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## Percutaneous Absorption of Butylparaben *in Vitro*. II.<sup>1)</sup> Effects of Micellar Trapping of the Drug and Percutaneous Absorption of Nonionic Surfactants

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The effect of surfactants on the percutaneous absorption of butylparaben from micellar solution was studied. Percutaneous absorption of butylparaben from aqueous solution containing the critical micellar concentration (cmc) of a surfactant was measured in order to determine the contribution of the free butylparaben. Percutaneous absorption of polysorbate 80 and octaethyleneglycol dodecyl ether was also measured in order to evaluate the effect of absorption of micelles, if any. The results indicated that the surfactants were scarcely absorbed, but the free concentration of butylparaben could not account for the total drug absorption from micellar solution. However, the amount of surfactant absorbed increased with increase in the surfactant concentration above the cmc, indicating that surfactant molecules in micelles could be absorbed. This suggests that some of the micelles were adsorbed and/or dissociated on the skin surface, so that the effective concentration of surfactant as well as that of butylparaben for percutaneous absorption would be increased.

**Keywords**—percutaneous absorption; butylparaben; polysorbate 80; octaethyleneglycol dodecyl ether; micelle

Surfactants have been shown to be capable of modifying the rate of absorption of drugs across biological membranes. One of the effects of surfactant is the entrapment of drug molecules in the micelles, resulting in a decrease in the concentration of free drug.<sup>2)</sup> It has been pointed out that only free drug molecules in the outer phase can participate in percutaneous absorption.<sup>3)</sup> In our previous paper,<sup>1)</sup> similar absorption of butylparaben (I) was observed in two systems, 0.015% I in distilled water and 0.1% I in 1% polysorbate 80 (II) aqueous solution. The concentration of free I in the latter system was considered to be much lower than 0.015% since the preservative activity of the latter, which was reported to be dependent on the concentration of free preservative,<sup>4)</sup> was considerably lower than that of the former.<sup>1)</sup> In the case of intestinal absorption, many studies have indicated that the drug entrapped in the micelles as well as the free drug participates in the absorption.<sup>2a,b)</sup> Few studies have been done, however, on the contribution of the drug molecules entrapped in the micelles in the case of percutaneous absorption. Thus, in this study, the contribution of I free from micellar entrapment was measured and compared with the total absorbed amount of I. Percutaneous absorption of nonionic surfactants, II and octaethyleneglycoldodecyl ether (III), was also measured in order to determine the possible contribution of the absorption of micelles as a whole.

### Materials and Methods

<sup>14</sup>C-Butylparaben (butyl *p*-hydroxybenzoate-carbonyl-<sup>14</sup>C),<sup>5)</sup> <sup>14</sup>C-polysorbate 80 (polyoxy-<sup>14</sup>C-ethylene sorbitan monooleate)<sup>6)</sup> and octaethyleneglycol mono-*n*-(<sup>14</sup>C) dodecyl ether<sup>6)</sup> were employed for the absorption study, their specific activities being 1.94 mCi/mmol, 0.5  $\mu$ Ci/mg, and 50 mCi/mmol, respectively. Compounds I and II were

TABLE I. System Compositions

System	Compound I % (w/v)	Compound II % (w/v)	Compound III % (w/v)
F <sup>a)</sup>	0.1	1.0	—
I <sup>a)</sup>	0.1	2.0	—
L	0.1	1.5	—
M	0.1	—	1.0
N	0.1	—	1.25
O	0.1	—	1.5
P	0.1	—	2.0
Q	0.004—0.024	$6.2 \times 10^{-3}$	—
R	0.002—0.015	—	$3.8 \times 10^{-3}$

a) Cited from ref. 1.

purchased from Wako Pure Chemical Industries Ltd. and compound III was purchased from Nikko Chemicals Co., Ltd. Compound I was of special grade while II and III were of reagent grade. All were employed without further purification.

Table I shows the system compositions employed in percutaneous absorption. Distilled water was used to dissolve I and II or III. Compound I was considered to be undissociated in distilled water, since its  $pK_a$  was reported to be about 8.5.<sup>7)</sup> Each system contained 50 I.U. of penicillin and 50  $\mu$ g of streptomycin/ml<sup>8)</sup> in order to avoid possible degradation of the radioisotope by microorganisms on the skin surface.

Percutaneous absorption *in vitro* through the skin of male guinea pig (Hartley strain, weighing *ca.* 350 g) was measured with a diffusion chamber according to the method described previously.<sup>1)</sup> Two ml of test solution which contained 2  $\mu$ Ci of radioactivity was applied to the epidermal side of the excised guinea pig skin. The dermal side receptor fluid was saline, which was passed through the chamber continuously at about 8 ml/h at 37°C. The eluted saline was collected in test tubes during 24 h and the radioactivity in each fraction was measured. Four experiments were done for each group. When the cumulative absorption was plotted against time, a steady-state increase was observed after about 10 h.<sup>1)</sup> The flux ( $J$ ) was determined from the slope of this steady-state increase as the absorbed amount ( $\mu$ g) per unit time (h) through unit area ( $\text{cm}^2$ ), which is also shown as follows.

$$J = \frac{PC \cdot D}{l} \cdot \Delta C \quad (1)$$

where  $PC$  is the partition coefficient of a drug between the vehicle and skin,  $D$  is the diffusion constant of a drug through the barrier membrane of thickness  $l$  and  $\Delta C$  is the concentration difference. The dialysis experiment was performed with a glass cell composed of two chambers (A, B), each having a cavity of 10 ml capacity. The dialysis membrane (cellulose)<sup>9)</sup> was placed between the two chambers. Chamber A contained I and surfactant in distilled water while chamber B contained distilled water only. The solutions of the two chambers were equilibrated at 37°C for 72 h. Then the concentration of I in chamber B was measured by spectrophotometry at 256 nm.<sup>10)</sup>

## Results

The concentration of free I in a micellar solution was measured with an equilibrium-type dialysis cell (Fig. 1). The concentration of free I decreased with increase in the surfactant concentration. In order to evaluate the contribution of the free I to the percutaneous absorption of I from micellar solution, percutaneous absorption of I was studied from systems Q and R (Fig. 2).

Since the cmc values of II and III were reported to be  $6.2 \times 10^{-2}$  g/l<sup>11)</sup> and  $3.8 \times 10^{-2}$  g/l,<sup>12)</sup> respectively, the amount of the surfactant cited in system Q or R should exist as monomers even in micellar solution. The flux of I increased with increase in the concentration of I similarly for both surfactant solutions. From Figs. 1 and 2, the flux of free I was calculated. Table II shows the total flux of I and the flux from free I.

Figure 3 shows the cumulative absorption of II. The absorbed amount of II was very low; when expressed as a percentage of the amount applied, the total amount absorbed during 24 h

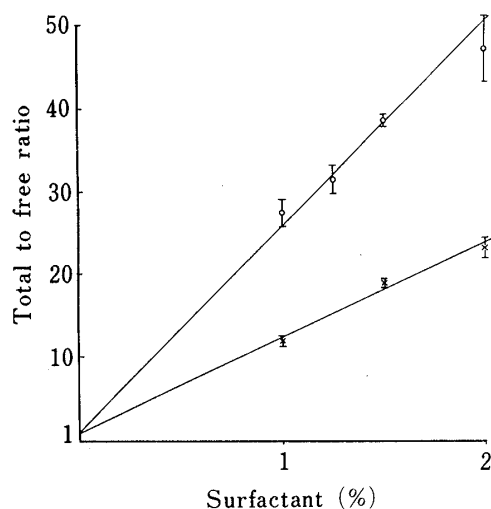


Fig. 1. Total to Free Ratio of 0.1% I in Aqueous Surfactant Solution

Each point represents the mean and standard deviation from three experiments. Key: —×—, II (systems F, I, L); —○—, III (systems M–P).

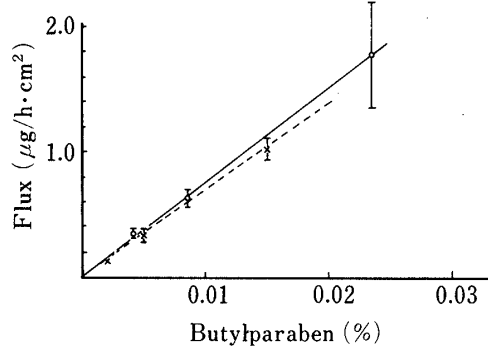


Fig. 2. Percutaneous Absorption of I from Systems Q and R

Each point represents the mean and standard deviation of the flux from four experiments. Key: —○—, system Q; —×—, system R.

TABLE II. Percutaneous Absorption of Butylparaben

System	Flux ( $\mu\text{g}/\text{h}\cdot\text{cm}^2$ )	
	Total <sup>a)</sup>	Free <sup>b)</sup>
F	$1.50 \pm 0.18$	$0.64 \pm 0.17$
L	$0.88 \pm 0.21$	$0.41 \pm 0.10$
I	$0.60 \pm 0.08$	$0.31 \pm 0.08$
M	$0.80 \pm 0.13$	$0.26 \pm 0.03$
N	$0.71 \pm 0.22$	$0.22 \pm 0.02$
O	$0.65 \pm 0.07$	$0.18 \pm 0.01$
P	$0.57 \pm 0.07$	$0.16 \pm 0.01$

a) Flux from micellar solution.

b) Flux calculated from Figs. 1 and 2.

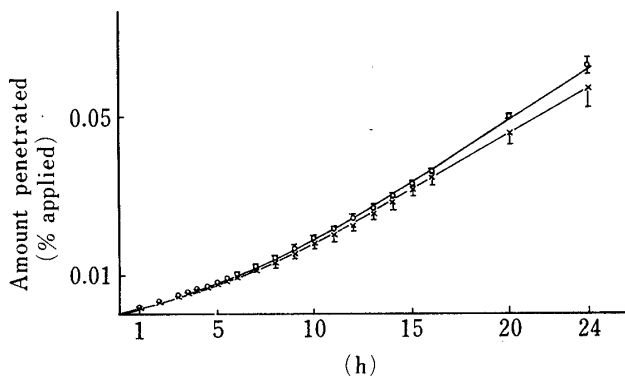


Fig. 3. Percutaneous Absorption of II from Systems F and I

Results are expressed as cumulative amount.

Each point represents the mean and standard deviation from four experiments. Key: —○—, system F; —×—, system I.

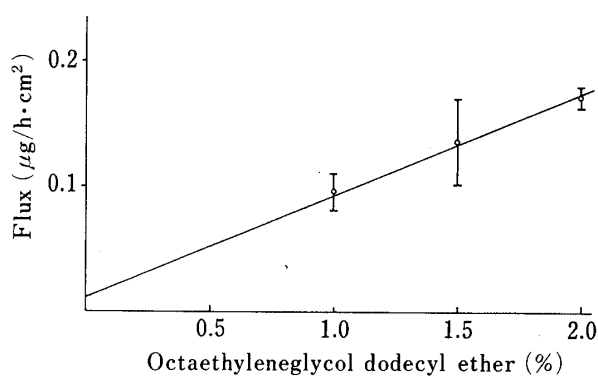


Fig. 4. Percutaneous Absorption of III from Systems M, O and P

Results are plotted as the flux versus concentration of III. The flux was measured from the steady-state absorption of III 8 to 10 h after topical application. Each point represents the mean and standard deviation from four experiments.

was only about 0.06%.

Even if micelles as a whole could be absorbed percutaneously, the absorbed amount of I (within the micelles) would be less than 0.06% of the applied amount of I, because not all of I was entrapped in the micelles. Since the absorbed amounts of I from systems F and I during 24 h were about 3.5 and 1.5% of the applied amounts, respectively,<sup>1)</sup> the value of less than 0.06% means that the contribution of absorption of micelles as a whole, if any, to the total absorption of compound I was less than 2 and 4% for systems F and I, respectively. Percutaneous absorption of III is shown in Fig. 4. The results are plotted as flux *versus* concentration of III. The absorbed amount of III increased with increase in the concentration of III, but was only 0.03—0.04% of the amount applied for each concentration of III. These results indicate that the surfactants employed in this study scarcely penetrated into the skin, so the contribution of the absorption of II and III to the absorption of I would have been very small.

### Discussion

Percutaneous absorption of I from micellar solution was observed to decrease with increase in surfactant concentration. It was reported that percutaneous absorption of benzocaine from micellar solution was proportional to the concentration of the free drug.<sup>3)</sup> However, the total flux of I through guinea pig skin could not be explained by the flux of I free from micellar entrapment.

The results in Figs. 3 and 4 indicate that percutaneous absorption of II or III was so small that the contribution of the absorption of micelles as a whole, if any, was negligible. Even though the surfactant would be partly metabolized during percutaneous absorption by the enzymes in the skin, especially in the epidermis,<sup>13)</sup> the metabolites would diffuse into the receptor fluid with the parent compound since the barrier for diffusion is the stratum corneum, below which drugs diffuse quite rapidly.<sup>14)</sup> It is noteworthy, however, that the absorbed amount of III increased with increase in the concentration of III (Fig. 4). Since micelles of nonionic surfactants are aggregates of 50—100 surfactant molecules, these entities are too large to diffuse as such into the skin, so the monomer molecules are the actual diffusing species.<sup>15)</sup> The concentration of monomer molecules does not increase any further when the added amount of surfactant exceeds its cmc. Therefore, the flux of percutaneous absorption of surfactant should level off above the cmc, which is inconsistent with the results in Fig. 4. This inconsistency suggests that surfactant molecules in the micelles also participate in part in percutaneous absorption.

Increased skin permeability resulting from the application of surfactant solution can be explained in some cases by the impairment of the barrier function by the surfactant. However, the skin irritation potential and keratin denaturation activity of nonionic surfactants were reported to be much less than those of ionic surfactants.<sup>16)</sup> In fact, pretreatment with 1% II solution for 24 h *in vivo* induced no increase in percutaneous absorption of I. Furthermore, our previous study showed that II had no effect on the lag time  $\tau$  in the percutaneous absorption of I (given by the intercept of the steady-state line on the time axis when the cumulative penetrated amount was plotted against time), suggesting that the diffusion of I through the skin was not altered by the surfactant.<sup>17)</sup> A similar value of  $\tau$  was also obtained in the case of I absorption from systems M—P in this study (data not shown).

From these results, the changes in the flux of I can be attributed to the changes in the *PC* values, as shown in Eq. 1. In other words, if the reported method to measure *PC* of a drug between skin and solvent<sup>18)</sup> could be applied to evaluate *PC* of I between skin and micellar solution including the contribution of I molecules entrapped in micelles, the flux of I could be estimated from the *PC* thus determined, as will be discussed in the following paper.<sup>19)</sup>

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