Hydration and Percutaneous Absorption IV: Influence of Hydration on *n*-Alkanol Permeation Through Rat Skin; Comparison with Hairless and Swiss Mice

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Abstract □ The effect of protracted aqueous contact of rat skin on its permeability to methanol, *n*-butanol, and *n*-hexanol was investigated. With the aid of small diffusion cells, sets of intermittent permeation experiments, each ~7 hr in duration, were performed on excised rat skin sections over periods lasting several days, and permeability coefficients were calculated as a function of the duration of the hydration. The permeability coefficient of methanol increased gradually to an asymptote 2.5 times higher than the initial value over the first 80 hr of immersion and then remained essentially invariant through an additional 70 hr. In contrast, the butanol permeability coefficient increased by only a small fraction (~25%) through the first 5 hr of hydration, and it remained at the higher value through to the end of the experiment at 80 hr. For more hydrophobic hexanol, the permeability coefficient increased by ~40% over the first 10 hr and then declined, returning to near the initial value by the second day. It was relatively constant past this point—up to 150 hr. When these data were compared with similarly obtained data from earlier studies involving two strains of mice, the Swiss mouse and a hairless mouse mutant, parallelism was noted in the behavior of the rat and Swiss mouse skins, which set them both apart from the behavior of the skin of the hairless mouse. The comparison suggests that, irrespective of animal species, the development of a thick coat of hair occurs with commensurate functional changes in the chemical barrier properties of the epidermis.

Keyphrases □ Permeability—hydration and percutaneous absorption, influence on *n*-alkanol through rat skin, Swiss and hairless mice comparison □ Hydration—percutaneous absorption, influence on *n*-alkanol permeation through rat skin, Swiss and hairless mice comparison □ Absorption—hydration, influence on *n*-alkanol through rat skin, Swiss and hairless mice comparison

The relative influences of moisture absorption and retention on different animal skins serves as one point of comparison of the skins and, therefore, is a factor to be considered when animals are to be used instead of human subjects in percutaneous absorption research. In recent studies the permeabilities of the skins of two strains of laboratory mice affected by protracted immersion in an isotonic saline medium were explored (1–3). Permeability, and its alteration by these extreme hydrating conditions, was shown to be dependent on the strain of mouse chosen and, within a strain, on the chemical structure of the permeant. This had mechanistic implications with regard to the functioning of the Swiss mouse and the hairless mouse ($SKh\text{-}hr^{-1}$) skins as chemical barriers. The strikingly different behaviors noted between these two strains were presumed primarily to be related to the disparity in abundance and prominance of hair and, possibly, to a change in the relative importance of the transepidermal and transfollicullar routes. It could be assumed further that the absorption behaviors of animal skins may be related more to the similarity of their coats than to the species involved, but this has yet to be proven.

Therefore, the present study was undertaken to assess the effect of hydration on the permeability of rat skin using several of the same reference permeants and under identical conditions as in the mouse experiments. The diffusional behaviors of the hairy skins of the Swiss mouse and Sprague-Dawley rat proved to be somewhat similar and set apart from that of the hairless mouse. This has implications for the choice of animals used for certain dermatological research.

EXPERIMENTAL

Chemicals—[^{3}H]methanol[1], [^{14}C]butanol[2], and [^{14}C]hexanol[2] were used as received. The radiochemicals were diluted with 0.9% sodium chloride irrigation medium[3] (saline) for the permeation experiments. The final chemical concentrations in the diffusional medium were $\leq 10^{-4}$ *M*.

Animals—Sprague-Dawley adult male rats (~300 g) were used. They had free access to food and water. All skins used in the permeation experiments were denuded of hair by the nondestructive and nonirritating procedure of close cropping with a pair of surgical scissors (2). Rats have a relatively large skin area, considering that the opening between diffusion cell compartments is on the order of 0.6 cm^2. Therefore, it was possible to take nine or more separate sections of skin from the dorsal surface of the animal, as indicated in the sketch in Table I. Differences in skin site were minimized by this technique, and only a few rats were needed to complete the study.

Permeation Procedure—Two-compartment glass diffusion cells (1) were employed to determine skin permeability. Ten skin sections were excised from the dorsal surface of one rat, sacrificed by administering sodium pentobarbital. The external medium of the diffusion cell was saline. The half-cell contents were stirred at 150 rpm. All experiments were carried out at 37°. Permeation was followed by monitoring the receiver chamber concentration for ~2 hr. The half-cell facing the stratum corneum was always the donor compartment, and the half-cell facing the dermis was always the receiver compartment. Therefore, net diffusion occurred from the stratum corneum to the dermis side. Complete hydration profiles were obtained on each piece of skin by running eight sequential experiments covering up to 150 hr of conditioning, with thorough rinsing done between the runs (1).

Radioisotopic Assay—The concentration of the radiolabeled permeant was determined by placing discrete samples in a scintillation solution[4] and assaying on a liquid scintillation counter[5]. Using the technique of dual labels, methanol–butanol and methanol–hexanol were paired. Methanol thus served as a common solute in both sets of the experiments providing a means of assuring membrane integrity.

Data Analysis—The data were plotted as receiver chamber concentration (in terms of counts per minute) as a function of time. The permeability coefficient was calculated from (3):

$$P = \frac{V}{A}\frac{(dc/dt)}{\Delta C} \qquad \text{(Eq. 1)}$$

where P is the permeability coefficient in centimeters per hour, A is the diffusional area (~0.6 cm^2), ΔC is the concentration difference across

[1] New England Nuclear Corp., Boston, Mass.
[2] International Chemical and Nuclear Corp., Irvine, Calif.
[3] Abbott Laboratories, North Chicago, Ill.
[4] Aquasol, New England Nuclear Corp., Boston, Mass.
[5] Beckman Liquid Scintillation Counter, Model LS 9000, Beckman Instruments, Inc., Fullerton, Calif.

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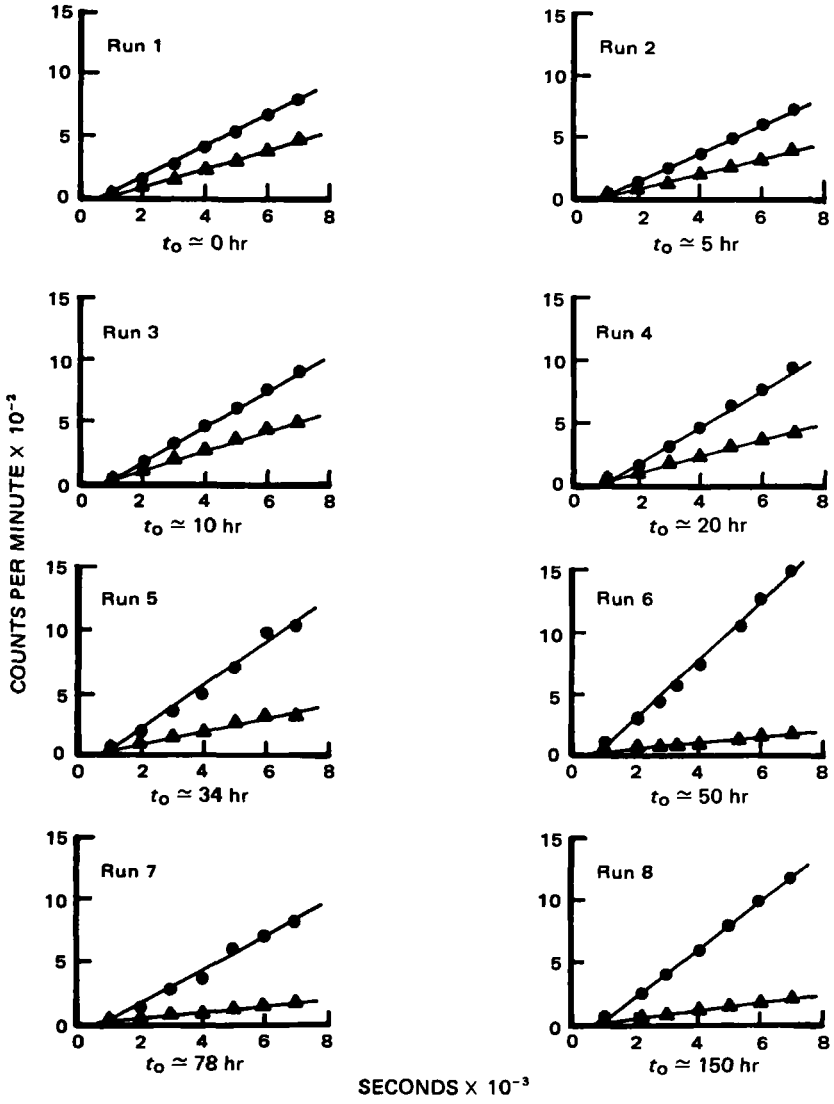


Figure 1—*Series of receiver compartment concentration (cpm)* versus *time profiles for a set of eight sequential runs on a single skin. This is one set of data from the methanol–hexanol series (see text). The initial time of hydration (t_0) for each run is indicated in the lower left-hand corner of each plot. Key: (●) methanol; (▲) hexanol.*

the membrane, which was taken to be equal to the donor concentration (cpm), V is the half-cell volume (1.4 ml), and dc/dt is the quasi steady-state slope (cpm/cm^3/hr).

RESULTS

Figure 1 contains a representative set of eight subplots obtained in sequential permeation experiments carried out on one piece of skin over 150 hr of hydration using [^{3}H]methanol and [^{14}C]hexanol as dual permeants. A linear relationship between the receiver concentration *versus* time is indicative of good approximation of steady-state transport conditions. Also, the lag times do not seem to change with hydration time. This is consistent with the permeation behavior reported for hairless (1, 3) and Swiss (2) mice. Slopes of these linear plots were used to compute permeability coefficients.

Table I contains the individual and average permeability coefficients of methanol as a function of hydration time. Both sets of methanol data, one run with butanol as the copermeant and the second run with hexanol as the copermeant, are presented. Tables II and III contain similar data for butanol and hexanol, respectively.

From data reported in Tables I–III, the percent change in permeability at any time relative to the permeability at time zero was computed (2). (The first diffusional run began immediately after placing a skin section in the diffusion cell.) These changes are reported in Figs. 2–4 for each of the permeants in terms of the percent change as a function of hours of hydration.

DISCUSSION

Effect of Hydration on Alkanol Permeation—The permeability coefficient of methanol through rat skin approaches an asymptote 2.5 times the initial value (150% hydration influence) as a function of time, with the limiting value essentially obtained at the 80-hr point (Fig. 2).

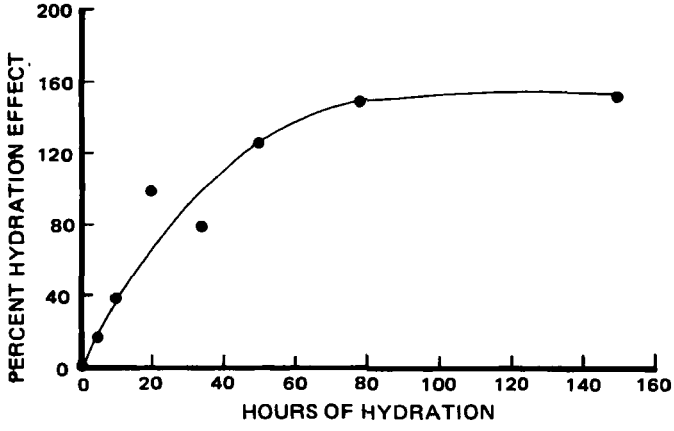
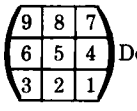


Figure 2—*Plot of percent hydration effects* versus *hydration time for methanol.*

Table I—Hydration Effect Data for Methanol

Hydration time, hr	$P \times 10^3$, cm/hr Skin Section No.[a] 1	2	3	4	5	Average ±*SD*
	Set 1 (butanol copermeant)					
~0	2.7	2.3	1.6	4.2	2.2	2.6 ±1.0
5	2.5	2.4	2.2	4.5	1.9	2.7 ±1.0
10	3.0	3.1	2.1	5.3	2.0	3.1 ±1.3
20	3.3	3.2	2.4	5.5	2.3	3.3 ±1.3
34	3.4	3.2	2.6	7.1	3.0	3.9 ±1.8
50	5.6	3.7	2.7	11.0	3.6	5.3 ±3.3
78	9.5	4.7	2.5	13.5	5.4	7.1 ±4.4
	Set 2 (hexanol copermeant)					
	6	7	8	9		
~0	1.6	1.6	3.8	2.1		2.1 ±0.8
5	1.6	1.7	5.1	2.4		2.7 ±1.6
10	2.0	2.0	6.2	2.8		3.3 ±2.0
20	2.1	2.2	7.0	2.9		3.6 ±2.3
34	2.5	2.5	7.6	4.8		4.4 ±2.4
50	—	—	7.4	3.0		5.2 ±3.1
78	3.3	3.6	7.2	—		4.7 ±2.2
150	5.8	4.7	—	—		5.3 ±0.8

[a] 

Dorsal surface of the rat.

With hairless mouse skin, the methanol permeability coefficient was unaffected by such conditioning (1). The behavior of rat skin to methanol, however, is qualitatively similar to that previously seen for the Swiss mouse (2). In the latter strain of mouse, the methanol permeability coefficient was increased by 80% (1.8 times) by hydration, with the effect leveling out by ~20 hr.

In the rat, the butanol permeability coefficient appeared to be increased marginally by hydration (20–25%) with the effect noted as early as 5 hr (Fig. 3). The permeability coefficient then appeared invariant up to 78 hr. This contrasts with the hairless mouse where a doubling of the parameter took place over the first 10 hr of experimentation (1) and with the Swiss mouse where a slightly greater than twofold increase was noted by 15 hr (2). In the Swiss mouse skin, the permeability coefficient appears to gradually and linearly decrease past 15 hr, so that the increase at the end of the second full day measured only 85%.

In the rat, hexanol permeation appears to have a unique hydration dependency (Fig. 4). Over the first 10 hr of saline immersion, the permeability coefficient appears to increase. There is, on the average, a 44% enlargement at the 10-hr point. The trend then reverses, with a systematic return to the initial, baseline value by 78 hr. In the hairless mouse hexanol behaves as butanol; there is a twofold increment which is largely complete by 10 hr. No data were obtained for hexanol passing through Swiss mouse skin.

The up and down type of pattern, apparent for butanol in the Swiss mouse and for hexanol in the rat, surfaces in the hairless mouse skin at an alkanol alkyl chain length of 8 (*n*-octanol). The absolute values of the alkanol permeability coefficients through rat skin increase from methanol to hexanol. Taking the permeability coefficients after 10 hr of conditioning as representative, the average values are 3.2×10^{-3} cm/hr for methanol, 6.4×10^{-3} cm/hr for butanol, and 13.5×10^{-3} cm/hr for hexanol. The ratio of the hexanol and methanol values of ~4 indicates a marginal dependency of permeation on hydrophobicity through the series. By way of comparison, the factor at 10 hr of hydration in the hairless mouse is >20, pointing to a more acute lipid–water partitioning reliance of the permeation process in this tissue (1).

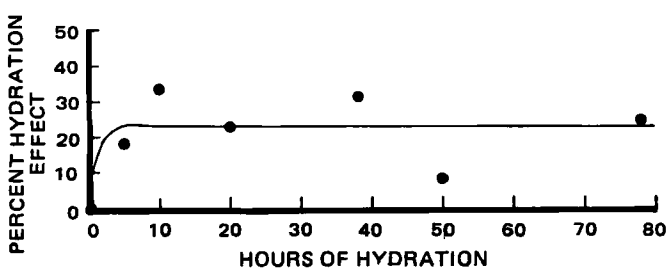


Figure 3—*Plot of percent hydration effects* versus *hydration time for butanol.*

Table II—Hydration Effect Data for Butanol

Hydration time, hr	$P \times 10^3$, cm/hr Skin Section No.[a] 1	2	3	4	5	Average ±*SD*
~0	4.2	5.0	3.7	7.1	4.0	4.8 ±1.4
5	4.8	5.9	4.9	8.6	4.2	5.7 ±1.7
0	7.3	7.1	6.6	7.3	3.6	6.4 ±1.6
20	5.9	6.1	7.7	6.2	3.8	5.9 ±1.4
34	4.7	6.6	6.8	8.5	5.0	6.3 ±1.5
50	4.9	5.8	5.9	5.9	3.7	5.2 ±1.0
78	5.0	8.7	5.5	4.8	6.1	6.0 ±1.6

[a] See Table I.

Table III—Hydration Effect Data for Hexanol

Hydration time, hr	$P \times 10^3$, cm/hr Skin Section No.[a] 6	7	8	9	Average ±*SD*
~0	9.0	8.3	10.5	9.8	9.4 ±1.0
5	8.8	11.2	15.0	13.0	12.0 ±2.6
10	11.2	11.9	18.4	12.6	13.5 ±3.3
20	9.4	10.8	14.6	13.1	12.0 ±2.3
34	8.3	10.5	14.0	11.0	11.0 ±2.3
50	7.8	8.8	12.0	7.8	9.1 ±2.0
78	6.3	9.6	10.1	10.6	9.6 ±1.9
150	10.0	10.3	—	—	10.2 ±0.2

[a] See Table I.

Significance of the Findings—It is difficult to interpret these collective observations, but the following factors affect the permeation process. The development of a coat of hair generally is accompanied by thickening of the skin, especially its dermal elements in which the follicles are anchored. This magnifies the diffusional importance of the living and functionally aqueous strata beneath the stratum corneum when these are a part of the membrane mounted in the diffusion cell. Thus, it is to be expected that the thicker skins of the furry animals will have earlier onset of rate control by the viable epidermal and the dermal strata with increasing hydrophobicity (as the alkyl chain is extended) and that the limiting rate of diffusion as aqueous tissue control is approached will also be lower; each appears to be the case.

This in part explains the more compressed range of permeability coefficient values for the alkanols in the full thickness Swiss mouse and rat skins. This influence of the thickened subcorneal elements creates a false impression of permeability of the skin in that in the living animal the peripheral blood flow collects permeant at the immediate undersurface of the epidermis, eliminating much of the diffusional resistance experienced in the nonblood perfused skins of *in vitro* measurement.

Strata thickness variations cannot explain the different hydration sensitivities of methanol permeation through the skin of the hairless mouse and the furry integuments as the permeability coefficients are small, signifying rate control by strata elsewhere in the skins. For the hairless mouse, stripping (4) and other experiments (5–7) establish the stratum corneum as the principal source of diffusional resistance for methanol. Also, both the alkanol alkyl chain length profile and the hydration effect data for the hairless mouse tissue indicate that this solute finds some pathway through the skin which is around rather than through the lipid domains of the horny layer. Thus, it is possible to conclude that the hairy epidermises function differently as barriers to methanol. The

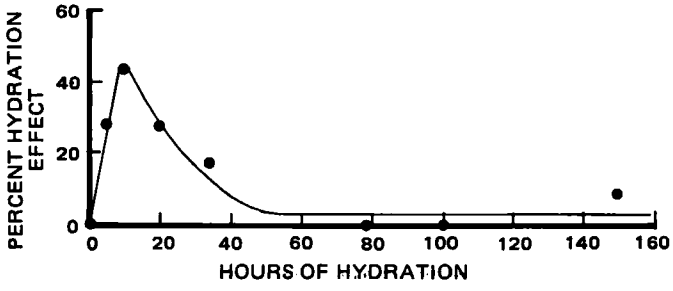


Figure 4—*Plot of percent hdyration effects* versus *hydration time for hexanol.*

difference centers around the increased prominence and number of hair follicles. Probably diffusion across the transfollicular shunt is greatly enhanced, to the point where it becomes the principal pathway. The horny layer of the epidermis is also affected by the formation of a thick coat of hair. Since the hairy mat restricts insensible perspiration, the stratum corneum associated with furry skin need not be as impermeable to water, and it is typically thinner and less well formed than when the surface is hairless. Thus, structural and compositional changes in the horny layer also provide a basis for explaining the differing hydration sensitivities. It is likely that both factors are important.

This study provides further evidence of intra- and interspecies complexities of animal skins as mass transport regulators. To a degree it demonstrates that skins covered with thick coats of hair do not behave diffusionally as hairless skins, whether of mouse or humans. Previous work (1–8) indicates a high degree of parallelism in the chemical barrier properties of hairless mouse and human skins. This study adds support for the use of hairless animals for research on percutaneous absorption, primary irritancy, topical drug delivery, *etc.*, when it is not possible or practicable to use human subjects.

REFERENCES

(1) C. R. Behl, G. L. Flynn, T. Kurihara, N. Harper, W. M. Smith, W. I. Higuchi, N. F. H. Ho, and C. L. Pierson, *J. Invest. Derm.*, **75**, 346 (1980).

(2) C. R. Behl and M. Barrett, *J. Pharm. Sci.*, **70**, 212 (1981).

(3) C. R. Behl, M. Barrett, G. L. Flynn, T. Kurihara, K. A. Walters, O. G. Gatmaitan, N. Harper, W. I. Higuchi, N. F. H. Ho, and C. L. Pierson, *ibid.*, **71**, 229 (1982).

(4) G. L. Flynn, H. H. Durrheim, and W. I. Higuchi, *ibid.*, **70**, 52 (1981).

(5) C. R. Behl, G. L. Flynn, T. Kurihara, W. Smith, O. Gatmaitan, W. I. Higuchi, N. F. H. Ho, and C. L. Pierson, *J. Invest. Derm.*, **75**, 340 (1980).

(6) G. L. Flynn, C. R. Behl, K. Walters, O. G. Gatmaitan, J. Kurihara, N. F. H. Ho, W. I. Higuchi, and C. L. Pierson, *Burns*, **8**, 47 (1981).

(7) C. R. Behl, E. E. Linn, G. L. Flynn, C. L. Pierson, W. I. Higuchi, and N. F. H. Ho, *J. Pharm. Sci.*, in press.

(8) R. B. Stroughton, in "Animal Models in Dermatology," H. Maibach, Ed., Churchill Livingstone, New York, N.Y., 1975, p. 121.

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NOTES

In Vitro Adsorption of Phenobarbital onto Activated Charcoal

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Abstract □ *In vitro* experiments were performed to determine the extent and duration of adsorption and desorption of phenobarbital onto and from activated charcoal in solutions of various pHs. The results of studies supported the evidence of the effectiveness of charcoal as an adsorbent. Adsorption was dependent upon the quantity of charcoal used. With amounts of charcoal ≥0.5 g, adsorption was complete within 60 min. Desorption was rapid, quantity dependent, and pH independent. The results of adsorption isotherms indicated no change in binding capacity of the drug from solutions of different pH.

Keyphrases □ Phenobarbital—*in vitro* adsorption onto activated charcoal, desorption, binding □ Adsorption, *in vitro*—phenobarbital onto activated charcoal, desorption, binding

The importance of activated charcoal cannot be overemphasized as an emergency treatment in drug poisoning. Activated charcoal given orally as a slurry can effectively adsorb and hold many drugs such as alkaloids, glycosides, and barbiturates (1). Acute barbiturate poisoning is common and it accounts for ~1500 deaths annually in the United States (2). Barbiturates are the second most frequent cause of poisoning in children (3).

Phenobarbital is one of the major barbiturates used in many products as a sedative–hypnotic and antiepileptic agent. It has a therapeutic range of 0.03–0.6 g daily in divided doses, and its potential for poisoning is great, having a fatal range of 1–10 g (4). Data collected on the use of phenobarbital in adsorption studies with activated charcoal can be useful.

A slurry of activated charcoal given 30 min after hypnotic doses of phenobarbital or glutethimide resulted in a plasma drug concentration at least 50% lower in treated than in untreated dogs, even when charcoal was allowed to pass through the entire GI tract (5). Adsorbed material is retained tenaciously throughout passage in the gut. There is a concern that part of the poison in the intestine may later be released because of less favorable pH conditions. It was found that the reduction in the amount of available poison is markedly high as compared to insignificant elution of the poison in the intestine (6). Administration of large amounts of activated charcoal is considered to be a routinely useful procedure. In view of the delayed emetic action of ipecac syrup, there is an increasing speculation that activated charcoal may be of more importance as an emergency treatment for accidental poisoning. Recent studies (7–9) have shown the importance of adsorption of different drugs onto activated charcoal.

The purpose of this study was to investigate and understand the extent of adsorption of phenobarbital sodium

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