Permeation of Skin and Eschar by Antiseptics I: Baseline Studies with Phenol

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Abstract □ To assess how the permeability of phenol is altered by thermal injury, it was first necessary to have baselines of comparison on normal skin. Using *in vitro* diffusion cells and the skin of the hairless mouse, [^{14}C]phenol was applied to skin in an aqueous medium with a reference copermeating species, [^{3}H]methanol, and 37° permeability coefficients of the pair were evaluated as functions of animal age, skin hydration, stripping of the skin, dermis isolation, and phenol concentration. Age proved to be of little consequence to permeability over a wide age range. Prolonged aqueous soaking of the skins was also without much effect. Stripping of the skin and isolating the dermis by soaking techniques allowed assessment of individual skin strata diffusional resistances. When applied to skin in trace radiochemical concentrations, phenol behaved diffusionally as an alkanol with a chain length of six. But at concentrations >2% w/v, phenol facilitated the permeation rates of itself and methanol; the effect was markedly concentration sensitive and only fractionally reversible. Concentration studies using silicone rubber membranes proved that the effects on the skin were the results of destroyed barrier integrity. At 6% phenol concentration there was an essentially instantaneous, 10-fold increase in the phenol permeability coefficient, raising it to two-thirds that observed with fully stripped skin. Overall, the data suggest that the stratum corneum is proportionally impaired as the phenol concentration is increased.

Keyphrases □ Permeation, skin—of phenol, baseline studies with hairless mice, effects of animal age, skin hydration, skin stripping, dermis isolation, and concentration □ Phenol—effects of animal age, skin hydration, skin stripping, dermis isolation, and concentration on skin permeation, baseline studies on hairless mice

Phenol is an industrial chemical with widespread use and is so frequently a contaminant of industrial waste streams that it is designated a priority pollutant by the Environmental Protection Agency (1). It is used in topical pharmaceuticals as a microbial preservative and other medical uses include local antipruritis, an anesthetic action which requires cutaneous penetration, and antisepsis. On contact, phenol apparently diffuses through skin readily; this has caused serious injuries in the workplace (2). Phenol has been associated also with untoward systemic responses when medicinally used and fatalities have been recorded in cases where its application has been over extensive areas of barrier-impaired skin (3). Therefore, whether the skin exposure occurs accidently or in therapy, diffusive passage of phenol through skin is of some considerable concern.

It was suggested in a recent report that "the lipophilic character of solutes (phenols) and their hydrogen bonding capacity are the two main structural features determining their penetration through the human epidermis" (4). Being only mildly hydrophobic, phenol's absorption was presumed to be rate-controlled by the stratum corneum. Increased phenolic permeation rates in concentrated solution have also been recorded and speculatively attributed to protein denaturation within the skin during transport (5). Higher phenol permeability was reported for skins damaged by either simple abrasion or burning (6).

In previous investigations from these laboratories (7–10), permeabilities of normal skin as well as burn-traumatized skin (11) of the hairless mouse have been examined using water and the *n*-alkanols as test permeating species. Three basic rate-controlling mechanisms of skin permeation were evident (10): (*a*) diffusion through aqueous pores (an aqueous shunt pathway), (*b*) diffusion through lipoidal components of the stratum corneum, and (*c*) diffusion controlled by the viable strata (epidermis and dermis) lying beneath the horny layer. These studies are continued here, for the first time using a chemical with known serious local and systemic consequences in humans. The purpose of this specific study was to obtain baseline data for the assessment of thermal alterations on the permeability of phenol through skin.

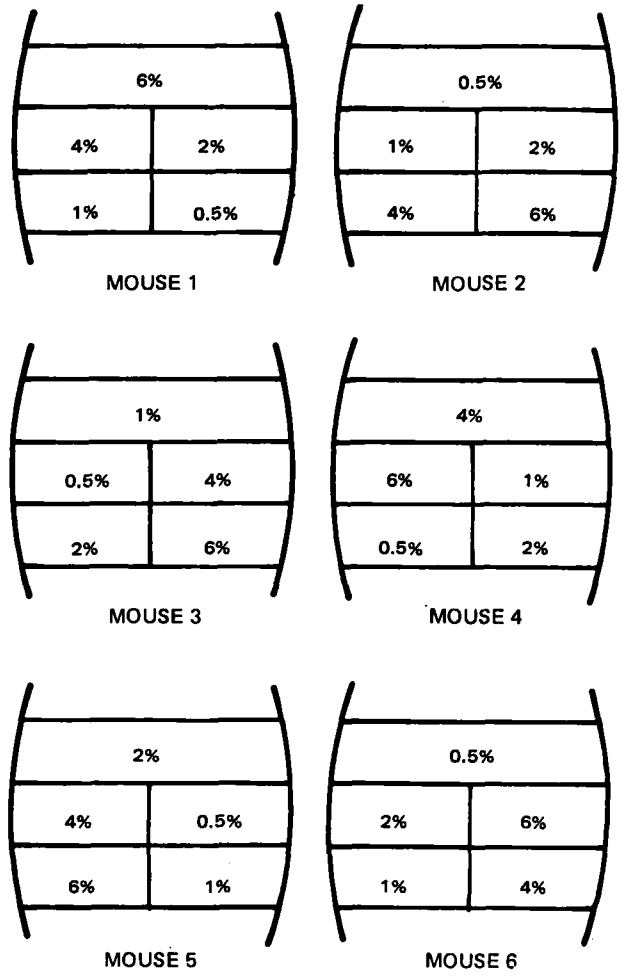

Figure 1—*Illustration of the manner of excising skin sections from the dorsal surfaces of mice for use in phenol concentration experiments. The numbers indicate the phenol concentrations; each concentration was studied six times.*

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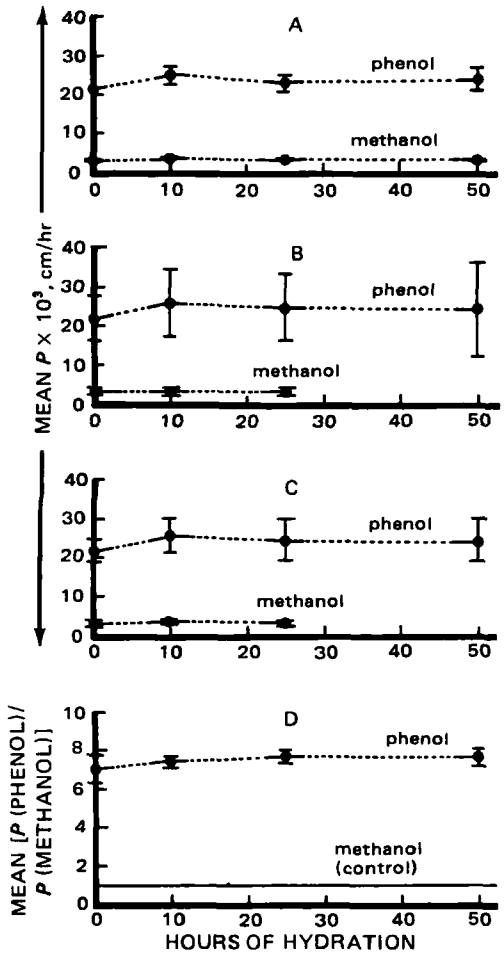


Figure 2—*Plots of average permeability coefficients of (A) the abdominal skins, (B) the dorsal skins, and (C) the average of both sites; and (D) the phenol–methanol permeability ratios as a function of hydration time. The bias represent the standard deviation for each mean value.*

EXPERIMENTAL

Chemicals—[^{3}H]methanol[1] and [^{14}C]phenol[1] were diluted with saline[2] to prepare solutions for the permeation experiments. Crystalline phenol[3] was used to prepare aqueous solutions of varying concentrations for the concentration-effect experiments. The final bulk chemical concentration of the permeating species was 10^{-4} *M* or lower except where unlabeled phenol was added to be the expressed concentrations in the concentration-effect studies.

Animals—Male hairless mice of SKh-hr^{-1} strain[4] were used. They were provided with free access to food and water. The bedding was changed at least once a week. The mice were housed individually in shoe box-type cages to prevent them from damaging one anothers skins.

Stripping and Dermis Isolation Procedures—Immediately following sacrifice by cervical dislocation, the abdominal and the dorsal surfaces of mice were stripped 5, 10, or 25 times with cellophane tape[5]. The animal was secured on a table and the skin was stripped by placing the tape on the animal surface and moving the thumb back and forth a few times, with as uniform pressure as was possible (12). A fresh piece of the tape was used for each stripping. Dermal sections were isolated by placing skin in the diffusion cell and soaking it for 24 hr (13). At this time the cell was opened and the epidermis was carefully lifted from the dermis with a pair of forceps. The cell was then reassembled for permeation experiments.

Radioisotopic Assay—Concentrations of the radiolabeled permeating species were determined using a liquid scintillation counter[6] and a commercial cocktail[7]. Permeation rates of both methanol and phenol were studied simultaneously using a technique involving dual labels.

Table I—Permeability Coefficients of Methanol as a Function of Mouse Age

Age, days	Number of Mice	$P \times 10^3 \pm SD$, cm/hr		
		Abdominal	Dorsal	Overall Average[a]
36	1	2.1	2.7	2.4
92	5	3.4 ± 0.5	2.9 ± 0.6	3.2 ± 0.6
340	6	2.2 ± 1.0	2.7 ± 0.3	2.5 ± 0.8
381	1	3.2	2.1	2.7
441	3	1.9 ± 0.8	1.6 ± 0.4	1.7 ± 0.6

[a] Average across sites.

Diffusion Cell and Permeation Procedure—A two-chamber glass diffusion cell (7–9) was employed to determine the skin permeability. The external medium of diffusion was saline, except in the concentration-effect studies where the donor compartment was filled with phenol solution of designated concentration. 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 compartment. Therefore, the net diffusion occurred from the stratum corneum to the dermis side.

Procedure of the Hydration Effect Studies—The effect of hydration on the skin permeability to methanol (reference solute) and phenol was studied for 50 hr of skin immersion in saline, using the abdominal and the dorsal skins obtained from each of five mice. A technique of sequential experiments developed earlier (9) was employed to obtain the entire hydration profile on each of the 10 skins. This was accomplished by carrying out four permeation experiments in succession on the same piece of the skin, with rinsing between experiments.

Procedure for Studying the Concentration Effects and Reversibility Using Skin and Silicone Rubber Membranes—Permeation of methanol and phenol was studied using phenol solutions with concentrations of 0.5, 1.0, 2.0, 4.0, and 6.0% (w/v). The receiver chamber always contained saline. To minimize mouse-to-mouse and site-to-site variation, each of the five concentrations was studied in six pieces of skins excised from five different dorsal locations of six mice. The manner in which the concentrations were rotated around the sites is depicted in Fig. 1. The permeation experiment was initiated within 0.5 hr of skin exposure to the phenol solution and was completed within an additional 2 hr. At ~2.5 hr of skin contact with the phenol solutions, the donor and receiver chambers were evacuated and cleansed with saline using three triple rinsings ~0.5 hr apart. A second permeation experiment was then conducted with methanol and phenol in trace radiochemical concentrations, using saline in both compartments. This experiment [referred to as a reversibility experiment (14) in the remainder of this paper] was aimed at determining the extent to which the accelerating effects of phenol were reversible. An identical procedure was followed in assessing concentration effects and the reversibility of such effects using 0.0254-cm silicon rubber membranes[8].

Data Analysis—The data were plotted with the receiver compartment concentration (in cpm) as a function of time. The permeability coefficient was calculated from (9):

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

where P is the permeability coefficient (cm/hr); A is the diffusional area (~0.6 cm^2); ΔC is the concentration difference across 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 steady-state slope (cpm/cm^3/hr) of a counts (concentration) *versus* time plot.

RESULTS

Tables I and II contain mouse skin permeability coefficients of methanol and phenol, respectively, as a function of mouse age. The choice of the ages and the numbers of the mice per age were dependent on the availability of animals within the colony.

[1] New England Nuclear, Boston, Mass. Supplier-estimated purity >98% in each case.
[2] 0.9% Sodium chloride irrigation; Abbott Laboratories, North Chicago, Ill.
[3] Mallinckrodt, Inc., St. Louis, Mo.
[4] Skin Cancer Hospital, Temple University, Philadelphia, Pa.
[5] Scotch Brand Cellophane Tape, 3M Co., Minneapolis, Minn.
[6] Beckman LS 9000. Beckman, Irvine, Calif.
[7] Aquasol, New England Nuclear, Boston, Mass.
[8] Silastic Medical Grade Sheeting, Dow Corning, Midland, Mich.

Table II—Permeability Coefficients of Phenol as a Function of Mouse Age

Age days	Number of Mice	$P \times 10^3 \pm SD$, cm/hr		
		Abdominal	Dorsal	Overall Average[a]
36	1	19.7	21.6	20.7
92	5	28.4 ± 4.5	22.4 ± 4.3	25.4 ± 5.3
124	5	17.5 ± 5.1	23.2 ± 5.1	20.3 ± 5.7
340	6	16.0 ± 5.2	20.6 ± 2.2	18.3 ± 4.5
381	1	20.8	17.7	19.3
441	3	12.6 ± 2.6	12.4 ± 0.8	12.5 ± 1.7

[a] Average across sites.

Figure 2 illustrates the mouse skin permeability coefficients of methanol and phenol, respectively, as a function of skin hydration time. The abdominal and the dorsal skins excised from five mice were used. The permeation experiments were run at 0, 10, 25, and 50 hr of hydration on each of the skins. The animals were weighed before sacrifice and their weights were consistent with an expected weight–age relationship (1), indicating that the animals were healthy. The data are plotted to indicate the standard deviation about each point. Figures 2A, 2B, and 2C present the average abdominal, dorsal, and combined values, respectively. Figure 2D presents the data as ratios of the permeability coefficients of phenol–methanol, a data manipulation previously shown to reduce animal-to-animal variability.

Table III contains the permeability coefficients of methanol and phenol through the abdominal and the dorsal skins stripped 5, 10, or 25 times. Five mice were used for each experimental condition. The data are provided in terms of the individual and mean permeability coefficients with standard deviations. Table III also contains data for dermis isolated by the soaking procedure (13). In this case, four permeability coefficients were gathered for each permeating species, two on each skin site.

Tables IV and V contain the permeabilities of methanol and phenol as a function of phenol concentration in the donor chamber. Also summarized in these tables are data from the reversibility experiments where both compartments of the diffusion cell contained saline. At each concentration, six pieces of the skin obtained from five different sites on the dorsal surfaces of six mice were used. The vital statistics of each animal are presented and individual as well as the mean permeability coefficients, with standard deviations, are listed. From these data, a relative enhancement of the permeabilities of methanol and phenol was computed as the ratio of the mean permeability coefficient at a given phenol concentration to that at the trace radiochemical concentration of phenol. These derived data are illustrated in Figs. 3 and 4 for both the high concentration and reversibility experiments.

Table VI contains permeability coefficients of phenol and four alkanols (methanol, ethanol, butanol, and hexanol) through 0.0127-cm silicone rubber (polydimethylsiloxane) membranes. For these experiments, the concentrations of the permeating species were $\leq 10^{-4}$ *M*. Table VII contains permeability coefficients of methanol and phenol through 0.0254-cm silicone rubber membranes as a function of phenol concentration. As in the case of the skin studies, results are presented for an initial experiment at the high phenol concentrations and a second reversibility experiment. In the latter, permeability was also measured from solutions containing only trace phenol concentrations. In the initial experiments, the phenol concentrations in the donor chamber were 0.5, 1.0, 2.0, 4.0, and 6.0% w/v. At each concentration three different silicone rubber membranes were used.

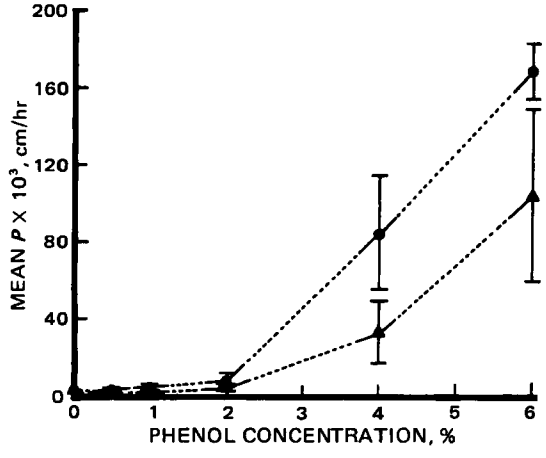


Figure 3—*Plots of the mean permeability coefficients of methanol obtained in the concentration-effect (●) and reversibility (▲) experiments. The bars represent the standard deviation for each mean value.*

Table III—Permeability Coefficients of Methanol and Phenol through Stripped Skins and the Dermis

Number of Strippings	Number of Mice[a]	$P \times 10^3 \pm SD$, cm/hr			
		Methanol		Phenol	
		Abdominal	Dorsal	Abdominal	Dorsal
0	5	3.4 ± 0.5	2.9 ± 0.6	28.4 ± 4.5	22.4 ± 4.3
5	5	154 ± 35	48.3 ± 36	210 ± 38	120 ± 44
10	5	342 ± 18	260 ± 64	354 ± 48	277 ± 49
25	5	339 ± 55	291 ± 31	318 ± 60	275 ± 25
Dermis	2	445 ± 81	395 ± 5	337 ± 101	301 ± 13

[a] All mice were 90–100 days of age.

DISCUSSION

Effect of Aging on the Permeability of Phenol—From the results of replicated experiments on mice of 36, 92, 124, and 340 days of age, the phenol permeability coefficient through hairless mouse skin underwent no appreciable change with age (Table II). A representative value for the 37° phenol permeability coefficient is 2×10^{-2} cm/hr. A 25° value for human skin (cadaver epidermis isolated using ammonia fumes) of 0.8×10^{-2} cm/hr has been reported (5), and when the temperature difference is taken into account (activation energies for structurally similar alkanols run ~15 kcal/mole), the human and mouse data are remarkably similar.

The average permeability coefficient for phenol of 1.25×10^{-2} cm/hr obtained at 441 days seems smaller than that found in younger animals. Here the value of using a copermeating-species marker with a different radiolabel becomes apparent, as the methanol average permeability coefficient at 441 days (Table I) is also of smaller magnitude than at younger ages. Extensive age-influence studies with the alkanols (10) indicate the methanol permeability coefficient to be invariant from ~90 days of age to ages exceeding 441 days. Thus it is concluded that the decline in permeability coefficients at 441 days is due more to animal variability than age. It is notable that phenol–methanol ratios of the mean permeability coefficients at 92, 340, and 441 days are 7.9, 7.3, and 7.4, respectively, showing that the relative permeability of the two chemicals remains essentially invariant even as the skins of different age groups of animals evidence variability. Permeability coefficients obtained on single sections of skin at other ages are in general accord with the values from the replicated runs. Based on these data, it was decided to use mice ~100 days old for the remainder of the study.

When the permeability coefficients for methanol and phenol are normalized to animal mass (permeability coefficient divided by body weight),

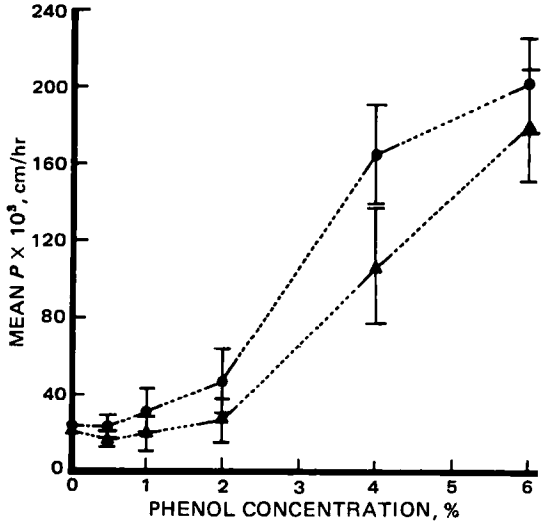
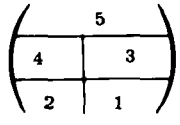


Figure 4—*Plots of mean permeability coefficients of phenol obtained in the concentration-effect (●) and reversibility (▲) experiments. The bars represent the standard deviation for each mean value.*

Table IV—Effect of Phenol Concentration on the Skin Permeability of Methanol [a]

Phenol Concentration (Donor), % w/v	$P \times 10^3$, cm/hr						
	Mouse [b]						
	1	2	3	4	5	6	Mean ± *SD*
Concentration Effect Experiment (phenol–saline):							
0.0							1.5 [c]
0.5	1.6(1)	2.4(5)	2.2(4)	5.1(2)	2.1(3)	2.4(5)	2.6 ± 1.2
1.0	4.2(2)	3.3(4)	1.1(5)	9.0(3)	3.3(1)	2.6(2)	3.9 ± 2.7
2.0	8.1(3)	5.5(3)	4.4(2)	13.0(1)	2.5(5)	7.7(4)	6.9 ± 3.7
4.0	92.5(4)	53.1(2)	81.1(3)	131.1(5)	51.7(4)	101.4(1)	85.2 ± 30.3
6.0	172.9(5)	159.6(1)	153.4(1)	193.7(4)	172.6(2)	164.0(3)	169.4 ± 14.1
Reversibility Experiment (saline–saline):							
0.0							1.5 [c]
0.5	1.1(1)	1.6(5)	1.4(4)	3.2(2)	1.4(3)	1.5(5)	1.7 ± 0.8
1.0	2.4(2)	1.8(4)	0.6(5)	5.7(3)	1.9(1)	1.9(2)	2.4 ± 1.7
2.0	4.1(3)	2.6(3)	1.9(2)	7.5(1)	0.8(5)	4.1(4)	3.5 ± 2.3
4.0	38.5(4)	19.7(2)	22.4(3)	62.1(5)	18.5(4)	37.7(1)	33.2 ± 16.7
6.0	115.8(5)	47.2(1)	70.4(1)	176.8(4)	95.3(2)	122.3(3)	104.6 ± 45.2

[a] Number in parentheses indicates the skin location at the dorsal site 

; mouse age is ~117 days. [b] Mice 1, 3, 4, and 5 each weighed 30.5 g; mice 2 and 6 weighed 31.5 and 30.0 g, respectively. [c] Data were abstracted from ref. 9.

the age patterns displayed in Fig. 5 are obtained. The mass-weighted values decline appreciably for both solutes over the initial year of age, in each case the fall in magnitude being approximately twofold. Thus, even though the skins of younger animals evidence similar mass-transfer coefficients as seen with older skins, the younger animals appear to be at greater risk to comparable area exposures of phenol and systemic accumulation of phenol would be seen to be inversely related to animal size. We believe such factors have bearing on the increased toxicity of phenol observed in infants (15, 16).

Effect of Anatomical Site on the Permeability of Phenol—Tables I and II contain permeability coefficients of methanol and phenol through the abdominal and dorsal skins. At no age is there a significant difference in values for the two sites and, moreover, for both permeating species the site of greater permeation rate changes from age to age. Therefore, it is concluded that within the age range studied there is no factorable site dependency to the permeability of these compounds. This conclusion is further supported by the hydration study data where permeability coefficients for the abdominal and dorsal skins remained unchanged during the full 50-hr hydration. The methanol data are consistent with the previous studies (10) where the permeabilities of the *n*-alkanols were shown to be site independent for animals older than ~50 days.

Influence of Hydration on the Permeability Coefficient of Phenol—Permeability coefficient values obtained for methanol and phenol as a function of hydration time are plotted in Fig. 3. There is no clear indication that the transport rate of either permeating species is altered by hydration. For methanol, this observation is fully consistent with previously published results (9). Since the phenol permeability coefficient through hairless mouse skin is, at the outset, similar to that of hexanol (Table VIII) and given the structural similarity of these two 6-carbon hydroxy compounds, it was expected there would roughly be a doubling of the permeability coefficient of phenol during hydration. Phenol permeability coefficients for fresh and extensively hydrated skins appear essentially the same, however, although the latter may be marginally larger. Exactly why there is increased facility of permeation for butanol, hexanol, and heptanol through hydration and not for the physicochemically similar phenol [the octanol–water partition coefficient of phenol is 29 (17) and lies between that of butanol, 7.6 (18) and hexanol, 76 (18)] is not understood. The aromatic compound has a larger diffusional cross-sectional area than the alkanols, which may be a factor limiting its access to presumably hydration-expanded lipoidal channels.

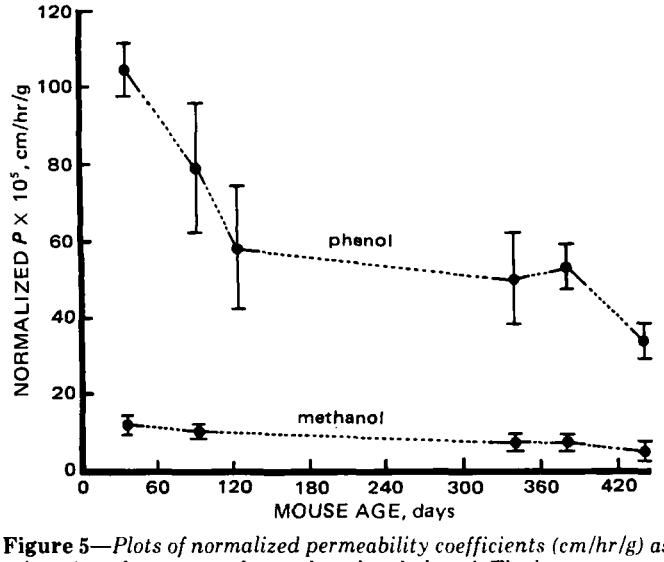


Figure 5—*Plots of normalized permeability coefficients (cm/hr/g) as a function of mouse age for methanol and phenol. The bars represent the standard deviation for each normalized value.*

Effect of Stripping and Dermis Isolation on the Permeability of Phenol—Stripping increases the permeability of both abdominal and dorsal skins to both methanol and phenol (Table III). The permeability coefficients obtained tend to level off at 10 strippings. In the earlier studies, it required more than 10 strippings to notice a comparable effect (8). The difference in observations may be due to greater care securing adhesion of the tape to the skin in the present study. The increased permeation rate on total stripping is far greater for methanol than phenol, which reflects the fact that the stratum corneum functions lipoidally to these compounds, offering higher resistance to methanol, the more polar of the two solutes. It is also evident (Table III) that abdominal permeabilities for partially and fully stripped skins invariably exceed those of the dorsal surfaces even though there is no apparent difference for the intact skins. It appears that the abdominal stratum corneum may be more easily removed and further, that the permeability of the remaining abdominal strata may be intrinsically greater than that of the dorsal skin. The latter observation is consistent with the abdominal skin being thinner than the dorsal skin (10).

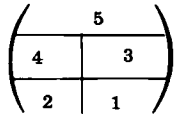
The abdominal dermis isolated by the soaking technique appears to be more permeable than the dorsal dermis, although the data are too limited to draw a firm conclusion (Table III). These data, together with the stripping and whole skin data, allow quantitative assessment of the diffusional resistances of the individual strata of the skin. The following equation, derived from the previously reported physical model (8, 10), can be used to compute the individual permeabilities of the stratum corneum, the viable epidermis, and the dermis:

$$\frac{1}{P_{WS}} = \frac{1}{P_{SC}} + \frac{1}{P_{EP}} + \frac{1}{P_D} \qquad \text{(Eq. 2)}$$

where P_{WS}, P_{SC}, P_{EP}, and P_D are the permeabilities of whole skin, stratum corneum, viable epidermis, and dermis, respectively. The values of

Table V—Effect of Phenol Concentration on Skin Permeability of Phenol [a]

Phenol Concentration (Donor), % w/v	$P \times 10^3$, cm/hr Mouse[b] 1	2	3	4	5	6	Mean ± *SD*
Concentration Effect Experiment (phenol–saline):							
0.0							20.3 ± 5.7[c]
0.5	18.6(1)	19.9(5)	20.9(4)	35.7(2)	18.6(3)	22.6(5)	22.7 ± 6.5
1.0	37.7(2)	25.6(4)	15.2(5)	53.6(3)	28.8(1)	23.1(2)	30.7 ± 13.4
2.0	52.4(3)	39.9(3)	33.0(2)	76.1(1)	27.4(5)	50.9(4)	46.6 ± 17.4
4.0	169.8(4)	131.7(2)	160.7(3)	196.6(5)	141.6(4)	192.0(1)	165.4 ± 26.2
6.0	182.3(5)	239.3(1)	184.5(1)	223.8(4)	199.9(2)	186.4(3)	202.7 ± 23.7
Reversibility Experiment (saline–saline):							
0.0							20.3 ± 5.7[c]
0.5	14.3(1)	15.0(5)	14.8(4)	23.3(2)	16.3(3)	17.2(5)	16.8 ± 3.4
1.0	20.9(2)	14.6(4)	11.5(5)	37.2(3)	12.6(1)	19.5(2)	19.4 ± 9.5
2.0	29.9(3)	19.8(3)	13.6(2)	45.6(1)	17.6(5)	35.0(4)	26.9 ± 12.1
4.0	113.0(4)	75.6(2)	79.6(3)	141.7(5)	90.8(4)	142.5(1)	107.2 ± 30.0
6.0	188.0(5)	139.6(1)	151.7(1)	214.8(4)	189.9(2)	201.9(3)	181.0 ± 29.3

[a] Number in parentheses indicates the skin location at the dorsal site 

; mouse age is ~117 days. [b] Mice 1, 3, 4, and 5 each weighed 30.5 g; mice 2 and 6 weighed 31.5 and 30.0 g, respectively. [c] Data were abstracted from Table II.

P_{WS} and P_D are direct experimental values (Tables I–III). P_{EP} can be estimated from:

$$\frac{1}{P_{25\times}} = \frac{1}{P_{EP}} + \frac{1}{P_D} \qquad \text{(Eq. 3)}$$

where $P_{25\times}$ is the permeability coefficient of the skins stripped 25 times (Table III). Results of these computations are reported in Table IX. The computed P_{SC} values for methanol or phenol for either anatomical site are essentially the *same as* P_{WS} observed experimentally. This indicates that the resistance offered by the whole skin is, in fact, almost entirely due to the stratum corneum. The P_{EP} values are unrealistically large, indicating that the viable epidermis presents little resistance to the transport of methanol or phenol. In other words, insofar as can be told through estimation of difference (Eq. 3), the dermis offers the bulk of the resistance to diffusion once the stratum corneum has been removed, mostly because it is far thicker than the epidermis. But it is compositionally and structurally dissimilar as well, and these differences may also be a factor. The condition of the viable epidermis of the stripped skin is unknown; it is also possible that it is either damaged or partially removed by the repeated strippings, minimizing its apparent residual resistance.

Comparison of Phenol and Alkanol Permeabilities in Synthetic Membranes—One means of placing the phenol permeability into an overall perspective is to compare it with that of the *n*-alkanols using a membrane that, relative to the skin, is well understood in terms of its diffusional properties and mechanism. Silicone rubber sheeting provides an essentially isotropic, hydrophobic medium ideal for such purposes. In this case the permeability coefficient of phenol compares favorably with that of butanol (Table VI). But for fresh skins, data for hexanol and phénol are comparable. Moreover, as previously pointed out, the octanol–water partition coefficient for phenol of 29 (17) is bracketed by those of butanol and hexanol (18). These combined observations have an elementary significance. While phenol is structurally similar to an alkanol, its aromatic character sets it apart in terms of intermolecular interactions and, where oil–water partitioning is concerned, its affinity for different oil phases is not consistently the same as that of any particular alkanol. Nevertheless, since the differences in intermolecular associations in water immiscible phases are subtle, its distributioning behavior tends to be reasonably close to that of the alkanols of comparable size. From the standpoint of skin permeation, the lipid phase of the stratum corneum behaves as expected, "seeing" phenol more or less as it "sees" the 6-carbon alkanol, at least until the hydration effects set in.

Table VI—Comparison of Phenol and Alkanol Permeability Coefficient Through 0.0127-cm Silicone Rubber Sheeting

Compound	Number of Experiments	$P \times 10^3 \pm SD$, cm/hr
Phenol	3	175 ± 13
Ethanol[a]	3	53.5 ± 2
Butanol[a]	3	180 ± 12
Hexanol[a]	3	761 ± 54
Octanol[a]	3	1090 ± 15

[a] Abstracted from unpublished studies of G. L. Flynn, C. R. Behl, T. Kurihara, W. Smith, J. Fox, H. Dürrheim, and W. I. Higuchi, University of Michigan, College of Pharmacy.

Effect of Phenol Concentration on the Permeabilities of Methanol and Phenol Through Skin and Silicone Rubber Membranes—The hairless mouse skin permeability coefficients of methanol increased slowly up to the 2% level and then rapidly accelerated with further increases in phenol concentration (Fig. 3). The permeability coefficient of 0.17 cm/hr observed at 6% phenol concentration is >100 times that of normal skin. However, it represents only a partial compromise of the barrier function of the stratum corneum to methanol as, based on stripped-skin data, an upper limit of ~0.28 cm/hr for the methanol permeability coefficient is possible (Table III). The reversibility data indicate that there is some restoration of the barrier to methanol. Almost half of the enhancement observed at 0.5, 1.0, 2.0, 4.0, and 6.0% phenol concentrations is reversible (Fig. 3).

Phenol also accelerated its own permeability when applied in high concentration. As with methanol the effects are nominal to ~2% phenol concentration and exaggerated thereafter (Fig. 4). In this case the data suggest an upper limit on the effect is being approached at the 6% concentration. This seems reasonable as the permeability coefficient of 0.203 cm/hr at 6% phenol concentration is approaching the value of 0.275 cm/hr noted for the stripped skin. The relative enhancement in the permeability at the 6% level is 10.0 and it is reversible only to 8.9. The relative enhancement for fully stripped skin is ~13.5. All these observations are consistent with the fact that the mouse skin stratum corneum is intrinsically more permeable to phenol than to methanol; therefore, chemical

Table VII—Effect of Phenol Concentration on the Permeability of Methanol and Phenol Through 0.0254-cm Silicone Rubber

Phenol Concentration (Donor), % w/v	Mean $P \times 10^3 \pm SD$, cm/hr[a] Methanol: Concentration Effect Experiment	Methanol: Reversibility Experiment	Phenol: Concentration Effect Experiment	Phenol: Reversibility Experiment
0.5	9.5 ± 1.6	9.2 ± 1.1	90.0 ± 3.0	83.1 ± 1.4
1.0	11.7 ± 1.5	12.3 ± 2.0	107.0 ± 9.0	96.7 ± 3.0
2.0	13.0 + 2.4	10.7 + 2.1	85.8 ± 11.4	94.1 ± 14.3
4.0	12.3 ± 0.1	12.0 ± 1.2	98.5 ± 0.6	93.2 ± 20.4
6.0	11.1 ± 0.6	12.7 ± 1.5	99.9 ± 8.1	89.6 ± 4.6
Overall Mean ± *SD*	11.5 ± 1.3	11.4 ± 1.4	96.4 ± 8.2	91.3 ± 5.3

[a] All results are the average of three independent experiments.

Table VIII—Comparison of Phenol and Alkanol Permeability Coefficients Through Hairless Mouse Skin

Compound	$P \times 10^3 \pm SD$, cm/hr	
	Unhydrated Skins[b]	Hydrated Skins
Phenol	21.5 ± 3.4	24.0 ± 6.1
Methanol[a]	2.0 ± 0.4	2.0 ± 0.4
Ethanol[a]	2.1 ± 0.1	2.1 ± 0.1
Butanol[a]	5.4 ± 1.1	10.8 ± 2.2
Hexanol[a]	19.4 ± 7.8	38.8 ± 15.6
Heptanol[a]	65.9 ± 24.4	98.9 ± 36.6
Octanol[a]	73.4 ± 10.1	97.8 ± 13.5

[a] Abstracted from refs. 7, 9, and 11. Values obtained on freshly mounted skin sections.

impairment has a lesser effect on the permeability of phenol. It is notable that, at the 0.5, 1.0, 2.0, and 4.0% concentrations where enough of the stratum corneum integrity remains to essentially provide sole control of permeation, recovery of the phenol permeability coefficient on washing was roughly of the same degree as observed for methanol.

Possible Causes of the Observed Concentration Effects—In the previous section it was implied that the accelerating effect of phenol on skin permeability was due to impairment of the stratum corneum, as was also assumed by previous researchers (4, 5). The observation that the reference compound, methanol, as well as phenol experienced increased permeability in the present studies strongly supports this assumption. Moreover, the maximum enhancements for these solutes were quantitatively similar to the enhancements observed by fully stripping the stratum corneum from the skins. Nevertheless, it is possible to rationalize the data in terms of physicochemical effects such as complexation in the donor medium. It would be necessary for phenol to form complexes both with itself and methanol capable of increasing partitioning into the skin, thereby facilitating permeation, to explain the results. This implausible effect is ruled out by concentration experiments performed with silicone rubber membranes (Table VII). These membranes were assumed *a priori* to be chemically inert to phenol, which subsequently proved to be the case. When methanol and phenol permeated the synthetic membranes at concentrations used in the skin studies (0.5–6.0% w/v), no concentration-induced alterations in the permeability of either solute were noted. Since the complexes, if formed, would likely also facilitate permeation of these hydrophobic films, and since no effect was observed, complexation as the basis of enhanced permeation can be discounted. It should be noted that the complete protocol of the concentration studies was carried out using the silicone rubber and that the reversibility experiments yielded essentially the same permeability coefficients as noted at high phenol concentrations. This further strengthens the conclusion that the acceleration of skin mass-transfer rates by phenol is unrelated to external factors such as molecular association.

Significance of These Studies—Previous work (7–14) has shown that permeability of the hairless mouse skin to the *n*-alkanols to be similar quantitatively and qualitatively to that of the human epidermis. The present studies show that phenol chemically alters the mouse skin stratum corneum, leading to increased mass-transfer rates, a phenomenon also observed in isolated human epidermis (5). Moreover, the concentration reported as the threshold concentration for damage in the human skin (1.5%) is virtually the same as observed with mouse skin. And the magnitudes of phenol-induced increases in skin permeabilities at given concentrations of phenol are similar in each case. This parallelism in behavior indicates fundamental similarities in the biological compositions and the constructions of the two tissues. The implications are that the mechanisms of permeation of phenol and chemical denaturation by phenol are nearly identical in the two species. This adds to a growing body of information which suggests that hairless mouse and human skins are comparable chemical barriers and lends credibility to the use of the hairless mouse model for topical drug delivery research.

As far as the concentration effects are concerned, all signs point to phenol having a specific ability to chemically alter the stratum corneum. When it does so, the partitioning dependency of the skin permeation process is effectively lost. This action is partially reversed when the phenol is rinsed out from the diffusion cell. Relatively recent evidence proves that there is a well-defined extracellular lipid domain in the stratum corneum, comprising 10% or more of its total volume (19, 20). It is tempting to assign the lipid pathway across the tissue to this region. This would necessarily mean, for compounds like the intermediate chain-length alkanols (C_3–C_8) and phenol which are in the partitioning sensitive region, that the path of least diffusional resistance is around the cellular building blocks of the horny layer, something that could only occur if the keratinized, intracellular contents are relatively impervious.

Table IX—Individual Permeabilities of Whole Skin, Stratum Corneum, Viable Epidermis, and Dermis to Methanol and Phenol

Membrane	$P \times 10^3$, cm/hr[a]			
	Methanol		Phenol	
	Abdominal	Dorsal	Abdominal	Dorsal
Whole skin	3.2	3.2	25.4	25.4
Stratum corneum	3.2	3.2	27.5	28.0
Epidermis	1400	1100	5800	3200
Dermis	450	400	340	300

[a] Computed from eqs. 2 and 3 and from the data given in previous tables. Epidermis and dermis data are reported to the nearest 100 and 10 unit, respectively.

It is hard to believe that phenol, even at 6% w/v, would have significant effect on the lipid medium, other than perhaps just concentrating there, and inconceivable that it could all but wipe out the partitioning dependency of this postulated pathway. On the other hand, consistent with its known abilities to denature proteins, phenol may act on the keratin within the cellular building blocks and on the cell wall proteins as well, denaturing and, perhaps in the case of the semicrystalline keratin helices, uncoiling them. Such effects could be partially reversible. If the suppositions are correct that the extracellular lipids are the partitioning pathway and that the destructive activity of phenol is primarily within the intracellular space, then phenol must act to open up an alternative polar shunt route across the cells. It follows that the extent to which this occurs is proportional to the phenolic concentration. The mechanism envisioned is consistent with a threshold concentration (~2.0%) for the effects of phenol.

Phenol at ≥5% concentration is doubly dangerous since the permeability coefficient is 10-fold enhanced. The absorption rate from a 5% compared with a 1% solution is not just 5 times greater but rather 50 times. This explains many of the rapid lethal poisonings detailed by Deichmann (2). At extremely high concentrations (>75% w/v) phenol is corrosive enough locally to coagulate the epidermis, retarding its self-absorption, so that such solutions are less toxic than 5% levels (14).

REFERENCES

(1) List of Priority Pollutants—1980, Environmental Protection Agency. Cincinnati, Ohio.

(2) N. L. Sax, in "Dangerous Properties of Industrial Raw Materials," 3rd ed., Reinhold, New York, N.Y., 1968, p. 1007.

(3) T. D. Cronin and R. O. Brauer, *J. Am. Med. Assoc.*, **139,** 777 (1949).

(4) M. S. Roberts, R. A. Anderson, and J. Swarbrick, *J. Pharm. Pharmacol.*, **29,** 677 (1977).

(5) M. S. Roberts, R. A. Anderson, J. Swarbrick, and D. E. Moore, *ibid.*, **30,** 486 (1978).

(6) M. V. Freeman, E. Alvarez, and J. H. Draize, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **9,** 273 (1950).

(7) H. Durrheim, G. L. Flynn, W. I. Higuchi, and C. R. Behl, *J. Pharm. Sci.*, **69,** 781 (1980).

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

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

(10) C. R. Behl, G. L. Flynn, T. Kurihara, W. Smith, N. Harper, O. G. Gatmaitan, C. L. Pierson, W. I. Higuchi, and N. F. H. Ho, in "Abstracts," vol. 9 (1), APhA Academy of Pharmaceutical Sciences, Washington, D.C., 1979, p. 110.

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

(12) R. Meyer, C. R. Behl, and G. L. Flynn, in "Abstracts," vol. 11 (1), APhA Academy of Pharmaceutical Sciences, Washington, D.C., 1981, p 67.

(13) 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, in "Abstracts," vol. 9 (2), APhA Academy of Pharmaceutical Sciences, Washington, D.C., 1979, p. 90.

(14) C. R. Behl, K. A. Walters, G. L. Flynn, and W. I. Higuchi, in "Abstracts," vol. 10 (1), APhA Academy of Pharmaceutical Sciences, Washington, D.C., 1980, p. 98.

(15) M. Feiwel, *Br. J. Dermatol.*, **81,** 113 (1969).

(16) R. L. Nachman and N. B. Easterly, *J. Pediatr.*, **79,** 628 (1971).

(17) F. A. Wilson and J. M. Dietschy, *Biochim. Biophys. Acta*, **363**, 112 (1974).

(18) N. F. H. Ho, J. Y. Park, W. Morozowich, and W. I. Higuchi, in "Design of Biopharmaceutical Properties and Prodrugs and Analogs," E. B. Rahe, Ed., APhA, Academy of Pharmaceutical Sciences, Washington, D.C., 1977, p. 136.

(19) P. M. Elias, J. Georke, D. S. Friend, *J. Invest. Dermatol.*, **69**, 535 (1977).

ACKNOWLEDGMENTS

This study was supported by National Institutes of Health Grant No. 5 R01 GM 24611.

Permeation of Skin and Eschar by Antiseptics II: Influence of Controlled Burns on the Permeation of Phenol

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Abstract □ The safe antiseptic use of phenol over the burn-traumatized surface depends on knowledge of how the systemic accumulation of phenol is affected by burn processes. To gain insight into the underlying permeation phenomenon, the diffusion of phenol and a reference cosolute, methanol, through both scalded and branded dorsal skin sections of the hairless mouse was studied as a function of burn temperature using *in vitro* diffusion cells. Temperatures up to 100 and 150° were used for scalding and branding, respectively, using a 60-sec exposure time. Permeability coefficients of the traumatized skins were assessed at 37° and compared with control values. Coefficients of both permeating species were not increased significantly by burn temperatures up to 70° applied either by scalding or branding, however, at higher temperatures exaggerated increases in permeation rates were noted. A limiting increase of ~7 times the control value was noted for phenol irrespective of the burn method. Permeability of methanol was altered even more dramatically and at 100° by scalding and 150° by branding was over 50 times the control rate. At 80 and 100° for methanol and at 80° for phenol, scalding produced larger increases in the permeability coefficients than branding. Since contact for 1 min at 60° is capable of producing a full-thickness burn injury, it is clear that eschar permeability to phenol immediately postburn is not related to the clinical degree of burning, but is a function of the thermal intensity (hotness) of the burn stimulus. Full-thickness wounds can be expected to have highly variable rates of systemic absorption as a direct consequence of the wide-ranging permeability possible for such burns, with the risks of topical application varying accordingly.

Keyphrases □ Permeability—of phenol and methanol, through burn-traumatized skin □ Phenol—permeability through burn-traumatized skin □ Methanol—permeability through burn-traumatized skin

Survival of patients with extensive deep partial-thickness (second-degree) and full-thickness (third-degree) burns depends, in part, on limiting microbial colonization of the wound surface. Topical antiseptics are used for this purpose since eschar is nonvascularized and all but inaccessible systematically. Virtually every known antiseptic chemical has been applied to burns, all too often with serious systemic consequences due to excessive transeschar adsorption. Phenol has proven to be one of these toxic agents (1, 2).

In previous studies using model permeating species originating from these laboratories, the permeation behavior of hairless mouse skin has been shown to be altered in unique ways by both scalding (3, 4) and branding (5, 6). Skin permeation rates for water and the *n*-alkanols were maximally increased two- to fourfold when the skins were burned at 60°, irrespective of burn duration. However, when skins were burned for 60 sec at various temperatures, large increases in the permeabilities were noted beginning at ~80°; the effect was greater the more polar the permeating species. In branding experiments, it was possible to use temperatures >100° and correspondingly larger permeability increases were observed for sensitive compounds. These studies provided basic insights into the conditions for and mechanisms of thermal alteration of skin permeation.

The permeability of phenol through hairless mouse skin in its normal state and in a stripped condition was also investigated in these laboratories (7). The overall behavior of this animal tissue to phenol was found to be similar quantitatively and qualitatively to the behavior reported for the human epidermis (8, 9), including exact agreement on the concentration level where self-acceleration of permeation rates due to chemical denaturation of the stratum corneum began. Since there are reports that the percutaneous absorption of phenol through thermally damaged tissue is greatly enhanced (1, 10), the present study was undertaken to quantitate this clinically serious limitation to the topical use of phenol. Specifically, this study was aimed at investigating the influences of incrementally increased burn temperature by scalding or branding on the permeation of phenol through skin.

EXPERIMENTAL

Chemicals—[^{3}H]methanol[1] and [^{14}C]phenol[1] were diluted with 0.9% sodium chloride irrigation[2] (saline) to prepare solutions for the permeation experiments. The final chemical concentrations of the permeating species in the external diffusion medium were $\leq 10^{-4}$ *M*.

Animals—Male hairless mice of SKh-hr^{-1} strain[3] were used. Their care was as described in the preceding paper (7).

Radioisotopic Assay—Concentrations of the radiolabeled permeating species were determined using a liquid scintillation counter[4] and a suitable liquid scintillator[1]. Permeation of both methanol and phenol was studied simultaneously using a technique involving dual labels (11).

Scalding Procedure—Immediately following sacrifice, the dorsal

[1] New England Nuclear, Boston, Mass. (Supplier-estimated purity >98% in each case.)
[2] Abbott Laboratories, North Chicago, Ill.
[3] Skin Cancer Hospital, Temple University, Philadelphia, Pa.
[4] Beckman Liquid Scintillation Counter, Model LS 9000, Beckman Instruments, Inc., Fullerton, Calif.

0022-3549/83/0400-0397$01.00/0