Standard curves constructed by plotting peak areas *versus* sample concentrations show good linearity in the concentration range for all substances, with correlation coefficients >0.9994 in all cases.

REFERENCES

(1) A. A. Fernandez, V. T. Noceda, and E. S. Carrera, *J. Pharm. Sci.*, **58,** 443 (1969).

(2) P. P. Ascione and G. P. Chrekian, *ibid.*, **59,** 1480 (1970).

(3) E. Addison and R. G. Clark, *J. Pharm. Pharmacol.*, **15,** 268 (1963).

(4) J. P. Sharma, G. D. Koritz, E. G. Perkins, and R. F. Bevill, *J. Pharm. Sci.*, **66,** 1319 (1977).

(5) A. G. Butterfield, D. W. Hughes, W. L. Wilson, and N. J. Pound, *ibid.*, **64,** 316 (1975).

(6) J. H. Knox and J. Jurand, *J. Chromatogr.*, **110,** 103 (1975).

(7) *Ibid.*, **186,** 763 (1979).

(8) J. H. Knox and A. Pryde, *J. Chromatogr.*, **112,** 171 (1975).

(9) A. P. De Leenheer and H. I. C. F. Nelis, *ibid.*, **140,** 293 (1977).

(10) T. D. Sokoloski, L. A. Mitscher, P. H. Yuen, J. V. Juvarkar, and B. Hoener, *J. Pharm. Sci.*, **66,** 1159 (1977).

(11) B. Hoener, T. D. Sokoloski, L. A. Mitscher, and L. Malspeis, *ibid.*, **63,** 1901 (1974).

(12) K. D. Schlecht and C. W. Frank, *ibid.*, **62,** 258 (1973).

(13) R. E. Majors, *Anal. Chem.*, **44,** 1722 (1972).

(14) C. R. Stephens, K. Murai, K. J. Brunings, and R. B. Woodward, *J. Am. Chem. Soc.*, **78,** 4155 (1956).

(15) J. Hermansson, *J. Chromatogr.*, **152,** 437 (1978).

(16) K.-G. Wahlund, *ibid.*, **115,** 411 (1975).

(17) A. Sokolowski and K-G. Wahlund, *ibid.*, **189,** 299 (1980).

(18) D. Westerlund and E. Erixon, *ibid.*, **185,** 593 (1979).

ACKNOWLEDGMENTS

The authors are grateful to Mr. S. Eriksson for skillful drawing of the figures and to Mr. B. Wiese for technical assistance.

Hydration and Percutaneous Absorption III: Influences of Stripping and Scalding on Hydration Alteration of the Permeability of Hairless Mouse Skin to Water and *n*-Alkanols

CHARANJIT R. BEHL *x, MICHAEL BARRETT, GORDON L. FLYNN, TAMIE KURIHARA, KENNETH A. WALTERS, OLIVIA G. GATMAITAN, NANCY HARPER, WILLIAM I. HIGUCHI, NORMAN F. H. HO, and CARL L. PIERSON

Received January 19, 1981, from the *College of Pharmacy and Medical School, University of Michigan, Ann Arbor, MI 48109.* Accepted for publication June 10, 1981. *Present address: Pharmaceutical Research, Roche Laboratories, Hoffmann-La Roche Inc., Nutley, NJ 07110.

Abstract □ The influence of hydration on the permeability of stripped and scalded skins of hairless mice was investigated *in vitro* using water and *n*-alkanols as test permeants. Irrespective of pretreatment, the permeation rates of water, methanol, and ethanol were unaffected by aqueous immersion of skin sections in a diffusion cell, consistent with earlier data on unprocessed skins. The permeation rates of butanol and hexanol also were insensitive to hydration, differing from earlier studies on normal, intact skin in which both solutes' rates doubled after 10 hr of soaking. Following both pretreatments, the permeability of octanol declined over the first 5–10 hr of maceration, but remained invariant thereafter. The decline was most pronounced for the scalded skins. With untreated skin, octanol permeability initially increased and then declined before assuming a constant value. This study indicates that the barrier properties of the epidermis and dermis are not particularly sensitive to extended hydration except in the case of octanol. Scalding at 60° for 60 sec rapidly hydrates the skin, altering tissue permeability to about the same extent as a 10-hr (or longer) immersion in water at 37°. Octanol's unique hydration profile is explained by locating the origin of permeability decline in tissue beneath the horny exterior of the skin.

Keyphrases □ Permeability—of hairless mouse skin to water and *n*-alkanols after stripping and scalding □ Absorption, percutaneous—influence of stripping and scalding on permeability of water and *n*-alkanols, hairless mouse skin □ Hydration—alteration of permeability of stripped and scalded mouse skin to water and *n*-alkanols

It is known that the permeability of intact hairless mouse skin is altered by aqueous maceration, and that increases in permeation rates upon extended immersion of the skin in saline are a function of chemical structure for small, nonelectrolyte penetrants. Thus, the processes of hydration are complex, and probably involve more than one isolated phase of the skin membrane. A more thorough understanding of such hydration phenomena will add to the mechanistic understanding of the skin's barrier behavior.

Recently, effects of hydration on hairless mouse skin permeability were examined using water and *n*-alkanols as test permeants (1). These *in vitro* studies showed that hydration-induced permeability increases were a function of the penetrant lipophilicity. The permeabilities of the polar solutes, water, methanol, and ethanol, were not changed by hydration, while the permeabilities of the moderately lipophilic compounds, butanol and hexanol, asymptotically doubled in 10 hr of hydration. Permeation rates of the more lipophilic heptanol also increased to an asymptote, but only by ~50% in 10 hr. Octanol, the most lipophilic solute, showed an initial increase of ~50% in 5 hr, but then declined by ~25% by the 10th hr, undergoing a net increase in permeability of ~25%.

The skin hydration studies were extended to the Swiss mouse using water, methanol, ethanol, and butanol as permeants (2). In contrast to the hairless mouse skin results, water permeability increased up to 30 hr of hydration, and showed signs of leveling off between 30 and 43 hr. The permeabilities of methanol and ethanol also increased, but plateaued by 15 hr. Permeation rates of butanol increased over the first 15 hr and then declined almost linearly up to 48 hr. The hydration effect profile differences between the Swiss mouse and its hairless counterpart are

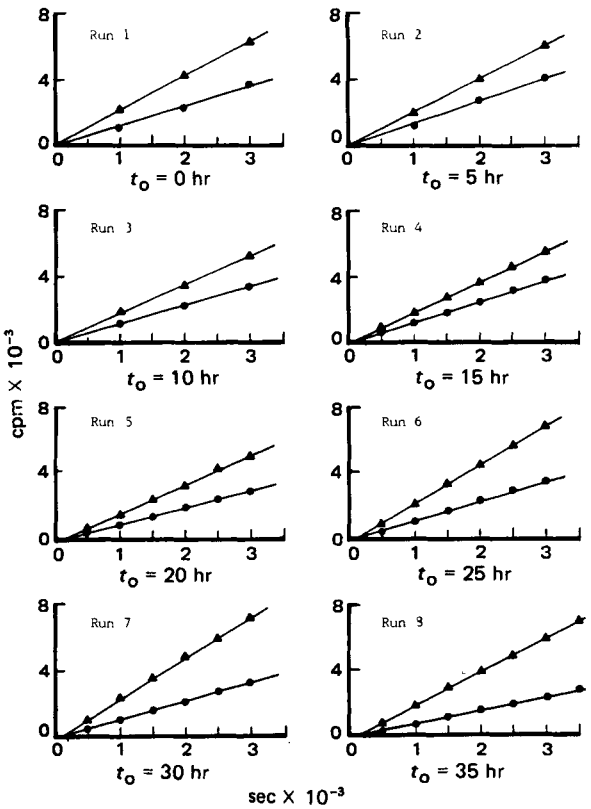

Figure 1—*Series of amount penetrated (cpm) versus time profiles for a set of eight sequential permeation runs on a single piece of stripped (25×) skin. The initial time of hydration for each run in terms of the total elapsed time (t_0) is indicated under each plot. This is one set of data for the methanol–butanol series. Key: ●, methanol; and ▲, butanol.*

apparently due to the abundant follicular presence in the Swiss species.

In both of the previous studies, unaltered full thickness skins consisting of the stratum corneum, the viable epidermis and the dermis were employed. It is well known that different strata of the skin control permeation rates of different solutes, depending on the relative oil/water partition coefficients of the permeants (3–5). Therefore, it is likely that different layers of the skin are responsible for the observed hydration-induced permeability increases or lack of increases. The present study was undertaken to study the influences of hydration on hairless mouse skins that were either scalded to alter thermally the properties of the stratum corneum (6) or stripped to completely remove the stratum corneum.

EXPERIMENTAL

Chemicals—[^{3}H]water[1], [^{3}H]methanol[1], [^{14}C]ethanol[2], [^{14}C]hexanol[2], [^{14}C]heptanol[2], and [^{14}C]octanol[2] were used as received. The radiochemicals were diluted into 0.9% sodium chloride irrigation medium[3] (saline) to prepare solutions for the permeation experiments. The final chemical concentrations in the diffusional medium were 10^{-4} *M* or lower.

Animals—Male hairless mice, SKH-hr^{-1} strain[4], were given free access to food and water. The mice were housed individually in shoebox cages to prevent them from damaging each other's skin; bedding was changed at least once a week. They were visually examined at least once a day to ascertain their general health.

[1] New England Nuclear, Boston, MA 02218.
[2] International Chemical and Nuclear Corp., Irvine, CA 92664.
[3] Abbott Laboratories, North Chicago, IL 60064.
[4] Skin Cancer Hospital, Temple University, Philadelphia, PA 19140.

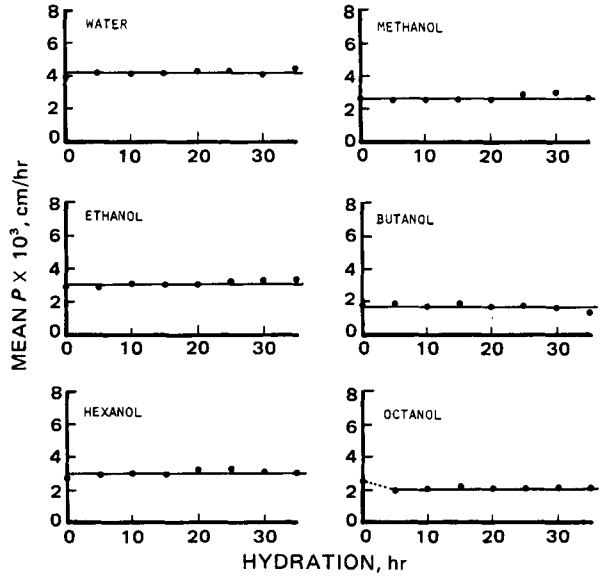

Figure 2—*Plots of average permeability coefficients (P) of the stripped (25×) skins versus hydration time for water, methanol, ethanol, butanol, hexanol, and octanol.*

Scalding Procedure—The dorsal surface of a mouse freshly sacrificed by spinal dislocation was scalded for 60 sec at 60° by immersing the dorsum in water contained in a jacketed apparatus through which water (60°) was perfused from a constant-temperature water bath (6). Both the abdominal and the dorsal surfaces were scalded before running the permeation experiments. Since scalding softens the stratum corneum, extra care was exercised in handling the skin. The skins were excised and mounted in diffusion cells within a few minutes after scalding, and the permeation experiment was started within 30 min.

Stripping Procedure—The abdominal surface of a mouse, freshly sacrificed by spinal dislocation, was stripped 25 times with adhesive tape (5, 7), using a fresh piece of tape each time. The abdominal surfaces were stripped with the adhesive tape because this site is easier to strip than the back. This choice did not affect the results because no site-to-site variations were observed in previous hydration-induced permeability alterations (1). Since removal of the stratum corneum causes the skin to shrink and fold over itself, making it difficult to handle and to excise from the animal, great care was observed in processing the stripped skins.

Radioisotopic Assay—The concentration of the radiolabeled permeant was determined by placing discrete samples in a cocktail[5] and assaying on a liquid scintillation counter[6]. Whenever possible, the technique of dual labels (1, 2, 4, 6–11) was used to economize the study and improve experimental precision.

Permeation Procedure—Two-compartment glass diffusion cells were employed to determine skin permeability. The mouse age was controlled (age 60 days) as much as possible, to avoid any age-related effects (4). The

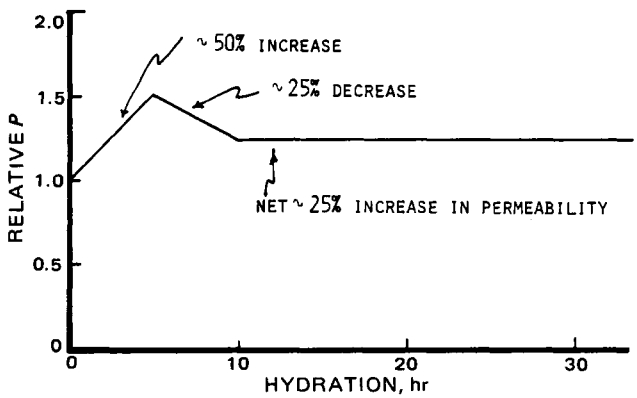

Figure 3—*Changes in octanol permeation rate as a function of hydration time. Data for this plot were abstracted from Ref. 1.*

[5] Aquasol, New England Nuclear, Boston, MA 02218.
[6] Beckman Liquid Scintillation Counter, Model LS 9000, Beckman Instruments, Fullerton, Calif.

Table I—Summary of Results of Stripped Abdominal Skins

Hydration, hr	$P \times 10^3$, cm/hr: Water[a] Mouse 1, 60 days; 31.0 g	Mouse 2, 60 days; 31.0 g	Mouse 3, 60 days; 26.0 g	Mean $P \times 10^3$ [b], cm/hr
~0	382.0	—	—	382.0
5	420.0	—	—	420.0
10	409.7	—	—	409.7
15	424.6	—	—	424.6
20	434.2	—	—	434.2
25	429.5	—	—	429.5
30	415.5	—	—	415.5
35	444.8	—	—	444.8
Mean ± *SD*				420.0 ± 18.9

Hydration, hr	Methanol: Mouse 1, 117 days; 37.0 g	Mouse 2, 117 days; 37.0 g	Mouse 3, 117 days; 35.0 g	Mean $P \times 10^3$ ± *SD*, cm/hr
~0	269.0	255.0	239.7	254.6 ± 14.7
5	254.8	258.0	238.8	250.5 ± 10.3
10	249.0	223.0	264.6	245.5 ± 21.0
15	274.0	247.1	251.7	257.6 ± 14.4
20	290.0	215.9	244.6	250.2 ± 37.4
25	[c]	289.4	291.9	290.7 ± 1.8
30	[c]	300.8	302.4	301.6 ± 1.1
35	[c]	277.1	259.7	268.4 ± 12.3
Mean ± *SD*				264.9 ± 20.7

Hydration, hr	$P \times 10^3$ cm/hr: Ethanol Mouse 1, 60 days; 31.0 g	Mouse 2, 60 days; 31.0 g	Mouse 3, 60 days; 26.0 g	Mean $P \times 10^3$ [b], ± *SD*, cm/hr
~0	276.5	303.3	261.2	280.3 ± 21.3
5	303.7	263.0	285.1	283.9 ± 20.4
10	317.5	344.6	273.0	311.7 ± 36.2
15	319.9	259.5	339.4	306.3 ± 41.7
20	328.8	259.4	336.2	308.1 ± 42.4
25	330.8	278.8	370.1	326.6 ± 45.8
30	333.9	291.5	370.1	331.8 ± 39.4
35	339.9	314.2	373.3	342.5 ± 29.6
Mean ± *SD*				311.4 ± 22.0

Hydration, hr	Butanol: Mouse 1, 117 days; 37.0 g	Mouse 2, 117 days; 37.0 g	Mouse 3, 117 days; 37.0 g	Mean $P \times 10^3$ ± *SD*, cm/hr
~0	181.0	188.0	189.7	186.2 ± 4.6
5	198.0	200.3	177.7	192.0 ± 12.4
10	190.0	155.0	188.2	177.7 ± 19.7
15	193.0	197.6	158.1	182.9 ± 21.6
20	195.0	147.5	153.1	165.2 ± 26.0
25	169.0	171.6	169.5	170.0 ± 1.4
30	157.0	154.7	156.3	156.0 ± 1.2
35	139.0	114.8	115.5	123.1 ± 13.8
Mean ± *SD*				169.1 ± 22.0

Hydration, hr	$P \times 10^3$ cm/hr: Hexanol Mouse 1, 60 days; 34.0 g	Mouse 2, 60 days; 25.0 g	Mouse 3, 60 days; 32.5 g	Mean $P \times 10^3$ [b], ± *SD*, cm/hr
~0	227.1	353.2	226.4	268.9 ± 73.0
5	205.1	411.0	262.2	292.8 ± 106.3
10	205.1	430.0	262.2	299.1 ± 116.9
15	219.8	417.8	223.4	287.0 ± 113.3
20	237.9	455.5	282.0	325.1 ± 115.0
25	258.1	436.0	273.4	322.6 ± 98.7
30	263.2	406.4	263.3	311.0 ± 82.6
35	261.0	411.7	255.5	309.4 ± 88.6
Mean ± *SD*				302.5 ± 19.6

Hydration, hr	Octanol: Mouse 1, 60 days; 35.0 g	Mouse 2, 60 days; 30.0 g	Mouse 3, 60 days; 27.0 g	Mean $P \times 10^3$ ± *SD*, cm/hr
0	244.4	216.0	283.7	248.0 ± 34.0
5	189.3	193.0	200.3	194.2 ± 5.6
10	230.9	188.9	194.1	204.6 ± 22.9
15	236.9	225.5	201.3	221.2 ± 18.2
20	231.1	195.6	195.0	207.2 ± 20.7
25	208.8	200.2	208.4	205.8 ± 4.9
30	224.2	207.8	198.5	210.2 ± 13.0
35	213.9	210.2	202.2	208.8 ± 6.0
Mean ± *SD*				207.4 ± 8.0

[a] Skins of Mouse 2 and Mouse 3 were damaged after ~5 hr of experiment. [b] The standard deviations represent mouse-to-mouse variability, not in the estimate of hydration-induced alterations (or lack of them). [c] The skins were damaged.

external medium of diffusion was normal saline. The half-cell contents were stirred at 150 rpm and all permeation experiments were carried out at 37°. The half-cell facing the stratum corneum was always the donor chamber and the half-cell facing the dermis was always the receiver chamber. Therefore, net diffusion was from the stratum corneum to the dermis side. Complete hydration profiles were obtained on each piece of skin by running six to nine sequential experiments, with thorough rinsing between runs (1).

Data Analysis—The data were plotted as receiver compartment concentration (in counts per minute) *versus* time. The permeability coefficients were calculated from (1):

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

where:

P = the permeability coefficient (centimeters per hour)
A = the diffusional area (~0.6 cm^2)
ΔC = the concentration difference across the membrane, which was taken to be equal to the donor concentration (counts per minute)
V = the half-cell volume (1.4 ml)
dC/dt = the quasisteady-state slope (counts per minute per cubic centimeter per hour)

RESULTS

Figure 1 contains a representative set of eight subplots obtained in sequential permeation experiments carried out on one piece of skin over 35 hr using [^{3}H]methanol and [^{14}C]butanol as dual permeants. A linear relationship between the receiver concentration *versus* time was obtained, indicating a good approximation of steady-state transport conditions. This permeation behavior is consistent with that reported for the intact, unaltered skins of hairless (1) and Swiss (2) mice. Slopes of these linear plots were used to compute permeability coefficients.

Table I contains a summary of results for the stripped abdominal skins. Three mice were used for each of the permeants, and eight sequential permeation experiments were run on each skin.

Data from sequential runs performed on scalded skins were processed as indicated in Fig. 1. One of the experiments, the methanol/butanol set, was of long duration (>40 hr) and involved nine separate experiments on each piece of tissue spaced at 5-hr intervals. Table II contains a summary of results for the scalded skins.

Two skins, one abdominal and one dorsal, were employed for each of the permeants, and [^{3}H]methanol was used as a copermeant with all alkanols. The individual permeability coefficients of the abdominal and the dorsal skins are quite similar, indicating that the data are not a function of the anatomical site. This observation is consistent with the earlier studies where the abdominal and the dorsal permeabilities were found to converge beyond the age of ~50 days (4).

Since methanol was used as a reference permeant, it is possible to normalize the data for ethanol, butanol, hexanol, and octanol to methanol permeability at a given hydration time. This procedure levels out animal-to-animal variability and results in more precise data. The standard deviations associated with the average ratios are much smaller than those associated with the average permeability values (Table II).

DISCUSSION

In vitro diffusion through membranes from body tissues or from synthetic materials is one of the most general means of characterizing the physicochemical attributes of physiological and synthetic barriers. With

Table II—Summary of Results of Pre-scalded Skins

Hydration, hr	$P \times 10^3$, cm/hr: Abdominal	Dorsal	Abdominal	Dorsal	Average $P \times 10^3 \pm SD$, cm/hr:		Average Ratio $\pm SD$
	Methanol[a]		Ethanol[a]		Methanol	Ethanol	P_{c_2}/P_{c_1}
~0	3.7	3.8	3.1	3.1	3.8 ± 0.1	3.1 ± 0.0	0.8 ± 0.0
5	3.4	3.4	2.8	2.9	3.4 ± 0.0	2.9 ± 0.1	0.9 ± 0.1
10	3.7	4.0	3.1	3.2	3.9 ± 0.2	3.2 ± 0.1	0.8 ± 0.0
15	4.0	4.2	3.4	3.5	4.1 ± 0.1	3.5 ± 0.1	0.9 ± 0.1
20	4.1	4.4	3.2	3.4	4.3 ± 0.2	3.3 ± 0.1	0.8 ± 0.0
25	4.1	4.6	3.2	3.3	4.4 ± 0.4	3.3 ± 0.1	0.8 ± 0.0
Mean ± *SD*					4.0 ± 0.4	3.2 ± 0.2	0.8 ± 0.1
Hydration, hr	Methanol[b]		Butanol[b]		Methanol	Butanol	P_{c_4}/P_{c_1}
~0	3.0	2.6	9.7	11.9	2.8 ± 0.3	10.8 ± 1.6	3.9 ± 1.0
5	3.3	2.7	9.7	11.5	3.0 ± 0.4	10.6 ± 1.3	3.8 ± 1.2
10	3.1	2.8	10.4	9.5	3.0 ± 0.2	10.0 ± 0.6	3.4 ± 0.0
15	3.8	2.7	10.3	12.9	3.3 ± 0.8	11.6 ± 1.8	3.8 ± 1.5
20	3.2	2.9	10.1	13.1	3.1 ± 0.2	11.6 ± 2.1	3.9 ± 0.9
25	3.0	2.9	9.4	13.5	3.0 ± 0.1	11.5 ± 2.9	3.9 ± 1.1
30[c]	—	2.8	—	12.7	3.0 ± 0.3[d]	11.3 ± 2.0[d]	3.9 ± 1.1[d]
35[c]	—	2.8	—	12.9	3.0 ± 0.3[d]	11.3 ± 2.1[d]	3.9 ± 1.1[d]
40[c]	—	2.7	—	12.7	3.0 ± 0.4[d]	11.3 ± 2.0[d]	3.9 ± 1.1[d]
Mean ± *SD*					3.0 ± 0.1	11.1 ± 0.5	3.8 ± 0.2
Hydration, hr	Methanol[e]		Hexanol[e]		Methanol	Hexanol	P_{c_6}/P_{c_1}
~0	2.6	3.0	42.0	53.2	2.8 ± 0.3	47.6 ± 7.9	17.0 ± 1.1
5	2.2	2.6	35.2	43.3	2.4 ± 0.3	39.3 ± 5.7	16.4 ± 0.5
10	2.5	2.6	36.1	40.3	2.6 ± 0.1	38.2 ± 3.0	15.0 ± 0.8
15	2.6	2.7	37.3	45.3	2.7 ± 0.1	41.3 ± 5.7	15.7 ± 1.8
20	2.8	2.7	40.7	45.3	2.8 ± 0.1	43.0 ± 3.3	15.7 ± 1.6
25	2.8	2.7	38.7	46.0	2.8 ± 0.1	42.3 ± 5.2	15.4 ± 2.3
Mean ± *SD*					2.7 ± 0.2	42.0 ± 3.3	15.9 ± 0.7
Hydration, hr	$P \times 10^3$, cm/hr: Abdominal	Dorsal			Average $P \times 10^3 \pm SD$, cm/hr:		Average Ratio $\pm SD$
	Methanol[f]		Octanol[f]		Methanol	Octanol	P_{c_8}/P_{c_1}
0	2.7	2.1	129.6	94.0	2.4 ± 0.4	111.8 ± 25.1	46.4 ± 2.3
5	2.2	2.8	87.5	100.2	2.5 ± 0.4	93.9 ± 9.0	37.8 ± 2.8
10	1.8	2.2	68.3	76.2	2.0 ± 0.3	72.3 ± 5.6	36.3 ± 2.3
15	2.2	2.3	78.7	77.0	2.3 ± 0.1	77.9 ± 1.2	35.5 ± 1.9
20	2.1	2.6	75.5	70.7	2.4 ± 0.4	73.1 ± 3.4	31.6 ± 6.2
25	2.2	2.8	78.7	80.6	2.5 ± 0.4	79.7 ± 1.3	32.3 ± 4.9
30	1.8	2.9	72.6	91.9	2.4 ± 0.8	82.3 ± 13.6	36.0 ± 6.1
Mean ± *SD*					2.4 ± 0.2	77.1 ± 4.3[g]	34.3 ± 2.2[g]

[a] Mouse age, 77 days; mouse weight, 20 g. [b] Mouse age, 54 days; mouse weight, 19.5 g. [c] The abdominal skins were damaged. [d] The abdominal data at 0, 5, 10, 15, 20, and 25 hr of hydration were averaged and combined with the dorsal data at 30, 35, and 40 hr of hydration. [e] Mouse age, 102 days; mouse weight, 33.0 g. [f] Mouse age, 102 days; mouse weight, 31.5 g. [g] Averages of data at hydration times of 10, 15, 20, 25 and 30 hr.

a judicious choice of permeants, the mass transfer mechanisms of the membranes can be determined and related fundamentally to permeant chemical structure. However, all such research presumes the membranes in question to be physically stable over the experimental time frame. However, diffusional experiments are by their nature lengthy, and over the extended membrane contact time with usually liquid external media there is ample opportunity for solvation to modify basic membrane integrity. Therefore, the effects of solvation must be considered during the assessment of barrier properties.

In the case of biological membranes, the media bathing the tissue is usually aqueous and made osmotically physiological with salts. It is the influence of such aqueous solutions that must be assessed. Since biological membranes are polyphasic, with each distinct phase presumably having a unique hydration sensitivity, a full understanding of hydration phenomena promises to be of mechanistically interpretive value. Certain skin preparations such as tape-stripped skin may be useful for simulating the influences of abrasive and disease related damage on the permeability of skin. Thus, the stability of processed skins to solvation are also of interest.

Effect of Hydration on Permeability of Stripped Skin—The skin becomes exceedingly permeable as a result of the stripping procedure used, which effectively removes the entire stratum corneum. Table III contains a comparative list of the average permeability coefficients of normal skins (abstracted from earlier studies) and stripped skins. The stripping procedure made the skin most permeable to water; a more than 300-fold increase was noted. Both methanol and ethanol demonstrate about half as much enhancement.

As the permeants become more lipophilic, the ratio drops almost exponentially, indicating a declining role of the stratum corneum with increased lipoidal characteristics of the permeants. The permeability coefficients for the alkanols through the stripped skin decline by only a factor of two from methanol to octanol, in contrast to the systematically increasing permeability coefficients of normal skin. The former pattern is *prima facie* evidence that transport of these compounds through the dermis and residual epidermis does not involve a lipid/water partitioning step. This evidence reaffirms conclusions previously made by these authors and by Scheuplein and Blank (12).

With the possible exception of octanol, there does not appear to be any change in permeability of the stripped skin sections to water or the *n*-alkanols over >35 hr of hydration (Fig. 2). It was previously reported (1)

Table III—Permeabilities of Stripped and Unaltered Skins of Hairless Mice

Compound	$P \times 10^3$, cm/hr: Skin[a] Unaltered[b]	Stripped	Ratio, $P_{stripped}/P_{unaltered}$
Water	1.3 ± 0.2	420.0 ± 18.9	323.0
Methanol	2.0 ± 0.4	269.4 ± 25.2	134.7
Ethanol	2.1 ± 0.1	311.4 ± 22.0	148.3
Butanol	10.8 ± 2.2	169.1 ± 22.0	15.7
Hexanol	38.8 ± 15.6	302.5 ± 19.6	7.8
Octanol	97.8 ± 13.5	207.4 ± 8.0	2.1

[a] Hydrated skins. [b] Abstracted from Ref. 1.

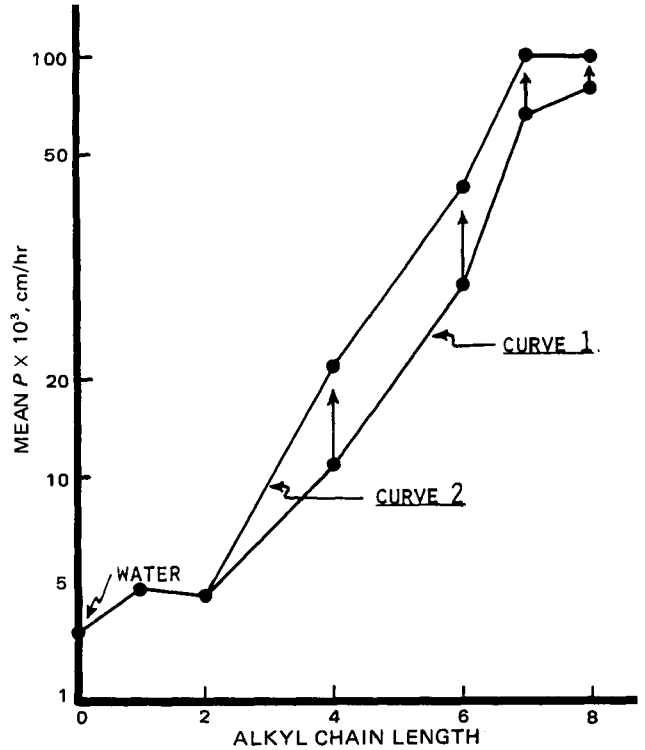

Figure 4—*Semilogarithmic plots of average permeability coefficients* versus *alkyl chain length for skins in their natural state of hydration (curve 1) and for skins which were exposed to saline in the diffusion cells for a minimum of 10 hr. Raw data for these plots were abstracted from Ref. 1.*

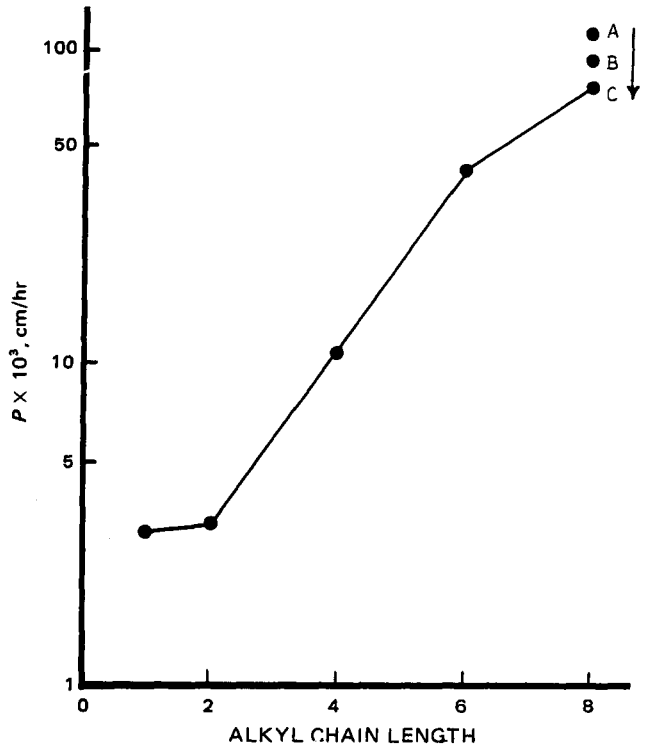

Figure 5—*Semilogarithmic plot of average permeability coefficients* (P *values*) versus *alkyl chain length for the scalded (60°; 60 sec) skins. Data for methanol, ethanol, butanol, and hexanol obtained at different times of hydration, were averaged. The individual permeabilities of octanol at ~0 and 5 hr of hydration are presented (points A and B, respectively). The remaining octanol data by hydration times of 10, 15, 20, 25, and 30 hr were averaged (point C).*

that the permeability coefficients of butanol and hexanol doubled over 10 hr of soaking. Considering the large increases in absolute permeability coefficients of the permeants caused by stripping and the hydration insensitivity of the membrane to their permeation after stipping, it appears that the effect of water with intact skin originates in the horny layer.

Because both butanol and hexanol increase exponentially on the log *P versus* alkyl chain length plot, it is evident that the effects are within the lipoid region of the stratum corneum. Whether they represent a physicochemical change in the diffusional medium or a physical rearrangement of the critical phase cannot be determined at this time. For the length of time they were carried out, the data for methanol are consistent with earlier studies where its permeation rate was found to be invariant in both abdominally and dorsally isolated dermis for hydration times of up to 800 hr (8).

The data for octanol may be interpreted differently. Its hydration–permeability profile, previously reported (1), is mechanistically revealing (Fig. 3). There is a 50% increase in permeation rates in the first 5 hr of hydration, and a 25% decrease by the 10th hr, followed by invariant permeability thereafter. To aid the discussion, data from previous hydration effect studies (1) were abstracted and are presented (Fig. 4) in terms of the alkyl chain profiles of the unhydrated skins (curve 1) and the hydrated skins (curve 2). Both curve 1 and curve 2 contain the following three segments: (*a*) a lower plateau evident for the polar solutes, water, methanol, and ethanol, signifying that their permeation occurs through some sort of highly polar, presumably aqueous pores present in the stratum corneum (4); (*b*) a steep rise in the permeability coefficients between ethanol and heptanol, which is linear when the data are obtained in the fully hydrated state (curve 2). [This segment demonstrates that the permeation process of these penetrants is controlled by their partitioning into the lipid regions of the stratum corneum (3, 4)]; and (*c*) the beginning of a second plateau near octanol, meaning that a third mechanism becomes important for the more hydrophobic compounds.

This mechanism has a permeation rate that is controlled by aqueous tissue resistance encountered in the viable epidermis and the dermis (3–5); *i.e.*, at an alkyl chain length of eight, the rate-controlling stratum changes from the horny layer to the viable epidermis and dermis. Based on this information and previous observations that octanol permeability increases somewhat with incremental stripping of the skin (7), it can be concluded that the control rate of octanol transport is significantly biphasic. Due to the wide difference in lipophilicity, it is reasonable that the stratum corneum and the dermis might influence the permeability of octanol (a transition compound) in an opposite manner, as a function of hydration time. Therefore, the initial increase in the permeability coefficients of octanol (Fig. 3) may be due to maceration of the stratum corneum. (Both competing processes occur simultaneously.)

If this argument is true, hydration of the stripped skin should slightly decrease its permeability to octanol. The hydration profile depicted in Fig. 2 indicates that this is probably the case. There seems to be some decrease in octanol permeability in the first 5 hr of hydration. This is less evident based on the stripped skin data alone but a statistically significant and similar effect is noted with scalded skin. It is curious that the other alkanols studied are not, as far as can be judged, similarly affected in stripped skin. At the present time, it is not possible to ascribe the ob-

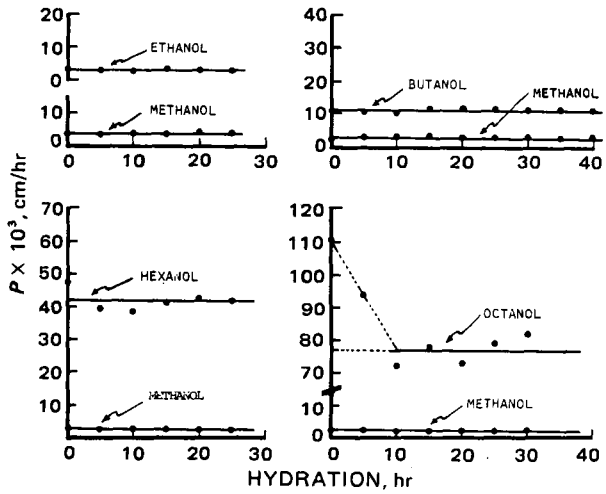

Figure 6—*Plots of average permeability coefficients* (P) *of the scalded (60°, 60 sec) skins* versus *time of hydration for methanol, ethanol, butanol, hexanol, and octanol. Methanol was used as a copermeant with all other alkanols to serve as a control solute.*

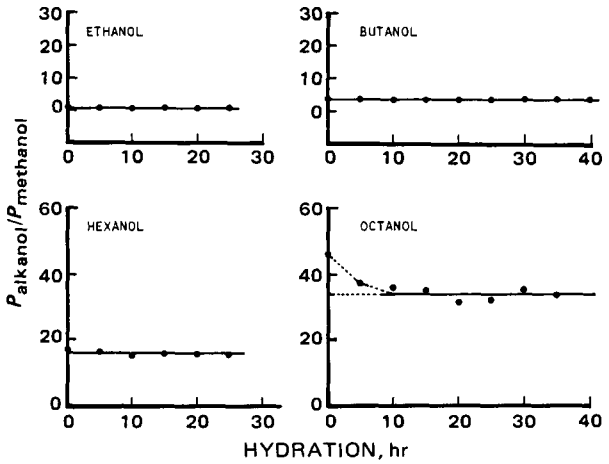

Figure 7—*Plots of normalized permeabilities (permeability of alkanol/permeability of methanol) of ethanol, butanol, hexanol, and octanol versus time of hydration.*

served octanol effect to any specific property of the molecule, *i.e.*, hydrophobicity or molecular size.

Effects of Hydration on Permeability of Scalded Skins—Figure 5 graphically illustrates the alkyl chain length profiles of the scalded skin permeabilities. Since no definite hydration–permeability trends are apparent through hexanol, data obtained at different hydration times were averaged for water, methanol, ethanol, butanol, and hexanol and these averages are plotted. Data for octanol were averaged only after 10 hr of hydration when its skin permeability coefficient was stabilized.

Octanol permeabilities at ~0 and 5 hr of hydration are also displayed in Fig. 5. A smooth sigmoidal curve is obtained when the points representing the fully equilibrated skin permeabilities are joined. This plot contains the three segments of curve 2 (Fig. 4) discussed previously, *i.e.*, the lower plateau, steep rise in the P values, and a second plateau. The absolute numbers plotted in Fig. 4 (curve 2) and in Fig. 5 are also, in agreement within ±50%. This close qualitative and quantitative parallelism between the two situations is significant and indicates that permeation in the normal, fully hydrated skin, and the scalded skin, occurs *via* the same mechanism.

There did not appear to be any significant increases or decreases caused by aqueous immersion in the permeability of scalded hairless mouse skin to the *n*-alkanols through hexanol. Neither the averages of the actual permeability coefficients (Fig. 6) nor their ratios to concurrently studied methanol permeability coefficients (Fig. 7) varied with hydration time. Water, methanol, and ethanol were previously shown to be unaffected by hydration in normal skin (1) but there were measurable hydration-associated increases in the permeabilities of butanol and hexanol (and heptanol). These were obliterated by scalding. The conclusion that scalding rapidly hydrates the skin was substantially reinforced by these extended studies. Moreover, the absolute permeability coefficients of the scalded skins were close in magnitude to those reported for fully hydrated normal skin. Therefore, 60-sec scalding not only rapidly hydrates the skin, but alters its permeability to about the same extent as does the 10 hr (or longer) aqueous soaking at 37°. This conclusion can aid in the interpretation of burned skin permeability data regardless of whether the skin is scalded (6, 9) or branded (10, 11).

Octanol appeared to behave differently. Its permeation rates gradually declined between 0 and 10 hr of hydration, and remained mostly unaltered thereafter. In view of the discussion and the theory of the switching-over of the rate-controlling mechanisms presented in the earlier section, it can be hypothesized that the observed decline probably was due to the effects of hydration on the epidermis–dermis. The data reported here further support the tentative interpretation presented to explain the up and down trends observed in octanol hydration–permeability profiles (1).

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. Dermatol.*, **75,** 346 (1980).

(2) C. R. Behl, M. Barrett, and G. L. Flynn, *J. Pharm. Sci.*, in press.

(3) H. H. Durrheim, G. L. Flynn, W. I. Higuchi, and C. R. Behl, *ibid.*, **69,** 781 (1980).

(4) C. R. Behl, G. L. Flynn, T. Kurihara, W. M. Smith, N. Harper, O. G. Gatmaitan, C. L. Pierson, W. I. Higuchi, and N. F. H. Ho, Basic Pharmaceutics Abstract 82, presented at the 126th Annual Meeting of the American Pharmaceutical Association, April 21–26, 1979, Anaheim, Calif.

(5) G. L. Flynn, H. H. Durrheim, and W. I. Higuchi, *J. Pharm. Sci.*, **70,** 52 (1981).

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

(7) R. Meyer, C. R. Behl, and G. L. Flynn, Presented at the 128th American Pharmaceutical Association Annual Meeting, Basic Pharmaceutics Section, St. Louis, Mo., March 29, 1981.

(8) C. R. Behl, G. L. Flynn, W. M. Smith, T. Kurihara, K. A. Walters, O. G. Gatmaitan, W. I. Higuchi, C. L. Pierson, and N. F. H. Ho, Basic Pharmaceutics Abstract 53, presented at the 27th Annual Meeting of APhA Academy of Pharmaceutical Sciences, Kansas City, Mo., Nov. 1980.

(9) G. L. Flynn, C. R. Behl, K. A. Walters, O. G. Gatmaitan, A. Wittkowski, T. Kurihara, N. F. H. Ho, W. I. Higuchi, and C. L. Pierson, Basic Pharmaceutics Abstract #52, presented at the 27th Annual Meeting of APhA Academy of Pharmaceutical Sciences, Kansas City, Mo., November 1980.

(10) C. R. Behl, G. L. Flynn, M. Barrett, K. A. Walters, E. E. Linn, Z. Mohamed, T. Kurihara, and C. L. Pierson, Basic Pharmaceutics Abstract 80, presented at the 127th Annual Meeting of the American Pharmaceutical Association, Washington, D.C., April 1980.

(11) C. R. Behl, G. L. Flynn, M. Barrett, E. E. Linn, C. L. Pierson, W. I. Higuchi, and N. F. H. Ho, Basic Pharmaceutics Abstract 81, presented at the 127th Annual Meeting of the American Pharmaceutical Association, Washington, D.C., April, 1980.

(12) R. J. Scheuplein and I. H. Blank, *J. Invest. Dermatol.*, *60*, 286 (1971).

ACKNOWLEDGMENTS

Presented, in part, at the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Washington, D.C. meeting, April 1980, and the APhA Academy of Pharmaceutical Sciences, Kansas City, Mo. meeting, November 1979.

Supported by National Institutes of Health Grant GM 24611.