Permeation of Buprenorphine and Its 3-Alkyl-Ester Prodrugs Through Human Skin

Audra L. Stinchcomb,[1,2] Anupam Paliwal,[1] Rajesh Dua,[1] Hirofumi Imoto,[1,3] Ronald W. Woodard,[1] and Gordon L. Flynn[1,4]

Received February 7, 1996; accepted July 25, 1996

Purpose. Homologous 3-alkyl-ester prodrugs (C_2 to C_4) of buprenorphine with decreased crystallinity have been synthesized and evaluated for transdermal delivery commensurate with opioid dependence treatment.

Methods. To assess the influence of derivatization on delivery, the permeation of the prodrugs through human skin was determined *in vitro*. Prodrug metabolism was measured in human blood and skin supernatant *in vitro* along with chemical hydrolysis controls. The prodrugs' octanol/water partition coefficients were measured.

Results. Without exception, the prodrugs were completely hydrolyzed on passing through the skin and appeared as buprenorphine in the receptor compartment. However, using saturation conditions, in no instance did the buprenorphine flux through skin from a prodrug solution exceed the flux of buprenorphine base itself *in vitro*. Moreover, the flux of the acetyl ester, the least hydrophobic of the prodrugs, was not significantly elevated upon stripping the skin. Whether in blood or the skin supernatant, the prodrugs hydrolyzed in an apparent first-order fashion and rate constants and half-lives were calculated.

Conclusions. We conclude from the results that the prodrugs' very high octanol/water partition coefficients (hydrophobicity) placed them in viable tissue layer controlled diffusion. Consequently, one does not derive the potential flux-increasing benefit of reducing crystallinity that was expected.

KEY WORDS: buprenorphine; transdermal delivery; prodrugs; cutaneous metabolism; partition coefficients.

INTRODUCTION

Improved pharmacotherapies for opioid addiction treatment are urgently needed to maintain opioid addicts in treatment and away from high-risk tuberculosis (TB) and HIV/AIDS transmission behavior associated with the intravenous abuse of this class of drugs (1,2). Buprenorphine, a synthetic partial agonist-antagonist opioid, has proven clinically effective in reducing illicit opioid use and maintaining patients in treatment when it is given sublingually at a high dose of 8 mg per day (3). Buprenorphine is also an analgesic in its own right and has been used i.v. and i.m. in human therapy at one-fourth or less of this dose for some time (4). Interestingly, all its present dosage forms are associated with rapid systemic delivery. A means of administration which meters the drug slowly into the body, smoothing out blood levels and obviating serum highs, is desirable. From this standpoint, a transdermal delivery system seems an ideal way to administer buprenorphine. Some researchers have suggested that a penetration enhancer formulated with buprenorphine·HCl can achieve a desired systemic analgesic effect (5). However, buprenorphine's high level of crystallinity as reflected in the melting point of its free base, 218°C, makes it unlikely it can be delivered transdermally at the dose required for opioid maintenance therapy. Prodrugs of buprenorphine having less crystallinity might be effective, however, and we have synthesized derivatives (Figure 1) with this reasoning in mind.

Previous studies showed the straight-chain 3-alkyl-ester prodrugs' decreased crystallinities and increased oil (hexane) solubilities as compared to the parent compound, buprenorphine (6). The branched-chain 3-alkyl-ester prodrug, isobutyl derivative, melts 106°C lower and is 250-fold more soluble in oil (hexane) than buprenorphine.

This study includes the homologous 3-alkyl-ester (C_2 to C_4) prodrugs' partition coefficients, hydrolysis rates and abilities to deliver buprenorphine through human skin *in vitro* as part of an evaluation of a transdermal buprenorphine prodrug approach for opioid dependence treatment.

MATERIALS AND METHODS

Materials

Buprenorphine base was purchased from AKZO (Diosynth), The Netherlands. The alkyl esters were synthesized directly from buprenorphine as previously described (6). Importantly, high levels of purity (>98%) were assured through elemental analysis (C, H, N), ^{1}H-NMR spectroscopy, HPLC and by the sharpness of melting points. Reagent-grade chemicals from Sigma Chemical Co. (St. Louis, MO) were used as received.

Chromatographic Procedure

Buprenorphine and its prodrugs were assayed by HPLC (Beckman) using UV detection at 215 nm. A C_8 Brownlee OSS Spheri-5 micron cartridge (220 × 4.6 mm) with a guard column was used. The mobile phase consisted of 85% acetonitrile:methanol (7:3) and 15% 0.01 M phosphate buffer at pH 5. The flow rate was set at 1.5 ml/min. Fifty microliters of sample were injected by a Perkin-Elmer ISS-100 autosampler. Standard curves exhibited excellent linearity over the entire concentration range employed in the assays.

Skin Preparation and Permeation Methods

Skin excised during abdominal reduction surgery from one patient was used for the skin permeation studies. Skin samples were harvested from the abdomen using a Padgett dermatome set to 250 μm; skin samples were frozen as received at −20°C for a maximum of four months. Frozen skin samples were thawed and used at the 250μm split-thickness for the diffusion studies. For one experiment, the skin was stripped repeatedly (35 times) with Scotch tape (3M, Minneapolis, MN) to effectively remove the entire stratum corneum (7). Successive fresh

[1] College of Pharmacy, The University of Michigan, Ann Arbor, Michigan 48109.
[2] Present address: School of Pharmacy, University of California, San Francisco, California 94143-0446.
[3] Present address: Sumitomo Pharmaceuticals Research Center, 3–45 Kurakakiuchi 1-Chome, Ibaraki-Shi, Osaka 567, Japan.
[4] To whom correspondence should be addressed.

0724-8741/96/1000-1519$09.50/0

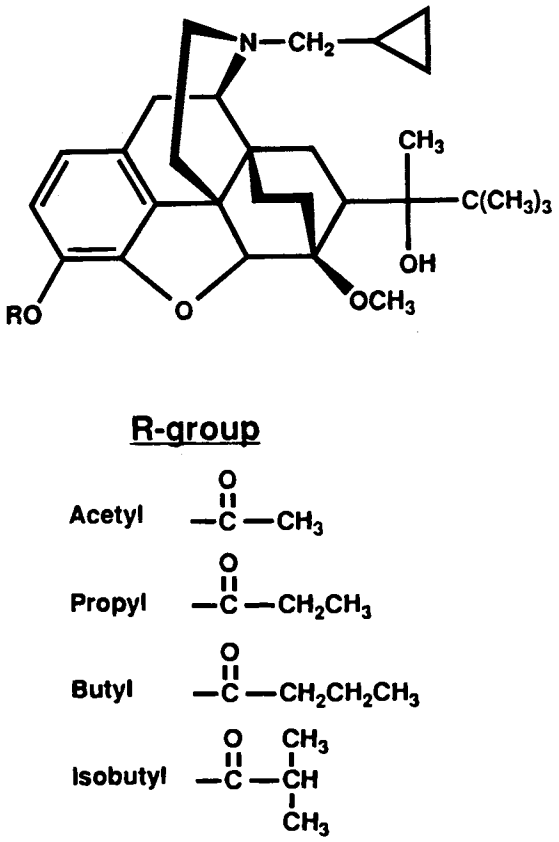

Fig. 1. Structures of buprenorphine and its alkyl esters.

pieces of tape were placed firmly against the skin surface and then peeled away. A standard five milliliter Franz cell was used for the skin permeation studies. The receptor fluid was isotonic phosphate buffer at pH 6.4. The full receiver volume was withdrawn as the sample and replaced with fresh 37°C buffer at predetermined intervals to help maintain sink conditions. The receiver samples were stored at 4°C until HPLC assay. The temperature of the diffusion cells was maintained at 37°C with a circulating water bath. The diffusion experiment was initiated by charging the donor compartment with 0.3 ml of drug suspended in light mineral oil (the drug suspension was shaken overnight to first attain saturation). The solubilities in mineral oil should be more than sufficient to keep low vehicle concentrations themselves from influencing the rate. In fact, as shown in Table III, the prodrugs' mineral oil solubilities are fifteen to sixty-six-fold higher than buprenorphine's mineral oil solubility (8).

Data Analysis

The permeation data were plotted as the cumulative amount of drug collected in the receptor compartment as a function of time. The flux value for a given run was calculated from Fick's First Law of diffusion:

$$\frac{1}{A}\left(\frac{dM}{dt}\right) = J_s = P\Delta C \qquad (1)$$

In this equation, J_S is the flux in the steady state in $\mu g/cm^2 \cdot h$; A is the area of the membrane, 0.785 cm^2; P is the effective permeability coefficient in cm/h; and ΔC is the concentration gradient across the membrane. Since build up in the receiver cell was kept to a minimum throughout the studies, the latter is well approximated by the donor concentration.

Kinetics of Prodrug Enzymatic Degradation in Skin Supernatant

The skin removed during abdominal reduction surgery from three patients was harvested as described in the skin permeation methods. It was frozen as received at −20°C for a maximum of four months. Frozen skin samples were thawed and used at the 250 μm split-thickness for the skin kinetics experiments.

An esterase-rich supernatant was created by solubilizing the esterases from the three patients' split-thickness skin in Dulbecco's Modified Phosphate Buffered Saline (9) (1:5, tissue weight:buffer volume) for four hours at 37°C. This procedure works well because of the high aqueous solubility of esterase enzymes. Three ml of the supernatant was decanted into each of four capped test tubes. The experiment was initiated by adding 1.5 μg of one of the prodrugs (either the acetyl, propyl, butyl, or isobutyl ester) dissolved in acetonitrile into the four separate tubes of supernatant at 37°C. No protein precipitation was observed at this concentration of acetonitrile (<1.2%). Each tube was vortexed for thirty seconds and 0.5 ml of the solution was added to 0.5 ml of chilled acetonitrile to quench the reaction as well as extract the compounds of interest and provide the zero time point for the experiment. The remaining solution was maintained at 37°C in a water bath. The reaction was monitored by withdrawing samples from the reaction mixture at various times, quenching the reaction as previously described, and assaying them. Because of intrinsic chemical instability of the esters, controls (no enzymes) were also monitored for the experiment duration to provide information about the non-enzymatic stability of the prodrugs. The samples were prepared for HPLC analysis by vortexing them for thirty seconds and then centrifuging them at 3300*g* for eight minutes. This experiment was repeated twice and carried out to four half-lives of degradation.

Kinetics of Prodrug Enzymatic Degradation in Blood

Whole blood containing heparin was obtained from one healthy human volunteer. One-third of the fresh blood was immediately used for the experiment, the other two-thirds was frozen at −20°C for eleven (experiment 2) or twelve (experiment 3) days. The experiment was initiated by adding sixty μg of one of the prodrugs (either the propyl, butyl, or isobutyl ester) dissolved in 50 μl of acetonitrile to three separate tubes of ten ml each warmed (37°C) whole blood. No protein precipitation was observed at this concentration of acetonitrile. Each tube was vortexed for thirty seconds, whereupon one ml of the solution was added to two ml of chilled acetonitrile to quench the reaction and extract the compounds of interest. This provided the zero time point for the experiment. Control reactions carried out simply in buffer under the same conditions were also monitored for the experimental duration to provide information about the non-enzymatic stability of the prodrugs. Samples were withdrawn from the blood-containing medium at various times and the reaction quenching procedure was repeated. The

samples were each vortexed for thirty seconds and the proteins were allowed to settle. The supernatant was analyzed directly by HPLC. The experiments were carried out to three half-lives of degradation.

Octanol/Buffer Partition Coefficients

Isotonic phosphate buffer pH = 6.4 and octanol were added to a large separatory funnel and shaken to obtain cosaturation of the phases. Further phase equilibrium was allowed by setting aside the funnel overnight. All steps were carried out at room temperature. An appropriate amount of drug was weighed into a vial and dissolved in a known amount of the water-saturated octanol. An equivalent amount of octanol-saturated buffer was then added to the vial and the phases shaken for thirty hours on a Vibrax. The vials were left undisturbed for at least one hour after shaking. The aqueous phase, final pH 6.25, was analyzed directly by HPLC. An exact volume was taken from the octanol phase and diluted with an appropriate amount of acetonitrile, and then analyzed by HPLC. Partition coefficients were calculated by determining the phase concentration ratios.

RESULTS

Skin Flux of Buprenorphine Base and Four Prodrugs

Plots of the cumulative amounts of buprenorphine permeated over time from saturated mineral oil solutions of either buprenorphine base or one of its prodrugs were constructed for data analysis (Figure 2). Lag times and steady-state fluxes calculated using the terminal, linear portions of the curves are summarized in Table I. The slopes through these regions, equal to J_S for a run, were determined using linear regression analysis. In all cases, the coefficients of determination for the lines were ≥ 0.98. The lag times reported in Table I were determined by extrapolating the steady-state curves to the X-axis, as shown in Figure 2. Donor solutions were analyzed at the end of the diffusion experiments for prodrug content; in all cases these solutions contained no less than 98% of intact prodrug. However, without exception the prodrugs were completely hydrolyzed on passing through the skin and appeared as buprenorphine in the receptor compartment. Buprenorphine base and the acetyl prodrug solutions had the highest fluxes of buprenorphine, while the other three prodrugs had fluxes approximately five times less.

Since it is possible that in the permeation experiments enzymatic hydrolysis was mediated by enzymes leached from the skin and into the receiver compartment, the chemical stability of the prodrugs in the receiver compartment was checked in independent experiments. The maximum amount of conversion of all the prodrugs to buprenorphine in the receptor compartment buffer at 37°C in the presence of skin was 26.4% in 19.5 hours (at least twice as long as the average sampling time), corresponding to a half-life of about 44 hours. Therefore, this enzyme leaching can not explain the one-hundred percent prodrug conversion seen in the flux experiments.

Cumulative amounts of buprenorphine permeated from acetyl prodrug saturated mineral oil solutions through stripped and normal human skin were also plotted as a function of time. The lag times and steady-state fluxes calculated using the terminal, linear portions of the curves from these plots are also summarized in Table I. The prodrugs were completely hydrolyzed on passing through the skin and appeared as buprenorphine in the receptor compartment. No statistically significant difference ($p > 0.05$) in the permeation of buprenorphine from the acetyl prodrug solutions through intact and stripped human skin was observed by t-test comparison.

Kinetics of Prodrug Enzymatic Degradation in Skin Supernatant

When solutions of the buprenorphine esters were exposed to leached enzymes, the prodrugs disappeared in an apparent first-order fashion, therefore plots were made of the natural logarithm of concentration depletion as a function of time. The observed rate constants and their corresponding half-lives calculated using the curves from these plots are summarized

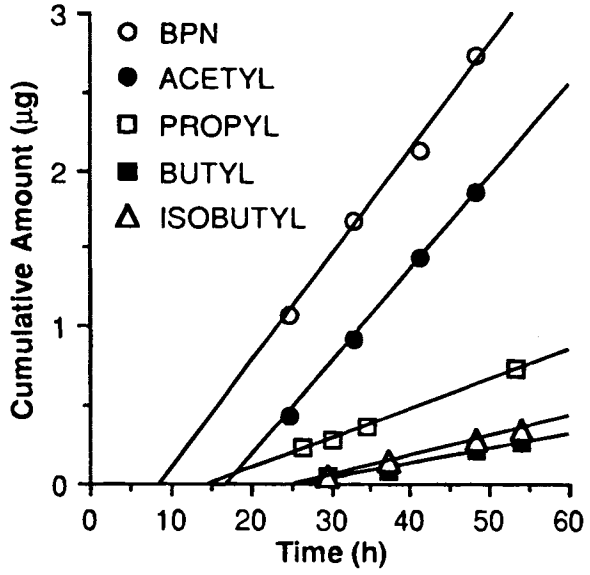

Fig. 2. Representative Permeation Profiles for the Diffusion of Buprenorphine from Five Saturated Solutions Through Human Skin at 37°C.

Table I. *In Vitro* Skin Flux and Lag Times of Buprenorphine and Four Prodrugs with Standard Deviations

Drug	Flux J_s ($\mu g/cm^2/h$)	Lag Time t_L (h)
Buprenorphine Base n = 6	0.093 ± 0.021	11.8 ± 4.8
Acetyl Prodrug Intact stratum corn. n = 4	0.076 ± 0.038	17.3 ± 2.2
Acetyl Prodrug Tape-stripped n = 3	0.061 ± 0.004	5.3 ± 4.6
Propyl Prodrug n = 3	0.023 ± 0.015	16.3 ± 1.5
Butyl Prodrug n = 3	0.015 ± 0.002	22.0 ± 4.6
Isobutyl Prodrug n = 3	0.015 ± 0.003	16.3 ± 9.0

Table II. Conversion Rates for Prodrugs in Skin Supernatant, Whole Blood, and DPBS at 37°C, Values Are the Mean of Three Experiments with Standard Deviations

	Skin Supernatant		Whole Blood		Dulbecco's Modified Phosphate Buffered Saline at pH 7	
	Rate Constant k_{obs} (min^{-1})	Half-life $t_{1/2}$ (hours)	Rate Constant k_{obs} (min^{-1})	Half-life $t_{1/2}$ (hours)	Rate Constant k_{obs} (min^{-1})	Half-life $t_{1/2}$ (hours)
Acetyl Prodrug	.0038 ± .0011	3.3 ± 1.2	not determined	not determined	.0010 ± .0001	11.6 ± 1.1
Propyl Prodrug	.0175 ± .0065	0.7 ± 0.2	.0057 ± .0005	2.0 ± 0.2	.0017 ± .0002	6.8 ± 0.7
Butyl Prodrug	.0212 ± .0049	0.6 ± 0.2	.0066 ± .0007	1.8 ± 0.2	.0024 ± .0001	4.8 ± 0.2
Isobutyl Prodrug	.0261 ± .0219	0.7 ± 0.5	.0027 ± .0007	4.4 ± 1.2	.0023 ± .0005	5.0 ± 0.9

in Table II. The slopes of the curves, which are equal to the rate constant, were determined using linear regression analysis. None of the coefficients of determination for the lines were less than 0.85. All chromatograms from the degradation experiments showed an increasing buprenorphine peak as the prodrug was depleted. The acetyl prodrug conversion rate was slow but nevertheless was about four-fold faster than its simple chemical hydrolysis. The other prodrugs had conversion rates in the enzyme-containing medium approximately ten-fold faster than their chemical hydrolysis rates.

Kinetics of Prodrug Enzymatic Degradation in Blood

The prodrugs also decomposed by first-order kinetics in the blood. The observed first-order rate constants and corresponding half-lives are summarized in Table II. Again linear regression analysis was used to get the best value of the slopes. In this case none of the coefficients of determination for the lines were less than 0.86. No significant difference nor quantitatively appreciable difference was seen in the rate constants of fresh *vs.* frozen blood experiments and therefore all the blood results were pooled.

Chemical Hydrolysis

The results of simple chemical hydrolysis of the prodrugs in Dulbecco's Modified Phosphate Buffered Saline, pH 7, are shown in Table II. As expected in this case, the prodrugs disappeared in a first-order fashion, the slopes of the ln C vs. time curves were determined using linear regression analysis. None of the coefficients of determination for the lines were less than 0.80.

Octanol/Buffer Partition Coefficients

The octanol/buffer partition coefficients (K_{oct}) for buprenorphine base and its prodrugs are summarized in Table III. These partition coefficients increase logarithmically with increasing alkyl chain length.

DISCUSSION

Even though the prodrugs are less crystalline and more oil soluble, the buprenorphine flux of the prodrugs from their saturated solutions never exceeded the flux of buprenorphine base. The failure of the prodrugs to deliver greater levels of buprenorphine under these circumstances is rooted in the permeation mechanism. The skin functions as a two-ply membrane, the stratum corneum and viable tissues beneath being physicochemically very dissimilar. All existing experience on skin permeation tells us that the stratum corneum functions to a first good approximation as a lipoidal barrier. Therefore drugs distribute into the interstitial, conduit lipids of the stratum corneum based on their lipophilicities. The viable tissues in series, on the other hand, act diffusionally as if they were a thickened, aqueous medium. Ordinarily the latter offers a low and relatively inconsequential resistance to diffusion relative to the stratum corneum. When this is the case fluxes of compounds from their saturated solutions are well aligned with the absolute oil solubilities of the compounds, the oil itself being somewhat immaterial. However, as one approaches the extremes of high lipophilicity, the aqueous tissue resistance becomes the dominant resistance. The theory, developed long ago in these labs, is very clear on this point (10). Aqueous tissue resistance was clearly demonstrated by finding no statistically significant difference ($p > 0.05$, t-test) in the permeation of buprenorphine from the acetyl prodrug solution through intact and stripped human skin. The significance of this finding is that gradients of compounds are mainly expressed across the aqueous stratum, the stratum corneum functioning as a reservoir between this stratum and the donor medium. Thus the flux from saturated solutions only reflects the trend in aqueous solubility. Buprenorphine is itself an unusually hydrophobic starting material for a prodrug series. Esterification only adds to the hydrophobicity. It is our surmise that the esterification, though changing the crystalline properties in the manner which was sought, also pushed the mechanism of transport further into aqueous tissue control. The flux from saturated solutions is thus expected to drop rather than rise. In fact, the flux of buprenorphine decreases with the increasing alkyl chain length of the prodrugs. This

Table III. Octanol/Buffer Partition Coefficients and Light Mineral Oil Solubilities (8) of Buprenorphine and Four Prodrugs

Drug	log K_{oct} at 25°C	Mineral Oil Solubility at 37°C, (mg/ml)
Buprenorphine	2.9	0.68
Acetyl Prodrug	3.5	19.8
Propyl Prodrug	3.7	not determined
Butyl Prodrug	4.1	10.0
Isobutyl Prodrug	4.2	45.1

change in mechanism of transport has also been the unfortunate fate of other promising prodrugs for transdermal delivery enhancement (11). Other researchers have shown that less crystalline/more hydrophobic prodrug series can show extreme transdermal delivery enhancement when starting from a hydrophilic parent drug. For example, Drustrup *et al.* found increased morphine transdermal flux from ester prodrug oils, the extreme of noncrystallinity (12).

The experiments in this study have demonstrated that the prodrugs are converted to buprenorphine in human blood and, directly and indirectly, in the presence of skin enzymes. Studied directly, these reactions appeared to be first-order to completion under all conditions. Beyond this, the isobutyl prodrug conversion rate is significantly slower ($p < 0.01$) than the straight-chain prodrugs' conversion rates in human blood by t-test comparisons. In fact, when the chemical hydrolysis contribution to the degradation of the isobutyl prodrug is subtracted from the total rate, virtually no enzymatic hydrolysis actually occurred. This demonstrates the potential ability of the bulky isobutyl branched chain to slow the attack of esterase enzymes. Despite this, the isobutyl compound did not escape from the skin intact in the course of the permeation experiments. Finally, the conversion rates for the propyl and butyl prodrugs in skin supernatant were significantly ($p < 0.05$) faster than the acetyl prodrug rate by t-test comparisons.

In previous work done in these laboratories, a series of hydrocortisone 21-alkyl-ester prodrugs also gave clear although indirect evidence of increased hydrolysis rates with increasing alkyl-ester chain length of simple alkyl esters when permeation studies were performed on dermis tissue in the hairless mouse (13). Moreover, at about the same time O'Neill and Carless (14) proved directly that hydrolysis rates increase with chain length to a maximum at a chain length of about five when the same hydrocortisone esters were incubated with hamster and guinea pig skin homogenates for thirty minutes. Thus, there appears to be an enzyme receptor/binding site partitioning dependency on hydrophobicity. Indeed, such hydrophobically driven reactions are actually quite common, when enzyme reactions are studied within congeneric families. Because of this, we anticipated that the enzymes might actually draw buprenorphine through the skin when sourced in the form of one or another esters. This would happen if the ester conversion was so rapid that significant conversion to buprenorphine takes place at and very near the stratum corneum's interface with the viable tissue, a phenomenon observed before with hydrocortisone (13). In effect, such rapid conversion and near this interface steepens the ester gradient expressed across the stratum corneum, in doing so overriding the influence of hydrophobicity. In this expectation we were clearly disappointed.

It should not go unnoticed or unappreciated that in these studies with buprenorphine 3-alkyl-esters we have shown that human skin metabolizes the compounds efficiently while in diffusive transit and that the enzymes in the blood are also very active in the prodrug conversion. Thus that small fraction of prodrug which gets through the skin would function as the parent compound, buprenorphine.

ACKNOWLEDGMENTS

The authors gratefully acknowledge support from Dr. Cynthia L. Marcelo, Dermatology Department, The University of Michigan, Ann Arbor, MI, the Upjohn Research Endowment Fund and the National Institute on Drug Abuse for grant #DA07575.

REFERENCES

1. C. R. Schuster. *British Journal of Addiction.* **84**:19–28 (1989).
2. M. K. Lehman. *Substance Abuse and Mental Health Services Administration News.* **1**:17–19 (1993).
3. R. E. Johnson, J. H. Jaffe, and P. J. Fudala. *JAMA.* **267**:2750–5 (1992).
4. A. Goodman Gilman, L. S. Goodman, T. W. Rall, and F. Murad. *Goodman and Gilman's The Pharmacological Basis of Therapeutics, Seventh Edition*, Macmillan Publishing Company, New York, 1985, pp. 523–524.
5. S. D. Roy, E. Roos, and K. Sharma. *J. Pharm. Sci.* **83**:126–130 (1994).
6. A. Stinchcomb, A. Paliwal, R. Dua, R. W. Woodard, and G. L. Flynn. *Pharm. Res.* **12**:1526–1529 (1995).
7. G. L. Flynn, H. Durrheim, and W. I. Higuchi. *J. Pharm. Sci.* **70**:52–56 (1981).
8. H. Imoto, Z. Zhou, A. L. Stinchcomb, and G. L. Flynn. *Biol. Pharm. Bull.* In press (1996).
9. S. W. Collier, N. M. Sheikh, A. Sakr, J. L. Lichtin, R. F. Stewart, and R. L. Bronaugh. *Toxicol. Appl. Pharmacol.* **99**:522–533 (1989).
10. G. L. Flynn and S. H. Yalkowsky. *J. Pharm. Sci.* **61**:838–852 (1972).
11. J. J. Fort, Z. Shao and A. K. Mitra. *Int. J. Pharma.* **100**:233–239 (1993).
12. J. Drustrup, A. Fullerton, L. Christrup and H. Bundgaard. *Int. J. Pharm.* **71**:105–116 (1991).
13. W. M. Smith. *An Inquiry Into the Mechanism of Percutaneous Absorption of Hydrocortisone and Its 21-n-Alkyl Esters.* Dissertation, The University of Michigan, 1982.
14. R. C. O'Neill and J. E. Carless. *J. Pharmacol.* **32** supp:10–11 (1980).