Influences of 1-Dodecylazacycloheptan-2-one on Permeation of Membranes by Weak Electrolytes. 1. Theoretical Analysis of Weak Electrolyte Diffusion through Membranes and Studies Involving Silicone Rubber Membranes

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Abstract □ The pH dependency of permeation of weak electrolytes allows inferences to be made about the barrier characteristics of membranes. The influences of enhancers on pH–permeation profiles promise further mechanistic enlightenment. To explore issues of weak electrolyte mass transfer, a steady-state mathematical model for a hydrophobic membrane with aqueous pores existing in series with aqueous phases, presently a popular depiction of the skin and other biological barriers, has been developed. The case in which there are no pores is then considered theoretically and in studies involving the mass transfer of benzoic acid across silicone rubber membranes. Specifically, the flux of [^{14}C]benzoic acid across Silastic sheeting as a function of pH was investigated. This isotropic membrane's behavior conformed to expectations drawn from the model in that the un-ionized species penetrated in proportion to benzoic acid's prevailing state of ionization, the membrane being all but impenetrable to the benzoate anion. The enhancer, 1-dodecylazacycloheptan-2-one (Azone), was then applied to the membrane in emulsions of increasing concentration. There were two important consequences of such application. First, the un-ionized species of benzoic acid partitioned into the emulsion droplets, lowering the activity of the permeant in the emulsion's continuous phase. Second, Azone was imbibed to a degree into the polymeric membrane, significantly altering the permeability of the silicone rubber of which it is composed. The former influence had to be carefully factored out in order to delineate Azone's intrinsic enhancing effects on the membrane. The silicone rubber membrane system served well as a model for study of the enhancing effects of Azone on a wholly hydrophobic barrier, establishing a basis for the analysis of the actions of enhancers such as Azone on more complex, multiphasic biological barriers.

Introduction

1-Dodecylazacycloheptan-2-one (Azone) enhances the permeability of lipid membranes to a variety of substances. In particular, a large number of *in vitro* and *in vivo* studies have been reported on various aspects of its ability to increase the permeability of skin.[1,2] Previously we studied Azone's enhancement of the permeation of hydrocortisone, a nonelectrolyte of 362.5 daltons (Da) molecular weight, through mouse and human skin.[3] Azone emulsions influenced both the kinetic and thermodynamic determinants of the skin's permeability.[3,4] In the present studies, we extend our investigation to the permeation of membranes by weak electrolytes, for pH profiles obtained for such permeants promise to be generally informative relative to barrier function.[5–7]

Silicone rubber sheeting was chosen as a model membrane lacking pores to begin the studies. Eventually we plan to rationalize the influences of Azone on the skin in the light of its influences on the permeation of this model membrane. This strategy is in keeping with past work from our laboratories in which we have successfully incorporated principles drawn from the behavior of simple membranes to set up models to better understand the functioning of far more complex biological barriers.[6,8–10] Benzoic acid, a monofunctional acid of pK_a 4.2 at 37 °C,[11] was chosen as the test compound. Its dissociation constant is high enough on the pH scale to allow study of the permeability of the membranes of interest at pH values where benzoic acid exists exclusively as an un-ionized species and where it is totally ionized.

Theoretical

General Concepts of Permeation of Laminated Barriers—The barrier system of interest consists of a membrane placed between two well-stirred aqueous phases, yielding a diffusional barrier minimally composed of three strata in series, the membrane and its flanking boundary layers. The membrane itself can be simple, i.e., a functionally isotropic field as found in silicone rubber, or complex, i.e. a layered structure with parallel pathways such as the skin.[5,6,12–14] Complexities such as the latter are inherent in most biological barriers, and they influence the magnitudes and dependencies of the mass transfer coefficients (permeability coefficients) determined for the membranes. Implicit here is the fact that the geometrical organization of phases within a membrane is critical to the membrane's function. Invariably, the mathematical expressions set out for complex membranes in the course of developing models for them are premised on specific investigator assumptions about the structure of the membrane. A membrane with aqueous pores (an aqueous pore pathway), for instance, would necessarily function differently than one with none and would require a different mathematical characterization.

We are interested in identifying and better defining the transport influences of aqueous channels which may exist within biological barriers. We are particularly interested in determining if such channels exist within the skin barrier. Suzuki et al.[8,9] and Ho et al.[10] proposed a mathematical model for drug transport across the gastrointestinal tract. The flux per unit area through n laminae in series in a steady state (or quasisteady state) can be described by

$$\left(\frac{dM}{dt}\right)_{\text{steady state}} = P\left(C_0 - \frac{C_{n+1}}{\kappa}\right) \quad (1)$$

where P (units usually = cm/h) is the overall mass transfer or permeability coefficient and C_0 and C_{n+1} are the concentrations in the so-called donor and receiver phases of a two-compartment diffusion cell, respectively.[12,13] The term κ is a dimensionless constant governed by the nature of the external phases. If the external phases are identical, $\kappa = 1$. Otherwise, κ takes on a more complex form. In this equation, as in the subsequent ones, dM/dt takes the units of mass/time/area (mg/h/cm^2).

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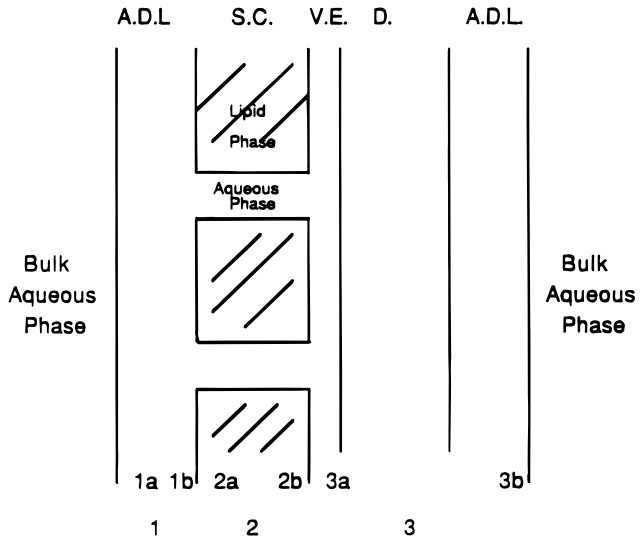

Figure 1—Schematic diagram for the skin with the stratum corneum as a two-phase permeability barrier. A.D.L. = aqueous diffusion layer; S.C. = stratum corneum; V.E. = viable epidermis; D. = dermis.

The overall permeability coefficient, P, of a series barrier is determined by the permeability coefficients of its individual layers, i.e.,

$$\frac{1}{P} = \sum_{i=1}^{n} \frac{1}{P_i} \tag{2}$$

The reciprocal, $1/P$, is the total diffusional resistance experienced by a permeant within the diffusion barrier and is the sum of the resistances of all individual laminae in series within the barrier, each in turn being the reciprocal of its individual permeability coefficient. For an isotropic layer, $P_i = D_i K_{0,i}/h_i$ where D_i and h_i are the diffusivity and thickness of the ith lamina, respectively, and $K_{0,i}$ is the partition coefficient for the permeant between that particular lamina and the donor phase. The partition coefficient thus provides a measure of the capacity of the ith lamina (phase) to transport (dissolve) the permeant relative to the capacity of the applied phase. Also, in the particular instance of the terminal hydrodynamic or boundary layer (stagnant diffusion layer in the receiver), $K_{0,n} = K_{0,n+1} = \kappa$, the partition coefficient between the receiver and donor external phases. Thus it can be seen that κ scales the thermodynamic activity of the permeant in the receiver phase to its activity in the donor phase.

Steady-State Permeation of a Weak Electrolyte through a Hydrophobic Membrane with Parallel Aqueous Channels—One model which has been put forth to describe the barrier properties of cornified epithelial membranes is a hydrophobic membrane penetrated by pores which is, in turn, attached to a cell mass (noncornified) acting physically as an aqueous slab in series.[6] Consequently, the diffusional resistances of the flanking boundary layers generated within the diffusion cell are folded in with the resistance of the aqueous cell mass to arrive at the total aqueous resistance of the barrier. A representative barrier construct with particular relevance to the skin is sketched in Figure 1.

Within chemically tolerable bounds, the pH's of the bulk phases external to a membrane can be varied at will, allowing one to express a proton gradient across the membrane by simply placing media of different pH's in the opposite compartments of the diffusion cell. Mass transfer of a permeant across the barrier then depends on the specific physicochemical nature of the membrane and, in the instance of a weak electrolyte, the pH of the external media. The latter determines the degrees of ionization of the weak electrolyte permeant in the fronting phases of the membrane. Penetration enhancers like Azone alter the rates of mass transfer of permeants across barriers in accordance with their physicochemical effects on the barrier phases themselves. Enhancers invariably influence partitioning of the permeant, and the un-ionized and ionized species necessarily respond differently to the effects of the enhancer. To account for such effects, we provide here a derivation for a membrane with aqueous pores, the equations differing slightly from the equations of Suzuki, Higuchi and Ho,[9] and Ho, Higuchi, and Turi,[10] in that the equations here account for tissue and hydrodynamic layers on the receiver side and nonsink conditions.

In our model, we assume that (1) a weak acid is the permeant (benzoic acid being the test compound); (2) its anionic and un-ionized species diffuse with equal facility through the aqueous regions of the barrier, including those penetrating the otherwise hydrophobic element of the membrane; (3) the un-ionized species alone partitions into and diffuses through the hydrophobic regions of the membrane; (4) the affinities of the aqueous regions for the un-ionized species are identical; (5) the membrane element of the total barrier has two continuous pathways, one hydrophobic and one functionally aqueous; (6) individual strata characteristics are fixed in place and stable; and (7) unit area. The first and last of these assumptions need no explanation. The second assumption, setting the aqueous diffusion coefficients of the un-ionized and ionized species to the same value, is not unreasonable given that the species are roughly equal in size. The third assumption necessarily means that the fractions of un-ionized species in the respective layers abutting the nonpolar membrane phase are responsible for the concentration gradient across this phase (in conjunction with appropriate partition coefficients). The fourth assumption allows a single partition coefficient to be used to describe interfacial concentrations between the hydrophobic phases of the membrane and its contiguous aqueous phases. Assumption 5 sets out the dual pathway characteristic of the membrane, while assumption 6 establishes that the identified phases, however heterogeneous, are fixed in their properties as functions of both depth and time. This allows one to incorporate integral rather than differential diffusion coefficients into the equations.

One can write independent differential equations for the fluxes of a weak acid across each of the three phases specified for the barrier. The flux across the initial boundary layer is described by the simple equation

$$\left(\frac{dM}{dt}\right)_1 = \frac{D_1^{0/-}}{h_1}(C_{1a}^{T} - C_{1b}^{T}) \tag{3}$$

where the permeability coefficient for the phase, P_1, is equal to $D_1^{0/-}/h_1$. The total flux across the central membrane element is the sum of the fluxes across its assumed two independent, parallel pathways and takes the substantially more complex form

$$\left(\frac{dM}{dt}\right)_2 = \alpha\left[\frac{D_2^0}{h_2}(C_{2a}^0 - C_{2b}^0)\right] + (1-\alpha)\left[\frac{D_2^{0/-}}{h_2}(C_{2a}^- - C_{2b}^-) + \frac{D_2^{0/-}}{h_2 K}(C_{2a}^0 - C_{2b}^0)\right] \tag{4}$$

The subscripts 1, 2, and 3 in these equations and the ones

that follow designate the respective layers of the trilaminate. In these equations D stands for diffusivity, h for thickness, and C for concentration. All concentrations are interfacial concentrations, and the subscripts ia and ib are used to designate the layer (i = 1, 2, or 3) and the interface of the layer (a = upstream interface, b = downstream interface), respectively. α represents the fractional volume of the hydrophobic phase of the central membrane element, making $(1 - \alpha)$ the fractional volume of the pore phase of this element. The superscripts 0, −, and 0/− refer to the un-ionized permeant (zero charge), to the anion, and to parameters for which the un-ionized and ionized forms take the same value, respectively. T superscripted on C designates the total permeant concentration, un-ionized plus ionized, at an interface. The individual contributions of the dual pathways through the central membrane element are separately accounted for. At each of the interfaces it makes with the aqueous strata, the concentration within the hydrophobic phase of the key membrane element is determined by the concentration of the un-ionized species in the adjacent medium, and this is true within the aqueous pores of the central membrane as well. Since the physicochemical characteristics of the aqueous phases are presumed to be the same, a common partition coefficient can be used to describe the o/w partitioning of the permeant at every level, i.e.,

$$C^0_{2a} = KC^0_{1b} = KC^T_{1b}X_{1b} \tag{5}$$

$$C^0_{2b} = KC^0_{3a} = KC^T_{3a}X_{3a} \tag{6}$$

For a monoprotic weak acid, the un-ionized fraction, X, at the various locations is given by either $1/(1 + K_a/[H^+])$ or $1/(1 + 10^{pH-pK_a})$. It follows that

$$\left(\frac{dM}{dt}\right)_2 = C^T_{1b}\left[\alpha\left(\frac{D^0_2}{h_2}\right)KX_{1b} + (1-\alpha)\left(\frac{D^{0/-}_2}{h_2}\right)\left(\frac{X_{1b}}{X_{2a}}\right)\right] - C^T_{3a}\left[\alpha\left(\frac{D^0_2}{h_2}\right)KX_{3a} + (1-\alpha)\left(\frac{D^{0/-}_2}{h_2}\right)\left(\frac{X_{3a}}{X_{2b}}\right)\right] \tag{7}$$

If $P^0_2 = \alpha KD^0_2/h_2$ and $P^{0/-}_2 = (1 - \alpha)D^{0/-}_2/h_2$ are the permeability coefficients for the hydrophobic phase and aqueous phase through the dual pathways of the membrane, respectively, then

$$\left(\frac{dM}{dt}\right)_2 = C^T_{1b}\left[P^0_2X_{1b} + P^{0/-}_2\left(\frac{X_{1b}}{X_{2a}}\right)\right] - C^T_{3a}\left[P^0_2X_{3a} + P^{0/-}_2\left(\frac{X_{3a}}{X_{2b}}\right)\right] \tag{8}$$

Finally, the equation for flux across the third layer in series takes the form

$$\left(\frac{dM}{dt}\right)_3 = \frac{D^{0/-}_3}{h_3}(C^T_{3a} - C^T_{3b}) \tag{9}$$

where the permeability coefficient through this phase is $D^{0/-}_3/h_3$. It should be noted that, in the instance of a biological barrier like the skin, h_3 would include the effective thickness (resistance) of any adhering cellular (aqueous) tissue in addition to the terminal hydrodynamic layer.

By definition, for the steady state (or quasisteady state),

$$\left(\frac{dM}{dt}\right)_{steady\ state} \equiv \left(\frac{dM}{dt}\right)_1 \equiv \left(\frac{dM}{dt}\right)_2 \equiv \left(\frac{dM}{dt}\right)_3 \tag{10}$$

Furthermore, invoking eq 2 we have

$$\frac{1}{P_{total}} = \frac{1}{P_1} + \frac{1}{P_2} + \frac{1}{P_3} \tag{11a}$$

a statement affirming that resistances in series are additive. The respective permeability coefficients for the model are

$$P_1 = P^{0/-}_1 \tag{11b}$$

$$P_2 = P^0_2X_{1b} + P^{0/-}_2\left(\frac{X_{1b}}{X_{2a}}\right) \tag{11c}$$

$$P_3 = P^{0/-}_3\left[\frac{P^0_2X_{1b} + P^{0/-}_2\left(\frac{X_{1b}}{X_{2a}}\right)}{P^0_2X_{3a} + P^{0/-}_2\left(\frac{X_{3a}}{X_{2b}}\right)}\right] = P^{0/-}_3\kappa \tag{11d}$$

The overall permeability coefficient arrived at is highly complex; it takes on a more manageable form under certain conditions. If the buffer capacities in the bulk aqueous phases are high, a uniform pH, the same as in the bulk phases, effectively exists across the aqueous diffusion layers. We can further assume that the pH of any cellular tissue abutting one of the external compartments adjusts to that of the bulk phase with which it is in contact. The pH values across the aqueous channels positioned within the lipoidal element of the membrane necessarily vary from point to point since the pH here is set by diffusion of the buffer species from the interfacing aqueous regions into the channels. However, if the pH's of the donor and receiver bulk aqueous phases are set to the same value, then $X_{1b} = X_{3a}$, $X_{1b} = X_{2a}$, and also $X_{2b} = X_{3a}$. It follows that $\kappa = 1$ and $P_3 = P^{0/-}_3$. The effective permeability coefficient becomes

$$P = \frac{1}{\frac{1}{P^{0/-}_1} + \frac{1}{P^0_2X_{1b} + P^{0/-}_2} + \frac{1}{P^{0/-}_3}} \tag{12}$$

The steady-state flux (per unit area) of a weak acid crossing the trilaminate having the membrane configuration set forth here is then

$$\left(\frac{dM}{dt}\right)_{steady\ state} = P(C^T_{1a} - C^T_{3b}) = P(C_{donor} - C_{receiver}) \tag{13}$$

The customary experimental analysis can be carried out to obtain the operative, overall permeability coefficient.

Behavior in the Absence of Aqueous Channels—A clear interpretation of data generated on membranes with pores depends on knowledge of how the membrane would behave without pores. Therefore, it is appropriate to first deal with this case. Polydimethylsiloxane (silicone rubber, Silastic) is a functionally isotropic material with a solubility parameter of 7.6, only slightly greater than that of a pure hydrocarbon (solubility parameter of hexane = 7.27). Permeation of the barrier created when this membrane is placed in the diffusion cell involves diffusion through the polymeric substance of the membrane and also diffusion through inescapable hydrodynamic boundary layers flanking the membrane. Setting α = 1 and $(1 - \alpha) = 0$ in eq 7 (no pores) leads to

$$\left(\frac{dM}{dt}\right)_2 = C^T_{1b}\left(\frac{D^0_2}{h_2}\right)KX_{1b} - C^T_{3a}\left(\frac{D^0_2}{h_2}\right)KX_{3a} \tag{14}$$

The expressions set out for $(dM/dt)_1$ and $(dM/dt)_3$ are the same as in the master derivation (eqs 3 and 9, respectively). Thus

$D_1^{0/-}/h_1$, $KD_2^0X_{1b}/h_2$, and $D_3^{0/-}(X_{1b}/X_{3a})/h_3$ are the individual permeability coefficients of the three defined strata, P_1, P_2, and P_3, respectively, and

$$\left(\frac{dM}{dt}\right)_{\text{steady state}} = \left[\frac{1}{\frac{h_1}{D_1^{0/-}} + \frac{h_2}{KD_2^0X_{1b}} + \frac{h_3}{D_3^{0/-}(X_{1b}/X_{3a})}}\right](C_{1a}^T - C_{3b}^T/\kappa) \quad (15)$$

where $\kappa = X_{1b}/X_{3a}$. It will again be recognized that the C_{1a}^T and C_{3b}^T represent the concentrations of the donor (upstream compartment) and receiver (downstream compartment) phases, respectively. When the donor and receiver pH's are equal, $X_{1b} = X_{3a}$, and eq 15 becomes

$$\left(\frac{dM}{dt}\right)_{\text{steady state}} = \left(\frac{1}{\frac{h_1}{D_1^{0/-}} + \frac{h_2}{KD_2^0X_{1b}} + \frac{h_3}{D_3^{0/-}}}\right)(C_{1a}^T - C_{3b}^T) \quad (16)$$

The concentration differential between these bulk phase concentrations is often represented simply by ΔC. Staying with unit area, it follows that

$$\left(\frac{dM}{dt}\right)_{\text{steady state}} = P\Delta C \quad (17)$$

A second case of theoretical interest is when the receiver phase pH is far greater than that of the donor phase. In this case $X_{3a} \ll 1$, $X_{3a} \ll X_{1b}$, and

$$\left(\frac{dM}{dt}\right)_{\text{total in the steady state}} = \left(\frac{1}{\frac{h_1}{D_1^{0/-}} + \frac{h_2}{KD_2^0X_{1b}}}\right)C_{1a}^T \quad (18)$$

An alkaline pH in the receiver compartment leads virtually to a sink condition at the downstream boundary of the membrane.

It is reasonable to assume that a molecule like Azone has little influence on diffusion through the aqueous phases because Azone is very insoluble in the aqueous phases. As a plasticizer, Azone can be expected to increase diffusivities in hydrophobic phases of a barrier. If it is highly soluble in such phases, it may alter the capacities of the phases to dissolve other substances as well. The latter effect can, in principle, have a positive or negative effect on the flux depending on the physicochemical attributes of the permeant. In the instance of the un-ionized species of benzoic acid, enhancement can be expected since benzoic acid, like Azone, is a mildly amphiphilic species and, as such, will tend to dissolve well in Azone.

Materials and Methods

Radiochemical—[^{14}C]Benzoic acid (ICN Radiochemicals, Irvine, CA) with a specific activity of 43 mCi mol^{-1} and radiochemical purity of 97−99% was used as the permeant. A stock solution was prepared by dissolving the solid (2.8 mg) in 5 mL of absolute ethanol.

Buffer Solutions—McIlvaine's buffer system (citric acid and dibasic sodium phosphate) was used between pH 2 and 5, and Sorenson's buffer system (monobasic and dibasic sodium phosphate) was used to establish pH's of 7 and 8. Concentrated NaOH was used to adjust pH to the desired values for the buffers at pH 3.5−7, and concentrated HCl was used for the buffers at pH 2 and 8. All buffers were adjusted to 300 mOsm by the addition of appropriate amounts of NaCl.

Azone Emulsions—Azone was provided by Nelson Research of Irvine, CA. The preparation of Azone−polysorbate 20 (Tween 20) emulsions was exactly as described previously[3] for experiments with hydrocortisone except that the aqueous phases were buffered to pH values between 2 and 8. Previously, 0.9% NaCl rather than isotonic buffers had been used.[3] The requisite amounts of Azone and polysorbate 20 were weighed into volumetric flasks, and buffer solution was added to volume. The mixtures were shaken and then homogenized twice with a hand homogenizer.

Partitioning—The procedure used to determine the partition coefficient of benzoic acid between Azone and water was the same as that previously described[3] except for the fact that the aqueous phase was buffered to pH 2 to suppress ionization. As a result, partitioning strictly involved equilibrium of the un-ionized form of benzoic acid. In brief, the partitioning was determined at room temperature using a 20 mL plungerless glass syringe clamped with its tip down. The barrel of the syringe was filled with 10 mL of buffered benzoic acid solution, and 5 mL of Azone was gently placed over the aqueous medium so as to avoid physical mixing. The lower, aqueous phase was magnetically stirred. The upper Azone phase was stirred from above using a propeller on a shaft. The phases were mixed continuously and sampled six times over 24 h. Steady concentrations in the phases were achieved from the third sampling time on, in approximately 8 h. The partition coefficient was calculated as the ratio of concentrations in the immiscible phases and averaged for the last four sampling times.

Silicone Rubber Membranes—Silastic Sheeting (Medical Grade Sheeting, Dow Corning Corporation, Midland, MI) of labeled thickness 0.020 in. (508 μm) was used. The membrane was cut into 2 cm squares, washed in a 0.1% detergent solution (Liqui-Nox, Alconox, New York, NY) for 0.5 min, and rinsed with hot tap water (40 °C) for 5 min and then distilled water for 2 min.

In the course of the experiments it was discovered that silicone rubber imbibes Azone, raising its permeability to benzoic acid profoundly. Therefore, in the experiments to determine the physicochemical effects of formulating benzoic acid in Azone emulsions, the membranes were presoaked in a 10% Azone emulsion stabilized with 0.1% polysorbate 20 in a buffer of the pH to be used in the specific diffusion experiment. The membrane was kept immersed at 37 °C in the emulsion medium for 24 h. Thereupon, the Azone-treated membranes were rinsed briefly in a stream of ethanol to remove all emulsion adhering to their surfaces. They were then rinsed with distilled water before being mounted them in the diffusion cells.

Diffusion Cells—Both our Side-Bi-Side diffusion cells (Crown Glass Co., Somerset, NJ) and their operation have been previously described in detail.[3] Briefly, each cell consists of two symmetrical, individually water jacketed half-cells (cylindrical chambers 3.4 mL each in volume). The water circulated through the cells to control temperature was maintained at 37 °C. Each chamber has one circular, open face of 0.5 cm diameter, which defines the area (~0.8 cm^2) offered for diffusion. Stirring of the media within the chamber was achieved by externally driven magnetic stirring bars placed in circular depressions of the chambers near the opening.

Permeation Experiments—After a cell was assembled, 25 μL of the [^{14}C]benzoic acid stock solution was placed into its donor half-cell. In the experiments with Azone-treated silicone rubber membranes, the donor medium for a given experiment was completed by the addition of 3.3 mL of one of the several strengths of Azone emulsion at one of the

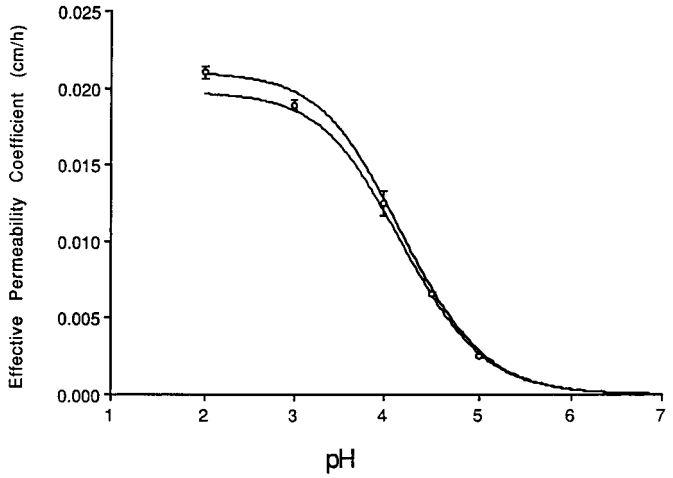

Figure 2—Silicone rubber membrane effective permeability coefficients for benzoic acid as a function of pH at 37 °C. Symbols represent experimental values (average ± standard deviation, $n = 3$). The lines represent predicted values $P = P_2^0 X_{1b}$. The lower line uses a value of P_2^0 from data fitting whereas the upper line takes the experimental permeability value at pH 2 to be P_2^0.

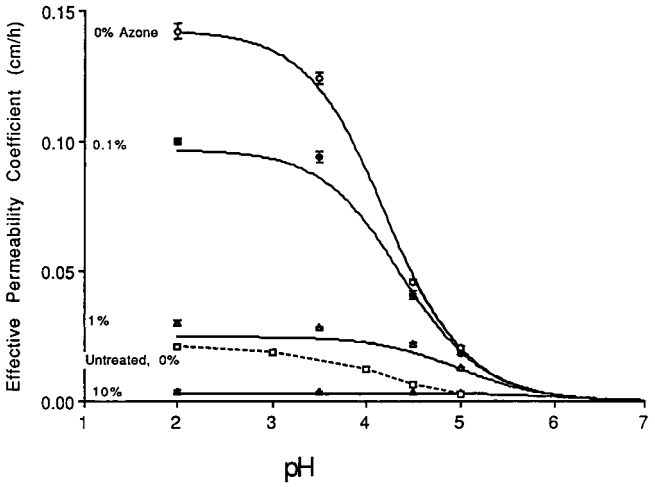

Figure 3—Effective permeability coefficients for benzoic acid permeability (37 °C) through silicone rubber membrane pretreated with 10% Azone emulsion with Azone emulsions of various concentrations as the donor media. Symbols represent experimental values (average ± standard deviation, $n = 3$). Continuous lines are predicted values (see Discussion).

designated pH's to the radiochemical concentrate. Prior to the addition of the emulsions to the cell, the emulsions were stirred with a magnetic stirring bar for a half-hour in a vessel immersed in a water bath maintained at 37 °C to bring them to temperature. Two 50 μL samples were taken before the start of the experiment to determine the initial benzoic acid concentration. The receiver was filled with 3 mL of buffer having the same pH as the donor emulsion. The experiment was commenced with the introduction of 3 mL of the radiochemically charged emulsion into the donor. Samples (200 μL) were taken periodically with replacement by an equal amount of buffer.

Analysis of Permeation Data—The cumulative radioactivity reaching the receiver (counts per min (CPM) per 0.5 mL) was plotted as a function of time. These cumulative CPMs were corrected for substance removed in the prior samples. The permeability coefficient is given by

$$P = \frac{V}{A}\frac{\mathrm{d}C/\mathrm{d}t}{C} \tag{19}$$

where $\mathrm{d}C/\mathrm{d}t$ is the steady-state or quasisteady-state slope of the cumulative receiver concentration versus time plot; C is the total benzoic acid concentration difference across the membrane as approximated by the initial donor concentration (sink conditions prevailed on the receiver side); V is the half-cell volume; and A is the actual diffusional area.

Results

The true partition coefficient of benzoic acid between Azone and water buffered to pH = 2.0 at room temperature (23 °C) was found to be 460. Based on previous results with hydrocortisone,[3,4] this immediately seemed sufficiently large for benzoic acid's thermodynamic activity in the donor medium to be reduced in the presence of emulsified Azone as the result of its partitioning into the Azone-rich phase.

Benzoic acid permeability coefficients obtained with untreated silicone rubber membranes as a function of pH are plotted in Figure 2. The lag times for the permeation runs ranged from 26 to 31 min and were unaffected by pH. Permeability coefficients for Azone-treated and stabilized silicone rubber membranes as functions of pH and composition of the Azone emulsions are provided in Figure 3. It can be seen that pretreatment with the 10% Azone emulsion for 24 h raised the permeability of the synthetic membrane a consistent 7−8 times over the pH range 3.5−5 (compare data for 10% Azone in donor to data for untreated membrane). The Azone pretreatment caused the lag times to drop to between 17−24 min but without any noticeable changes in the physical dimensions of the membrane.

Discussion

Contribution of the Hydrodynamic Layers—The total resistance of the hydrodynamic boundary layers adjacent to the membrane surface can be estimated for our experimental system. The total resistance is given by

$$\frac{1}{P_{\mathrm{bl}}^{\mathrm{total}}} = \left(\frac{1}{P_{\mathrm{bl}}^{\mathrm{d}}} + \frac{1}{P_{\mathrm{bl}}^{\mathrm{r}}}\right) = \frac{h_1}{D_1^{0/-}} + \frac{h_3}{D_3^{0/-}} = \frac{2h_i}{D_i^{0/-}} \tag{20}$$

where the subscript i simply designates one or the other of the two external phases and the superscripts d and r stand for the donor and receiver compartments, respectively. It should be pointed out that, given the symmetry of the diffusion cell, the boundary layers on each of its sides should be of equivalent thickness. A fair idea of the actual boundary layer dimensions in our cell can be garnered from Yu et al.[15] These investigators used a diffusion cell of slightly smaller dimensions (their cell had a 0.317 cm^2 permeation area and 1.2 mL compartmental volumes), with its half-cells stirred with small propellers. Yu et al. determined the effective boundary layer thickness by following the dissolution of benzoic acid from a smooth pellet covering the opening of one half-cell of their system into an acidified aqueous medium at 37 °C. Since an aqueous diffusion coefficient for benzoic acid of 1.4×10^{-5} cm^2/s had earlier been reported by Higuchi et al.,[16] and since its solubility in the system was known, they were able to estimate the boundary layer thickness directly from the dissolution rate. An approximate value of 0.01 cm (100 μm) was reported for the circumstance when the stirring rate in the half-cell was 150 rpm. Using this estimate, each individual diffusion layer resistance in our diffusion cell would be on the order of 0.2 h/cm (h_i/D), yielding a combined boundary layer resistance of about 0.4 h/cm. This value corresponds to an upper limit permeability coefficient for the diffusion cell system of 2.5 cm/h (value if only boundary layers

were present). In our experimental case of highest permeability, namely, the pH = 2 situation with the Azone-treated membrane, this allocation of boundary layer resistance accounts for only about 6% of the total experimental resistance observed. We therefore safely conclude that the transport process, as we measured it, is membrane controlled, with boundary layer influences all but absent.

Application of the Physical Models—Using a nonlinear least squares method (pseudo-Gauss–Newton method with routine PAR in BMDP Statistical Software),[17] model equations were fitted to the experimental permeability coefficients obtained as a function of pH, with the pH of the external phases being the independent variable. Since the standard deviation decreases as the permeability value decreases, the fitting was done by using the reciprocal of the variance (square of the sample standard deviation) as weighting factor.[18] Moreover, for data obtained using untreated silicone rubber membranes, boundary layer resistances, the estimated contributions of which should be well within experimental error, were ignored (set to 0). When this is done, we have

$$P = P_2^0 X_{1b} + P_2^{0/-} \tag{21}$$

clearly a straightforward simplification of eq 12. When this modified form of eq 12 is fitted to the permeability data for the untreated membrane, P_2^0 takes a value of 0.0197 ± 0.0004 cm/h and $P_2^{0/-}$ factors out as 0. Using these values, the predicted permeability as a function of pH is plotted through the data in Figure 2. Also plotted on this figure, a slightly higher curve, is the predicted shape of the pH–permeability profile obtained upon using the actual experimental value of P_2^0 determined at pH = 2.0. Either way, the agreement of theory with the experimental data is excellent. It is concluded that the untreated silicone rubber membrane behaves as a hydrophobic, partitioning barrier with respect to the permeation of benzoic acid. Permeation of ionized benzoic acid is ruled out.

A similar fitting of eq 21 to data obtained with the Azone-treated membranes yields values of P_2^0 and $P_2^{0/-}$ of 0.151 ± 0.006 cm/h and $2.80 \times 10^{-5} \pm 114 \times 10^{-5}$ cm/h, respectively. The large standard deviation for the latter testifies to its highly unreliable character. As a practical matter, the value is 0. Since the permeability ratio for treated to untreated membrane is constant at about 7.5 over the pH range studied, we conclude that an Azone-treated membrane, while far more permeable than one that is not treated, is also acting as a hydrophobic barrier which thermodynamically excludes the benzoate anion. Without exception, the data show that it is un-ionized benzoic acid which is responsible for the flux of the compound across these membranes. No other conclusion could have possibly been intuitively credible.

Effects of Azone Emulsions on Permeation—We previously demonstrated that hydrocortisone's permeation of the skin is reduced by its partitioning into the Azone-rich internal phase of the Azone/water emulsions used to apply it to skin.[3,4] We can expect the same to be true for a weak electrolyte like benzoic acid placed up against the silicone rubber membrane, but only in terms of partitioning of the un-ionized fraction in the aqueous medium. Given that the ionized species does not contribute to the flux of benzoic acid across the silicone membrane, the flux of benzoic acid is expected to depend directly on the state of ionization in the aqueous phases (an equivalent pH was maintained on both sides of the cell). This is, of course, determined by pH. In the previous work with hydrocortisone, the intrinsic permeability coefficient of the compound measured from Azone emulsions was determined from C_2, the prevailing concentration of hydrocortisone in the aqueous phase of the emulsions.[3,4] For benzoic acid, the appropriate concentration would actually be the concentration of un-ionized species in the aqueous phase of an emulsion, $C_2^0 = C_2/(1 + K_a/[H^+])$, where C_2 is now the total aqueous concentration of the acid and K_a is the acid dissociation constant. These concentrations can be related to the total concentration in the emulsion, $C_{o/w}^T$, through

$$C_2 = \frac{Co/w^T}{[(K_{o/w} - 1)\delta + 1]} \tag{22}$$

where δ is the volume fraction of Azone in the emulsion and $K_{o/w}$ is the apparent partition coefficient. The latter, in turn, takes the form $K_{o/w} = K_{o/w}^0/(1 + K_a/[H^+])$, where $K_{o/w}^0$ is the true partition coefficient for the un-ionized species between the oil (Azone) and aqueous phases of the emulsion. It follows that

$$C_2 = \frac{Co/w^T}{\left[\left(\frac{K_{o/w}^0}{\beta} - 1\right)\delta + 1\right]} \tag{23}$$

where $\beta = 1 + K_a/[H^+]$. Upon substituting eq 23 into eq 19, one obtains

$$P_n = \frac{V}{A}\frac{\left(\frac{dC}{dt}\right)}{C_2}[K_{o/w}^0\delta + (1 - \delta)\beta] = P_e[K_{o/w}^0\delta + (1 - \delta)\beta] \tag{24}$$

where P_e is the effective permeability coefficient. The intrinsic permeability coefficient of the Azone-treated silicone rubber membranes can be gleaned from the permeability coefficient obtained at pH = 2 (0.142 ± 0.003 cm/h), where benzoic acid's ionization is completely suppressed, after correction for the hydrodynamic layers. The corrected value is 0.151 cm/h; this value was used to calculate the theoretical curves in Figure 3 using independently determined values of δ and pH. The agreement between theory and experiment is again excellent, although there is a tendency for the experimental values to lie above the curves, especially at lower pH's. The presence of the emulsifier, polysorbate 20, in the aqueous phase of the emulsions lowers the activity of benzoic acid below that obtained when it is formulated in pure water, and we reason that this effect accounts for the small theory–experiment differences which were observed.

The data obtained with Azone present are interesting when looked at in another light. Enhancement of permeation can be kinetic and experienced in diffusion coefficients and/or thermodynamic and experienced in increased partitioning into membranes, the latter of which would steepen gradients. It seems clear from lag times that there is an effect on diffusivity, for these drop from about 28 min on the average in the absence of Azone to about 20 min on the average with the enhancer present. However, the reduction in lag time is nowhere near what it would have to be to explain the approximately 7.5-fold increase in flux. As diffusivity is inversely proportional to the lag time, only a 40% increase in flux might in fact be expected. We therefore conclude that Azone increases the solvency of the membrane for benzoic acid. Moreover, the 7.5-fold magnitude of the enhancement indicates that the solvency effect has the greater influence on benzoic acid's transport across the silicone rubber membrane. We believe this to be the first time that the two concerted activities of enhancers, increased diffusivity and increased solvency, have been so clearly experimentally differentiated.

Touitou and Abed[19] previously researched benzoic acid's permeation of both silicone rubber membranes and hairless mouse skin as a function of pH; they reported a 4-fold drop in

permeability upon increasing the pH from 2 to 5.3. We see roughly twice this response over the same pH range. Touitou and Abed were unable to detect benzoic acid in the receiver chamber of their diffusion cell after 8 h when the pH of the donor compartment was set to 6.5. Figure 2 reveals that the permeation rate would indeed be very slow at this pH. They concluded, as we do here, that benzoic acid permeates silicone rubber only in its un-ionized form. Notably, Garrett and Chemburkar[20] reached this same conclusion concerning silicone rubber membranes when studying the permeation of these membranes by 4′-aminopropiophenone. Thus, for those who might not have accepted a strictly theoretical argument ruling out the permeation of ions through molecularly continuous hydrophobic barriers, we now offer unequivocal experimental evidence that this is the case. Obviously, ions are thermodynamically constrained from partitioning into the hydrophobic phases of membranes. Corollary to this, if one finds a measurable ionic flux across a biological membrane, one must conclude that an alternative pathway to the hydrophobic phases exists in the membrane to make this possible.

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