Absorption of solvent-deposited weak electrolytes and their salts through human skin in vitro

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ABSTRACT

Permeation of a weak acid (benzoic acid) and a weak base (propranolol) in various stages of ionization through human skin *in vitro* was measured from 0-72 h following solvent deposition of radiolabeled doses ranging from 11-11,000 nmol/cm^2 and 1.93-1930 nmol/cm^2, respectively. For the twenty combinations tested for each compound, mean permeation into the receptor fluid over 72 hours ranged from 1.5-40.7 percent of dose for benzoic acid and 1.3-35.5 percent of dose for propranolol. For all but the lowest doses, permeation increased with increasing fraction of nonionized permeant in the dose solution. Generally, this trend became stronger as the dose increased. Recovery of radioactivity averaged 94.3 ± 5.5% for propranolol and was independent of ionization state and dose. Recovery of radioactivity for benzoic acid ranged from 40 to >100%, increasing with fraction nonionized and with dose. These effects can be qualitatively explained in terms of the low permeability of ionized species through stratum corneum, the volatility of free benzoic acid, and a buffer capacity of the stratum corneum deposition region on the order of 10-20 nmol/cm^2.

Keywords: buffer capacity, percutaneous absorption, organic salts , skin permeability, topical delivery, weak electrolytes

1. Introduction

It is well known that the steady-state skin permeability of weak organic acids and bases applied to skin in aqueous solutions is a strong function of their ionization state (Chantasart et al., 2013; Roy and Flynn, 1989, 1990; Smith and Irwin, 2000). For the weak bases fentanyl and sufentanyl, Roy and Flynn estimated the human skin permeability of the free base to be 100- to 200-fold higher than that of the protonated amine (Roy and Flynn, 1990). For weak acids, the permeability difference may be even higher, based on their behavior in *n*-octanol and phospholipid membranes (Avdeef, 2003). The pH dependence of steady-state skin permeability for both acids and bases is reasonably, if imperfectly, described by current computational models (Baba et al., 2017; Kasting et al., 2019).

Less is known about the skin permeation of partially ionized weak electrolytes deposited on skin in finite doses. Neutral forms may precipitate as solids or liquids, depending on their melting point. Salts are more likely to deposit as solids, as they are generally high melting. Both have the potential to modify the pH in the upper layers of the stratum corneum (SC), which we have elsewhere termed the "deposition zone" (Kasting and Miller, 2006). The extent to which the pH is altered from its natural value of 5.0-5.5 will depend on the pK_a, ionization state and dose of the permeant, the presence of any other buffering agents in the dose solution, and the buffer capacity of the deposition zone. A recent example of a study in which this phenomenon may have come into play is the finite dose study of the skin permeation of 56 cosmetic relevant compounds conducted by Cosmetics Europe investigators (Hewitt et al., 2019).

In order to shed light on this matter, we conducted an in vitro human skin permeation study of benzoic acid, propranolol and two salts thereof using radiochemical methods. The counterion for benzoic acid was Na^+ and for propranolol was Cl^-. Five combinations of each acid/conjugate

base pair dissolved in an ethanol:water vehicle and applied to human skin at four logarithmically-spaced doses, yielding 40 distinct test conditions. Measurements included permeation of radioactivity into the receptor solution from 0-72 h and distribution of radioactivity into the skin wash, epidermis, dermis and receptor solution at the conclusion of the study. Clear trends with both dose and ionization state were established for each permeant. Results are expressed in terms of an effective buffer capacity of the skin following topical application of weak electrolyte solutions. In this paradigm, skin buffer capacity is defined as the threshold dose of weak electrolyte at which permeation changes from no dependence on initial composition (at very low doses) to a strong dependence (at high doses).

2. Materials and methods

2.1. Materials

Benzoic acid, [7-^{14}C] (55 mCi/mmol; 1 mCi/mL) was purchased from American Radiolabeled Chemicals (St. Louis, MO, USA). The supplier-reported radiochemical purity was greater than 99%. Unlabeled benzoic acid and sodium benzoate, purity 99.5 and 99%, respectively, were purchased from Alfa Aesar (Ward Hill, MA, USA). D,L-propranolol, HCl salt [ring-^{3}H] (24 Ci/mmol; 0.5 mCi/mL) was purchased from Vitrax (Placentia, CA, USA). The supplier-reported radiochemical purity was greater than 98%. Unlabeled D,L-propranolol HCl, purity ≥ 99%, and calcium-free Dulbecco's phosphate-buffered saline were purchased from Sigma-Aldrich (St. Louis, MO, USA). Solvable™ (a tissue solubilizer) was purchased from Perkin-Elmer (Shelton, CT, USA). Deionized (DI) water, resistivity 18 MΩ-cm, was obtained from a Millipore® filtration system.

Unlabeled propranolol free base was prepared from the hydrochloride salt according to methods previously described (Paker-Leggs and Neau, 2008). Briefly, sodium hydroxide was

added to a solution of propranolol HCl in water until the pH was 11.4. The precipitate was washed with water and then dried in an oven. A melting point (93 ºC; lit 96 ºC) was taken to estimate purity. The yield was 1.21 g free base from 1.55 g propranolol HCl (89%). Physical properties of the test materials, obtained from a variety of sources, are shown in Table 1.

Table 1. Physical properties of the test permeants. Source: Experimental values at 25 °C reported in EpiSuite™ Vers. 4.1 (US_EPA, 2009) unless otherwise noted.

Property	Units	Benzoic Acid	Sodium Benzoate	Propranolol	Propranolol HCl
FW		122.12	144.10	259.34	295.81
log K_{oct}[a]		1.87	-	3.48	-
P_{vp}[b]	torr	7.00×10^{-4}	3.67×10^{-9} [c]	9.44×10^{-8} [c,d]	3.16×10^{-13} [c]
S_w[e]	g/L	3.4[f]	556[g]	0.062	50[h]
T_m[i]	ºC	122.4	>300	96	164
T_b[j]	ºC	249.2	465[c]	435[c,k]	544[c]
ρ[m]	g/cm^3	1.321[l]	1.44[h]	1.09[c,g,k]	unknown
pK_a		4.19	-	-	9.42
pH of a saturated solution in water		2.8[h]	8[l]	9.4[c, n]	5.1[c, n]

[a]log of octanol water partition coefficient. [b]vapor pressure. [c]calculated value. [d]The EDETOX database reports a value of 3.82×10^{-8} torr, original source unknown. [e]water solubility. [f]Additional values between 2.64 and 3.54 reported by Aquasol. [g]value at 20°C.[h]PubChem, National Institutes of Health, https://pubchem.ncbi.nlm.nih.gov/. [i]melting point. [j]boiling point. [k]ChemSpider, ACD Laboratories, www.chemspider.com. [l]Merck Index (Budavari, 1996). [m]density. [n]calculated from proton balance equations (Sinko, 2011) using the above water solubilities and pK_a's.

Excised split thickness human cadaver skin from the posterior leg (5 donors) having a nominal thickness of 400 μm, was procured from The New York Firefighters Skin Bank (New York, NY, USA). The skin was preserved in RPMI 1640 with 10% glycerol, oxacillin sodium and gentamicin and kept at −70 ºC until use.

2.2. In vitro permeation test (IVPT)

Methods employed in the IVPT have been described previously (Merritt and Cooper, 1984). Briefly, the skin was thawed, rinsed with DI water, and mounted directly on modified Franz diffusion cells (0.79 cm^2). No additional sealant was required as the skin itself forms a tight seal. The receptor solution was calcium-free Dulbecco's phosphate buffered saline, pH 7.4, to which

0.02% sodium azide was added to inhibit microbial growth. A magnetic stir bar was placed in the receptor compartment prior to placing the cells in thermostatted heating/stirring modules that maintained the receptor fluid at 37°C, leading to a skin surface temperature of 32°C. A skin integrity test was performed with $^{3}H_2O$ as described by Kasting et al. (Kasting et al., 1994). Cells in which greater than 2 μL/cm^2 of $^{3}H_2O$ permeated into the receptor fluid within 1 h following a 5 min exposure were excluded from the study.

Three experiments were performed for each of the two permeants, ^{14}C-benzoic acid and ^{3}H-propranolol. In each experiment, 20 different test formulations were applied to the skin from a single donor, n = 1-3 per formulation for benzoic acid and n = 2-4 per formulation for propranolol. (Skin from one of the donors was used for both permeants.) The 20 dosing solutions consisted of four dose levels for each of five ionization levels: 0, 25, 50, 75, and 100 mole percent free acid or base, with the remaining amount being the salt form, sodium benzoate or propranolol hydrochloride. They were prepared by fully dissolving appropriate amounts of unlabeled free acid or base and its salt into an ethanol:water solution (composition below) containing the radiolabeled material, followed by thorough mixing. The radiochemical concentration of each test formulation was sufficient to deliver a radiochemical dose per diffusion cell ranging from 1 μCi for a 20 μL dose to 0.25 μCi for a 5 μL dose.

For benzoic acid, the four dose levels 11000, 1100, 110, and 11 nmol/cm^2 were applied in 20, 10, 5, and 5 μL, respectively, of 1:1 v:v ethanol/water solution. Over the course of the three experiments, each treatment was applied to 6 skin samples. For propranolol, dose levels of 1930, 193, 19.3, and 1.93 nmol/cm^2 were applied in 20, 10, 5, and 5 μL, respectively, of 3:1 v:v ethanol/water solution. Each treatment was applied to a total of 8 skin samples.

Experimental design considerations were as follows: The ethanol/water solvent was

selected to ensure adequate solubility for the free acid and base legs of the study along with facile equilibration of ionized and neutral species. The mixture also lowered the surface tension with respect to water alone, which allowed the solutions to spread more easily on the skin. Propranolol was much less water soluble than benzoic acid and required a higher volume fraction of ethanol. The dose volume was capped at 20 μL per 0.79 cm^2 cell to ensure rapid dry down of the dose solutions, thus minimizing the impact of the solvent on skin permeability. The maximum dose for each compound was selected as the maximum concentration of each agent that was soluble in the test solvent at room temperature, in order to avoid inadvertent precipitation in the dose solutions. A logarithmic progression of decreasing doses was selected in order to provide a wide range of specific doses, as motivated by finite dose diffusion models (Kasting and Miller, 2006).

The heating/stirring modules, each containing six unoccluded diffusion cells, were placed in a fume hood, allowing evaporation of both the vehicle and permeant. The sash was raised to 18" at which the air flow velocity was measured to be 0.874 m/s. The entire receptor solution (4.5 mL) was collected at 2, 4, 9, 24, 32, 51, and 72 h post-dose. After the terminal receptor sampling, the surface of the skin was twice washed with a dilute soap solution prepared from commercially available Dial® Gold antibacterial hand soap with moisturizer (1 part) and tap water (9 parts). The wash was performed by applying a 0.3 mL aliquot of the soap solution to the skin with a pipette and briefly and gently rubbing with a dose applicator (a thin, rounded glass rod) for 30 s. The solution was removed by transfer pipette and collected in a vial. The skin was then rinsed again in the same manner using 0.5 mL tap water. Each successive wash or rinse sample was collected into the same vial. The surface of the skin and the inside of the cell top was brushed with a cotton swab for 5 seconds and the swab was added to the vial containing the rinses.

The exposed portion of the skin was dissected to obtain epidermis and dermis samples,

which were dissolved at 55 ºC in 1 mL and 2 mL of Solvable™, respectively. The top and bottom sections of the Franz cell were briefly rinsed with ethanol, and the rinse was added to the wash sample. Tissue and wash sample analyses were performed for a known dose of ^{14}C-benzoic acid or ^{3}H-propranolol added to control samples. The recovery was insignificantly different from the dose in all cases except when ^{3}H-propranolol was applied to wash samples. The propranolol wash samples were corrected accordingly, being adjusted upward by a factor of 1.4.

Receptor solution data were averaged for each treatment for each donor using a quasi-logarithmic data transformation (Kasting et al., 1994) and are reported as percent of dose permeated. The transformation removed positive skewness. Log transformations or variations thereon are particularly useful for ionic permeants, which have even more asymmetric distributions than neutral permeants (Liu et al., 1993). Wash and tissue samples were averaged with equal weight within each donor. All of the data were then averaged with equal weight across donors to obtain the reported results.

2.3. Statistical significance

Pairwise comparisons between treatments were made via two-tailed t-test (GraphPad, www.graphpad.com/quickcalcs/ttest1). A p-value ≤ 0.05 was considered to be statistically significant.

3. Results

3.1. Benzoic acid

Cumulative permeation of radioactivity associated with benzoic acid into the receptor solutions, expressed as a percent of dose, is shown in Figure 1. Permeation generally increased with increasing percentage of free acid. This effect was not observed at the lowest dose (11 nmol/cm^2), but became more pronounced as the dose increased. The small deviation to this trend

for the 110 nmol/cm^2 dosage (75 vs 100% free acid) was not statistically significant. At 72 hours post-dose, permeation ranged from 1.5 to 41% of the applied dose for the highest dose (11000 nmol/cm^2) and from 21 to 33% for the lowest dose. The percentage of dose permeated was greater for the 110 nmol/cm^2 dose than the 11 nmol/cm^2 dose for the two highest free acid solutions and was less for the three lowest.

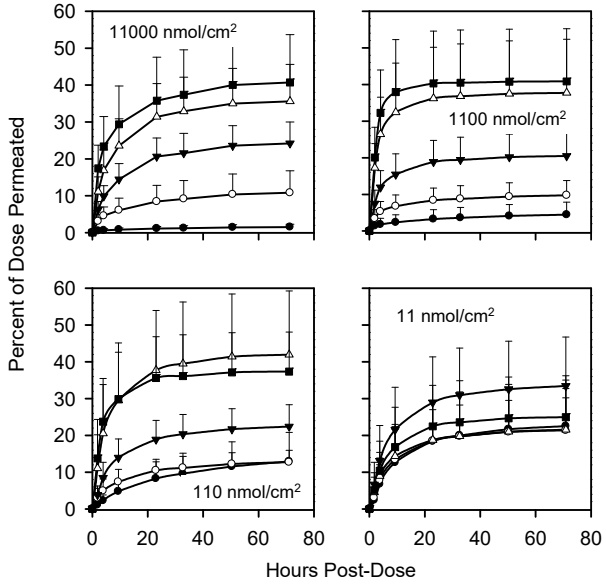

Figure 1. Cumulative amounts of ^{14}C-benzoic acid collected in receptor following doses of 11000, 1100, 110, and 11 nmol/cm^2 of benzoic acid/sodium benzoate mixtures in 1:1 ethanol/water solutions. Key: ■ 100% free acid; Δ 75% free acid; ▼ 50% free acid; ○ 25% free acid; ●) 0% free acid. The error bars (shown in the upward direction only) represent the standard error of the mean of three donors and six total replicates per treatment. The lines are a guide to the eye.

Figure 2 shows that permeation increased strongly with increasing percentage of free acid for the two higher doses and was approximately dose-proportional. The two lower doses showed departures from this behavior. The 110 nmol/cm^2 dose showed small variations for both 0% and 100% free acid compositions that led to a nearly sigmoidal curve; the departure from monotonic behavior for the 100% composition was not statistically significant as noted above. At 11 nmol/cm^2 permeation was essentially independent of ionization state.

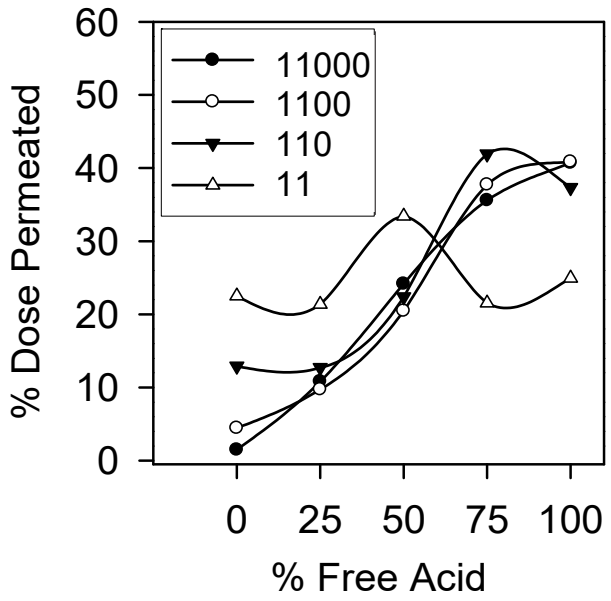

Figure 2. The 72 hour ^{14}C-benzoic acid data from Fig. 1 plotted as % free acid versus % of dose permeated. The lines are a guide to the eye.

The maximum permeation rate was achieved between 0 and 2 h post-dose for all treatments. There was no evidence of a time lag except possibly for the two lowest free acid treatments at the lowest dose, which had a time lag of up to one hour. A more detailed flux analysis may be found in the Discussion.

The average radioactivity mass balance for each treatment after 72 h is shown in Figure 3. Relative errors in the individual sample categories were comparable across treatments. Pooled relative standard errors (RSE) of the mean for the wash, epidermis, dermis and receptor samples were 18%, 42%, 42% and 44%, respectively. Because epidermis and dermis (and in some cases receptor solution or wash) contained little radioactivity, the absolute errors, SE = (RSE/100) × mean, associated with these compartments were very small. Some recoveries at the 11000 nmol/cm^2 dose reached or exceeded the theoretical dose applied, but most were substantially less than 100%. Amounts in the epidermis and the dermis ranged from about 0.4 to 3.5%, with no strong dependence on either dose or percentage of free acid in the dosing solution. The total amount of radioactivity recovered increased with increasing dose, but decreased with increasing percentage of free acid in the dosing solution.

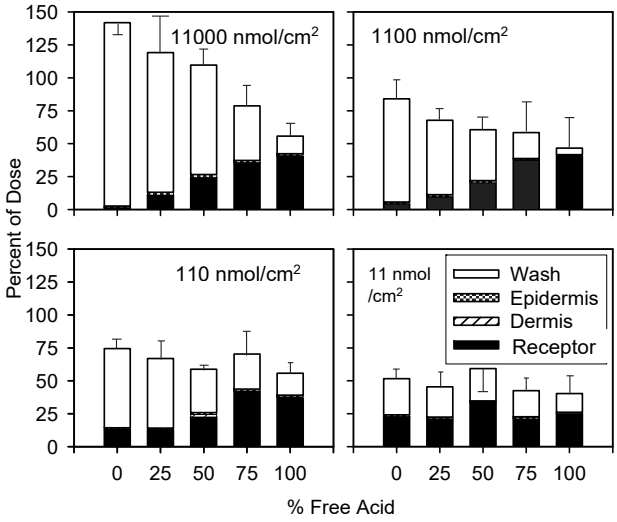

Figure 3. The distribution of radioactivity associated with ^{14}C benzoic acid after 72 hours. Error bars represent standard errors of the total mass balance for each dose and composition. Errors for the separate compartments are discussed in the text.

The cells dosed with the highest quantity of benzoic acid (11000 nmol/cm^2) at all compositions had an easily visible precipitate on the surface during the first day after application. This was particularly noticeable for one of the donors because the skin was darker. In some cases, the precipitate appeared to be thinner in some places, but accumulated in the crevices. By 50 h post-dose the observable precipitate had significantly diminished, especially for the cells dosed with 100% free acid. Little or no surface material was apparent on the cells to which lower doses were applied.

3.2. Propranolol

The results for propranolol (Figs. 4 and 5) were qualitatively similar to those for benzoic acid, but differed in some details. Permeation generally increased with increasing percentage of free base. The small deviations to this trend for 75 vs 100% free base levels at several dosages including 1930 nmol/cm^2 were not statistically significant. This effect was strong for the 1930 and 193 nmol/cm^2 doses, moderate for the 19.3 nmol/cm^2 dose and absent for the 1.93 nmol/cm^2 dose. At 72 h post-dose, permeation ranged from 1.3 to 36% for the highest doses and from 12 to

17% for the lowest dose. There were no significant differences between the highest two doses in percentage permeation into the receptor solution after 72 h, whether compared by average permeation over all compositions (18.0 vs. 18.2%) or within a specific composition.

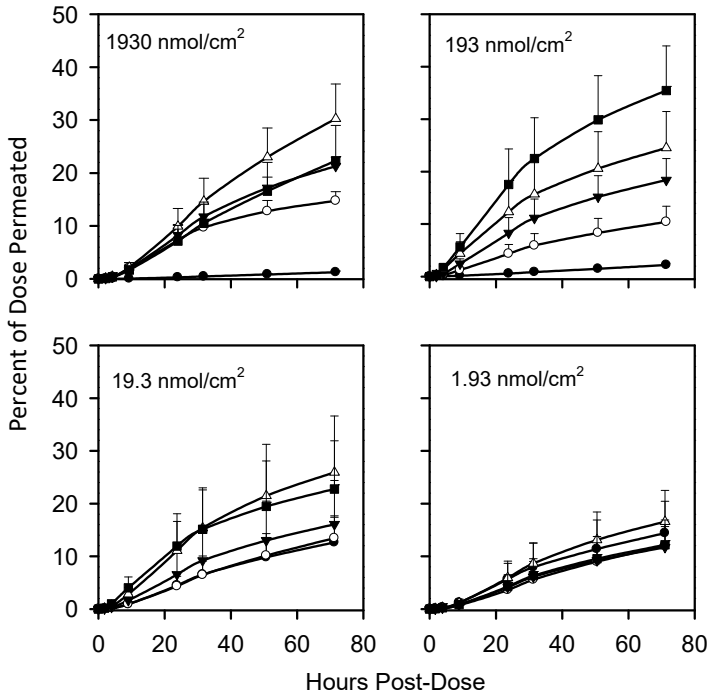

Figure 4. Cumulative amounts of ^{3}H-propranolol collected in receptor following doses of 1930, 193, 19.3, and 1.93 nmol/cm^2 of propranolol mixtures in 3:1 ethanol/water. Key: ■ 100% free base; Δ 75% free base; ▼ 50% free base; ○ 25% free base; ●) 0% free base. The error bars (shown in the upward direction only) represent the standard error of the mean of three donors and a total of 8 replicates per treatment. The lines are a guide to the eye.

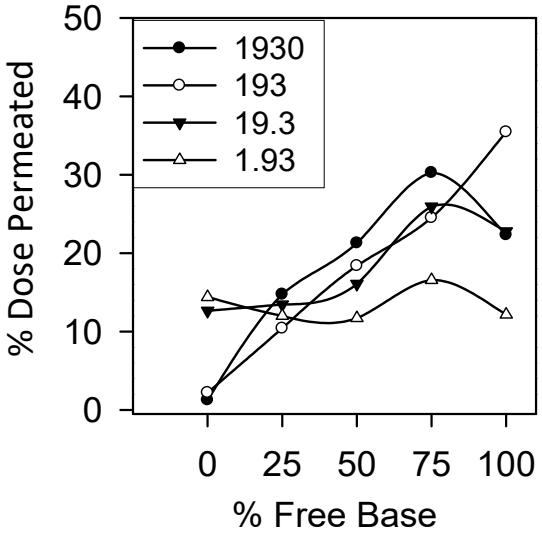

Figure 5. The 72 hour ^{3}H-propranolol data from Fig. 4 plotted as % free base versus % of dose permeated. The lines are a guide to the eye.

Maximum permeation rates were obtained much later than those for benzoic acid, usually occurring between 24 and 32 h post-dose. These values increased with increasing percentage free base for all but the 100% free base formulation. The time lag ranged from about 1 to 9 h and generally decreased with increasing percentage of free base in the dose solution. This effect was not observed at the lowest dose, but became more apparent as the dose increased.

The average radioactivity mass balance for each treatment 72 h after application is shown in Figure 6. Relative errors in the individual sample categories were comparable across treatments. Pooled RSEs of the mean for the wash, epidermis, dermis and receptor samples were 13%, 34%, 30% and 32%, respectively. The percentage of dose in the epidermis and the dermis at 72 h post-dose was substantially higher than that of benzoic acid, ranging from 3 to 24%. The total amounts recovered varied from 86 to 106%, with no substantial dependence on either free base percentage or total dose. The average recovery across all 20 treatments was (94.3 ± 5.5)%.

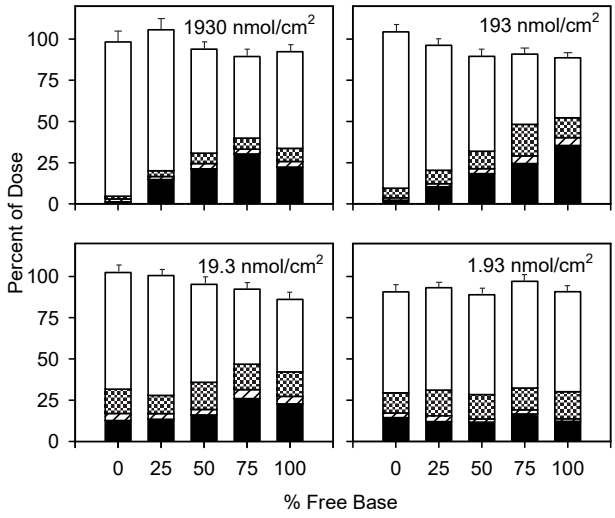

Figure 6. The distribution of radioactivity associated with ^{3}H-propranolol after 72 h. The key is the same as in Fig. 3. Error bars represent standard errors of the total mass balance for each dose and composition. Errors for the separate compartments are discussed in the text.

4. Discussion

The comments that follow reflect investigators' interpretation of the results presented.

4.1. Volatility

Free benzoic acid has a vapor pressure in the semi-volatile range (about 40% that of the mosquito repellent DEET (Kasting et al., 2008)), whereas sodium benzoate and both forms of propranolol have vapor pressures lower by at least four orders of magnitude (Table 1). Therefore, it is not unexpected that a substantial loss of radioactivity occurs from the ^{14}C-benzoic acid formulations following a 72 h exposure on skin mounted in unoccluded Franz diffusion cells and placed in a fume hood (Fig. 3). It is worth noting, however, that the loss of radioactivity occurred in compositions formulated with substantial amounts of sodium benzoate, up to and including 100%, i.e. 0% free acid. Furthermore, the impact of the initial composition on evaporation decreased with decreasing dose on the skin. The fact that compositions containing 100% sodium benzoate, a nonvolatile salt, suffered substantial evaporative loss establishes that a certain fraction of sodium benzoate was converted to free benzoic acid during the course of the 72 h exposure. It is evident that the skin buffered the effective pH of the deposited dose back toward the natural pH of the SC (5.0-5.5) during the exposure period. In this pH range, the nonionized fraction of benzoic acid in an aqueous environment such as the corneocyte phase of the SC is 0.05-0.14. The dose dependence of evaporation of this composition suggests that the magnitude of the pH drift depends on the ratio of the buffering capacity of the deposited dose to that of the region of the SC into which the dose is deposited. At very low doses, the SC dominates and volatility is independent of the initial ionization state of the applied weak electrolyte.

Recovery of radioactivity associated with ^{3}H-propranol averaged 94.3% and was virtually

independent of dose and ionization state, as would be expected for nonvolatile materials (Fig. 6).

4.2. pH drift, nature of the precipitate and potential solvent effects

The pH of the dose solutions must drift during dry down for one or more reasons. In all cases, the ethanol component of the solvent evaporates more quickly than water due to its higher vapor pressure; thus the solutions become more aqueous than hydroalcoholic as dry down proceeds. For both solvents evaporation proceeds much more rapidly than penetration into the skin – see, for example, (Gajjar and Kasting, 2014) who show that a maximum of 0.5% of an ethanol dose applied to skin under similar conditions to those in the present study permeates to the receptor solution. This change in solvent composition combined with the ever-increasing solute concentration leads to a drift of apparent pH. More importantly, as the solution becomes more aqueous, the neutral form of the dissolved solute begins to precipitate, as it is much less water soluble than the salt. When benzoic acid precipitates (or evaporates – see Sect. 5.1), the pH of the remaining solution must rise. Conversely, when propranolol precipitates, the pH must fall. Thus the fraction of neutral solute left in the solution will decrease in both cases. With rapid evaporation of the solvent, the salt solubility limit is soon reached and the salt must precipitate. We contend that, with highly volatile solvents such as water and ethanol, the composition of the precipitates will eventually approximate the composition of the initial solution, as a consequence of stochiometry. The skin permeation data bear this out.

The use of ethanol:water solutions for this study was questioned during review of this manuscript. We offer the following comments. Hydroalcoholic gels based on either ethanol or isopropanol have been employed in topical products for decades and are well accepted by both consumers and regulatory agencies. In topical applications typical of consumer use only a small fraction of the alcoholic component permeates through the skin (Gajjar and Kasting, 2014). In

their extensive study of the effects of ethanol in combination with other solvents on the skin permeation of methyl paraben, Oliveira et al. (Oliveira et al., 2012) state "The results showed that the presence of EtOH had little effect on the skin flux of methyl paraben compared with the corresponding saturated solutions. Formulations incorporating the volatile solvent were clearly more efficient, in line with the data obtained with silicone membranes." We carefully distinguish small, unoccluded applications of ethanolic solutions from large or occluded applications of ethanol:water mixtures, which can swell the skin (Berner et al., 1989a) and cause substantial increases in its permeability (Berner et al., 1989b). As a final remark, we note that the ethanol:water solvent was common across compositions for each test solute in the present study. It is therefore unlikely to have contributed to the composition-related differences in skin permeation reported here.

4.3.Dose and ionization state dependence of permeation

Both benzoic acid and propranolol showed dose-proportional permeation and a strong dependence of permeation on ionization state when tested at the two highest doses in their respective studies (1100 and 11000 nmol/cm^2 for benzoic acid; 193 and 1930 nmol/cm^2 for propranolol, Figs. 2 and 5). These doses are substantially larger than the saturation doses for the SC deposition zone, M_{sat} = 64 nmol/cm^2 and 128 nmol/cm^2, respectively, calculated for the nonionized form of these compounds according to the method described in (Kasting and Miller, 2006) and later summarized by (Dancik et al., 2013). This comparison suggests that a large portion of the test permeants precipitated on the skin surface as a solid deposit when applied at these doses. Nevertheless, permeation of the free acid or base species proceeded efficiently for both compounds until either the dose was exhausted (benzoic acid) or the experiment was terminated (propranolol). Using the nomenclature suggested in (Kasting et al., 2022), we

tentatively classify these compounds as "soft solids", i.e. they have a sufficiently high lipid solubility and dissolution rate that they continue to permeate the skin from well dispersed solid precipitates.

The permeation of smaller doses of both benzoic acid and propranolol showed either weaker dependence or no dependence on the initial ionization state (11 and 110 nmol/cm^2 for benzoic acid; 1.93 and 19.3 nmol/cm^2 for propranolol, Figs. 2 and 5). The average percentage permeation over 72 h across ionization states for the lowest dose was (24.8 ± 5.1)% for benzoic acid and (13.4 ± 2.1)% for propranolol. A comparison of Figs. 2 and 5 suggests that the effective buffer capacity of the accessible region of the SC, according to the manner in which the compounds are deposited, is approximately 10-20 nmol/cm^2. Whether this "accessible region" corresponds to the SC deposition zone as defined in (Kasting and Miller, 2006) or to some other property of the tissue is an open question.

4.4. Literature comparisons of permeation and skin buffer capacity

Finite dose absorption of benzoic acid (applied as free acid) through skin has been studied several times in vitro (Hotchkiss et al., 1992; Scheuplein and Ross, 1974) and in vivo (Roskos et al., 1989; Wester and Maibach, 1976). Steady-state permeability of propranolol from aqueous solutions as a function of pH has been measured (Chantasart et al., 2013), as has finite dose absorption of propranolol hydrochloride from topical gels (Zhang et al., 2015). To facilitate comparisons with these data, doses in this section will be expressed in mass rather than mole units. In this metric, the range of doses tested in the present study was 1.34-1340 μg/cm^2 for benzoic acid and 0.50-500 μg/cm^2 for propranolol.

A prominent feature of the present data is that there was little evidence that the percent of dose absorbed for compositions containing greater than 50% free acid or base ever began to

decrease with increasing dose, although the higher doses substantially exceeded the calculated M_{sat} values of 7.8 μg/cm^2 (benzoic acid) and 33 μg/cm^2 (propranolol). Such a decrease was observed (from 36.7% at 3 μg/cm^2 to 14.4% at 2000 μg/cm^2 in 72 h) in Wester et al.'s human *in vivo* experiments with free benzoic acid, using acetone as the deposition vehicle (Wester and Maibach, 1976). A plot of these data versus our in vitro data is shown in Figure 7. The plot suggests a difference in the buffering capacity of the SC in vivo and in vitro over the 72 h duration of the studies (note that Wester and Maibach's study ran for 120 h, but benzoic acid absorption was complete by 72 h). According to this hypothesis, the living skin has more capacity to return pH to its natural value than excised skin. In our studies, for large doses, the excised skin pH was evidently dominated by that of the dose solution (estimated pH 2.8 for free benzoic acid, c.f. Table 1) over the 72 h exposure time. This concept draws considerable support from earlier work on skin buffer capacity, which has been defined in terms of repeated exposures to strong acid and alkali and the gradual drift of skin surface pH back to its natural value (taken to be ~6.0 in the literature cited here) (Levin and Maibach, 2008; Zhai et al., 2009; Zheng et al., 2012). In both instances, living skin is found to have higher buffering capacity than excised skin.

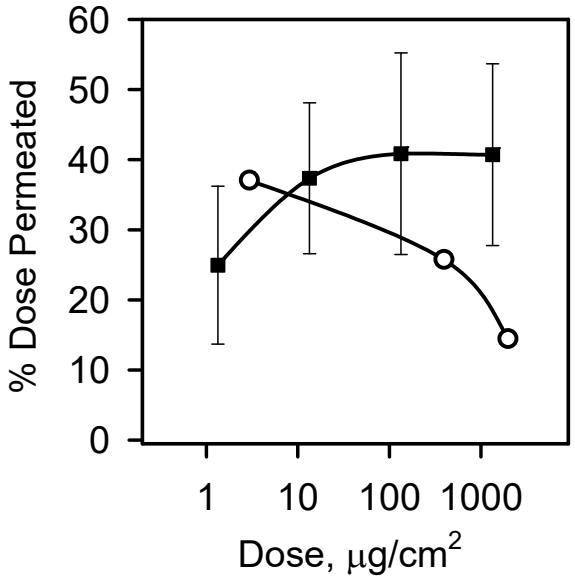

Figure 7. Cumulative amounts of free benzoic acid permeated through human skin in 72 h. The filled squares (■) represent in vitro data from the current study. The open circles (○) represent amounts absorbed in humans in vivo (Wester & Maibach, 1976). The lines are a guide to the eye.

Permeation of free benzoic acid at 1.3 and 13 μg/cm^2 in the present study fell within the range of that observed for a dose of 4 μg/cm^2 applied to the ventral forearms of young and old human subjects in vivo (Roskos et al., 1989). The time course of absorption in these studies was very similar. The 1.3 μg/cm^2 result was furthermore in general agreement with an in vitro study conducted by Hotchkiss and coworkers (Hotchkiss et al., 1992) in which 0.3 μg/cm^2 of free benzoic acid was applied to full thickness human skin. However, the time course of permeation in the Hotchkiss study was greatly delayed versus the present study. The delay may well have resulted from Hotchkiss' use of unperfused, full thickness human skin, which is often 2-3 mm thick.

Finally, Scheuplein and Ross followed the absorption of tracer levels of ^{14}C-benzoic acid and other radiolabeled compounds through human skin in vitro following application in small volumes of acetone. Benzoic acid doses were in the range 6.9-69 nmol/cm^2, comparable to the lower two doses in the present study. Maximum absorption rates ranging from 3-48 nmol/cm^{2}h were achieved between 1 and 2 h post dose and were found to be approximately dose-proportional. Peak fluxes for 100% free benzoic acid formulations in the present study were in the range 0.32-905 nmol/cm^{2}h and occurred between 0 and 2 h post dose for the higher doses and between 2 and 4 h for the lower doses (data not shown). They were found to have a power law relationship of the form $J_{max} \propto (\text{Dose})^{1.15\pm0.08}$. Peak fluxes for the smaller doses were lower than those reported by Scheuplein and Ross, most likely because they were averaged over a broader time interval. The greater than dose-proportional relationship of J_{max} to dose suggested that the higher doses of benzoic acid enhanced their own permeation. This effect may have counteracted the expected decrease in percentage permeation at doses above M_{sat}, resulting in the approximately dose-proportional permeation curves seen in Fig. 2.

Direct comparisons with finite dose skin permeation of propranolol and its salts are rare. Chantasart, Li and coworkers studied the steady-state absorption of propranolol and other β-blockers from aqueous solution as a function of pH (Chantasart et al., 2013) and also the finite dose absorption of propranolol hydrochloride from topical gels (Zhang et al., 2015). They found the pH dependence of propranolol permeability to be substantially less than that predicted from the pH-partition hypothesis, in agreement with results on other weak bases (Roy and Flynn, 1989, 1990). We found an approximately 28-fold difference in the amounts of propranolol free base and propranolol hydrochloride permeating the skin in 72 h, in qualitative agreement with (Chantasart et al., 2013). However, the maximum flux obtained with high doses of propranolol free base of 8.3 nmol/(cm^2h) in our study (data not shown) is much smaller than the value of 180 nmol/(cm^2h) observed for steady-state flux of propranolol from a saturated aqueous solution at pH 8.0, (Chantasart et al., 2013). The difference in skin hydration levels between these studies can account for some of difference in flux, typically a factor of 3-4 (Dancik et al., 2013). Peak fluxes achieved for the 100% free base formulations in the present study followed a power law relationship of the form $J_{max} \propto (\text{Dose})^{1.08\pm0.10}$, i.e. almost dose-proportional, in agreement with the 72 h permeation results shown in Fig. 4.

4.5. *Scope*

Investigators recognize that there is a considerable body of literature concerning skin permeation of weak electrolytes not considered in this study. For example, the impact of counterion selection on permeation including the possibility of ion pair formation using organic counterions (Hadgraft and Valenta, 2000; Valenta et al., 2000) and the additional considerations regarding effective topical formulations of weak electrolytes, e.g. diclofenac (Brunner et al., 2012; Brunner et al., 2005; Haltner-Ukomadu et al., 1019) are not discussed. Both are important

elements of formulation design. The present study concerns simple hydroalcoholic compositions of a weak acid and a weak base and their sodium and hydrochloride salts, respectively. These compositions are not expected to ion pair in skin lipids or to be used therapeutically to treat skin disorders. The focus of this article is on skin buffer capacity.

5. Conclusions and recommendation

The permeation of a solvent-deposited weak acid (benzoic acid) and a weak base (propranolol) through excised human skin 0-72 h post dose was measured as a function of dose and ionization state. Permeation of both compounds increased strongly with increasing fraction of free acid or base for doses greater than 100 nmol/cm^2. The effect was less pronounced for lower doses and absent for doses less than 19 nmol/cm^2. Analysis of the low dose results suggests that the buffer capacity of the accessible region of the SC, for electrolytes deposited on skin in this manner, is in the range 10-20 nmol/cm^2. This measure of skin buffer capacity is quite different from the commonly cited method of observing the return of skin surface pH to its natural value following challenge with a strong acid or base (Levin and Maibach, 2008; Zhai et al., 2009; Zheng et al., 2012).

Permeation of both compounds continued well after the dose solvent had dissipated, despite the fact that both the free acid/base and the salts employed are solids at skin temperature. There was no evidence of dissolution-limited absorption in the dose range tested. Both cumulative permeation and maximum flux of propranolol base were nearly dose-proportional, whereas the maximum flux of benzoic acid (which was tested at a 6-fold higher dose) was more than dose-proportional, suggesting that it enhanced its own permeation at high doses.

Based on these results, we recommend that investigators designing finite dose IVPTs for the purpose of predicting the skin disposition of small quantities of weak electrolytes, e.g. amines and

carboxylic acids, from fully formulated products carefully consider not only the dose, but also the pH and buffer capacity of the test formulations. All three factors play a significant role in the disposition of the test permeant. Use of conditions leading to test permeant ionization states other than those found in the product formulations may lead to misleading results. At the very least, the results will be difficult to interpret.

Conflict of interest

The authors declare no conflict of interests.

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It is a pleasure to contribute this experimental study to this special issue of IJP honoring Dr. Ken Walters. Ken was an experimental scientist at heart, deeply rooted in the study of topical and transdermal delivery. He was also a consummate organizer and a man of great warmth and humor – a highly sought after debate team member in Barrier Function GRCs. We will miss his warmth and perspective in the skin sciences area.

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