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PERCUTANEOUS ABSORPTION ENHANCEMENT BY NONIONIC SURFACTANTS

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ABSTRACT

The influence of nonionic surfactants (polysorbates) on hydrocortisone penetration through hairless mouse skin in vitro has been determined. Permeation was quite slow from purely aqueous media containing surfactants following finite dose application. However, if the vehicle contained high propylene glycol concentrations (40% or more), inclusion of a surfactant increased permeation rate significantly. Similar effects were noted following

application of a large donor volume (infinite dose). Synergism was attributed to enhancement of surfactant absorption by the stratum corneum leading to changes in skin barrier resistance. With vehicles containing a surfactant, penetration was higher after finite dose application due to compositional changes within the vehicle.

INTRODUCTION

It is generally appreciated that surfactants can affect the rate of skin penetration of various substances. Bettley[1] showed that, among surfactants themselves, anionics penetrated the skin faster than cationics which, in turn, were faster than nonionics. The use of ionic surfactants in topical products is limited by their significant skin irritation potential. Nonionics are preferred as they tend to be less irritating, and have consequently found wide use in dermatologic products and cosmetics.

Studies of the effect of nonionic surfactants on percutaneous absorption of drugs and other substances have shown that when the solution is not saturated, surfactant addition generally reduces the skin permeation rate. This has been attributed to a reduction of drug thermodynamic activity as a

consequence of solubilization or complexation. Dalvi and Zatz demonstrated effects of this type with purely aqueous solutions of benzocaine[3]. However, Shahi and Zatz[5] had observed that polysorbate 80 increased hydrocortisone flux from isopropanol-water mixtures. It was postulated that the nature of the medium could influence the interaction between nonionic surfactants and the skin barrier.

Further investigation using lidocaine solutions in propylene glycol-water vehicles supported this hypothesis[4]. The effect of nonionic surfactants was a function of the propylene glycol concentration. At 80% propylene glycol, steady state flux was increased approximately three times by polysorbate 20 or polysorbate 60.

Another variable examined in this study was the mode of application. When thin layers of the formulation were applied and allowed to remain under open conditions, simulating the usual clinical situation, a reduction in flux toward the tail end of the experiment due to drug depletion from the donor was expected. However, we also found that variation in skin surface temperature and vehicle composition due to evaporation affected the flux patterns. In systems that underwent evaporation, two peaks were observed in a plot of flux vs. time while a single peak was observed

from vehicles that absorbed water. Surfactant-induced enhancement of flux was most pronounced in the vehicle containing 80% propylene glycol, the highest concentration tested.

In the present study, penetration experiments were carried out using hydrocortisone, a more polar molecule, to determine the influence of permeant structure on surfactant effects and to gain insight into the mechanism of surfactant enhancement of permeation. All vehicles contained water with varying concentrations of propylene glycol.

MATERIALS AND METHODS

Materials

Hydrocortisone (Merck Chemical Div., Merck and Co., Rahway, NJ), propylene glycol U.S.P. (J. T. Baker, and Co., Phillipsburg, NJ), alcohol U.S.P. (Chemical Group Inc., Newark, NJ), and the polysorbates (ICI Americas Inc., Wilmington, DE) were used as received. (4- ^{14}C) hydrocortisone (New England Nuclear, Boston, MA) had a radiochemical purity of >97% as reported by the supplier. It was obtained as a solution in 9:1 benzene:ethyl alcohol with a specific activity of 55 mCi/mmol.

Preparation of Hydrocortisone Stock Solution

0.8 mL of the solution of labeled hydrocortisone was transferred to a 25 mL volumetric flask and the solution was evaporated to dryness on a water bath. After adding 4 mL of 1.25% hydrocortisone solution in ethyl alcohol, the mixture was agitated to dissolve the labeled compound. Ethyl alcohol was used to make the volume of the final solution 25 mL. This solution contained 0.2% w/v hydrocortisone and 640 nCi/mL radioactivity. The labeled material was 0.192% of the total hydrocortisone content. The stock solution was stored in the refrigerator.

Preparation of Donor Systems

After bringing the stock solution to room temperature, the proper amount of solution was transferred to a vial and evaporated to dryness in a water bath. To the residue, appropriate quantities of cosolvent were added (by weight) and the mixture was stirred till solution was effected. The required amount of water was then added to the solution by weight. This was followed by the weighed quantity of surfactant (if present). The surfactant concentration used was 1% by weight. Mixing was continued until a homogeneous system resulted. These solutions were made one day prior to the actual experiment and were stored overnight in a

refrigerator. On the day of the experiment, they were removed from the refrigerator, warmed to room temperature before applying them to the skin in the diffusion apparatus.

Procedure for Penetration Experiments

Membrane preparation, experimental apparatus, sampling and analysis of receptor solutions have been described[4]. In finite dose experiments, donor volume was 0.011 mL/cm^2 and the donor compartment was left open to the atmosphere. Donor volume was 0.275 mL/cm^2 in infinite dose experiments; the donor compartment was sealed to prevent evaporation.

RESULTS AND DISCUSSION

Data Treatment

Scheuplein[5] has reported a lag period of approximately 24 days for hydrocortisone through human skin. A value of approximately one day was obtained with mouse skin[3]. The lag time through hairless mouse skin is expected to be intermediate between these values. We did not achieve steady state in our experiments, which were usually carried on for 16 hours.

Shahi and Zatz[3] showed that large standard deviations result if cumulative amount penetrated is

averaged for replicates and that the best way to obtain a central value for a group of replicates was to average the steady state slopes. Analysis of data under non-steady state conditions is more complex. With application of thin layers of donor (finite dosing), depletion causes changes in flux with time[6].

We chose a nontraditional method of calculating average penetration rate from finite dose data. Our approach is illustrated in Fig. 1, which contains a typical penetration curve for hydrocortisone. Experimental points corresponding to penetration of about 10% and 25% of the applied dose were connected (dashed line in Fig. 1) and the slope of this line, which approximates the actual slope of the curve, gave us a penetration rate. It was felt that waiting until 10% of the dose reached the receptor permitted establishment of a pseudo steady state without extensive depletion of the donor. Penetration rates calculated in this way are close to the maximum rate for the system in question.

Slopes for each vehicle were averaged and compared to those for other treatments using single factor ANOVA[7]. If the differences in rate were significant, Student-Newman-Keul multiple range tests were performed at $\alpha = 0.05$.

With certain of the vehicles, penetration did not proceed far enough to employ the method described

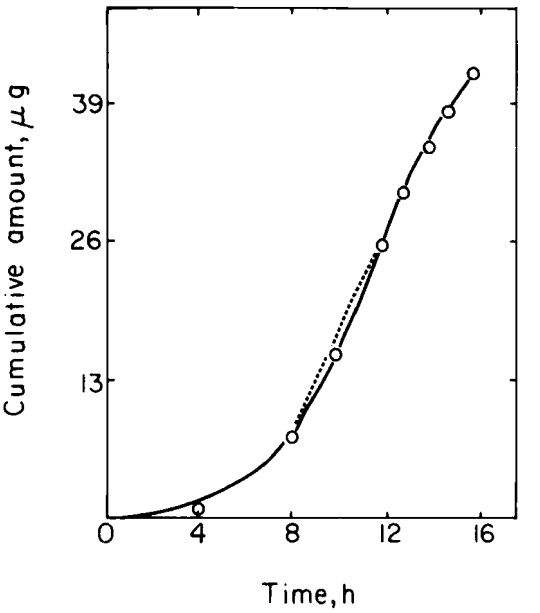

FIGURE 1.
Hydrocortisone penetration from 0.2% solution containing 80% propylene glycol and 1% polysorbate 60, finite dose.

above. In such cases, the slope of the terminal portion of the penetration curve was used. We also report average values for the amount of steroid reaching the receptor in 12 hours.

Finite Dose Studies

Figure 2 shows the effect of propylene glycol concentration on hydrocortisone penetration from vehicles containing no surfactant. Penetration rate generally increased as propylene glycol concentration was reduced. The total amount penetrated was also a

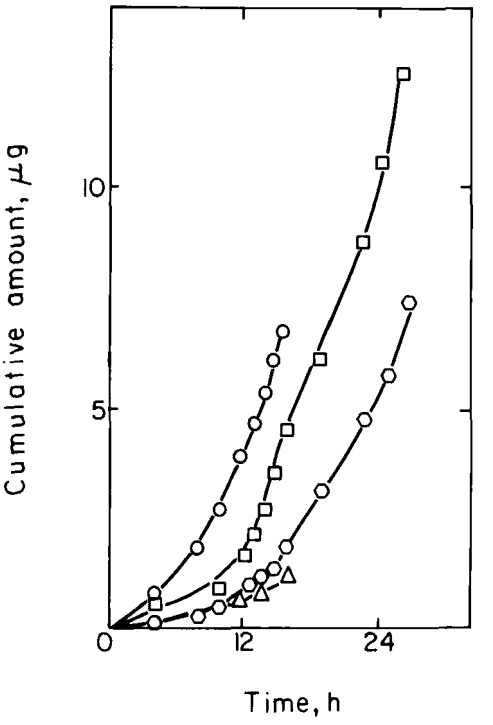

FIGURE 2.
Effect of propylene glycol concentration on penetration of hydrocortisone, 0.2%, finite dose.
○ 20% propylene glycol; □ 40% propylene glycol;
△ 60% propylene glycol; ⬡ 80% propylene glycol

function of propylene glycol content. At 80% propylene glycol, 27 hours of exposure was required for about 10% of the dose to be transported through the skin.

Similarly, very small quantities of hydrocortisone penetrated from aqueous suspension containing dissolved surfactant but no propylene glycol (Fig. 3). Only small differences were observed as a function of hydrophobic chain length of the surfactant.

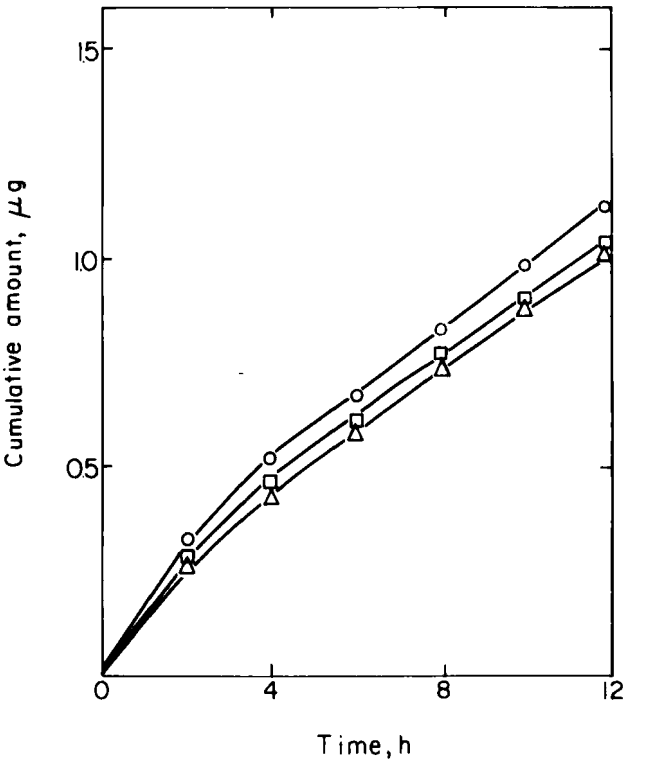

FIGURE 3.
Effect of nonionic surfactants at 1% concentration on hydrocortisone penetration from aqueous dispersion, finite dose. ○ polysorbate 20; □ polysorbate 40; △ polysorbate 60

Skin penetration was significantly enhanced from vehicles containing both propylene glycol and a nonionic surfactant. Mean penetration rates are collected in Table 1 except for the system containing 60% propylene glycol and no surfactant. In this case, we felt that experiments were not carried out long enough to yield a meaningful value. Table 2 contains values for the percent of applied dose reaching the

TABLE 1. Mean Rates of Penetration from 0.2% Hydrocortisone Solutions Following Finite Dosing.

Surfactant	Penetration Rate (S.D.), µg/hr		
	Propylene Glycol Concentration		
	40%	60%	80%
None *	1.7 (0.45)	---	0.8 (0.39)
Polysorbate 20	2.1 (0.21)	2.6 (0.60)	4.7 (1.8)
Polysorbate 40	3.7 (1.02)	3.2 (0.45)	3.9 (0.94)
Polysorbate 60	4.3 (1.06)	3.8 (0.88)	4.2 (0.83)
Polysorbate 80	4.1 (0.51)	4.5 (0.44)	4.3 (0.50)

* Calculated from terminal data points.

TABLE 2. Hydrocortisone Penetration at 12 hours, Finite Dosing.

Surfactant	Percent of Dose Penetrated			
	Propylene Glycol Concentration			
	0%	40%	60%	80%
None	---	1.2	0.8	0.9
Polysorbate 20	4.5	23.9	24.6	35.5
Polysorbate 40	2.7	39.2	28.5	30.9
Polysorbate 60	4.2	39.6	33.1	32.6
Polysorbate 80	---	34.5	38.1	24.9

TABLE 3. Results of Statistical Analysis.

Comparison	Conclusion
1. Polysorbate 20 and 60	
A. no propylene glycol	No difference
B. 40% propylene glycol	Penetration increased with longer chain length
C. 60% propylene glycol	
D. 80% propylene glycol	No difference
2. Propylene glycol conc.	
A. Polysorbate 20	Penetration increased with p.g. concentration
B. Polysorbate 40	No difference
C. Polysorbate 60	No difference
D. Polysorbate 80	No difference
3. Polysorbate 60 and 80	No difference

receptor in 12 hours. Conclusions based on statistical analysis are shown in Table 3. Table 4 shows the time required to achieve penetration of 10% of the dose. This provides an indication of the time that must elapse before significant permeation occurs. Results may be summarized as follows:

1. Rates of hydrocortisone penetration increased with increasing propylene glycol in systems containing

TABLE 4. Time Required for Penetration of 10% of Dose Following Application of 0.2% Hydrocortisone Solution under Finite Dose Conditions.

Surfactant	Time, hr		
	Propylene Glycol Concentration		
	40%	60%	80%
None	19	>16	27
Polysorbate 20	9	9	8.5
Polysorbate 40	7.5	9	9.5
Polysorbate 60	6.5	8	8.5
Polysorbate 80	7	7.5	9

polysorbate 20. There was no significant effect of propylene glycol concentration (within the range studied) in the presence of the other surfactants.

These findings may be contrasted with the pattern found in the absence of added surfactant, namely an inverse relationship between penetration and propylene glycol concentration. There is a synergistic effect between surfactant and propylene glycol, which was also noted in our previous work with lidocaine[4].

2. With vehicles containing 40% and 60% propylene glycol, hydrocortisone penetration rate increased as

the fatty acid chain length of the surfactant increased from 12 to 18 carbon atoms (polysorbate 20 to 60). This effect was not seen in vehicles containing 80% propylene glycol.

3. There was no effect on hydrocortisone penetration due to unsaturation of the surfactant fatty chain (polysorbate 60 vs. polysorbate 80).

4. The time for penetration of 10% of the dose of hydrocortisone was shortened significantly in the presence of surfactant at each propylene glycol concentration (Table 3). There were no significant differences in this value as a function of surfactant chemistry.

Infinite Dose Studies

Following infinite dose application, some of the experimental curves exhibited a terminal linear portion, while, in many cases, the slope continued to rise during the course of the experiment. From examination of the cumulative curves (Figs. 4, 5) it is obvious that hydrocortisone penetration was increased in the presence of two nonionic surfactants, polysorbate 20 and polysorbate 60. Penetration curves for vehicles containing a surfactant were similar at

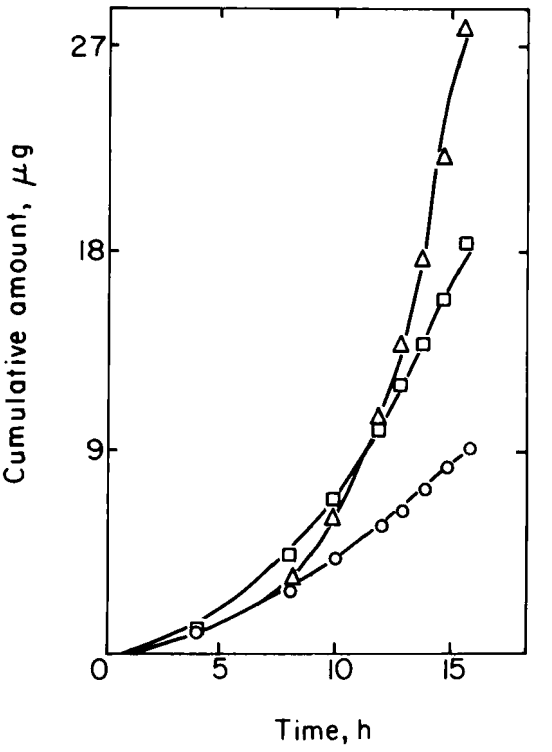

FIGURE 4.
Effect of nonionic surfactants on hydrocortisone penetration under infinite dose conditions, 40% propylene glycol. ○ no surfactant; □ polysorbate 20; △ polysorbate 60

both propylene glycol concentrations (40 and 80%). However, in the absence of surfactant the penetration rate was significantly lower from the vehicle containing 80% propylene glycol (Fig. 5) than from the 40% propylene glycol vehicle (Fig. 4) so that the apparent degree of permeation enhancement was greater at the higher propylene glycol concentration.

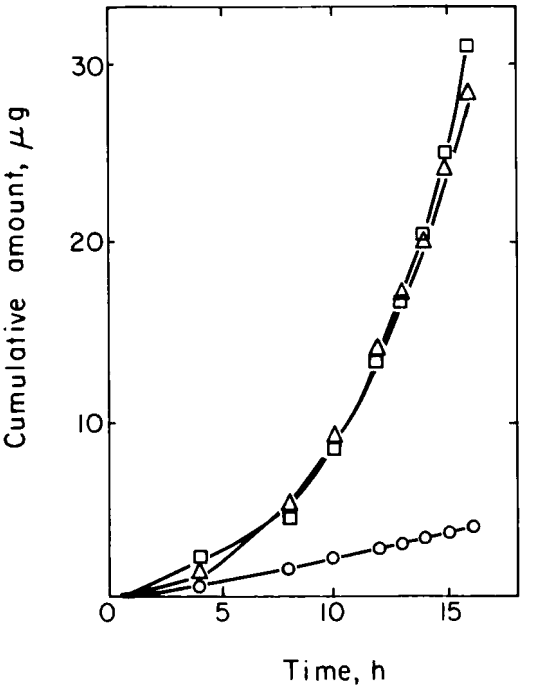

FIGURE 5.
Effect of nonionic surfactants on hydrocortisone penetration under infinite dose conditions, 80% propylene glycol. ○ no surfactant; □ polysorbate 20; △ polysorbate 60

Enhancement Mechanism

Studies of surface tension indicated that the critical micelle concentration of polysorbates was increased in the presence of propylene glycol, the effect being more pronounced at higher propylene glycol concentrations[4]. This would cause a rise in monomer concentration within the vehicle, and, assuming that only the monomer is capable of penetrating the stratum corneum, a proportional increase in surfactant absorption into the skin. The result would be increased

interaction with the skin leading to structural changes, and therefore increased drug penetration. It is also possible that drug penetration enhancement is due to alterations in stratum corneum structure caused by the combination of surfactant and propylene glycol.

Because of differences in vehicle surface tension, we can not rule out the possibility that the skin surface is wet incompletely by some of the formulations. In vehicles containing 40% propylene glycol, the addition of a surfactant lowers surface tension by several dyn/cm. Under infinite dosing, the large volume applied to the skin surface insures total coverage of the skin by the vehicle. However, the same is not true with a finite dose, and if the applied film were to contract into discrete droplets, a decrease in penetration rate would result. Because of their lower surface tension, vehicles containing surfactant might wet the skin better, hence increasing penetration.

However, it is unlikely that wetting was a major factor in our results. For one thing, hydrocortisone penetration was quite slow from vehicles containing a surfactant but no propylene glycol. Secondly, there were substantial increases in penetration from vehicles containing surfactants and propylene glycol applied under infinite dose conditions, where wetting effects are unimportant.

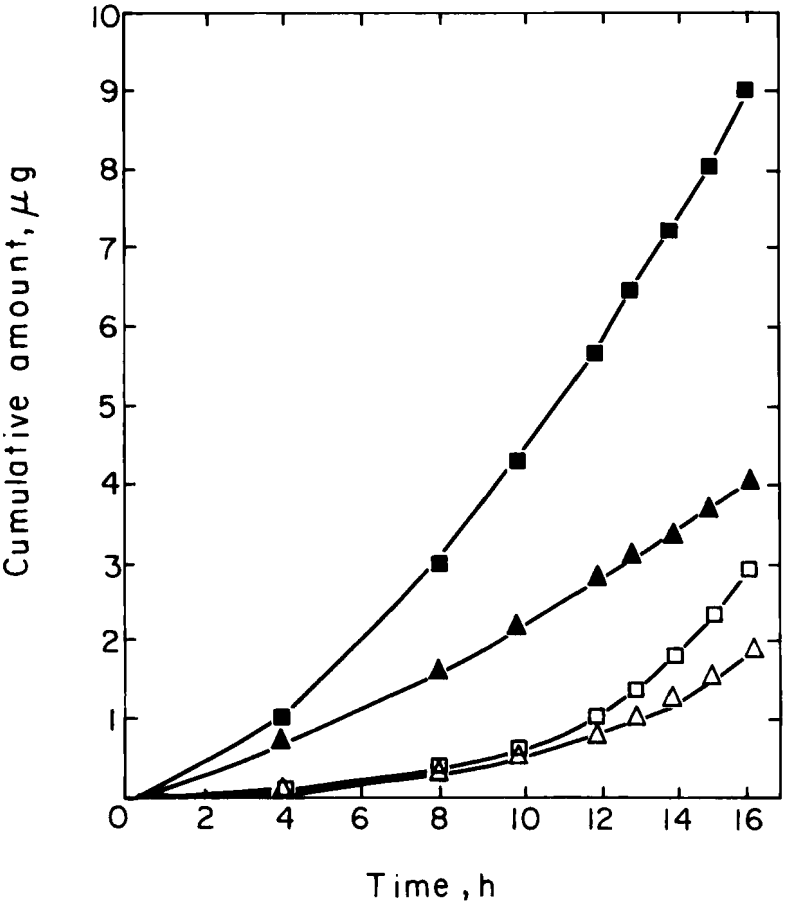

FIGURE 6.
Effect of application mode on hydrocortisone penetration from donors containing no surfactant.
40% propylene glycol: ■ infinite dose; □ finite dose
80% propylene glycol: ▲ infinite dose; △ finite dose

Comparison of Finite and Infinite Dose Results

Penetration curves following the different application modes (finite and infinite dose) are compared in Figs. 6-8. In the absence of surfactant, penetration was more rapid following application under infinite dose conditions than from a finite dose (Fig.

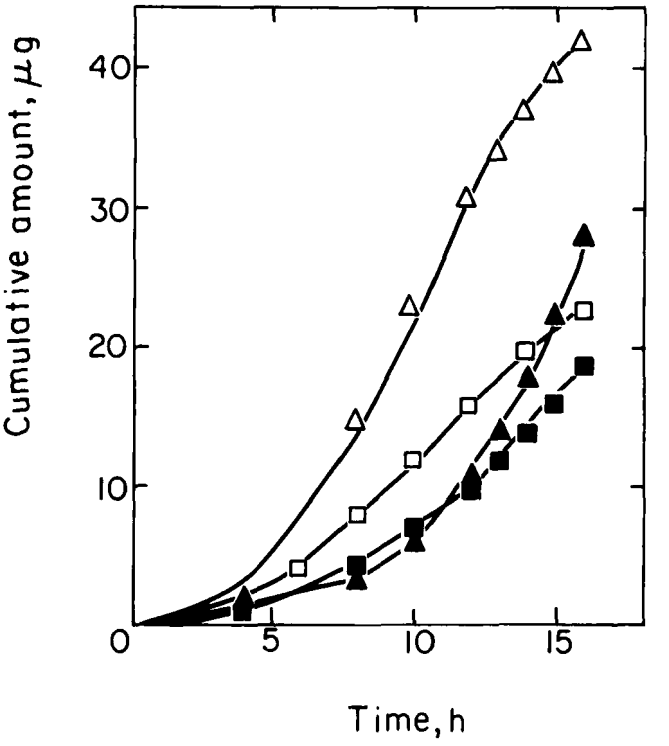

FIGURE 7.
Effect of application mode on hydrocortisone penetration from donor systems, 40% propylene glycol. polysorbate 20: ■ infinite dose; □ finite dose
polysorbate 60: ▲ infinite dose; △ finite dose

6). Exactly the opposite was found when vehicles containing surfactants were compared (Fig. 7, 8).

Although any explanation for these differences would be speculative at the present time, we can list some of the factors which might be involved. These are differences in skin surface temperature, drug depletion, differential wetting, and concentration enrichment within the donor.

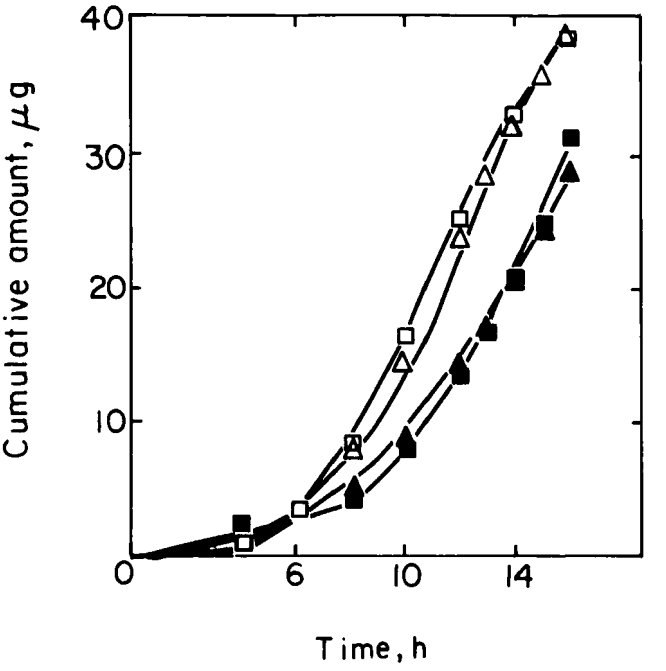

FIGURE 8.
Effect of applicaion mode on hydrocortisone penetration from donor systems, 80% propylene glycol. polysorbate 20: ■ infinite dose; □ finite dose polysorbate 20: ▲ infinite dose; △ finite dose

The finite dose allows normal transpiration of moisture; with occlusion, skin temperature is higher. This makes the flux in the infinite dose situation higher. Depletion of steroid from the donor as a result of partitioning and permeation would do the same thing.

Only with vehicles that contain no surfactant could differential wetting come into play. Incomplete wetting might reduce the contact area of a finite dose, but would have no effect on a large donor volume, thus favoring penetration from the infinite system.

With finite dose systems, the thin layer of donor in contact with the skin gradually becomes enriched in the steroid because of skin transport of the solvents, both of which are more rapid than hydrocortisone permeation. This increases the concentration gradient across the skin membrane and therefore the penetration rate. The same effect does not occur in the infinite dose situation. Because of the relatively large volume of donor, only minor changes in hydrocortisone concentration would take place due to permeation of water and propylene glycol.

For vehicles that do not contain a surfactant, it seems likely that differences in skin surface temperature, and possibly skin wetting are responsible for the greater permeation from an infinite dose. With a surfactant present, skin wetting differences would be eliminated. Apparently, the effect of steroid enrichment within the vehicle is sufficient to overbalance the influence of those factors which work in the opposite direction.

CONCLUSIONS

We have observed significant penetration enhancement of hydrocortisone through hairless mouse skin from vehicles containing low concentrations of

nonionic surfactants (polysorbates) in combination with propylene glycol. Similar results were obtained with lidocaine[4], but the increase in hydrocortisone permeation rate was more striking. This may have been due to the lower intrinsic permeability of the steroid or a difference in penetration pathway.

There was some indication from our studies that the degree of this interaction was dependent on surfactant fatty chain length. As a corollary of this explanation, it is suggested that one reason that nonionic surfactants appear to be noninteractive in aqueous solution is their very small monomer concentration (low critical micelle concentration).

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