Percutaneous Absorption of Butylparaben through Guinea Pig Skin *In Vitro*

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Abstract □ Percutaneous absorption of aqueous butylparaben through guinea pig dorsal skin was studied using a diffusion chamber. Polysorbate 80 increased the solubilized concentration but decreased penetration of the preservative. Polyethylene glycol 400 also reduced the amount of penetration. Propylene glycol was less effective than polyethylene glycol 400. Preservative activities of these systems on several microorganisms were evaluated on agar plates. The relationship between the butylparaben penetration and preservative activity is discussed.

Keyphrases □ Percutaneous absorption—butylparaben, guinea pig, *in vitro*, effect of surfactants, relation to preservative activities □ Butylparaben—percutaneous absorption, effect of surfactants, guinea pig, *in vitro*, relation of absorption and preservative activities □ Surfactants—effect on percutaneous absorption of butylparaben, guinea pig, *in vitro* □ Preservatives—butylparaben, percutaneous absorption, effect of surfactants, relation of absorption and preservative activities, guinea pig, *in vitro*

The preservative activity of a system is dependent on the aqueous concentration of the preservative (1–3). This fact suggests that organic compounds employed as preservatives will be somewhat solubilized in the aqueous phase in systems such as foods, ointments, and cosmetics. Such organic preservatives have affinities to both water and oil and are expected to penetrate into the skin easily (4, 5).

Many studies have concerned the percutaneous absorption of preservatives, especially hexachlorophene (6–9), and their preservative activities in various systems (3, 10). However, few reports concerning butylparaben (I) have been published, despite its wide use as a preservative, and there are few reports on the relationship between preservative activity and percutaneous preservative absorption.

Although recent reports stated that esters of *p*-hydroxybenzoic acid did not pass through a natural membrane 15 hr after application (11), these substances must penetrate into the skin since they induce allergic reactions (12, 13). A whole body autoradiography study of hairless mice (14) showed that I was percutaneously absorbed from several ointments.

In this paper, percutaneous absorption *in vitro* and preservative activity of I in an aqueous system were measured; the relationship between the preservative activity and the percutaneous preservative absorption is discussed.

EXPERIMENTAL

^{14}C-Butylparaben[1] was obtained with a specific activity of 1.65 mCi/mmole. Propylene glycol, polyethylene glycol 400, and polysorbate 80 were reagent grade[2].

Vehicle compositions are listed in Table I. A 2-ml aliquot of each system was used for the diffusion study.

Male guinea pigs (Hartley strain), ~320 g, were used. The dorsal hair was removed with a hair clipper immediately prior to the experiment. The residual hair was 0.5 mm long. The adipose tissue was removed after the dorsal skin was excised from the sacrificed animal. The skin sample was then punched into a 3-cm diameter disk.

The disk was placed in a modified diffusion chamber (15). The area of the donor side was 3.14 cm^2, and the volume of the receptor space was 4.4 ml. The dermal side receptor fluid was saline and flowed through the chamber continuously at ~8 ml/hr. The eluted solution was fractionally collected in test tubes. The chamber was placed in a controlled 37° water bath throughout the experiment.

Aliquots (1 ml) of eluted I were pipetted from each test tube into a vial containing 10 ml of Bray's scintillation fluid (16). The radioactivity was measured by a liquid scintillation counter[3].

Preservative activities were studied according to a modified method of Ishizeki (17). The test sample was mixed with each solution of *Escherichia coli* (IFO[4] 3043) plus *Staphylococcus aureus* (IFO 3061), *Bacillus subtilis* (IFO 3024), *Pseudomonas aeruginosa* (IFO 3445), *Aerobacter aerogenes* (IFO 3320), *Aspergillus niger* (IAM[5] 3001) plus *Penicillium citrinum* (IAM 7316), and *Candida albicans* (IFO 0583). After several intervals of up to 7 days at 32°, each mixture was transferred onto an agar plate[6]. For *P. aeruginosa* and *A. aerogenes*, glucose was added to the agar; for *A. niger* plus *P. citrinum* and *C. albicans*, Sabouraud medium[6] was employed.

Colonies were counted after 24 hr of incubation at 37° for bacteria and after 48 hr of incubation at 30° for fungi and yeasts. Preservative activities were evaluated by counting the days before all colonies disappeared. The score was 8 when no colonies were found on the plate zero day after addition of the test sample. The score decreased with time; when colonies were still observed after 7 days, the score was 0.

RESULTS AND DISCUSSION

The penetration flux, J, of a solute through the skin is expressed as:

$$J = \frac{Q}{At} = \frac{(D)(PC)(C_v)}{l} \qquad \text{(Eq. 1)}$$

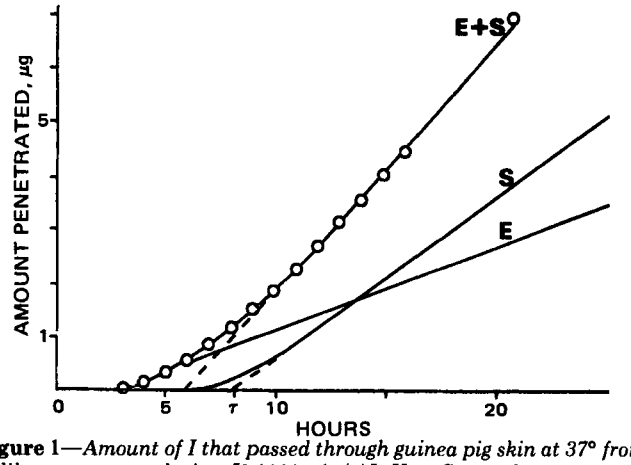

Figure 1—*Amount of I that passed through guinea pig skin at 37° from a dilute aqueous solution [0.002% (w/v)]. Key:* S, *steady-state penetration through epidermis;* E, *early penetration through appendages;* S + E, *observed amount of penetration; and* τ, *lag time.*

[1] Daiichi Pure Chemicals Co., Tokyo, Japan.
[2] Wako Pure Chemical Industries, Tokyo, Japan.
[3] Model LSC-601, Aloka Co., Mitaka, Tokyo, Japan.
[4] Institute for Fermentation, Osaka, Japan.
[5] Institute of Applied Microbiology, Tokyo, Japan.
[6] Eiken Chemical Co., Tokyo, Japan.

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Table I—Vehicle Composition

System	Compound I, % (w/v)	Polysorbate 80, % (w/v)	Propylene Glycol, % (v/v)	Polyethylene Glycol 400, % (v/v)
A	0.015	—	—	—
B	0.02	—	10	—
C	0.02	—	—	10
D	0.02	—	20	—
E	0.02	—	—	20
F	0.1	1.0	—	—
G	0.1	1.0	10	—
H	0.1	1.0	—	10
I	0.1	2.0	—	—
J	0.1	2.0	10	—
K	0.1	2.0	—	10

where Q is the amount of solute that diffuses across the area, A, in time, t; PC is the skin–vehicle partition coefficient of the solute; D is the solute diffusion constant in the skin of thickness l; and C_v is the solute concentration in the vehicle (18, 19). The permeability constant, k_p, which represents a penetration rate, is given by J/C_v.

Figure 1 shows the total amount of I absorbed from an aqueous vehicle through the skin as a function of time. Transient penetration through appendages becomes less important than the steady-state penetration through an unbroken membrane over time (18). In Fig. 1, however, the amount of early penetration, E, was comparable to the steady-state penetration, S. In the present study, therefore, the observed penetration was explained as the sum of two diffusion processes ($E + S$) (20).

A lag time, τ, was given by the intercept of the line S on the t-axis. The lag time is the time required for the solute to pass transepidermally through skin of thickness l with the diffusion constant D (20):

$$\tau = \frac{l^2}{6 \times D} \qquad \text{(Eq. 2)}$$

The penetration of I increased with time from Systems A–K (Tables I and II and Figs. 2 and 3). The average thickness of the skin samples from 22 animals without subcutaneous fat was 0.134 cm (SD 0.017 cm). This value was used for the diffusion constant calculations.

Percutaneous solute absorption is reportedly enhanced by solvents such as dimethyl sulfoxide and chloroform–methanol (21–24) because, perhaps, of stratum corneum changes (23, 24). Although there was a small D value deviation in the 11 systems in the present study ($D = 3.63 \pm 0.47 \times 10^{-4}$ cm^2/hr), no correlation was found between the system compositions and D values. The I partition coefficient varied markedly with the systems. Propylene glycol reduced the value significantly, but the effect was much less than that of polyethylene glycol 400. The effect of polysorbate 80 on the decrease of the partition coefficient was much more drastic. When polyethylene glycol 400 was coexistent with the surfactant, further reduction of the partition coefficient occurred. Propylene glycol addition had no effect.

The percentage of the solute passing through the skin from the system correlated well with the partition coefficient, indicating that the system–skin partition coefficient determined the penetration flux.

Preservative activities were evaluated qualitatively. Since the solubility of I in water is 0.015% (w/v), the I concentration in System A was slightly less than that in Systems B–E. The System E score was relatively high, but the preservative levels of these five systems were comparable (Table III). This finding suggested that there were no interactions between the solubilizers and I that would deactivate the preservative.

Table II—Values of Diffusion Constant (D), Permeability Constant (k_p), and Partition Coefficient (PC) of the Steady-State Penetration, and Percentage Penetration of the Total Amount Applied

System	D, $\times 10^{-4}$ cm^2/hr	k_p, $\times 10^{-4}$ cm/hr	PC	Penetration of Total Amount Applied, %
A	3.43	70.82	2.77	23.70
B	4.53	53.70	1.59	20.22
C	4.26	16.76	0.53	5.70
D	3.38	26.88	1.07	9.54
E	3.04	7.52	0.33	1.95
F	3.28	9.36	0.38	3.64
G	3.34	12.04	0.48	3.54
H	3.46	7.08	0.27	2.17
I	3.39	4.28	0.17	1.40
J	3.51	4.60	0.18	1.47
K	4.31	1.98	0.04	1.10

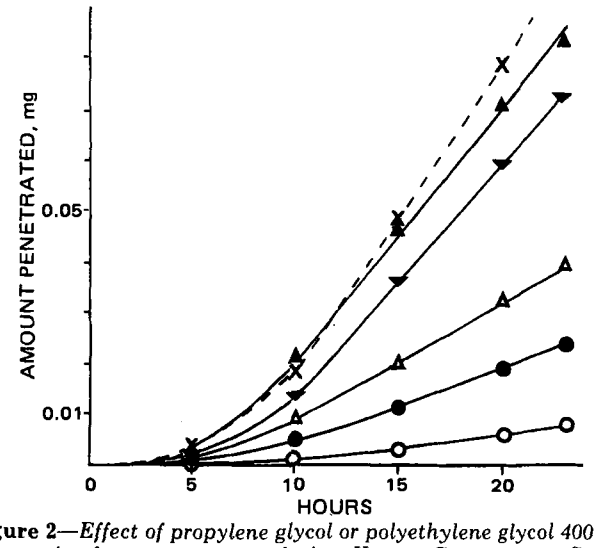

Figure 2—*Effect of propylene glycol or polyethylene glycol 400 on I penetration from an aqueous solution. Key: ▼, System A; ▲, System B; ●, System C; △, System D; ○, System E; and ×, supposed amount of penetration from 0.02% aqueous I solution.*

The increase in I concentration with polysorbate 80 did not improve preservative activity. On the contrary, the activity was reduced (Table III). Since the preservative activity is dependent on the effective aqueous I concentration (1), these results indicate that the surfactant increased the amount of I trapped in micelles and reduced the I concentration in the outer phase (3, 25).

Since the partition coefficient is also regarded as an index of mutual affinity between the preservative and vehicle (26), a low partition coefficient reflects the tendency of the preservative to remain in the vehicle. Therefore, the addition of propylene glycol or polyethylene glycol 400 to water, which increased the I solubility, decreased the partition coefficient. If the aqueous solubility were 0.02% (w/v) and the concentration were not increased by the solubilizers, the penetrated amount would be reduced in Systems B–E (Fig. 2). System A contained only 0.015% I, so the addition of solubilizers decreased the partition coefficient and increased I concentration. The latter was larger than the former in System B (Fig. 2), whereas the decrease in the partition coefficient was much more effective in Systems C–E. Polyethylene glycol 400 induced a greater decrease in the penetrated amount (or partition coefficient) than propylene glycol, as shown by a preliminary experiment. The saturated I concentration in 10% aqueous polyethylene glycol 400 was 0.050% (w/v) while that in 10% aqueous propylene glycol was 0.032% (w/v). These values were obtained by spectrophotometry[7] at 256 nm. An increase in

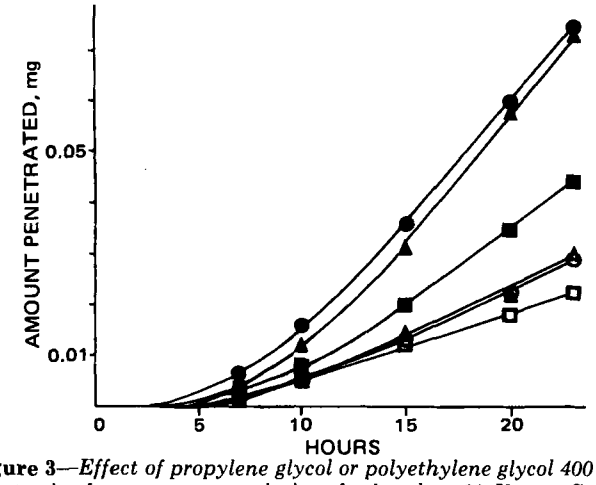

Figure 3—*Effect of propylene glycol or polyethylene glycol 400 on I penetration from an aqueous solution of polysorbate 80. Key: ●, System F; ▲, System G; ■, System H; ○, System I; △, System J; and □, System K.*

[7] Model 139 UV-VIS spectrophotometer, Hitachi Co., Tokyo, Japan.

Table III—Preservative Score

System	*A. niger* plus *P. citrinum*	*C. albicans*	*B. subtilis*	*E. coli* plus *S. aureus*	*A. aerogenes*	*P. aeruginosa*	Total Score
A	0	7	7	7	6	0	27
B	0	3	7	7	7	0	24
C	0	3	7	7	3	3	23
D	0	3	7	6	7	3	26
E	0	6	8	7	7	7	35
F	0	3	7	0	0	0	10
G	0	3	6	0	0	0	12
H	0	6	6	7	7	7	33
I	0	0	6	0	0	0	6
J	0	0	7	0	0	0	7
K	0	0	7	6	0	0	13

solubility means an increase in affinity to the system, which results in a decrease in the partition coefficient.

The preservative activity increased following addition of polyethylene glycol 400 to the surfactant solution (Table III), indicating that the I concentration in the outer phase increased due to alteration of the aqueous phase–micelle partition coefficient. The increase in I concentration in the outer phase (Table III) and the decrease in the amount passing through the skin (Fig. 2) caused by polyethylene glycol 400 help explain the percutaneous absorption results for Systems F–K.

The effect of the polyethylene glycol 400 addition in Fig. 3 was not so great as that in Fig. 2, where the surfactant was not present. Although the value of the skin–vehicle partition coefficient was reduced by the addition of polyethylene glycol 400, the effective concentration in the outer phase, which can contribute to penetration (19), was increased. Thus, the product of the effective outer phase concentration and the partition coefficient showed only a slight decrease in the penetrated amount.

The effect of propylene glycol was not so great as that of polyethylene glycol 400 on either permeability or preservative activity. Propylene glycol induced only a small increase in I concentration in the outer phase, which was counteracted by a small decrease in the partition coefficient, resulting in a smaller permeability change.

Some other factors also affected penetration from a surfactant solution since the amount of penetration from System F was similar to that from System A, although the preservative activities of these two were significantly different. Malik *et al.* (27) suggested that a surfactant increased the drug–skin affinity but that the excess addition of a surfactant decreased drug absorption, an effect also seen in Systems F and I in this study. Ostrenga *et al.* (28) suggested that the whole vehicle was absorbed or that the vehicle was separated on the skin after application. These reports reflect the difficulty of comparing the amount of penetration from systems with and without surfactant on the basis of the concentration and partition coefficient only. It was possible, however, to evaluate the effect of propylene glycol and polyethylene glycol 400 in each system on the basis of these two factors.

Although the addition of excess surfactant is an effective way to prevent a preservative from penetrating into the skin, such a system was inactive on microorganisms. Moreover, it is very dangerous to apply a large amount of a preservative on living skin since the compositions of the system change with time due to water evaporation and the secretion of sebum and sweat. The results in this study suggest that both the increase in preservative activity and the reduction of percutaneous preservative absorption can occur following the addition of a solubilizer, *i.e.*, polyethylene glycol 400, to the system.

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