Permeation of Hairless Mouse Skin I: Experimental Methods and Comparison with Human Epidermal Permeation by Alkanols

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Received July 31, 1979, from the *College of Pharmacy, University of Michigan, Ann Arbor, MI 48109.* Accepted for publication February 1, 1980.

Abstract □ The permeation of intact hairless mouse skin by alkanols was studied. The method is described, and data for the quasi-steady-state and nonstationary-state aspects of mass transfer are given. Partitioning data also are presented. The permeability coefficients increased exponentially up to a carbon chain length of about eight (octanol). There was a marked temperature dependency ($E_a \simeq 19$ kcal for the series), and the partitioning was biphasic, increasing exponentially for alkanols larger than butanol. These data are compared with literature data on human skin tissues, and great similarity in all facets of behavior is noted.

Keyphrases □ Permeation—*n*-alkanols through intact hairless mouse skin and human skin, effect of temperature and carbon chain length, biphasic partitioning □ Partition coefficients—*n*-alkanols in intact hairless mouse skin and human skin, effect of temperature and carbon chain length, biphasic partitioning □ Alcohols, normal—permeability coefficients in hairless mouse skin and human skin, effect of temperature and carbon chain length, biphasic partitioning

The present studies were initiated to answer questions about the usefulness of the hairless mouse for developing clinically relevant insights into percutaneous absorption and for evaluating topical products intended for humans. Scattered reports allude to a barrier property similarity between hairless animal skins, particularly porcine and mouse, and the human integument. However, a high degree of sameness in permeability with respect to human skin has yet to be substantiated for any animal skin, although close parallelism in the permeation of select drugs between human and hairless mouse skin has been reported (1). More data are needed to generalize such unrelated observations into a broad conclusion.

A second, narrower basis for this work was the establishment of permeation baselines for the hairless mouse in preparation for the evaluation of the general consequences of burning and other traumatizing treatments on the permeation of skin tissue. Therefore, the present studies represent the development of analytical procedures suitable for studying mass transport through mechanically, chemically, and physically processed skins aimed at the quantitative definition of the effects of injury on percutaneous absorption. The permeation of select homologous *n*-alkanols (methanol through octanol) through intact hairless mouse skin is reported since these results can be compared with literature data on human epidermal permeation of the same compounds (2–6).

EXPERIMENTAL

Chemicals—The following homologous *n*-alkanols were used: [^{14}C]-methanol[1], [^{3}H]ethanol[1], [^{14}C]*n*-propanol[2], [^{3}H]*n*-butanol[1], [^{14}C]*n*-pentanol[2], [^{14}C]*n*-hexanol[1], [^{14}C]*n*-heptanol[2], [^{14}C]*n*-octanol[1], [^{14}C]*n*-nonanol[2], and [^{14}C]*n*-decanol[1].

The alkanols were diluted in 0.9% NaCl for irrigation[3], henceforth referred to as normal saline, and the actual permeation solutions were prepared from these stock solutions. The preparation of a stock solution was individualized to each alkanol since each compound was received with a different radioactivity. In every case, the stock solution was brought to ~50 μCi/ml. The alcohol concentrations were $\leq 10^{-4}$ *M*, precluding any direct effect of the alcohols on the membrane.

Diffusion—Small diffusion cells were machined from a polycarbonate plastic or were prepared from tempered glass in local shops. These cells were in the form of two cylindrical half-cells that had an inside diameter of ~1.0 cm and were ~2.5 cm deep, yielding a half-cell volume of ~1.5 ml. Each half-cell was equipped with two ports; one, ~2.5 cm long, was to accommodate the shaft of the stirring device; and the other, 1 cm long, was used for sampling and filling of the compartments. One half-cell contained a solid plastic or glass cylindrical extension of ~2.5 cm, which allowed it to be clamped rigidly in place in the constant-temperature bath. Each half-cell also was fitted with a flange at the open end to allow tight sealing of the membrane (Fig. 1*a*).

The cell halves were assembled, with the full-thickness, freshly excised, abdominal mouse skin between the half-cells, using a No. 18 ball-joint clamp (Fig. 1*b*). The assembled cell then was immersed in a constant-temperature bath, with the cell ports being the only components above the surface. The cell contents were stirred by motors mounted above the cell system[4]. The stirrers were small propellers placed in close proximity to the membrane surface. The stirring propeller was mounted on the end of the stirring shaft prior to clamping the half-cells since the propeller diameter was larger than the porthole. The stirrer shaft was attached to the motor by tightly fitting pieces of polymeric tubing. Care was taken to center the stirrer exactly in the cell to keep it from contact with the walls. The stirring speed of 150 rpm was checked after each assembly. By using a drop of dye solution, it was found that the mixing was gentle but adequate to distribute the color homogeneously in <2 min.

The diffusion cell was designed to have a large membrane surface area to half-cell volume ratio (~0.5) and also to have a small half-cell volume so that a minimal amount of radiolabeled compound was needed for each experiment. Construction materials were selected that had little tendency to adsorb the alkanol permeants but that also would allow viewing of the events inside the cell compartments. The cells were tested for tightness of the seal around the membranes, and no leakage was observed.

Membrane—The membranes were full-thickness skin taken from the abdominal surface of the hairless mouse, HRS/J strain[5]. Hairless mice (males), 4–6 weeks old, were sacrificed by snapping the spinal cord at the neck. A rectangular section of abdominal skin, several centimeters in each dimension (Fig. 2), was excised from the animal with surgical scissors. Since the skin was not firmly attached to the viscera, it was lifted easily from the animal after the incision was made. Adhering fat and other visceral debris were removed carefully from the undersurface with tweezers.

Prior to skin removal, a uniform circle was made on the abdomen marking the precise skin section to be positioned between the half-cells. After the excised skin was trimmed into an oversized, rough circle, it was mounted between the half-cells with the marked section centered. Stretching of the skin, as evidenced by distortion or expansion of the circular outline, was corrected, and the cell halves were held fast by a spring clamp. The dotted circular section in Fig. 2 indicates the area of skin used. The cell compartments then were filled with normal saline to keep the membrane moist. The areas available for diffusion between

[1] International Chemical and Nuclear Corp., Irvine, CA 92664.
[2] California Bionuclear Corp., Sun Valley, CA 91352.
[3] Abbott Laboratories, North Chicago, IL 60064.
[4] Model CA115V constant-speed motor (150 rpm), Hurst Manufacturing Co., Princeton, Ind.
[5] Jackson Laboratories, Bar Harbor, ME 04609.

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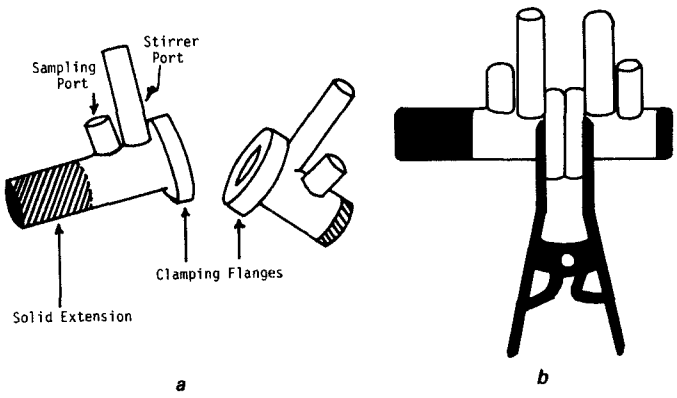

Figure 1—*Sketches of the unassembled and assembled diffusion cell showing the stirring ports, sampling ports, and other essential details.*

compartments were 0.713 cm^2 for the plastic cell and 1.094 cm^2 for the glass cell.

Permeation—The assembled saline-filled cell was immersed in a constant-temperature bath, the stirrer shafts were attached to the motor, and stirring was commenced. The contents were mixed for 30 min, at which time the compartments were evacuated with a syringe and refilled with fresh saline. Then the compartments were evacuated a second time, refilled, evacuated a third time, and finally refilled with normal saline preequilibrated to the experimental temperature. After mixing for 2 min, a sample of saline was withdrawn from the receptor compartment and counted to ensure that there was no residual activity from previous runs.

At this point, the donor compartment was charged with radioactive alkanol or alkanols (dual-absorption procedure), and the runs were commenced. Fifty microliters of concentrated solutions of the labeled alkanols in normal saline constituted the charge. The cell contents were stirred and, at predetermined times, samples were withdrawn from the respective compartments and transferred immediately using disposable Eppendorff[6] pipets to vials containing 10 ml of scintillation cocktail[7] for radiochemical assay[8].

In one set of experiments, aimed at determining the influence of pH on the permeation rate, isotonic buffers were used instead of normal saline (Table I). In the pH dependency experiments, the half-cells were rinsed with buffer in the final two rinses and filled with buffer prior to charging the donor compartment and initiating a run.

The moment of charging the donor half-cell marked the beginning of the diffusion run, and the receptor samples were referenced to this time. However, to allow for mixing to a uniform concentration, a minimum of 2 min was allowed to elapse before the initial donor sample was withdrawn. This sample was used as a close estimate of the initial donor cell concentration.

The overall procedure was designed such that depletion of the donor phase concentration was never an appreciable fraction of the donor concentration. There was no problem meeting this condition for water or the alkanols up to a chain length of seven. However, because partitioning from the aqueous medium into the lipoid phase of the tissue grows exponentially, a point is reached for longer chain length alkanols where the amount of alkanol retained in the membrane is a significant fraction of the total amount that has entered the membrane during the entire experiment. This problem is more acute when small donor and receiver volumes are involved, as is the case here, and a necessary condition for "stripping" the permeability coefficient from pseudo-steady-state slopes is violated.

The problem began with octanol in these studies, but the error induced was estimated not to be large enough to warrant discarding octanol results from the overall trend[9]. However, under the specified appropriate circumstances, the permeation enters a pseudo-steady state from which the permeability coefficient of each alkanol can be ascertained readily. If the concentration in the donor compartment changed significantly during the 2-hr run, as occurred for alkanols above a chain length of six, the donor compartment contents were replaced totally with fresh permeant solution periodically, as necessary, to maintain the concentration as essentially invariant.

Table I—Buffer Compositions [a]

Buffer Component	pH 3	pH 6	pH 8
$NaH_2PO_4 \cdot H_2O$	13.8 g	13.8 g	0.7 g
1.0 *N* HCl	11.7 ml	—	—
1.0 *N* NaOH	—	16.0 ml	—
NaCl	2.9 g	3.2 g	2.0 g
Na_2HPO_4	—	—	13.5 g

[a] All buffers were brought to a volume of 1000 ml with water.

In the initial experiments, the permeability of a membrane remained essentially unchanged for up to 12 hr using the lower molecular weight alkanols (*i.e.*, methanol and ethanol). Therefore, as many as four or five runs could be accomplished on a given piece of skin as long as the residual radioactive species from previous runs were fully washed out. Between runs on a given piece of skin, the cell volume was exchanged 12 times on each side with fresh saline. The skin, still mounted in the diffusion cell, was kept in contact with fresh saline for 45 min and then rinsed twice more with saline solution. The entire cleanup operation involved 14 rinses with an intermittent 45-min period of equilibration. The compartments then were refilled, the donor compartment was recharged, and a new run was commenced.

In subsequent extensive work (to be reported later), it was observed that invariant permeability as a function of the duration of skin immersion in the saline medium was strictly true only for water and the most polar alkanols, methanol and ethanol. For the higher homologs, there was up to a twofold change in the permeability coefficients over the initial 10 hr of skin hydration. Invariance was seen for all compounds after 10 hr of immersion. Partly because of the order of sequencing runs (lower alkanols preceding higher) and because of the small magnitude of change within compounds as compared to between compounds, these hydration-induced permeability alterations had no significant effect on the actual values or on the conclusions presented in this paper.

In the present studies, the permeation rates were assessed for selected *n*-alkanols through octanol at 20, 25, and 37°. The 20 and 25° data represent individual runs on each even-numbered alkanol. The 37° data represent averages of multiple runs for each alkanol on the uninterrupted homologous series from C_1 to C_8. The permeability data were plotted as counts (amount of permeant) collected in the receptor compartment as

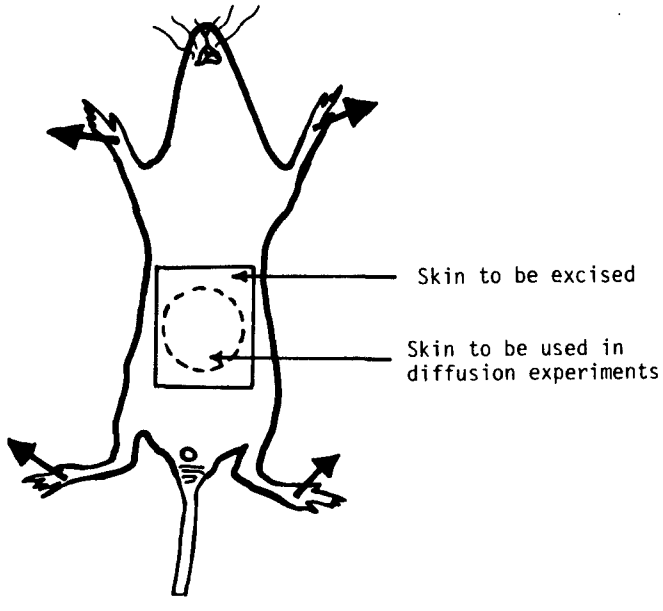

Figure 2—*Sketch of the hairless mouse indicating the exact section of skin excised for the diffusion cell studies.*

[6] Brinkmann Instruments, Westbury, NY 11590.
[7] Aquasol, New England Nuclear, Boston, MA 02118.
[8] Model LS 200 liquid scintillation counter, Beckman Instruments, Fullerton, Calif.
[9] Techniques were used to check the validity of this procedure and will be reported later. It was found that for octanol and above, the pseudo-steady-state procedure introduced an error, but this error was <15% with octanol, so these data are reported. The influence of membrane retention will be discussed quantitatively and theoretically in future reports.

Table II—Partitioning of the *n*-Alkanols into Full-Thickness Hairless Mouse Skin and Human Stratum Corneum

Alkanol	Partition Coefficient, cm^3/g: Hairless Mouse[a], 37°	Human Stratum Corneum[b], 25°
Methanol	5.5	0.6
Ethanol	5.4	0.9
n-Propanol	6.0	1.1
n-Butanol	6.6	2.5
n-Pentanol	7.7	5.0
n-Hexanol	10.7	10 0
n-Heptanol	24.0	30.0
n-Octanol	40.8	50.0
n-Nonanol	86.7	—

[a] Based on the weight of the full-thickness tissue and distribution from normal saline. [b] Obtained from Ref. 2. These data are for human stratum corneum and were obtained using dilute aqueous solutions rather than dilute saline solutions of the alkanols.

Table III—Permeability Coefficients of Full-Thickness Hairless Mouse Skin at 20, 25, and 37° and Human Epidermis at 25°

Alkanol	$P \times 10^3$, cm/hr: Hairless Mouse Skin 20°	37°	25°[a]	Human Epidermis[b], 25°[a]
Methanol	0.41	2.6 (0.46)[c]	0.93	0.5
Ethanol	0.72	4.8 (0.43)	1.23	0.8
n-Propanol	—	5.4	—	1.2
n-Butanol	2.9	14.6 (0.81)	4.4	2.5
Butanol, pH 3	—	10.23	—	—
Butanol, pH 6	—	10.66	—	—
Butanol, pH 8	—	11.75	—	—
n-Pentanol	—	22.0	—	6.0
n-Hexanol	5.25	48.0 (1.2)	8.6	13.0
n-Heptanol	—	92.9	—	32.0
n-Octanol	18.9	97.2 (2.4)	31.5	52.0
n-Nonanol	—	—	—	60.0
n-Decanol	—	—	—	80.0

[a] The 25° data were placed side by side for comparison. The hairless mouse permeation solutions were made in normal saline. [b] Obtained from Refs. 2 and 4. These data were obtained using dilute aqueous solutions. [c] Standard deviations of multiple runs.

a function of time. Correction was made for sampling, which was done by replacement using normal saline. The permeability coefficient for a given run was calculated from:

$$J_T = PA\,\Delta C \qquad \text{(Eq. 1)}$$

where J_T is the total flux and also the slope of plots of the amount penetrated *versus* time (counts per minute); P is the permeability coefficient (centimeters per hour); A is the diffusional area (square centimeters); and ΔC is the concentration differential across the membrane, which was taken to be equal to the donor phase concentration (counts per cubic centimeter).

The concentrations were expressed in terms of counts from a 50-μl sample. Thus, the permeability coefficient is calculated from:

$$P = \frac{J_T}{A\,\Delta C} \qquad \text{(Eq. 2)}$$

and since $J_T = dM/dt = V(dC/dt)$:

$$P = \frac{V\left(\frac{dC}{dt}\right)}{A\,\Delta C} \qquad \text{(Eq. 3)}$$

where V is the half-cell volume and the volume of the receptor compartment (cubic centimeters) and dC/dt is the steady-state slope in counts per minute per cubic centimeter. The computed permeability coefficients were converted to centimeters per hour by multiplying by 60.

Skin Partitioning—The skin–water partition coefficients of the alkanols also were assessed. These values were evaluated crudely by equilibrating the alkanol solutions with a known mass of skin, 20–50 mg, weighed immediately after removal from the animal. These skin pieces were immersed in 2 ml of normal saline containing the radiolabeled alkanol. The samples were equilibrated in a shaker bath at 37° overnight, which was sufficient to attain equilibrium.

Distribution of the alkanols between the aqueous phase of the tissue was estimated by the method of Scheuplein (2) as the difference between the initial aqueous phase concentration and the aqueous phase concentration at equilibrium. The relationship used was:

$$K = \frac{(C_0 - C_e)/W_T}{C_0/V_{aq}} \qquad \text{(Eq. 4)}$$

where C_0 is the initial alkanol solution concentration, C_e is the solution concentration at equilibrium, W_T is the tissue weight in grams, and V_{aq} is the solution phase volume (2 ml) in milliliters. Therefore, the partition coefficients are in cubic centimeters per gram. Given the obvious heterogeneity (multiphasic nature) of the tissue, no attempt was made to factor in density to obtain unitless values.

RESULTS

The data for the partitioning of the *n*-alkanols at 37° between full-thickness hairless mouse skin and saline are tabulated in Table II. The partition coefficients appeared to increase only slightly up to a chain length of five but then increased exponentially from pentanol to nonanol. The slope of the plot in the exponential growth region was ~0.285, corresponding to an increase in the partition coefficient by an average factor of 1.9 per chain unit (per methylene unit) past an alkyl chain length of five. Straightforward attempts to obtain a partition coefficient for decanol were unsuccessful since the small skin pieces absorbed essentially all of the C_{10} compound, making the estimate unreliable. The exponential trend was presumed to continue to C_{10}, and the experimental procedure was not adjusted to obtain a firm value for this compound.

Raw permeation profiles of the receptor phase concentration in moles per liter as a function of time for *n*-butanol are displayed in Fig. 3 for runs carried out at 20, 25, and 37°. Similar data were collected at 20 and 25° for select, mostly even homologs through *n*-octanol and at 37° for the uninterrupted homologous series from C_1 to C_8. Permeability coefficients were calculated from the terminal slopes of such plots, and lag times also were estimated graphically. The estimated permeability coefficients at each temperature are summarized in Table III, which also includes *n*-butanol data at 37° obtained using isotonic buffers at pH 3, 6, and 8.

An Arrhenius-like plot of the permeability coefficients is given in Fig. 4, where log P is plotted *versus* the reciprocal of temperature. In Fig. 5, the permeability data are plotted as log P *versus* alcohol chain length. Several significant trends are immediately evident from these data. There was a dramatic temperature dependency for the permeation process. Within the accuracy of the data, the apparent activation energy did not appear to vary among the homologs and was ~19 kcal/mole based on the slopes of statistically fitted lines through the data in Fig. 4. In both Figs.

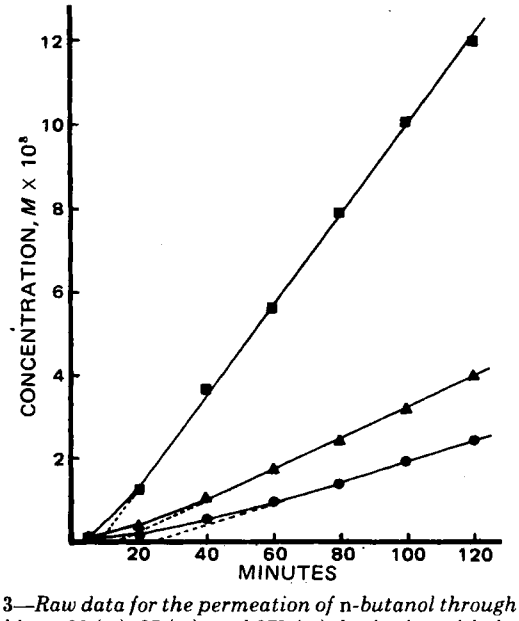

Figure 3—*Raw data for the permeation of* n-*butanol through hairless mouse skin at 20* (●), *25* (▲), *and 37°* (■), *beginning with donor solutions at* ~10^{-5} M. *Changes in diffusivity through the tissue as a function of temperature are indicated by shortened lag times (dotted extrapolations to the time axis) and increased slopes with increased temperature.*

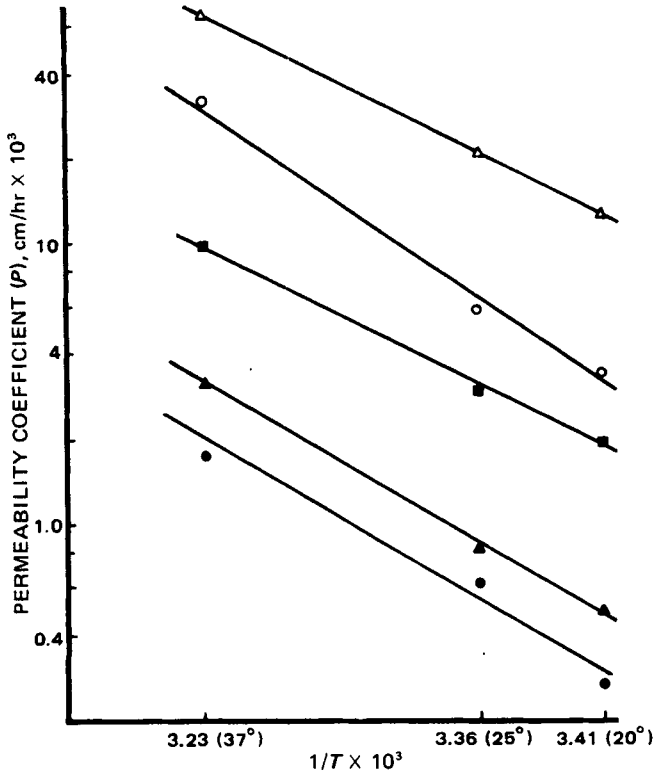

Figure 4—*Arrhenius plot of permeability coefficients for select (mostly even) alkanols. These data indicate an activation energy for the permeation process on the order of 19 kcal/mole. Key: ●, methanol; ▲, ethanol; ■, butanol; ○, hexanol; and △, octanol.*

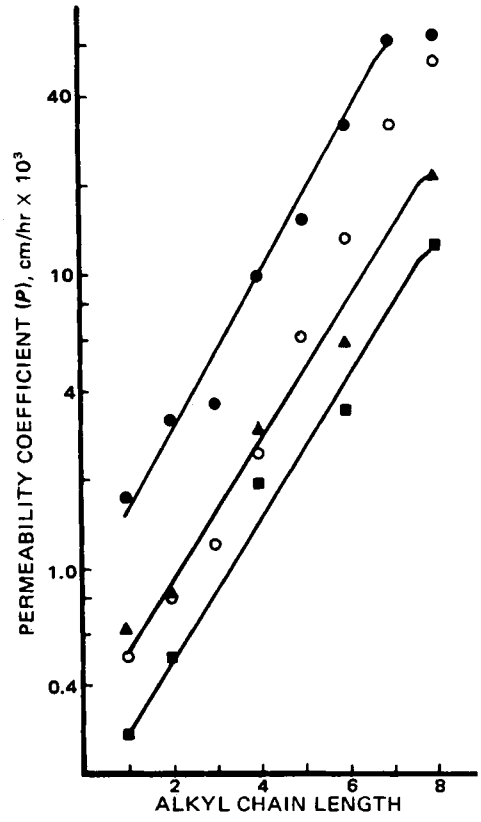

Figure 5—*Semilogarithmic plot of hairless mouse skin permeability coefficients against alkyl chain length for the alkanols at 20 (■), 25 (▲), and 37° (●) and human epidermis permeability coefficients (2, 4) at 25° (○). A general exponential increase in the permeation rate is evident from C_1 to C_8 in both hairless mice and humans, and the 25° data for the two species are in surprisingly close agreement.*

3 and 6, the lag times appear to shorten with increasing temperature, which is consistent with increasing diffusivity in the tissues. The lag times are considered as rough estimates since they are sensitive to placement of the steady-state line through the data. Nevertheless, the expectation that the lag times will be shorter at higher temperatures is supported by the data, even with this uncertainty. It also appears that the lag times tend to increase with increasing length of the alkyl chain (Fig. 6).

DISCUSSION

A nonhuman skin membrane that is sufficiently similar to the human integument is needed so that it can be substituted for human skin in *in vitro* percutaneous absorption and topical bioavailability studies. The human membrane is not procured as easily as an animal membrane, and its properties vary from sample to sample due to differences in the age, sex, race, and health of its donor (generally a cadaver) even if it is removed from the same anatomical site (7). It is not available on demand and must be stored frozen for variable periods (2), a condition that probably induces some physicochemical alteration. Furthermore, preparation of the excised human membrane for permeability studies is relatively complicated, requiring special equipment and demanding skill because the prepared membrane must be of uniform thickness and free of holes and other defects (2, 8, 9). These are surmountable but difficult experimental problems.

Alternatives to using excised human skin for percutaneous absorption assessment are *in vivo* studies in humans, such as the vasoconstriction assay for steroids, and the use of excised animal skins in *in vitro* diffusional systems. Studies on living human subjects are costly and time consuming due to the necessary safeguards, including approved clinical protocols that must be processed through local human use committees and the Food and Drug Administration. Thus, an animal membrane that behaves like human skin and is easily obtainable and relatively invariant from animal to animal could simplify the process of screening topical drugs. The development of critical structure–permeation relationships and of operationally superior drug delivery systems would be forwarded.

Montagna (10) pointed out that no animal skins sufficiently resemble human skin to serve as a general model. Instead, the skins of animals exhibit extraordinary variation and specialization. However, certain animal skins may be likened to human skin in one specific property or another, and an animal may prove suitable for a particular type of study. In terms of anatomical features that contribute to the unique chemical barrier properties of human skin, such as sparse hair distribution and a thickened epidermis with a highly cornified outer layer, the skins of essentially nude mammalian species have been suggested as suitable approximations. The pig, minipig, and hairless mouse are among the useful species; of these, only the latter is sufficiently small and inexpensive to make animal sacrifice for individual experiments practical.

The hairless mouse has another important and experimentally exploitable feature. The skin over both the dorsal and abdominal surfaces is loose and nonadhering to the viscera, making it possible to remove skin membranes of uniform thickness (several square centimeters in area) without recourse to microtomy. Thus, the hairless mouse has distinct advantages as an experimental animal for skin permeation studies *in vitro*. However, to be useful in research and development, it must behave at least qualitatively and, ideally, quantitatively like human skin as a chemical barrier.

The present studies deal with the question of similarity by comparing the permeabilities of hairless mouse skin and human skin to a homologous series of compounds, the *n*-alkanols, between 20 and 37°. These compounds were studied previously over a similar temperature span in human skin (2–6). The extent of barrier parallelism should be evident in permeability coefficients at a given temperature, in temperature dependency, and in partitioning.

Partitioning—In Table II, the partitioning of the alkanols into full-thickness hairless mouse skin is compared with the most recent literature values for humans (5). The comparison is made between the 37° distribution coefficients in mouse skin and the 25° distribution coefficients in human stratum corneum free of epidermal cells. Thus, the tissues are substantially different, with the mouse skin containing a significant living volume fraction (the living epidermis, the full dermis, and a subdermal basement membrane). Also, the literature values were obtained with water as the bathing medium, while the present data were obtained using normal saline. Nevertheless, the comparison is useful, particularly when considered along with permeation data.

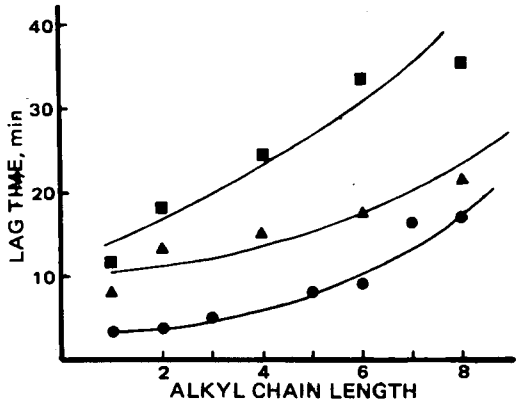

Figure 6—*Plot of lag times against alkyl chain length for data obtained at 20 (■), 25 (▲), and 37° (●). Both temperature and molecular size (alkyl chain length) effects are apparent. Lines through the data serve only to draw attention to the temperature groups and do not represent a best fit.*

In each case, the lower alkanols, methanol through propanol or butanol, did not exhibit a marked chain length sensitivity in phase distribution, with a value of slightly less than 1 for the human stratum corneum and a value of somewhat less than 6 for the hairless mouse tissue. The insensitivity suggests that the lower alkanols distribute into a polar phase in the tissue. One might speculate that this phase is imbibed water in human callus, which hydrates and swells up to 30% when immersed, and imbibed, cellular, intercellular, and interstitial water in the hairless mouse skin. Values greater than unity in the hairless mouse indicate that the tissue fluids or other tissue components have a greater affinity for the alkanols than the bathing saline phase. Whether the increased affinity is due to an actual solubility difference, adsorptive tissue binding, or another manner of tissue incorporation is unclear. What is clear is that the phenomenon responsible is not energetically dependent on hydrophobic factors since there is no chain-length effect (11, 12).

Partition coefficients above C_4 tended to increase rapidly; for the *n*-hexanol through *n*-nonanol homologs, the increase was exponential. Table II indicates that human data for the stratum corneum behave similarly; in each tissue, an approximately twofold increase in the partition coefficient per methylene group was experienced in the exponential region. The overall pattern of behavior (little or no chain-length effect for short homologs and an exponential growth with chain length for long homologs) is characteristic of a bi- or multiphasic tissue, with one phase (or collection of phases) polar and essentially aqueous and the other phase (or collection of phases) hydrophobic. In the intact hairless mouse skin, the hydrophobic phases would include the nonpolar elements of the stratum corneum and the Malpighian layer cell membranes, nonpolar elements of the dermis, fatty subdermal deposits, and, possibly, elements of the basement membrane.

Not only are the overall data trends for partitioning similar between human stratum corneum and full-thickness hairless mouse skin, but there also is an order of magnitude agreement in the values, despite the obvious differences in the tissues. In fact, at middle to long chain lengths, the agreement between the tissues is surprisingly close. Thus, the physicochemical natures of the tissue components apparently are similar.

Steady-State Permeability—The partitioning data suggest some gross physicochemical parallel between human stratum corneum and hairless mouse skin. However, this similarity need not correspond to a similarity in membrane barrier properties. Therefore, studies were begun to evaluate the permeability of hairless mouse skin to the alkanols. In Table III, permeability coefficient data are given as a function of temperature and pH for the various homologs studied. Also included are the 25° permeability coefficients obtained for heat-separated human epidermis (2). The hairless mouse data at 25° are tabulated immediately to the left of the human data for comparative purposes.

From the 37° data on butanol in isotonic buffers at pH 3, 6, and 8, it can be seen that pH had essentially no effect on membrane permeation over the wide pH range studied. Therefore, strict pH control (buffers) was not necessary in the remaining studies since the pH of saline solutions bathing the mouse skin for extended periods always was well within the specified pH range (the measured values were about pH 5 on both sides of the membrane).

In Fig. 4, the permeability coefficients for the C_1, C_2, C_4, C_6, and C_8 homologs are plotted semilogarithmically against the reciprocal of temperature. The average activation energy for these compounds based on the statistically evaluated slopes is ~19 kcal/mole with an uncertainty of several kilocalories per mole. This average activation energy is somewhat larger than the values obtained by Blank *et al.* (4) for the heat-separated human epidermis. Their average value for the alcohols ethanol through pentanol was 16.4 ± 2 kcal/mole and, above 25°, was comparably larger for hexanol through octanol. For the more nonpolar alkanols, biphasic temperature dependency was noted; below 25°, the activation energy was estimated to be about 10 kcal (4). In the present studies, this low temperature region was not investigated. Generally, the temperature dependencies for human epidermis and hairless mouse skin exhibited a marked similarity, with the animal membrane appearing slightly more temperature sensitive.

The data from Table III are treated graphically in Fig. 5 to illustrate the alkyl chain length dependency of the permeation process. At each temperature, the permeability coefficient initially increased exponentially as the alkyl chain was extended, except at 37° where octanol appeared to enter boundary layer control. The latter behavior is consistent with a series barrier consisting of a lipid or nonpolar phase of high diffusional resistance, presumably the stratum corneum, in series with an essentially aqueous phase or phases. At short chain length, the nonpolar barrier (stratum corneum) is rate determining, and the differences from compound to compound reflect differences in partitioning into this rate-limiting membrane phase. The permeability coefficient equation takes the following general form in this region (12, 13):

$$P = \frac{D_{sc}(K_{sc/aq})_0\, 10^{\pi n}}{h_{sc}} \qquad \text{(Eq. 5)}$$

which recognizes the exponentially increasing nature of the stratum corneum–water partition coefficient. Here D_{sc} is the effective stratum corneum diffusivity, $(K_{sc/aq})_0$ is a hypothetical zero chain length partition coefficient, h_{sc} is the tissue thickness, and n is the alkyl chain length. The π value in the exponent is the incremental increase in log P per unit increase in alkyl chain length. The slope of the plot at 37° indicates that the π_{CH_2} value (or incremental partitioning constant value) is ~0.263, and it appears to be of similar magnitude at 20 and 25°. The π_{CH_2} value for human stratum corneum may be obtained similarly from the data given previously (2–6) and is about 0.333, higher than but in reasonable agreement with that for the mouse skin. Values of π_{CH_2} can be expected to range to 0.62, the specified value for perfectly nonpolar, water-immiscible phases such as heptane or carbon tetrachloride (11).

The alkanol range where partition coefficients seemingly are invariant with chain length is a range where an exponential increase in permeability due to partitioning phenomena is observed. At first appearance, this finding may seem incongruent, but the respective observations in fact are internally consistent. Whereas the relative solubility in the stratum corneum and other lipid phases is small, the amounts partitioning into aqueous phases will determine the apparent partition coefficient. However, where the relative solubility in the stratum corneum is small, the molecular diffusional current across this stratum will be small and the stratum corneum will be the rate-determining tissue in the series. The limiting slope of the partitioning on the semilog plot, 0.285 (Fig. 5), is in very close agreement with the value of 0.263 obtained as the π_{CH_2} value for the permeability experiments. The difference is in the direction of an expected slight diffusion coefficient effect due to the increased molecular volume as the alkyl chain becomes longer.

Nonstationary State—The nonstationary-state data obtained in the present studies were crude. Slight differences in estimating the steady-state slopes led to large differences in estimating the lag time intercepts, particularly at short chain lengths where the slopes were relatively shallow. Nevertheless, several important patterns of behavior may be noted. The lag time for a given compound was dependent on temperature; lag times decreased as temperature was increased. This trend is consistent with a significant activation energy for the effective diffusivity in the membrane. The temperature dependency of the permeability coefficient seen in Fig. 4 is largely dependent on the temperature dependency of the diffusion coefficient, which directly affects the lag times. The increasing lag time with increasing molecular size may be a size effect on diffusivity or may be the result of increasing membrane binding (13).

CONCLUSIONS

The hairless mouse skin is an easily prepared membrane for permeation studies. Moreover, based on the permeability of homologous alcohols, the barrier properties are qualitatively and in some regards quantitatively similar to those of human epidermis. The membrane behaves as a complex barrier with a highly impermeable lipid phase (the stratum cor-

neum), in series with the viable tissues of the epidermis and connective tissue of the dermis. On the basis of this evidence, the hairless mouse might prove generally useful in sorting out membrane contributions to the relative activities of topical drugs and in evaluating the influence of formulation on the activity of a given topical therapeutic compound.

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Pharmacokinetic Studies of Propoxyphene I: Effect of Portacaval Shunt on Systemic Availability in Dogs

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Received August 20, 1979, from the **Department of Pharmaceutics and the* ‡*Department of Laboratory Animal Science, State University of New York at Buffalo, Amherst, NY 14260.* Accepted for publication February 6, 1980.

Abstract □ The elimination kinetics and systemic availability of *d*-propoxyphene were determined in dogs before and after construction of a portacaval shunt. For this purpose, the drug was administered intravenously and orally, in aqueous solution, according to a balanced experimental design. The total plasma clearance of propoxyphene ranged from 14.4 to 31.8 ml/min/kg before the shunt and decreased appreciably in two of four dogs after shunting. There was no significant change in the serum protein binding of the drug. The systemic availability increased from an average of 25% before to an average of 54% after shunting ($p < 0.05$). The ratio of areas under the plasma concentration–time curve of norpropoxyphene to propoxyphene was not significantly affected by the shunt when propoxyphene was administered intravenously; it decreased substantially after shunting when the drug was administered orally. Pharmacokinetic analysis indicates that orally administered propoxyphene is subject to prehepatic as well as hepatic first-pass elimination in dogs. The magnitude of the first-pass effect is similar to that in humans. These results suggest that the dosage of orally administered propoxyphene should be reduced in patients with portacaval shunt or with cirrhosis of the liver.

Keyphrases □ Pharmacokinetics—propoxyphene, effect of portacaval shunt on systemic availability, dogs □ Propoxyphene—pharmacokinetics, effect of portacaval shunt on systemic availability, dogs □ Systemic availability—propoxyphene, effect of portacaval shunt, dogs

The analgesic propoxyphene (*d*-propoxyphene) is one of the most frequently prescribed drugs in the United States. About 31 million prescriptions for it were issued in 1978 (1). Large doses can be fatal and often have been used to commit suicide. Propoxyphene also has been implicated in accidental deaths. A number of these deaths apparently occurred as a result of the consumption of propoxyphene in quantities only slightly larger than the upper limit of the recommended dosage, usually together with alcohol and/or other central nervous system (CNS) depressants (1).

BACKGROUND

Propoxyphene is subject to a pronounced first-pass effect in that only a small fraction of the absorbed dose enters the general circulation in unmetabolized form (2–4). Moreover, there are very large and apparently relatively consistent interindividual differences in the systemic availability of propoxyphene in humans (2–6). This fact suggests that certain accidental (as opposed to suicidal) poisonings due to propoxyphene may result from an unfortunate coincidence of a large (probably larger than prescribed) dose, an unusually small first-pass effect relative to the population average, and ingestion of one or more CNS depressants. For this and other obvious reasons, it is important to determine the factors responsible for interindividual differences in the first-pass effect on propoxyphene.

An orally administered drug may be subject to hepatic as well as prehepatic (intestinal) first-pass effects. The relative contribution of these two sites of biotransformation is important in assessing the reasons for interindividual differences in the systemic availability and for predicting the possible effect of surgically induced or endogenous portacaval shunts (*i.e.*, shunts due to hepatic cirrhosis). There are indirect indications, based on pharmacokinetic analysis of systemic availability relative to total clearance, that propoxyphene may be subject to some prehepatic biotransformation in humans (3, 4).

It is not possible to determine the magnitude of the prehepatic biotransformation of a drug such as propoxyphene by comparing its systemic availability in normal subjects and in patients with surgically constructed portacaval shunts. Such shunts are used to relieve variceal bleeding secondary to portal hypertension caused by hepatic cirrhosis and other pathological conditions associated with obstruction of portal blood flow. Liver disease and the drugs taken by the recipients of a portacaval shunt may modify the metabolic clearance of propoxyphene and thereby introduce an additional variable that cannot be controlled or defined readily because propoxyphene is not available for intravenous injection. Moreover, patients with portacaval shunts can develop various degrees of collateral circulation. Therefore, it is necessary to conduct studies on a suitable animal model.

Some published evidence suggested that the dog, like humans, exhibits a pronounced first-pass effect on orally administered propoxyphene (7). Demethylation to norpropoxyphene is a major elimination pathway of propoxyphene in both species (8). The dog is large enough for the convenient surgical construction of a portacaval shunt. Other investigators successfully used dogs to study the first-pass effect on orally administered drugs before and after a portacaval shunt (9).

In the present investigation, propoxyphene was administered both orally and intravenously to dogs before and after a portacaval shunt, according to a balanced experimental design. Propoxyphene was administered intravenously before and after surgery because the shunt may affect the plasma clearance of a drug (9). There is conflicting evidence concerning the dose dependence of the first-pass effect on propoxyphene in humans (2–4, 10, 11). The intravenous dose in this study was lower than the oral dose so that the areas under the concentration–time curve produced by both routes of drug administration would be similar.

0022-3549/80/0700-0786$01.00/0