements must be made over several (typically 5–7) days. Most experiments have been performed with the application site unprotected and nonoccluded. In the published literature, reporting on this technique, therefore, there are no examples for which a successful mass balance between applied dose and (corrected) excretion levels has been possible.

However, one must qualify these criticisms by asking, What would be the state of our knowledge in the absence of the in vivo evaluations that have used this technique? The answer, quite simply, is: very limited. There have been a few studies in which chemical assays have been performed in blood and urine following topical administration, but the number is very small. Furthermore, the doses were of a therapeutic magnitude and the analysis procedures were invariably already in place. On the other hand, the radiotracer procedure has permitted a large human in vivo database to be established on a wide range of penetrants (e.g., steroids, pesticides, cosmetics, hair dyes) (42,43,46,61–63). This information, while subject to the foregoing limitations, currently offers the "benchmark" evaluation of skin penetration in man. Despite some recent provocative and challenging remarks (66) concerning the relative usefulness of in vitro over in vivo measurements of percutaneous absorption, there remain too many ill-defined parameters in in vitro methodology (67) for the arguments to be generally persuasive. Thus, although the standard procedure is certainly not without limitation, it has been, and continues to be, immensely valuable. Nevertheless, improvements are possible and unknowns persist; the remainder of this section will consider possible refinements and one important, and immediate, unresolved issue.

B. Refinements

1. *"Reservoir" Technique*

In three recently published papers (68–70), Rougier, Dupuis, and their colleagues have presented evidence (obtained in both hairless rat and man) for a relationship between so-called stratum corneum reservoir function and in vivo percutaneous absorption. The initial study (68) in the hairless rat compared (a) the total body distribution after 96 hr of 10 substances, having a wide range of physicochemical properties, following a 30 min-topical application in a 95:5 v/v ethanol/water vehicle, with (b) the corresponding amount of the same substances that could be found in the stratum corneum by tape-stripping after, again, a 30-min topical exposure. The penetrants were labeled with ^{14}C or ^{3}H and were analyzed by liquid scintillation counting. In terms of penetration, it was found that there was a factor of 50 between the best penetrant (benzoic acid) and the least absorbed compound (dexamethasone) (see Table 2). Comparison of these values with the levels measurable in the stratum corneum demonstrated high correlation (Fig. 8). Thus, a major implication of the work was that the simple, short exposure procedure followed by immediate tape-stripping would provide a facile prediction of in vivo penetration over a longer period.

A logical sequential step was then performed: evaluation of the technique in man (69). Following identical procedures, the experiment was conducted in human subjects using four different doses of benzoic acid delivered in an ethylene glycol vehicle containing 10% Triton-X-100. For comparative purposes, parallel control measurements were also made in the hairless rat. Benzoic acid was chosen because it penetrates human skin well and is almost totally excreted in the urine, thereby facilitating the determination of % dose absorbed. The results are summarized in Figure 9, which contains three important features. First, penetration in the rat is approximately twice that in man. Second, the stratum corneum content of benzoic acid in man after a 30 min-exposure is linearly related to the amount found to penetrate over the subsequent 4 days. Third, the linear relationship between these quantities, previously determined from the absorption of 10 very different substances in the hairless rat (68), is not significantly different from that which holds in man for benzoic acid over a 10-fold range in applied concentration. The results of this second study support, therefore, the initial conclusion obtained in the hairless rat and suggest, in addition, a commonality between predictive relationships in an animal model (the hairless rat) and man.

The most recent publication (70) from the same laboratory showed that there is no unique feature to the previously exclusive use of a 30-min skin contact time. In the hairless rat, using benzoic acid,

Table 2 Percutaneous Absorption Assessed by the "Reservoir" Technique[a]

Chemical[b]	Total penetration 96 hr after topical application (nmol/cm^2 ± SD)	Amount in stratum corneum 0.5 hr after application (nmol/cm^2 ± SD)
Benzoic acid	26.6 ± 0.7	17.6 ± 1.5
Benzoic acid[c]	79.3 ± 3.6	48.1 ± 5.1
Acetylsalicylic acid	6.7 ± 0.7	5.2 ± 0.6
Dihydroepiandrosterone	5.3 ± 0.2	2.0 ± 0.4
Sodium salicylate	5.0 ± 0.7	3.9 ± 0.3
Testosterone	4.3 ± 0.5	2.1 ± 0.2
Caffeine	3.7 ± 0.3	2.8 ± 0.3
Thiourea	3.2 ± 0.5	2.6 ± 0.3
D-Mannitol	2.6 ± 0.2	2.2 ± 0.4
Hydrocortisone	0.9 ± 0.1	0.9 ± 0.2
Dexamethasone	0.6 ± 0.1	0.4 ± 0.0

[a]Six animals were used per chemical.
[b]Topical dose was 200 nmol/cm^2 except as noted in note c.
[c]Dose = 450 nmol/cm^2.
Source: Adapted from Ref. 68.

nicotinic acid, aspirin, and theophylline, linear relationships between penetration, recovery, and application time have been established (Table 3). Exposure periods of 0.5, 2, 4, and 6 hr were investigated. The correlation between 4-day penetration and 30-min stratum corneum levels for the four compounds studied was not significantly different from those reported before for various compounds in the rat and for benzoic acid at different applied doses in man (68,69).

The persuasive nature of these combined results is indisputable. The apparent generality of the observations, and the coincidence between the data in hairless rat with those in man, offer well-validated predictive potential. However, limitations of the approach do exist and require discussion. First, the procedure yields a single time-point measurement that can provide only an estimation of total absorption: kinetic details of the rate and extent of absorption are

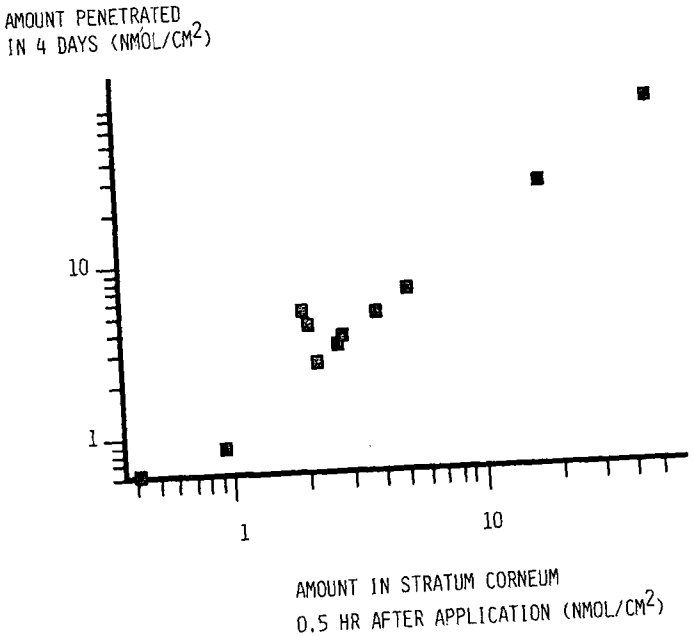


Figure 8 Total percutaneous penetration (nmol/cm^2) 4 days after topical application compared to amount (nmol/cm^2) in stratum corneum 30 min after administration for the compounds listed in Table 2. Linear regression of the data yields: $y = 1.64x - 0.5$, $r = 0.998$. (Adapted from Ref. 68.)

lost. Next, the technique is somewhat invasive, requiring stratum corneum removal by the tape-stripping process. Third, with only a 30-min application time, validation of the procedure has required the administration of large doses of radioactivity (so that absorption can be quantified by later tissue and body fluids analysis), which are probably unacceptable for routine studies in man. In addition, the brevity of the application period amplifies the uncertainty associated with the prediction estimation on the basis of the small difference between two large numbers. Modifications to the technique that would alleviate some of these shortcomings may be envisaged and an example follows. Nevertheless, in overall conclusion, it may be suggested that these experiments warrant cautious optimism and represent an encouraging advance.

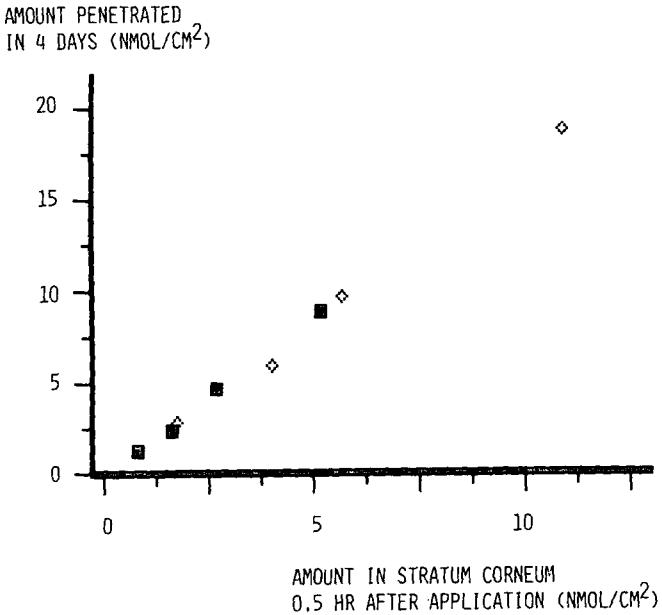


Figure 9 Correlation between total penetration of benzoic acid after 4 days with amounts recovered in the stratum corneum 30 min after application at 4 doses (125, 250, 500, and 1000 nmol/cm^2) in the rat (diamonds) and man (squares). Linear regression of the data points yields: $y = 1.83x - 0.52$, $r = 0.998$. (Adapted from Ref. 69.)

2. "Mass Balance" Technique

Recently, in our laboratory, a modification of the standard Feldmann and Maibach (41–46) in vivo approach has been explored. The procedure developed addresses some of the perceived "reservoir technique" limitations identified above. Experiments have so far been conducted with three steroidal penetrants: testosterone, estradiol, and hydrocortisone. The ^{14}C-labeled chemicals have been applied in acetone to the ventral forearm of male volunteers. Chemical and radioactivity doses were 4 μg/cm^2 and 1 μCi/cm^2, respectively; the area of application was 2.5 cm^2. After evaporation of the vehicle, the application site was covered with a polypropylene Hilltop chamber, which was affixed to the skin with adhesive tape.

Table 3 Influence of Application Time on the Assessment of Percutaneous Absorption by the "Reservoir" Technique[a]

Chemical[b]	Application time (hr)	Total penetration 96 hr after topical application (nmol/cm^2 ± SD)	Amount in stratum corneum 0.5 hr after application (nmol/cm^2 ± SD)
Theophylline	0.5	1.9 ± 0.1	2.5 ± 0.3
	2	7.0 ± 1.4	
	4	12.3 ± 1.2	
	6	25.1 ± 4.8	
Aspirin	0.5	8.1 ± 1.7	5.2 ± 0.6
	2	25.7 ± 1.9	
	4	53.6 ± 2.4	
	6	82.7 ± 14.0	
Nicotinic acid	0.5	15.7 ± 2.5	9.7 ± 0.9
	2	44.8 ± 11.7	
	4	123.5 ± 25.5	
	6	180.0 ± 61.5	
Benzoic acid	0.5	18.8 ± 2.6	10.5 ± 1.0
	2	78.6 ± 5.2	
	4	145.3 ± 12.7	
	6	185.2 ± 22.6	

[a]Five hairless rats were used per time point.
[b]Dose = 1000 nmol/cm^2.
Source: Adapted from Ref. 70.

The subjects collected their urine for 7 days postapplication; four fractions were collected on day 1 (0–4, 4–8, 8–12, and 12–24 hr), one per day, thereafter. After 24 hr, the occlusive chamber was removed and submitted to liquid scintillation counting. The application site was washed with a standardized procedure (soap solution, water, soap solution, water, water) and all washings were collected and assayed for residual surface chemical. For the remaining 6 days of the study, the administration site was again covered with a (new) chamber. An essentially identical protocol has also been performed following a multiple dosing regimen (71): daily topical doses of the same three compounds were administered over a 14-day period. The first and eighth doses were ^{14}C-labeled and urinary excretion for 7 days after each of the "hot" doses was followed. In this case, the 24-hr washing procedure was also performed daily, and a new chamber was provided on each occasion.

The results of these preliminary experiments are summarized in Figure 10, which plots the disposition of the ^{14}C-labeled chemical doses as a function of penetrant log (octanol/water) partition coefficient ($K_{O/W}$). It was found that the data from the single- and multiple-dose studies were indistinguishable and they have, therefore, been pooled. Surface wash recoveries for the multiple-administration regimen represent only those collected at 24 hr post-^{14}C administration; negligible radioactivity was present in the washings obtained on days 3–8 and 10–14. The data, as a whole, show that: (a) the sequestration of chemical by the polypropylene chamber is relatively constant and does not appear to be sensitive to $K_{O/W}$, (b) 24-hr surface wash recovery decreases linearly with increasing log $K_{O/W}$, (c) percutaneous absorption, determined as total (corrected) ^{14}C-urinary excretion level, increases linearly with increasing log $K_{O/W}$, (d) there appears to be a significant inverse correlation between surface wash recovery and percutaneous absorption, and (e) there is excellent mass balance between applied dose and postadministration disposition.

We believe, therefore, that this in vivo refinement is advantageous. The benign nature of the covering device and its apparent lack of specificity in binding are useful attributes. The ability to achieve mass balance in this type of experiment is an important improvement. The potential predictability of the surface wash measurement for percutaneous absorption and the correlation of these parameters with a physicochemical parameter of the penetrant is promising. Radiolabeled drug administration remains necessary, and kinetic data will be obtainable only if several small doses are applied at t = 0 and then removed at various times thereafter. Also, thus far, occlusion of the application site has been used. While this may be the most relevant situation for TDD, it will be interesting to observe whether the procedure is as effective if the dosing site is protected

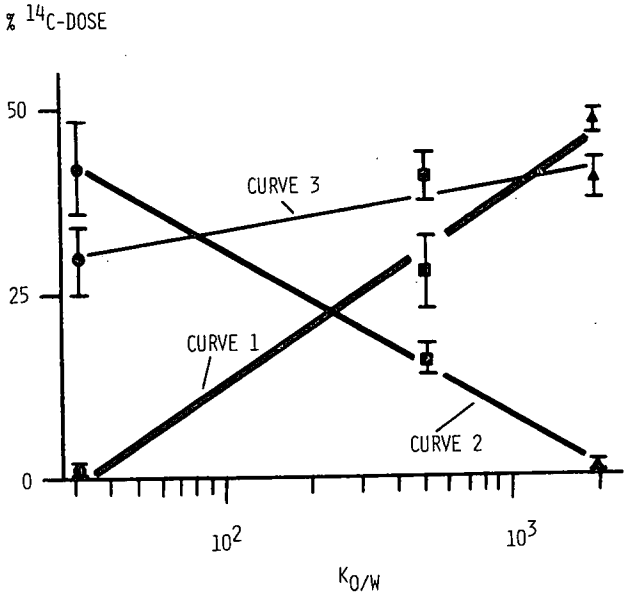


Figure 10 Disposition of testosterone (triangles), estradiol (squares), and hydrocortisone (circles), in vivo in man after topical application: curve 1, percutaneous absorption; curve 2, amount removed by washing; curve 3, chemical sequestered on polypropylene chamber. (Data presented at A. Ph. A. Academy of Pharmaceutical Sciences 39th National Meeting, Minneapolis, October, 1985, by D. A. W. Bucks, J. R. McMaster, H. I. Maibach, and R. H. Guy.)

rather than enclosed (e.g., with a ventilated chamber). It seems reasonable to speculate, however, that further validation of this approach with a wider variety and increased number of chemical species may establish a significantly improved technique for in vivo percutaneous absorption evaluation.

C. Effect of Aging on Skin Penetration

That human skin changes dramatically with increasing age is patently obvious on visual inspection. Physiological and biochemical changes within the tissue have been identified and investigated (72). However, we do not know whether, or how, the penetration barrier of the skin changes in the aged. At the opposite end of the age

spectrum (i.e., in the neonate) some experimental data exist and have been recently reviewed (15–19). On the other hand, for the elderly, who represent the subject population most likely to take advantage of the benefits of TDD (long-term, chronic therapy offering excellent compliance and reduced side effects), the question of altered barrier function has been minimally studied.

The few investigations, which have been performed in vivo in man or in vitro on excised human skin, have produced inconsistent conclusions. Percutaneous absorption in vivo has been reported to be lower in aged subjects (73,74), yet, in vitro, penetration through skin excised from elderly cadavers was significantly higher than that through stratum corneum removed from young donors (73). Transepidermal water loss does not appear to change with age in adulthood (75). The role of microcirculation clearance as an integral part of percutaneous absorption has been investigated because of the perceived increasing comprimisation of dermal perfusion with advancing age (76,77). Removal rates of intradermally injected materials have been found to both increase and decrease as a function of age with no clear consensus evolving to explain the disparate observations. All too often, work in this field has been characterized by small subject populations, poorly defined procedural techniques, and experiment selections that preclude straightforward comparisons and unambiguous conclusions. A more detailed review of the literature has recently been compiled (78).

Presently, in our laboratory, a preliminary in vivo, percutaneous absorption study in two elderly populations ("young-old," 65–75 years; "old-old," > 75 years) has been initiated. Four "model" penetrants (benzoic acid, hydrocortisone, estradiol, and testosterone), which demonstrate a wide range of penetrability characteristics and physicochemical properties, have been considered. Control data on "young" adults (18–35 years) have been published (42,43).

Using the standard radiochemical approach described earlier (41–46), the results collected in Table 4 have been obtained. For testosterone and estradiol, the two most lipophilic compounds, penetration in the three subject groups is essentially equivalent. In contrast, for hydrocortisone and benzoic acid, the less lipophilic and more water-soluble chemicals, absorption is significantly less in both elderly cohorts than in the young "controls." Thus, there appears to be good reason to suspect that percutaneous absorption does change with age and that the effect is sensitive to the physicochemical properties of the penetrant.

Why should the barrier function change toward compounds that are more hydrophilic than lipophilic? It is known that aged stratum corneum is considerably dryer than the young adult horny layer and that it contains a lower lipid content (72,75). A reduced presence of water implies that aged skin provides a less attractive environment

Table 4 Comparison of Percutaneous Absorption in "Young" and "Aged" Human Subjects[a]

Chemical	log K[a]	Cumulative % dose excreted in 5 days		
		Young	Young-old	Old-old
Testosterone[b]	3.31	13.2 ± 3.0 (n = 17)	10.6 ± 5.7 (n = 8)	15.2 ± 8.4 (n = 7)
Estradiol[b]	2.70	10.6 ± 4.9 (n = 3)	11.5 ± 3.5 (n = 6)	9.0 ± 5.6 (n = 6)
Hydrocortisone[c]	1.93	1.87 ± 1.6 (n = 15)	0.67 ± 0.58 (n = 8)	0.86 ± 0.50 (n = 9)
Benzoic acid[c]	1.87	42.6 ± 16.5 (n = 6)	27.5 ± 11.6 (n = 8)	23.1 ± 7.0 (n = 9)

[a]K = octanol/water partition coefficient (103).
[b]Penetration in the aged cohorts was not significantly different from that in the "young" group ($p > 0.05$).
[c]Penetration in the aged cohorts was significantly less than that in the "young" group ($p < 0.05$ or better).
Source: K. V. Roskos, R. S. Hinz, H. I. Maibach, and R. H. Guy, presented at the APhA Academy of Pharmaceutical Sciences 39th National Meeting, Minneapolis, October, 1985.

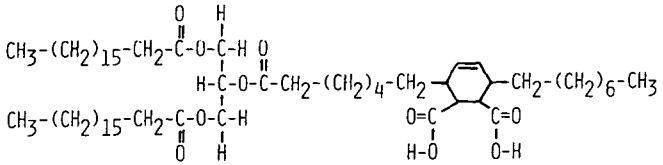


Figure 11 Chemical structure of the triglyceride Glyceridacid 100 (79–81).

to less lipophilic moieties. The diminished lipid content provides a reduced dissolution medium for chemicals administered to the skin surface. Such a change, one might predict, would differentiate against the absorption of compounds of relatively low lipid solubility—that is, agents with smaller rather than higher oil/water partition coefficients, namely, hydrocortisone and benzoic acid. A lowered water content and a smaller lipid capacity may not, however, affect stratum corneum uptake and passage of highly lipophilic molecules such as testosterone and estradiol.

Substantiation of these conclusions awaits further experiments with an expanded range of penetrants. In addition, close attention should be paid to recent investigations into a unique triglyceride skin softener (79–81) (Fig. 11). This agent shows a marked ability to improve the smoothness of the skin even at low water content and has potential, therefore, as a treatment for the relief of xerosis or dry skin in the elderly. While the mechanism of action remains speculative, X-ray data imply an important effect on the lipid structure within the horny layer. Specifically, a stabilization of the lamellar arrangement of intercellular lipids is supported.

Thus, probing the barrier function at both macroscopic and molecular levels as a function of age is now realistic. With the changing demographic pattern of Western civilization and the increasing awareness of the potential benefits that TDD can provide to elderly patients, the results of these rather new avenues of investigation are particularly important and pertinent to future geriatric chemotherapy.

IV. KINETIC MODELING OF TRANSDERMAL DRUG DELIVERY

The objective of model development in TDD is to provide a predictive tool that may be used prospectively, at an early stage, in the screening of candidate drugs. A suitable simulation does not need to be exactly quantitative, nor is it reasonable to expect that exact

coincidence between prediction and in vivo results can be routinely obtained. However, an acceptable model should identify high-priority compounds and should be able to indicate how "borderline" chemicals may be formulated to enable efficacious delivery via the skin.

The simplest theoretical calculation involves application of the steady-state mass balance equation:

$$IN = Cl \cdot c_{ss} \tag{2}$$

where IN (μg/hr) is the TDD input rate, Cl (cm^3/hr) is the drug clearance, and c_{ss} (μg/ml) is the target steady-state plasma concentration. If, for example, the TDD device was a "membrane-controlled" system (1–6,9,11–14), from which the drug was released with a zero-order kinetic rate constant k^0 (μg/cm^2/hr), then

$$IN = A \cdot k^0 \tag{3}$$

where A is the area of the patch.

Equations (2) and (3) can be used to determine whether a feasible IN can be accomplished. For example, for the antihypertensive drug clonidine, a suitable target c_{ss} would be 0.75 ng/ml and the literature reports a clearance of about 1.2 L/hr for a 70-kg individual. It follows that:

$$IN = (1.2 \times 10^3)\ (0.75)\ \text{ng/hr} = 9\ \mu\text{g/hr}$$

Hence, a 5-cm^2 device programmed to release clonidine at a little less than 2 μg/cm^2/hr should provide a steady, pharmacologically efficacious level of drug. Such a system has indeed been developed (11–13), approved by the FDA, and is now marketed as Catapres-TTS by Boehringer-Ingelheim.

However, the predictive approach based on a steady-state calculation is deficient in two ways. First, the predicted $A \cdot k^0$ product may not be feasible; that is, either an impossibly large area or input rate may be necessary to achieve the desired c_{ss}. Second, the calculation provides no indication of the time required to attain the target c_{ss}. For transdermal delivery, a classic estimation of this time based on the drug's biological half-life will often be inappropriate because percutaneous absorption is usually slower than the elimination process [i.e., "flip-flop" kinetics (82) pertain].

To resolve these difficulties, it is necessary to model the entire time course of transdermal delivery and, thus, it is essential to describe theoretically the kinetics of percutaneous absorption. As a first step, we identify the key physical events involved in the TDD process:

1. Transport within the delivery system
2. Drug partitioning from the device into the stratum corneum
3. Transport across the stratum corneum
4. Drug partitioning from the lipophilic stratum corneum into the (much more aqueous) viable epidermis
5. Transport across the viable epidermis
6. Uptake by the cutaneous capillaries (epidermal–dermal junction) and systemic distribution

Admittedly, this list does not include all possible interactions between drug and skin (83,84), but it does incorporate all the transport and partitioning steps necessary to move the drug from the device into the bloodstream. Transport (or diffusion) and partitioning are the key words here and must influence significantly the form of any effective model for TDD. The most rigorous approach to simulating this sequence of steps would involve, minimally, solution of Fick's second law of diffusion, with appropriate boundary conditions, for the device, the stratum corneum, and the viable epidermis. While this is theoretically feasible and has, in fact, been performed to various levels of sophistication (85–92), a complete treatment is mathematically complex, may require numerical solutions to the differential equations, and loses, as a result, a degree of insight and biophysical relevance.

An alternative approach involves the application of linear kinetics (93–102). While this methodology lacks rigorous purity, it does offer, we believe, an acceptable first approximation and it retains important physicochemical significance. Additionally, the model permits important, but experimentally inaccessible or intractable, questions to be addressed theoretically. Currently, the simulation has developed to the extent represented schematically in Figure 12. Details have been described fully in the literature (93,96–98,101,102) and only a brief recapitulation is given here. Input kinetics from the device are described by $f(k_{IN})$: for a so-called membrane-controlled patch, such as the clonidine-containing Catapres-TTS (11–13) (Fig. 13), $f(k_{IN})$ consists of a first-order component (k^1) accounting for drug release from the contact adhesive and a zero-order contribution (k^0) representing the membrane-determined flux of drug from the reservoir (1). The parameter k_r reflects the fact that there will be competition for drug between the patch and the stratum corneum. A well-designed patch will minimize k_r. However, it is clear that k_r will, for example, become progressively more significant for drugs of relatively low lipophilicity (log $K \leq 0$). Drug transport across stratum corneum and viable epidermis is characterized by two first-order rate constants, k_1 and k_2, respectively. These parameters are proportional to the corresponding diffusion coefficients across the two tissue layers and can be estimated from the drug molecular weight (M) via Eq. (4):

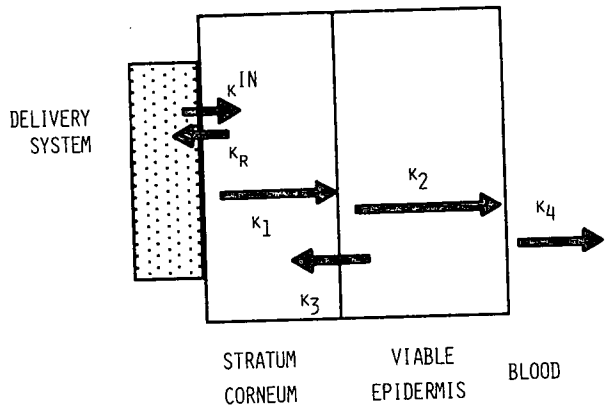


Figure 12 Kinetic model for transdermal drug delivery and percutaneous absorption (20,97,98,101,102).

$$k_i = C_i M^{-1/3} \qquad (i = 1, 2) \tag{4}$$

where C_i are known constants (97). The k_3 parameter reflects the relative rapidity with which the drug partitions from the stratum corneum to the viable tissue. Thus, for very lipophilic compounds, k_3 will be large and the transfer from stratum corneum to viable epidermis will be slow. The ratio k_3/k_2 measures, as a result, an "effective" stratum corneum/viable tissue partition coefficient for the drug and it has been empirically shown [by fitting in vivo percutaneous absorption data (97,98)] that

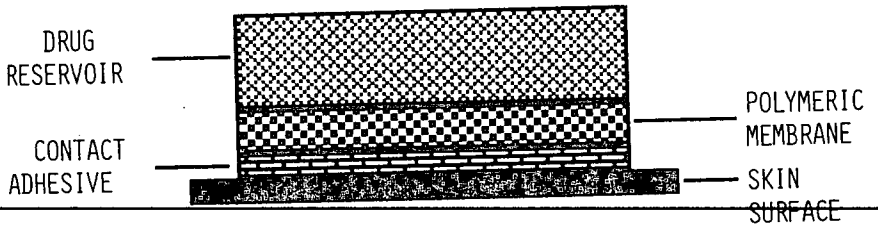


Figure 13 Schematic diagram of a membrane-moderated transdermal drug delivery system (9,20).

$$k_3/k_2 = K/5 \quad (5)$$

where K is the octanol/water partition coefficient of the drug. The empiricism of Eq. (5) should be emphasized—there is no a priori reason why a correlation of k_3/k_2 with K should exist. We suspect that other oil/water partition coefficients may be more appropriate; however, there is, of course, a very large database of log K (103), and this is the major reason for its use in this predictive approach. Last, k_4 (or a more complex function, if necessary) describes the drug's clearance from the systemic circulation. A prediction of k_4 is not possible; it must be measured following intravenous administration of the compound.

A series of differential equations describes the kinetics of the transdermal absorption process represented by Figure 12. These rate equations are easily solved to give the following expression for the drug concentration in blood (C_p) as a function of time (t) (98, 101,102):

$$C_p = f(k^I) + f(k^0) \quad (6)$$

where

$$f(k^I) = \frac{Mk^I k_1 k_2}{V}\left[\frac{\exp(-\alpha t)}{(\beta-\alpha)(\alpha-\omega)(\beta-\mu)} + \frac{\exp(-\beta t)}{(\alpha-\beta)(\beta-\omega)(\beta-\mu)} + \frac{\exp(-\omega t)}{(\alpha-\omega)(\omega-\beta)(\omega-\mu)} + \frac{\exp(-\mu t)}{(\alpha-\mu)(\mu-\beta)(\mu-\omega)}\right] \quad (7)$$

and

$$f(k^0) = \frac{Ak^0 k_1 k_2}{V}\left[\frac{1}{\alpha\beta\varepsilon} - \frac{\exp(-\alpha t)}{\alpha(\alpha-\beta)(\alpha-\varepsilon)} - \frac{\exp(-\beta t)}{\beta(\beta-\alpha)(\beta-\varepsilon)} - \frac{\exp(-\varepsilon t)}{\varepsilon(\varepsilon-\alpha)(\varepsilon-\beta)}\right] \quad (8)$$

Equations (7) and (8) describe, respectively, the first-order and zero-order contributions to transdermal input from a device of the type illustrated in Figure 13, and

M = amount of drug in the "priming" contact adhesive

A = surface area of the device

$\omega\mu = k^I k_1$; $(\omega + \mu) = k^I + k_r + k_1$

$\alpha\beta = k_2 k_4$; $(\alpha + \beta) = k_2 + k_3 + k_4$

$\varepsilon = k_1 + k_r$

Predictions of the C_p versus t profile following transdermal delivery have been successfully performed for nitroglycerin and clonidine using appropriate values for the parameters required (Table 5) (101,102). These simulations have been discussed extensively in previous publications (20,101,102), and Figure 14 summarizes the correlation between theory and human in vivo experiment. The agreement is good and shows that physicochemical characterization of transcutaneous kinetics, in conjunction with device release rate(s) and limited pharmacokinetic data, can be employed successfully to model and to predict TDD. More recently (104–106), plasma levels of estradiol, scopolamine, and timolol following TDD have been published, and the kinetic model has again been able to simulate the results in a self-consistent fashion (107). Figure 15 illustrates these latest analyses using estradiol as an example.

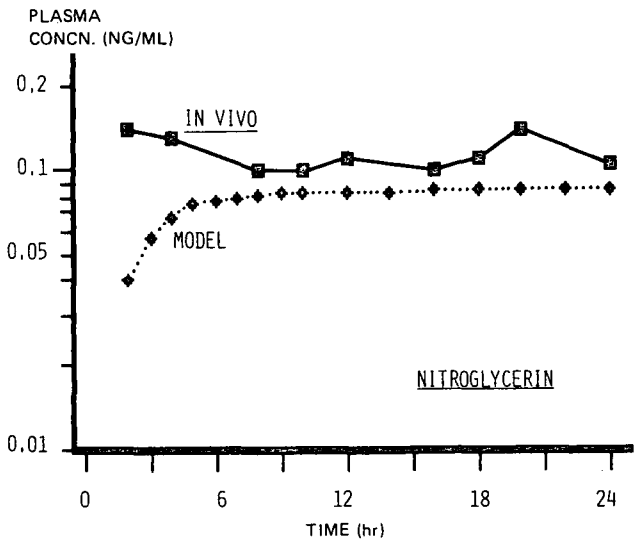
A secondary aim of an effective model for TDD is to permit examination of experimentally inaccessible or poorly defined variables of the percutaneous absorption process. Two examples, in particular, may be cited: (a) the impact of cutaneous metabolism on the systemic availability of transdermally delivered drugs, and (b) the desirable properties required of putative penetration enhancers as a function of the physicochemical properties of the delivered drug.

The first case has been explored both for drug biotransformation by cutaneous enzymes within the viable epidermis (108) and for drug degradation on the skin surface by microorganisms (109). The former situation requires that the degree of effect be explored by assuming a range of metabolic transformation rate constants (108). These parameters, however, have not been quantified experimentally and the significance of the theoretical findings awaits, therefore, suitable accurate measurements of enzyme activity in skin. The latter situation has been addressed specifically for nitroglycerin making use of microbial degradation kinetics of the drug determined in culture (109). The simulations show that microorganisms on the skin surface elicit their maximal effect on drug delivered to the skin with first-order kinetics either from the contact adhesive of a transdermal delivery system or from a thinly spread film of topical ointment. The result for TDD is to delay the attainment of the target steady-state plasma concentration, an effect that can be circumvented by increasing the magnitude of the "loading" dose contained within the patch adhesive. Experimentally, in rhesus monkeys, a cutaneous "first-pass effect" of about 16–21% has been demonstrated for nitroglycerin (110). Given the sensitivity of nitroglycerin to metabolism, one might speculate that the magnitude of this effect reflects a maximum value. However,

Table 5 Pharmacokinetic and Physicochemical Parameters Utilized by the Kinetic Model (20,97,98,101,102) to Calculate the Predicted Curves in Figures 14 and 15 for Nitroglycerin, Clonidine, and Estradiol

	Nitroglycerin[a]	Clonidine[b]	Estradiol[c]
Molecular weight	227.1	230.1	272.4
log K	2.05	0.83	2.49
Half-life (hr)	0.04	8.5	0.05
V (L)	231	147	4.81
A (cm^2)	10	5	20
M (mg)	2	0.47	0.1
k^0 ($\mu g/cm^2/hr$)	36	1.6	0.21
k^I (hr^{-1})	1.3	1.3	1.3
k_r (hr^{-1})	10^{-4}	10^{-4}	10^{-4}
k_1 (hr^{-1})	0.15	0.15	0.14
k_2 (hr^{-1})	2.36	2.35	2.22
k_3 (hr^{-1})	53	3.2	222
k_4 (hr^{-1})	18.2	0.08	13.9

[a]Parameters from Ref. 102.
[b]Parameters from Ref. 101.
[c]Parameters from Ref. 107.

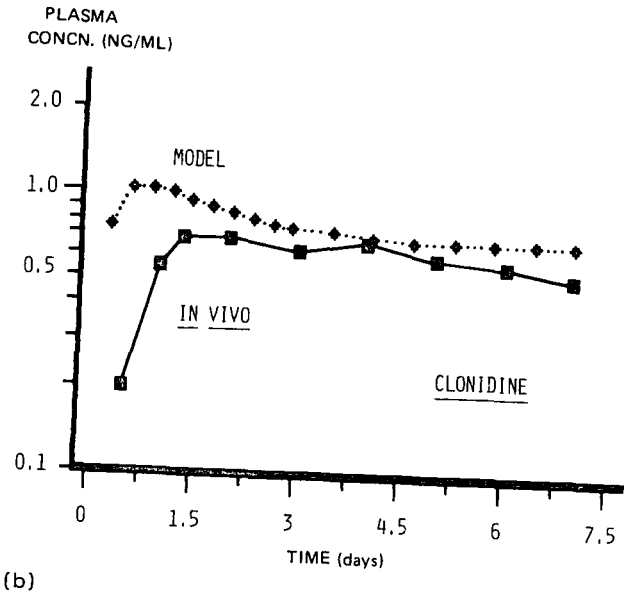


(a)

Figure 14 Comparison between theoretical predictions of the kinetic model (Fig. 12) (101,102) with in vivo plasma concentration versus time profiles for nitroglycerin and clonidine following transdermal delivery (9,11). The parameters used to calculate the simulated curves are given in Table 5. Time is measured in hours (a) and in days (b).

this hypothesis remains essentially untested, and the location of nitroglycerin metabolism (surface versus epidermal) is unknown. Considerably more work in this area is required, therefore, before the full significance of the model predictions can be assessed and acted upon.

The second case, the effect of penetration enhancers on TDD, has recently been examined by using the kinetic simulation to probe the action of "model" promoters as a function of the physicochemical properties of the penetrant (111). Two prospective enhancers, which act specifically in skin, have been considered: the first (PE1) increases the drug diffusion coefficient (D_S) across the stratum corneum by 10-fold; the second (PE2) increases D_S by the same amount but also reduces the effective stratum corneum/viable tissue partition coefficient of the drug by an order of magnitude from its



(b)

Figure 14 (continued)

unperturbed value. Enhancer PE1 acts specifically to reduce the diffusional resistance of the stratum corneum, therefore, while PE2 demonstrates an additional, more subtle, role by which the lipophilic nature of the horny layer is somewhat masked. In terms of the model (Fig. 12), PE1 increases k_1 by 10-fold, PE2 again raises k_1 by a factor of 10 but also reduces k_3/k_2 by an order of magnitude.

Figure 16 shows that the action of these parameters is sensitive to the oil/water partitioning characteristics of the drug. We consider two drug molecules (D1 and D2) being delivered from a 10-cm^2 transdermal device that releases the active species with zero-order kinetics at 20 $\mu g/cm^2/hr$. The drugs have identical molecular weights (250), biological half-lives (0.5 hr), and volumes of distribution (100 L). They differ only in their respective octanol/water partition coefficient (K):

D1, log K = 1 D2, log K = 3

The simulations presented in Figure 16 show that PE1 is effective for the relatively hydrophilic drug D1 but is ineffectual in promoting

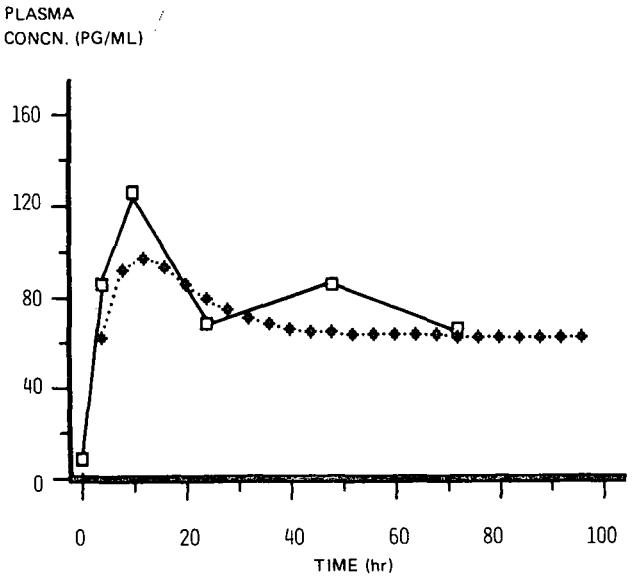
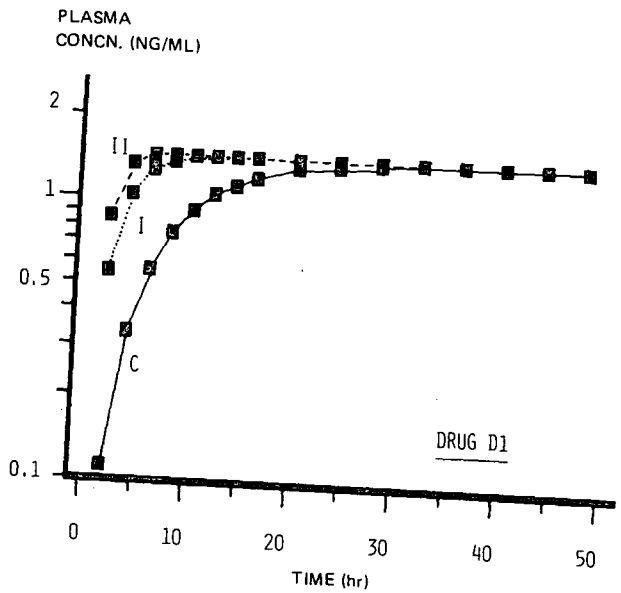


Figure 15 Theoretical prediction (diamonds) of the plasma levels of estradiol as a function of time (107) following transdermal delivery compared to in vivo data (squares) (104). The parameters used to calculate the simulations are listed in Table 5.

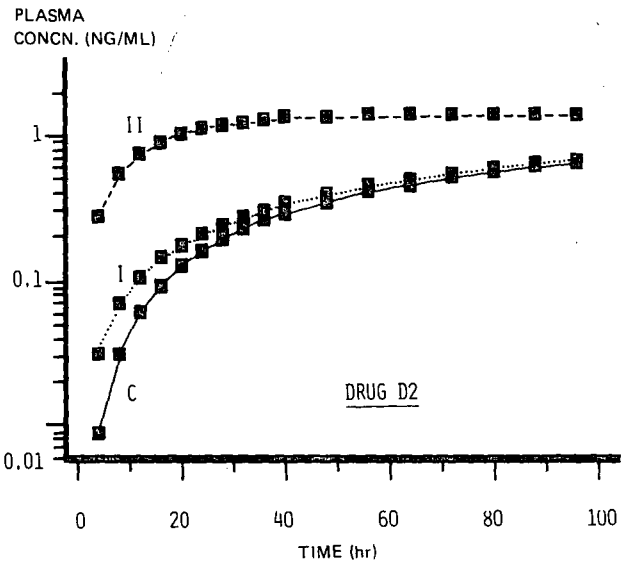
penetration of the more lipophilic agent D2. The reverse differentiation is shown by PE2, which elicits minimal action on D1 but significantly enhances absorption of D2. It is apparent, therefore, that the desirable properties of a penetration enhancer may change depending on the physicochemical nature of the drug being delivered. For a relatively hydrophilic drug (log $K \leq 1-1.5$), it is important for the enhancer to lower the diffusional resistance of the stratum corneum. For more hydrophobic compounds (log $K \leq 2$), however, the enhancer must, in some way, promote the rate of stratum corneum to viable tissue partitioning. In this case, speeding the rate of stratum corneum diffusion is less important because transfer of drug *out* of the horny layer is the slowest step in the absorption process.

At the present time, the mechanisms of action of the various agents proposed as penetration enhancers are not well defined (27–29). The search for more effective promoters, therefore, lacks an obvious rationale. We suggest that theoretical investigations of the type discussed here may permit a more logical approach to the understanding



(a)

Figure 16 The predicted effects of two "model" percutaneous penetration enhancers (PE1 and PE2) on the plasma concentration versus time profiles of two drugs (D1 and D2) delivered transdermally (111). Curves C are the controls (no enhancer), curves I and II are those resulting from transdermal delivery in the presence of PE1 and PE2, respectively.



(b)

Figure 16 (continued)

and identification of compounds that can effectively and controllably facilitate percutaneous absorption.

V. SUMMARY

The in vivo evaluation of TDD represents a crucial stage in the development of a therapeutic device. Design of the appropriate test experiment and utilization of a relevant experimental system are of fundamental importance, therefore. The objective of this chapter has been to identify methodologies for assessing TDD in vivo and to elucidate the procedures that may prove most informative and indicative of potential success. There have been useful recent advances in the approaches to measure percutaneous absorption in vivo, and these procedures should be given careful attention when planning for TDD evaluation. The advantages and limitations of various animal models are now more clearly defined and, while there remains no "ideal" system, there is now no excuse for choosing a poorly representative

species. Finally, our comprehension of the basic physicochemical and biological criteria, which determine the rate of drug passage across the skin, is becoming more sophisticated. We believe that in the foreseeable future it will be increasingly possible to define chemical structure/skin penetration relationships. Hence, we envisage the achievement of reliable predictions of TDD in vivo, from basic physical chemical and pharmacokinetic information.

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