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IN-VITRO TRANSDERMAL PERMEATION OF OXYCODONE: (I) EFFECT OF PH, DELIPIDIZATION AND SKIN STRIPPING

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ABSTRACT

The effect of pH, skin stripping and delipidization on the in-vitro transdermal permeation of a weak base analgesic, oxycodone (pKa=8.53), was studied using hydrodynamically calibrated Valia-Chien diffusion cells. Saturated oxycodone.HCl solutions in citrate-phosphate buffers ranging from pH 4 to 10 were used as the donor solution. Skin samples from the abdominal and dorsal sites of hairless rats, abdominal site of hairless mouse, rabbit pinna ear, as well as human cadaver skin were used in permeation studies. The pHs at which maximum flux attained varied from 6.5 to 7.5 depending upon animal model. The permeabilities of protonated form through intact skin of all the animal models used, was about 7-15 fold lower than that of nonionic form. The unexpected high permeation rate at pHs ranges 4 to 6.5 across human cadaver could be attributed to the possible damage upon storage. The skin stripping and delipidization process appeared to increase the permeation rates of oxycodone and the degree of enhancement is dependent upon the pH in the donor compartment.

INTRODUCTION

Percutaneous absorption of chemicals is generally regarded a diffusional process, with the rate-limiting barrier being the nonviable stratum corneum. The rate and extent of absorption of a drug via the skin depends on many factors, like the physicochemical characteristics of the drug applied, the concentration of drug in the delivery system, the nature of the vehicle in which the drug is placed, etc.

Although the use of transdermal therapeutic system (TTS) has revolutionized the concept of systemic delivery through the skin (1-6), its use has been limited to unionized lipophilic compounds based on their permeation characteristics. The use of ionic hydrophilic compound for TTS has met little success in the past. The stratum corneum is the main barrier limiting the passive transepidermal diffusion of ionized molecules. The transfollicular and transappendageal (skin pores) routes are considered to constitute the major penetration pathway for the ionized molecules(7-8); however, the surface area occupied by these pathways is relatively small. The pathways of penetration of ionic species through stratum corneum may be also via intercellular routes (9). The removal of intercellular lipids from stratum corneum could significantly affect transdermal transport, especially for ionic species.

Animal skin has been used to study the basic parameters in transdermal permeation. These include rats, rabbits, pigs, guinea pigs, hairless mice, hairless dogs and rhesus monkeys (10-18). In most cases, the permeability of a drug in human skin is lower than that in animal skin samples. The relative permeability values for *in vitro* percutaneous studies depends to a large extent on the nature of the penetrants and the thickness as well as the biophysical properties of the stratum corneum. In

addition, the lipid composition in the stratum corneum was considered to be one of the critical factors governing differences in percutaneous transport (19).

The purpose of this study was to investigate the effect of skin stripping and delipidization on the percutaneous absorption of oxycodone across skin samples at various pHs. The barrier properties due to the skin treatments for nonionic and ionized forms were compared.

EXPERIMENTAL

Materials Oxycodone hydrochloride, a gift from Penick Corporation (Lyndhurst, New Jersey), was used as obtained. Polyethylene Glycol 400 (PEG 400, HPLC grade), methanol, acetonitrile, sodium sulfate, sodium chloride, citric acid(anhydrous), sodium phosphate dibasic(anhydrous) were purchased from Fischer Scientific Company (Fair Lawn, New Jersey). Hairless mice (HRS/J strain), male, 5-7 weeks old were obtained from Jackson Laboratories (Bar Harbor, Maine). Hairless rats (HRS strain), male, 350-400 gm weight were obtained from the Institute of Pathology Walter Reed Army Medical Center (Washington D.C.). Rabbits (New Zealand white rabbit), male, 6-7 lbs weight were obtained from Sunrise Laboratory Animals (Whitehouse Station, New Jersey).

Intact Skin Preparation Hairless mouse was sacrificed by cervical dislocation technique. The skin was obtained from the abdominal site of a male hairless mouse using surgical scissors right after the mouse has been sacrificed. Hairless rat was sacrificed by intrapulmonary injection of euthanasia solution (T-61,Hoechst, Somerville, New Jersey,USA) of 0.5 to 2 ml, depending upon the size of the animal. The skin from abdominal and dorsal site of a hairless rat were obtained using surgical scissors

right after the rat has been sacrificed. Rabbit was sacrificed by ear vein injection of euthanasia solution 0.5 to 2 ml. Then both inner-pinna ear skin were peeled off immediately after sectioning the rabbit ears. Human cadaver skin was defrosted in the normal saline solution for about 1 hour and then dried with Kimwipe before use. The skin was mounted and clamped tightly between the two compartments of each diffusion cell.

Skin Delipidization The freshly excised intact skin of hairless rat was mounted on each diffusion cell with stratum corneum side facing the donor compartment and clamped tightly between the two half-cells. Methylene chloride (3.5ml) was filled in the donor compartment to extract lipids while no vehicle was in the receptor compartment at this moment. After extraction for 1/12. 1/2, 1, 2, 4, 6, and 8 hours, the methylene chloride solution was withdrawn and discarded. Trace of methylene chloride on the surface of skin was evaporated by a stream of nitrogen. Drug-saturated buffer suspension was then placed in the donor compartment and 40% PEG 400 solution was placed in the receptor compartment. Thereafter, the permeation of oxycodone across the delipidized skin was conducted.

Stripped or Viable Skin Preparation The stratum corneum was stripped off by well developed stripping technique (20). The cellophane tape was placed firmly on the abdominal skin of a sacrificed mouse, and then peeled off. The procedure was repeated, in multiples of two strippings, for up to 26 strippings.

Skin Permeation System

A hydrodynamically calibrated *in vitro* skin permeation cell (Crown Glass Company) (21) was used to study the permeation of oxycodone through various animal skins.

Oxycodone suspension prepared by suspending an excess amount of drug crystals in citrate-phosphate buffers of various pHs was

introduced into the donor compartment. Excess drug crystals in the donor solution were present to assure the saturated concentration throughout the course of experiment. Forty percent V/V PEG 400 aqueous solution was used as the dermal solution in the receptor compartment to maintain sink conditions during the skin permeation studies. The temperature of the donor and the receptor solution were thermostatically controlled at 37° C by a circulating water bath. At predetermined time intervals, a 1 ml sample was withdrawn from the receptor solution, which was replaced immediately with the same volume of drug-free receptor solution to keep a constant volume in the receptor compartment. Sample (1 ml) was assayed by a high performance liquid chromatography.

Solubility Determination

The solubility of oxycodone.HCl in citrate-phosphate buffers at various pHs was determined by equilibrated excess amount of oxycodone.HCl in buffers using a shaker bath at 37° for 3 days. After filtering the solution, the concentration of oxycodone.HCl in this saturation was determined by HPLC.

HPLC Assay

A high performance liquid chromatographic system consisted of a reciprocation pump (model 6000, Waters Assoc. Milford, Mass.), an injector (model 6uk, Waters Assoc.), a programmable UV detector (model 783, Spectros Inc.), a reverse-phase u-Bondapak C-18 column with a guard column containing 37-50 um Bondapak C_{18}/Corasil packing material (15cm x 3.9 mm I.D.), and a chart recorder (Fischer Recordall, Series 5000) was used in the study.

A combination (36:64) of acetonitrile and phosphate buffer (pH=4.0) was employed as the mobile phase. The UV detector, operated at the wavelength of 254 nm and the operating range of 0.01 AUFS, had a detection limit of 0.05 mcg/ml.

The concentration of oxycodone in the sample solutions was determined by measuring the peak height of oxycodone and computing the concentration from standard curve.

RESULTS AND DISCUSSION

Effect of pH on Solubility and Permeation

Figure 1 shows the effect of pH on the aqueous solubility of oxycodone. It can be seen that the solubility of nonionic oxycodone (B) remains fairly constant at pHs above 6.5 while the total solubility and the solubility of its protonated form or ionic form(BH^+) exhibit their maximum values at pH 4 and then decrease with the increase in pH.

The permeation profiles of oxycodone through various animal skin with the donor solution controlled saturated at pH 9.5 where most of the drug are in nonionic form are shown in Figure 2. The results indicate that the penetration rate of oxycodone through hairless mouse skin was the highest among the animal species tested, which could be largely attributed to its thinner stratum corneum (Table I). It is expected that the skin permeation rate is inversely proportional to the thickness of the skin or the thickness of the stratum corneum. The effect of pH in donor solution on the transdermal permeation of oxycodone was studied (Figure 3). Except for the unexpected high permeation rate through human cadaver at pH 4, it was found that there is no significant difference in permeation rate in each animal model at pH ranges studied. The slightly higher permeation rates at low pHs could possibly be due to the penetration of protonated form. Comparing the solubility-pH with flux-pH profiles, It was observed that the solubility of ionic form decreased 45 fold from pH 4 to pH 7.5 while the fluxes through the intact skin were barely affected by pH or the total solubility in the donor

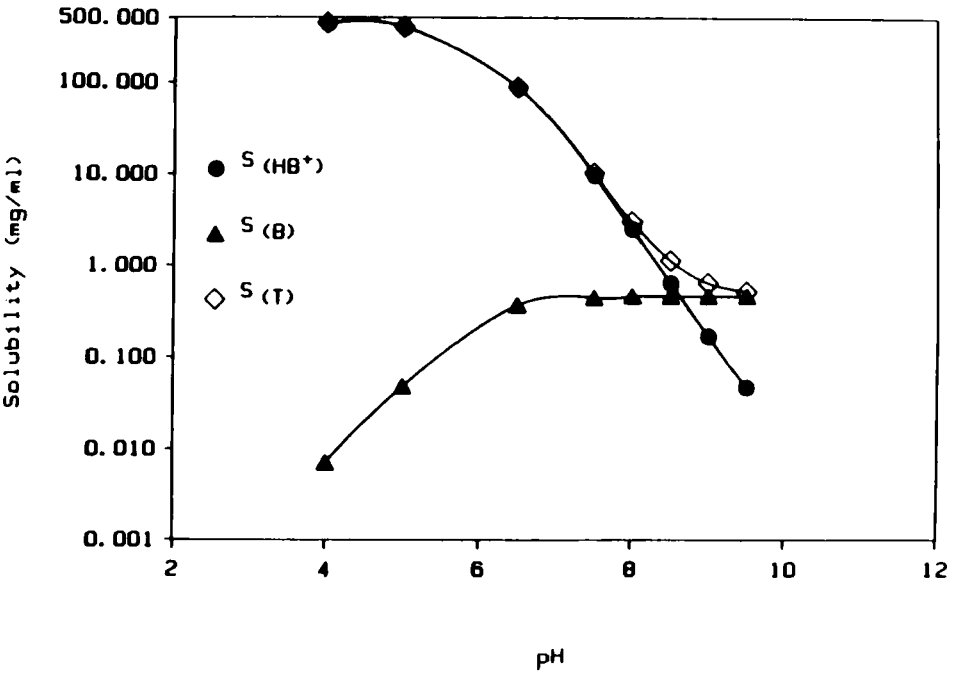

Figure 1: The pH-solubility profiles for (●)ionic form BH^+ and (▲) nonionic B of oxycodone.

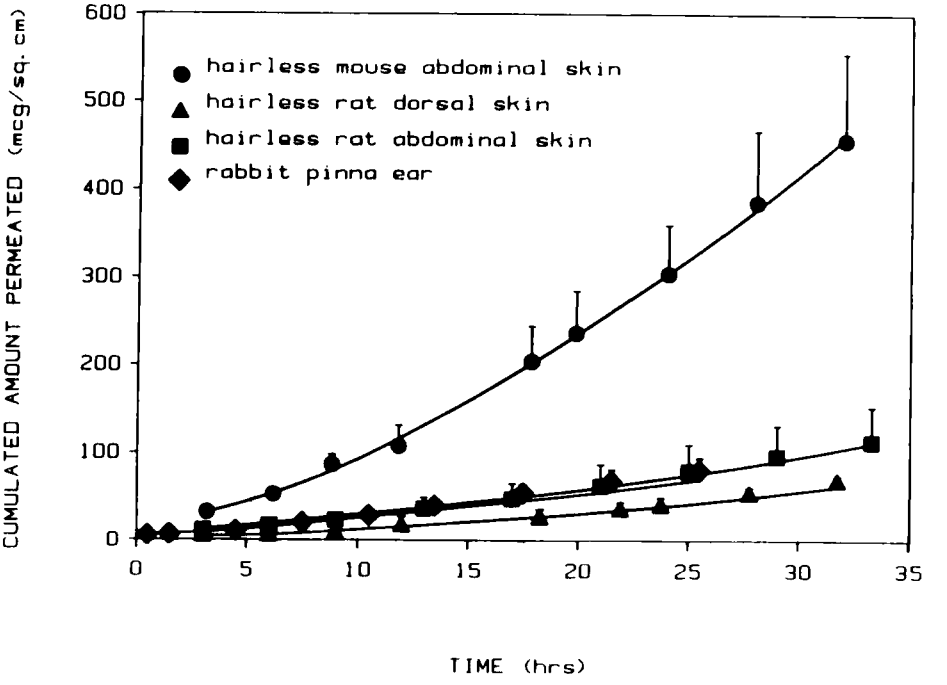

Figure 2: The transdermal permeation of oxycodone across: (●) hairless mouse skin , (▲) hairless rat dorsal skin, (■) hairless rat abdominal skin and (◆) rabbit pinna ear skin.

Table I: The thickness of human cadaver and animal skin.

Species	Stratum Corneum (μm)	Whole Skin (μm)
Human cadaver (16*)	19.8 ± 0.70	2900 ± 280
Hairless mouse (36) (abdominal)	9.9 ± 0.40	380 ± 20
Hairless rat (12) (abdominal)	40.8 ± 0.80	2090 ± 70
Hairless rat (12) (dorsal)	70.56 ± 0.92	3080 ± 580
Rabbit ear (36) (Pinna-inner)	20.30 ± 0.60	290 ± 220

* number of replicates.

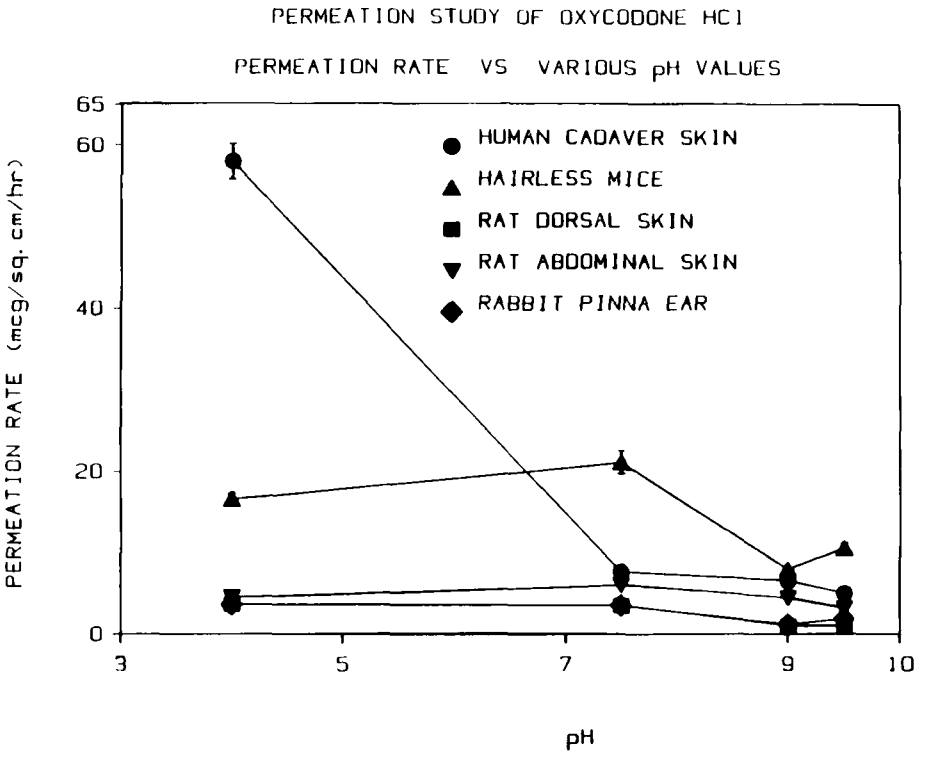

Figure 3: The effect of pH on transdermal permeation of oxycodone through different animal models.
(●) Human cadaver skin, (▲) Hairless mouse abdominal skin, (▼) Hairless rat abdominal skin, (■) Hairless rat dorsal skin, (◆) Rabbit pinna-inner ear.

compartment. The result suggests that the permeation characteristics are different between ionic and non-ionic species.

Skin Permeabilities and In-vitro Animal Model Comparison

The simplest way of modeling the process of skin permeation was to assume that Fick's law of diffusion was applicable. It has been used to explain almost every aspect of percutaneous permeation including skin species differences.

The principal physicochemical parameters of the permeant that influence the transport rate across skin are partition coefficient (K_p), diffusion coefficient (D) and the thickness of the skin (h). In other word, permeability (P), which is expressed as

$$P = D \cdot Kp/h \qquad (3)$$

characterizes permeation process. In the present study, the permeation proess is likely to be complicated by the simultaneous presence of both ionic and nonionic species, which possess different permeation characteristics. If it can be assumed that each species migrates through skin independently by simple diffusion and not interfered by the presence of the other species, we can correlate the total steady-state skin permeation flux (J_T) with the sum of the specific permeation rate of ionic and nonionic species:

$$J_T = C_B P_B + C_{BH^+} P_{BH^+} \qquad (1)$$

where P_B and P_{BH^+} are the permeabilities and C_B and C_{BH^+} are the concentration of nonionic and ionic forms of oxycodone, respectively. The specific permeabilities of nonionic and ionic form of oxycodone (P_B and P_{BH^+}) can be obtained from the

Table II: The apparent permeability of ionic form (P_{BH^+}) and nonionic form (P_B) of oxycodone across intact skin of various animal models.

Animal Models	Permeabilities (cm/hr x 10^{-3})			
	P_{BH^+}	P_B	r	P_B/P_{BH^+}
Hairless Mouse abdominal	2.3	17.5	0.92	7.61
Hairless Rat abdominal	0.5	7.3	0.89	14.61
Hairless Rat dorsal	0.5	1.8	0.98	3.6
Rabbit Pinna ear	0.3	5.8	0.38	19.40

intercept and the slope using modified Swarbrick Eqn. for weak base (22).

$$J_T/[B] = P_B + (P_{BH^+})\ ([H^+]/Ka) \qquad (2)$$

The results (Table II) indicate that the permeability of oxycodone base (P_B) is about 7.4 and 14 fold as high as that of protonated oxycodone (P_{BH^+}) in hairless mouse skin and hairless rat skin, respectively. The barrier characteristics of stratum corneum to ionic species is thus demonstrated. A poor correlation (r=0.38) between $J_T/[B]$ and $[H^+]/Ka$ (Eqn.2) was obtained for rabbit pinna-inner ear. This could be due to the presence of a layer of wax film on the surface of the ear surface.

Table III lists the normalized skin permeabilities of oxycodone after corrected by its thickness of stratum corneum. A smaller variation in the normalized permeabilities between animal species was observed as expected. The residual variation may be

Table III: - Normalized permeabilities of ionic and non-ionic form of oxycodone

	Normalized Permeability ($cm^2/hr \times 10^{-7}$)	
Species	P_{BH^+}	P_B
Hairless mouse	22.8	173.2
Hairless rat (abdominal)	20.4	297.8
Hairless rat (dorsal)	35.3	127.0
Rabbit ear (pinna-inner)	6.1	117.7

attributed to the resistance from viable skin and the differences in biophysiochemical and structure properties of skin among various species, such as lipid content of the skin.

The in-vitro transdermal permeation of oxycodone across human cadaver skin at various pHs was also studied and compared with animal models tested (Figure 3). It was found that the abdominal site of hairless rat skin could be a good animal model for human cadaver except for permeation in low pH ranges at which the ionic form is predominant in the solution. The unexpected high permeation rate through human cadaver skin at pHs ranging from 4 to 6.5 could be attributed to a possible damage which might have occurred in the structure of the stratum corneum while stored frozen(23). This damage may involve the keratinized cells and/or the intercellular lipid matrix. It is conceivable that water could crystallize out at low temperatures and thereby damage the cells or disrupt the intercellular matrix leading to the change of the physical state of lipids of the stratum corneum and larger intercellular space. Consequently, the resistance to the permeation of ionic species is reduced since the permeation of ionic form is mainly via intercellular route (7,8). The

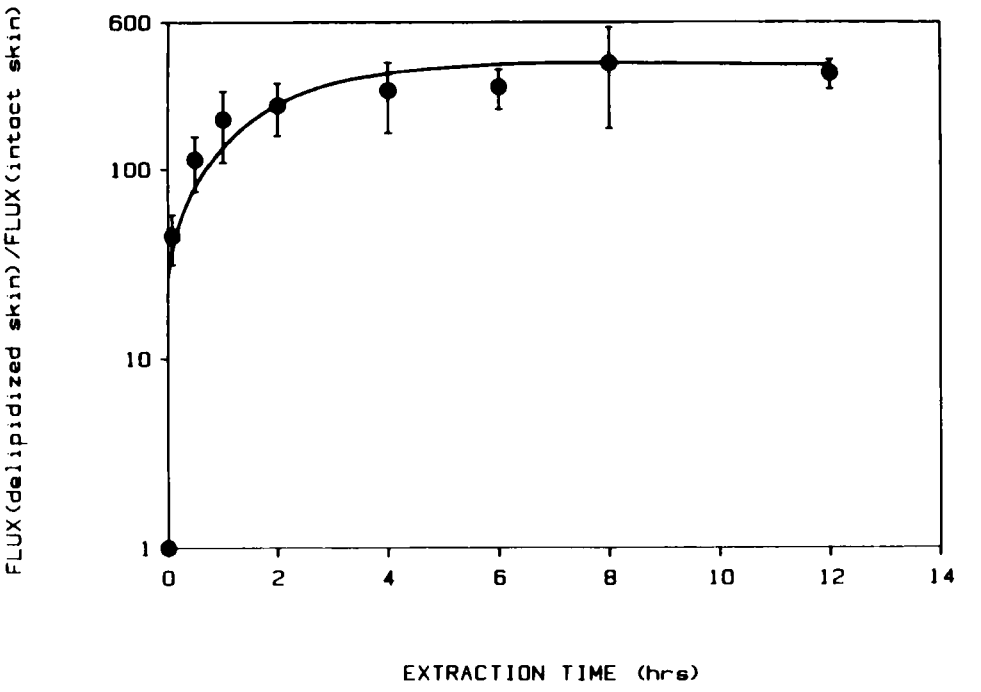

Figure 4: The effect of lipid extraction time on the enhancement of transdermal permeation of oxycodone at pH 5.

improper handling of storage process of human cadaver skin therefore could enhance the penetration of ionic form of oxycodone, especially at pH below 6.5 where the concentration of ionic form of oxycodone is extremely high.

Effect of Skin Delipidization on Transdermal Permeation of Oxycodone

The stratum corneum may be regarded as two-phase lipid/protein heterogeneous membrane in which the lipid is continuous. The role of lipids in the barrier capacity of the hairless rat abdominal skin was investigated through delipidization process. The permeation of oxycodone at pH 5 was markedly enhanced after only 5 minutes of lipid extraction and the degree of enhancement was about 45 fold higher than that of

Table IV: - The effect of pH on the transdermal permeation of oxycodone through delipidized and stripped hairless rat abdominal skin

pH	Enhancement Factor I*	Enhancement Factor II**
5.0	271.00	887.84
6.5	270.88	518.34
7.5	29.65	38.96
8.5	27.18	29.73

* Enhancement Factor I = Flux(delipidized)/Flux(intact)

**Enhancement Factor II = Flux(stripped)/Flux(intact)

intact skin (Figure 4). This might be due to the removal of the lightly thin film on the visible skin surface. The permeation rate was increasingly enhanced with the extraction time, and reached plateau after about 4 hours of delipidization, which was about 300 times higher than that of intact skin. It clearly illustrates that the lipids extracted from the skin by delipidization plays a significant role in the barrier capacity of the skin.

Table IV shows the effect of pH on the enhancement of percutaneous penetration through delipidized skin. The results demonstrate that the fluxes were drastically increased with the decrease in pH. The flux was about 270 fold higher than that of intact skin at pH 5, and only 30 fold of enhancement was observed at pH around 7.5. As discussed previously (Figure 2), the fluxes through intact hairless rat skin did not change significantly with pH or the total solubility in the donor compartment. Hence, the higher enhancements in permeation rate due to the

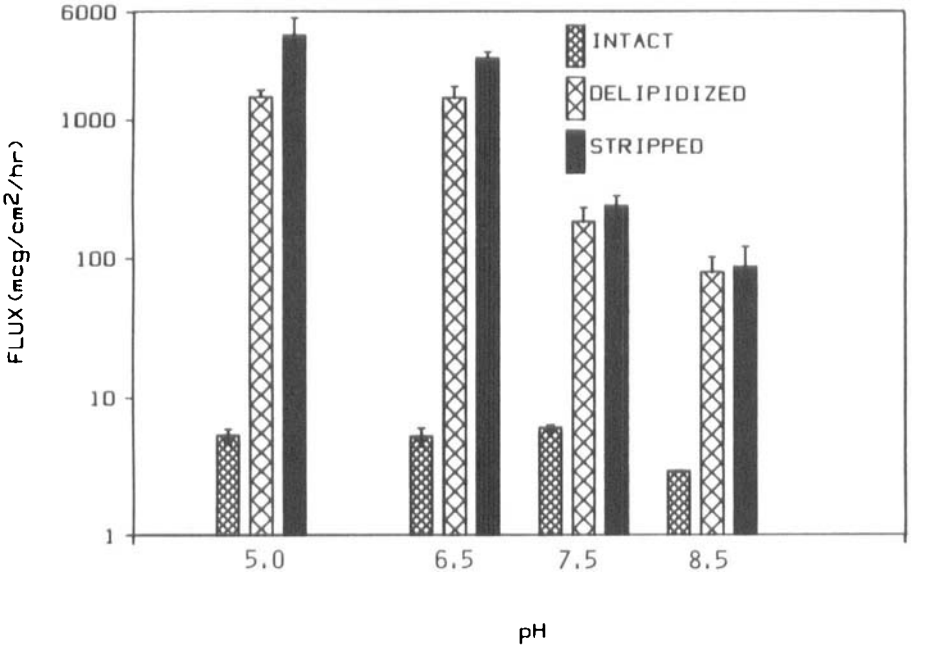

Figure 5: The effect of pH and skin treatment on transdermal permeation of oxycodone through the hairless rat abdominal skin:
(▩) intact skin, (▨) delipidized skin and
(■) stripped skin.

delipidization process at low pH ranges are mainly contributed from the concentration of ionic species. The degree of enhancement through delipidization is dependent upon the percentage of ionization of the penetrants. This finding fairly agrees with Doshi's work on steroids, in which it was demonstrated that the effect of delipidization on transdermal permation increased with hydrophilicity of the penetrants (24).

Effect of Skin Stripping on Oxycodone Permeation

Figure 5 shows the effect of pH on transdermal permeation of oxycodone through stripped hairless rat abdominal skin and

compared with those through intact and delipidized skin. It can be seen that the permeation rate was enhanced through skin stripping process and the degree of enhancement remarkedly decreased with the increase in pH; the enhancement values were about 888 times and 30 times as high as that through intact skin at pH 5 and 8.5, respectively (Table IV). The difference in the degree of enhancement to a large extent was due to the solubility of ionic form. In comparison of the delipidization and skin stripping process, the difference between these two treatments is the extra removal of the protein domain via skin stripping process. Figure 5 shows that there was little difference in the enhancement of permeation rates of both treatments at pH 8.5. On the other hand, the skin stripping had more influences on the penetration process as pH declined. According to Raykar's recent report on solutes uptake by protein and lipid domains of human stratum corneum, lipophilic compounds are uptaken mainly by the lipid domain while hydrophilic compounds are mainly uptaken by the protein domain of stratum corneum (19). The removal of protein domain by skin stripping, which may be considered the major part for the uptake of protonated oxydcodone, thus played a more important role for permeation at low pH ranges when oxycodone is highly protonated.

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