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Hydration and Percutaneous Absorption II: Influence of Hydration on Water and Alkanol Permeation through Swiss Mouse Skin; Comparison with Hairless Mouse

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Abstract □ *In vitro* permeation studies with biological membranes often involve long, aqueous maceration of the tissue. The present investigation examined the possible effects of hydration on barrier integrity of Swiss mouse skin, using water, methanol, ethanol, and butanol as permeants and a previously developed procedure involving multiple, sequential permeation runs on each piece of skin. The permeation rate of water increased almost linearly up to 30 hr of hydration and then tended to level off. Transport rates of methanol and ethanol increased asymptotically and then plateaued at ~15 hr. These results contrast with earlier findings on hairless mouse skin where the permeabilities of these three compounds were unaffected by aqueous immersion. The permeation rate of butanol also increased during the first 15 hr of hydration but gradually declined over the next 25 hr. This result again contrasts with the hairless mouse species in which butanol permeability doubled in 10 hr and then plateaued. The species differences in the hydration profiles appear related to the vastly dissimilar pellages and, in the Swiss mouse, may indicate greater involvement of the transfollicular pathway.

Keyphrases □ Hydration—effect on water and alkanol permeation through Swiss mouse skin, comparison with hairless mouse □ Absorption, percutaneous—effect of hydration on water and alkanol permeation through Swiss mouse skin, comparison with hairless mouse □ Permeability—effect of hydration on water and alkanol absorption through Swiss mouse skin, comparison with hairless mouse

A previous study (1) on the influence of long aqueous immersion (hydration) on the permeability properties of hairless mouse skin used a method involving sequenced, *in vitro* diffusional experiments on each skin membrane. Hydration-related alterations of the permeation behaviors of a series of compounds were systematically investigated, and the observed effects were related to the physicochemical properties of the permeants and mass transport mechanism. The permeabilities of water, methanol, and ethanol were not affected by immersion of the skin in normal saline. The permeabilities of butanol and hexanol doubled and reached asymptotes in 10 hr of hydration. The permeation rate of heptanol only increased by ~50%, while octanol showed an initial 50% increase followed by a 25% decline to assume an invariant rate with a net 25% hydration-induced alteration.

In view of the experimental and possible mechanistic significance of the hydration-induced permeability alterations, the present study compared the influence of hydration on the skin permeability of the Swiss mouse, to that of the hairless mouse. The results obtained proved the skins of the two species to be exceedingly different in their chemical barrier properties, both in terms of absolute rates of permeation of the low molecular weight alcohols and in their hydration sensitivities.

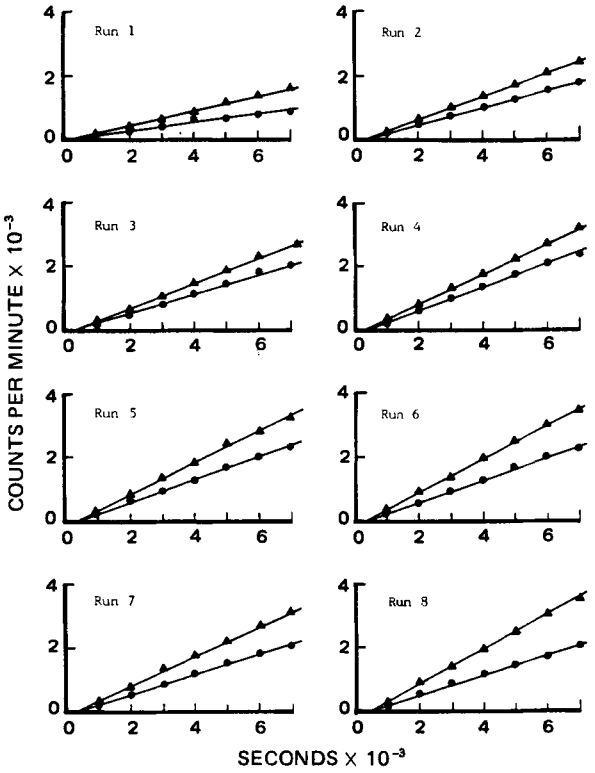

Figure 1—*Series of receiver concentration* versus *time profiles for eight sequential permeation runs on a single skin. This figure is one set of data from the methanol (▲)–butanol (●) series detailed in the text. Key: Run 1, t_0 = 0 hr; Run 2, t_0 = 5 hr; Run 3, t_0 = 10 hr; Run 4, t_0 = 15 hr; Run 5, t_0 = 20 hr; Run 6, t_0 = 25 hr; Run 7, t_0 = 30 hr; and Run 8, t_0 = 43 hr.*

EXPERIMENTAL

Chemicals—[^{3}H]Water[1], [^{3}H]methanol[1], [^{14}C]ethanol[2], and [^{14}C]-butanol[2] were used as received. The radiochemicals were diluted into 0.9%

[1] New England Nuclear, Boston, MA 02218.
[2] International Chemical and Nuclear Corp., Irvine, CA 92715.

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Table I—Summary of Hydration Effect Data for Water

Hours of Hydration	$P \times 10^3$, cm/hr Mouse 1 (33.0 g)	Mouse 2 (38.0 g)	Mouse 3 (33.0 g)	Mouse 4 (36.5 g)	Mouse 5 (35.5 g)	Average $P \times 10^3 \pm SD$, cm/hr
0	5.1	2.2	5.6	1.9	5.3	4.0 ± 1.8
5	6.1	2.8	6.2	6.3	4.0	5.1 ± 1.6
10	6.3	3.6	6.6	7.4	5.9	6.0 ± 1.4
15	7.1	4.5	7.4	8.3	9.5	7.4 ± 1.9
20	8.1	5.1	8.9	10.6	11.9	8.9 ± 2.6
25	8.6	5.7	9.3	10.6	13.7	9.6 ± 2.9
30	8.9	8.6	10.5	11.7	14.9	10.9 ± 2.6
48	9.7	8.2	11.6	11.4	17.7	11.7 ± 3.6

Table II—Summary of Hydration Effect Data for Methanol

Hours of Hydration	$P \times 10^3$, cm/hr Mouse 1 (34.5 g)	Mouse 2 (36.0 g)	Mouse 3 (38.0 g)	Mouse 4 (34.0 g)	Mouse 5 (33.0 g)	Average $P \times 10^3 \pm SD$, cm/hr
0	2.6	5.7	9.8	6.3	2.7	5.4 ± 3.0
5	4.2	7.1	10.0	8.5	2.7	6.5 ± 3.0
10	5.6	7.7	10.8	9.8	3.2	7.4 ± 3.1
15	7.3	9.6	12.2	12.1	3.9	9.0 ± 3.5
20	7.9	9.8	12.4	12.7	4.1	9.4 ± 3.5
25	7.6	10.7	13.2	13.3	4.2	9.8 ± 3.9
30	7.6	11.0	11.9	12.9	4.1	9.5 ± 3.6
43	7.2	11.0	13.4	13.0	4.2	9.8 ± 4.0

sodium chloride irrigation solution[3] (saline) for permeation experiments. The final chemical concentration in the diffusional medium was $\leq 10^{-4}$ *M*.

Animals—Male Swiss mice[4], albino out bred of Skh:Icr strains, had free access to food and water, and bedding was changed at least once a week.

Hair Removal—All skins used in the diffusion experiments were denuded of hair. Usual depilation methods, *i.e.*, shaving and chemical depilation, were avoided because they might seriously alter the barrier properties (2, 3). Hair from the abdominal surface of the freshly sacrificed mouse was removed by the nondestructive and nonirritating procedure of close cropping with a pair of surgical scissors.

Permeation Procedure—Two-compartment glass diffusion cells (1) were employed to determine skin permeability. All membranes sandwiched between the half-cells were full-thickness skin sections excised from the abdominal surfaces of Swiss mice, freshly sacrificed by spinal cord dislocation. Mouse age was tightly confined (~180 days) to avoid age-related effects (4). The external medium of the diffusion cell was saline, and the half-cell contents were stirred at 150 rpm. All experiments were carried out at 37°. The concentration in the receiver chamber (dermis side) was monitored with time for ~2 hr.

Complete hydration profiles were obtained on each skin by carrying out eight sequential experiments, with rinsing between runs (1). The

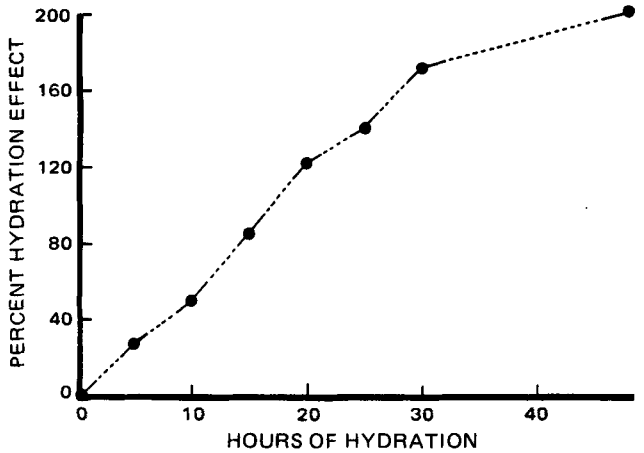

Figure 2—*Plot of hydration effects* versus *hydration time for water.*

[3] Abbott Laboratories, North Chicago, IL 60064.

[4] Skin Cancer Hospital, Temple University, Philadelphia, PA 19104.

Table III—Summary of Hydration Effect Data for Ethanol

Hours of Hydration	$P \times 10^3$, cm/hr Mouse 1 (33.0 g)	Mouse 2 (38.0 g)	Mouse 3 (33.0 g)	Mouse 4 (36.5 g)	Mouse 5 (35.5 g)	Average $P \times 10^3 \pm SD$, cm/hr
0	3.7	1.7	4.3	3.5	2.7	3.2 ± 1.0
5	4.4	2.7	4.7	—	4.8	4.2 ± 1.0
10	4.8	3.2	4.8	4.6	5.9	4.7 ± 1.0
15	5.2	3.3	5.3	5.4	8.1	5.5 ± 1.7
20	5.3	3.5	5.9	4.0	10.1	5.8 ± 2.6
25	5.0	4.3	5.2	4.3	11.0	6.0 ± 2.8
30	5.6	4.2	5.5	4.9	11.4	6.3 ± 2.9
48	4.7	3.6	5.7	4.8	10.9	5.9 ± 2.9

Table IV—Summary of Hydration Effect Data for Butanol

Hours of Hydration	$P \times 10^3$, cm/hr Mouse 1 (33.0 g)	Mouse 2 (38.0 g)	Mouse 3 (33.0 g)	Mouse 4 (36.5 g)	Mouse 5 (35.5 g)	Average $P \times 10^3 \pm SD$, cm/hr
0	8.3	6.1	8.7	9.0	5.7	7.6 ± 1.5
5	14.4	10.4	12.8	15.5	8.2	12.3 ± 3.0
10	15.7	11.6	14.6	16.8	8.7	13.5 ± 3.3
15	18.8	14.7	16.6	20.6	10.9	16.3 ± 3.8
20	20.3	13.5	14.8	19.7	9.7	15.6 ± 4.4
25	19.1	14.0	13.7	19.6	10.1	15.3 ± 4.0
30	18.6	14.6	11.8	19.5	9.5	14.8 ± 4.3
43	16.3	11.9	13.4	18.3	8.9	13.8 ± 3.7

permeant concentration was determined radioisotopically using a liquid scintillation counter[5] with Aquasol[1] as the cocktail. A technique of dual labels was employed to study the permeation of ^{3}H- and ^{14}C-labeled penetrants simultaneously (4).

Data Analysis—The data were plotted as the receiver compartment concentration as a function of time. Each permeability coefficient was calculated from (1):

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

where

P = permeability coefficient (centimeters per hour)
A = diffusional area (~0.6 cm^2)
ΔC = concentration difference across the membrane, which was taken to be equal to the donor concentration (counts per minute per cm^3)
V = receiver half-cell volume (1.4 ml)
dC/dt = steady-state slope (counts per minute per cubic centimeter per hour)

RESULTS AND DISCUSSION

Figure 1 contains eight subplots obtained in sequential permeation experiments carried out on one piece of skin over 43 hr with [^{3}H]methanol and [^{14}C]butanol as the dual permeants. Under the experimental conditions, the linear relationship between the receiver concentration and time (which develops in each run) is representative of a quasi steady-state

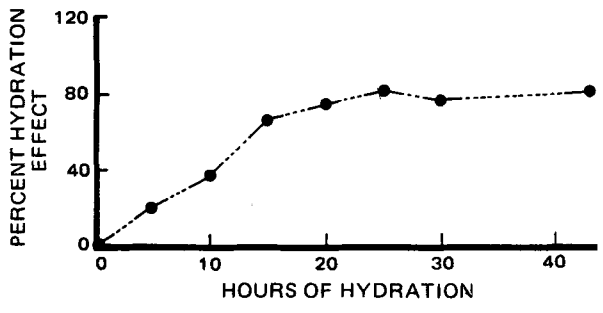

Figure 3—*Plot of hydration effects* versus *hydration time for methanol.*

[5] Model LS 9000, Beckman Instruments, Fullerton, Calif.

Table V—Summary of Hydration Effects

Hours of Hydration	Percent Hydration Effect[a]			
	Water	Methanol	Ethanol	Butanol
0	0.0	0.0	0.0	0.0
5	27.5	20.4	31.3	61.8
10	50.0	37.0	46.9	77.6
15	85.0	66.7	71.9	114.5
20	122.5	74.1	81.3	105.3
25	140.0	81.5	87.5	101.3
30	172.5	75.9	96.9	94.7
43[b] or 48[c]	192.5	81.5	84.4	81.6

[a] Equation 2. [b] Methanol and butanol. [c] Water and ethanol.

transport process. Slopes of these linear segments were used to compute the permeability coefficients (Eq. 1).

Five mice were used for each of the four penetrants, and eight sequential experiments were run on each skin, yielding a total of 160 diffusion experiments. Permeability coefficients computed from 20 figures analogous to Fig. 1 are reported in Tables I–IV for water, methanol, ethanol, and butanol, respectively. Rather large animal-to-animal variations in the permeability coefficients were found for all four permeants. Similar variability was observed in earlier studies with the hairless mouse skin (1, 4). However, within a given piece of skin, permeabilities changed systematically and stereotypically for a given compound. The "percent hydration effect" was calculated from:

$$\text{percent hydration effect} = \frac{P\text{ (at a given hydration time)} - P\text{ (at zero hydration)}}{P\text{ (at zero hydration)}} \times 100 \quad \text{(Eq. 2)}$$

These derived numbers are given in Table V. To illustrate the hydration influences, the results (percentage changes) were plotted as a function of hydration time (Figs. 2–5).

Of all the permeants studied in the Swiss mouse skin, water experienced the largest increase in permeability during the aqueous immersion. Figure 2 shows that the percentage increase in permeability rose almost linearly up to 30 hr of immersion but then showed signs of leveling off. The hydration sensitivity for water was markedly different than that reported for the hairless species (1) where water penetration rates were not significantly altered by the soaking treatment. Methanol's permeability also increased with time of immersion but only up to ~20 hr. Beyond this point, the net hydration increase remained essentially invariant at 78.3 ± 3.8% (average of data at 20, 25, 30, and 48 hr of hydration). Ethanol exhibited similar behavior, with an asymptote value of 87.5 ± 6.7% (average of data at 20, 25, 30, and 48 hr of hydration). The close parallelism in the hydration profiles of methanol and ethanol may imply that both solutes penetrate the Swiss mouse skin *via* a common route. The results represent a great departure from hairless mouse data (1), which indicate no hydration-induced alterations for either methanol or ethanol.

Butanol's permeability through Swiss mouse skin also rose during the first 15 hr of aqueous immersion. Data gathered beyond this time are difficult to interpret since butanol's permeability appears to decline between 15 and 43 hr in an almost linear manner. The absolute permeability coefficients at 15 and 43 hr are not statistically separable. However, the lack of a statistically supported difference may be the result of interanimal variability since all five Swiss mouse skins showed an approximate 20% decline in butanol's permeability coefficient over 15–43 hr. Moreover, the butanol data were obtained using the dual-label procedure with methanol as the copermeant, and the permeability coefficient

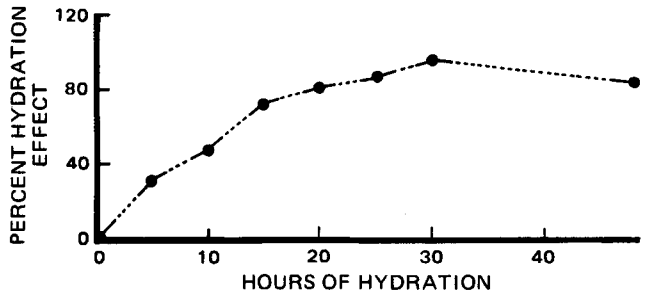

Figure 4—*Plot of hydration effects* versus *hydration time for ethanol.*

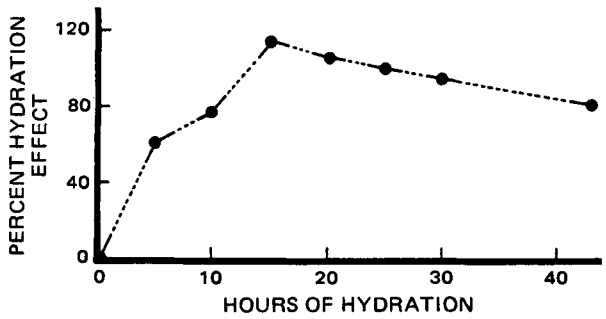

Figure 5—*Plot of hydration effects* versus *hydration time for butanol.*

for methanol was invariant past ~20 hr. It was tentatively concluded that when hydrating conditions are sustained past 15 hr, there is a small but real depreciation of the permeability coefficient of butanol. For hairless mouse skin, butanol's permeability coefficient doubled over the initial 10-hr immersion and then remained constant (1). Thus, with the lower alkanols and water, the skin of the Swiss species behaved qualitatively differently from that of hairless species.

Possible Explanation of Observed Hydration Profiles—*Water, Methanol, and Ethanol*—It was pointed out (4) that the polar solutes (water, methanol, and ethanol) do not appear to permeate the skin of the hairless mouse by the main lipoidal pathway, presumably located within the stratum corneum, but use an alternative parallel diffusional route of either transfollicular or transepidermal origin. In the mature hairless mouse, most follicles are cystic, the atrophied remains of an early coat of hair, and are not visually prominent. However, the Swiss mouse is abundantly covered with actively cycling hair follicles containing prominent hairs. Given this difference between the two species, it might be expected for a follicularly placed polar pathway that hydrophilic permeants would have greatly enhanced permeabilities through Swiss mouse skin. The permeability coefficients for water, methanol, and ethanol were indeed several times larger in the Swiss species than in the hairless species, particularly when compared in the fully hydrated states.

The qualitative differences in hydration sensitivities between the two types of mice are of even greater significance. Water gradually plasticized some critical phase of the Swiss mouse skin and opened it to more facile diffusion of the small polar solutes. In contrast, the permeabilities of water, methanol, and ethanol through hairless mouse skin were unaffected by long immersion. Quantitative considerations aside, there is obviously some fundamental difference in tissue structure and the barrier mechanism. These changes may be due to an enhanced follicular mechanism in the Swiss mouse. In this case, gradual hydration and softening of the sebaceous medium would explain the overall effects. However, the skin is integrated tissue and increased follicular density and hair prominency have been associated with a less well-formed horny layer. Thus, the critical changes possibly are associated with different degrees and qualities of cornification.

Butanol—Permeation of butanol was reported (1,4) to occur transepidermally by partitioning into the lipoid components of the stratum corneum of hairless mouse skin. This process is believed to occur in the Swiss species as well. Therefore, the hydration-related permeability changes in the Swiss species could be due to some modification of the lipoidal pathway in the stratum corneum. However, the exact mechanism for either species is unknown.

Significance of Present Studies—The influence of hydration needs to be considered in the design of skin permeation studies, and the exact state of hydration should be reported. This study suggests a possible means of factoring transepidermal and the transfollicular pathways. Given the possible association of the hair follicles and hydration-induced alterations in skin permeability, clinically different results of the occlusive dressing might be expected between hairy and nonhairy human surfaces.

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Stability-Indicating High-Performance Liquid Chromatographic Analysis of Lidocaine Hydrochloride and Lidocaine Hydrochloride with Epinephrine Injectable Solutions

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Received December 12, 1980, from *Astra Pharmaceutical Products, Inc., Worcester, MA 01606.* Accepted for publication March 24, 1981.

Abstract □ A reversed-phase, high-performance liquid chromatographic (HPLC) procedure, which is specific and quantitative for lidocaine hydrochloride, epinephrine, and methylparaben, was developed for the analysis of lidocaine hydrochloride and lidocaine hydrochloride with epinephrine solutions for injection. Epinephrine sulfonic acid and adrenochrome are separated in this system. Also separated are lidocaine and methylparaben and their respective degradation products, 2,6-xylidine and *p*-hydroxybenzoic acid. The analysis requires that three detectors (two UV and one electrochemical) be connected in series. By using this arrangement, lidocaine hydrochloride and methylparaben are quantitated by UV at 254 and 280 nm, respectively, while epinephrine is quantitated electrochemically. The method is simple, accurate, precise, and rapid. No sample preparation or internal standard is necessary, and only a 2-μl sample volume is required for analysis. Chromatographic conditions include a μBondapak CN column and a mobile phase of 0.01 *M* 1-octanesulfonic acid sodium salt, 0.1 m*M* edetate disodium, 2% acetic acid, 2% acetonitrile, and 1% methanol in water.

Keyphrases □ High-performance liquid chromatography—specific and quantitative stability-indicating procedure for lidocaine hydrochloride, epinephrine, and methylparaben □ Lidocaine hydrochloride—injectable solutions of epinephrine using stability-indicating high-performance liquid chromatography □ Epinephrine—high-performance liquid chromatographic procedure for assaying components of injectable solutions □ Methylparaben—high-performance liquid chromatography for assaying components of injectable solutions

A literature review indicated that the various colorimetric and fluorometric methods utilized for the analysis of epinephrine in local anesthetic solutions all have problems associated with them (1, 2). The colorimetric methods generally are not stability indicating. Epinephrine sulfonic acid and bisulfite interfere with the development of the color, resulting in a lack of specificity for intact epinephrine.

A fluorometric procedure, based on the trihydroxyindole reaction first observed by Loew (3) and later modified (4, 5), is specific for epinephrine but is subject to many variables such as time, temperature, pH, presence of bisulfite, and the composition of the final alkaline/ascorbate reagent. The current USP (6) method for assaying epinephrine in lidocaine hydrochloride injectable solutions is a fluorometric procedure; although the method is specific for epinephrine, its application requires a great deal of experience and technique. The fluorometric procedure for the analysis of epinephrine in lidocaine hydrochloride injectable solutions has been automated (7) but is subject to interferences.

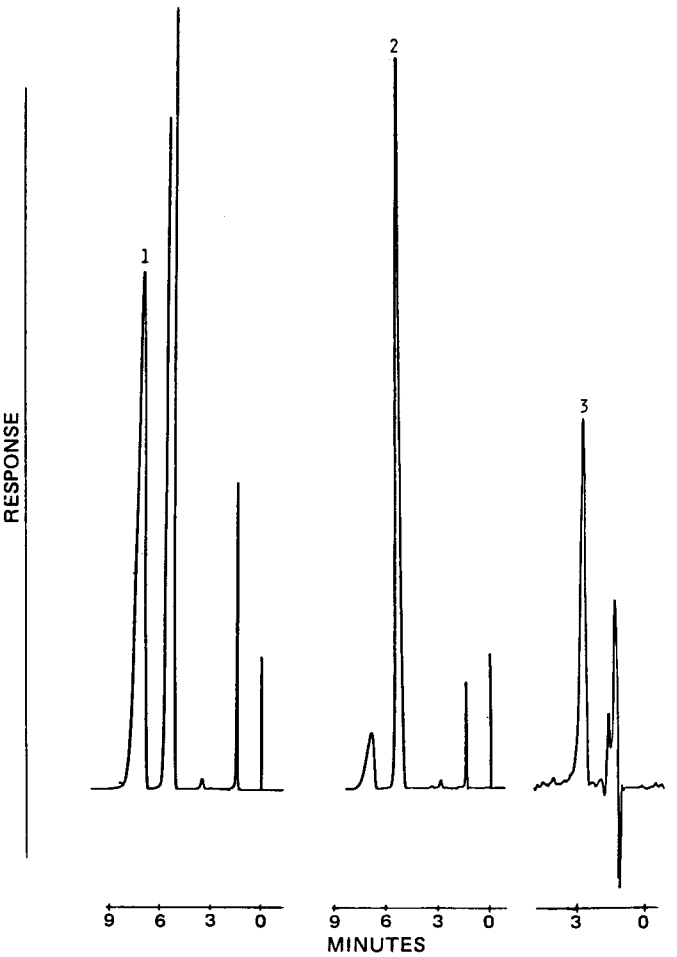

Figure 1—*Representative chromatograms of a 2-μl injection of a 2% lidocaine hydrochloride with 1:100,000 epinephrine solution, showing simultaneous detection of lidocaine (1) with UV detector at 254 nm, methylparaben (2) with UV detector at 280 nm, and epinephrine (3) with electrochemical detector at +0.90 v.*

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