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Distribution of salicylic acid in human stratum corneum following topical application in vivo: a comparison of six different formulations

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Abstract

Distribution of salicylic acid in human stratum corneum from treatment of six different formulations was assessed by quantitation of drug content in sequentially tape-stripped stratum corneum after a single 2-h dose was applied unoccluded to skin on the ventral forearm of four female subjects. The profile and total amounts of stratum corneum removed in 20 tape-strips varied among different types of formulations. With or without normalization by the total stratum corneum weights removed, the extent of drug delivery to the stratum corneum decreased in the following order: SA (5%) > > SAC (10%), Duofilm (16.7%) > TSSS (2%) > SAO (10%), Salic (2.5%), the percentage in parentheses indicating the salicylic acid concentration in each formulation. The greatest topical bioavailability was observed for the alcoholic solution containing glycerol (SA). The 10% collodion formulation (SAC) was found to deliver an amount of salicylic acid into the stratum corneum 2-fold greater than 10% ointment formulation (SAO). Use of absorption ointment (TSSS) also increased the uptake of salicylic acid into the stratum corneum in comparison with formulations based on simple ointment (SAO) and oil in water (o/w) cream (Salic). The partitioning of salicylic acid from collodion formulations (SAC and Duofilm) appeared to be concentration-independent. The results of this study indicate that topical bioavailability of salicylic acid in the stratum corneum varies substantially among different formulations.

Keywords: In vivo; Formulation; Salicylic acid; Stratum corneum; Tape-stripping

1. Introduction

Salicylic acid is a well-established keratolytic and has been used in concentrations of 0.5–60% in almost any base, including ointment, liquid, cream or plaster, for the treatment of acne, psoriasis, warts, ichthyosis and other hyperkeratotic

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disorders (Chren and Bickers, 1990; Tong and Vickers, 1993; AHFS Drug Information, 1996). Historically, the bioavailability of salicylic acid topical formulations has been evaluated based on its keratolytic effect and clinical efficacy (Baden and Alper, 1973; Davies and Marks, 1976; Nook, 1987). Various methods have been proposed to measure the effect of salicylic acid on the rate of cell loss from the epidermal surface, including analysis of released proteins (Christensen et al., 1978), disappearance of dansyl chloride fluorescent staining (Roberts et al., 1980), silver nitrate staining (Roberts et al., 1980; Nook, 1987), histological examination of skin biopsies (Davies and Marks, 1976; Roberts et al., 1980) and forced desquamation techniques (Roberts et al., 1980). Although these techniques may be useful to demonstrate the keratolytic effect of salicylic acid in the stratum corneum, they do not reveal any information regarding the efficiency of salicylic acid delivery from different formulations and its local concentration in the target site. Because of the tedious nature and frequent impracticality of such studies, very limited effort has been made to evaluate the mechanism and extent of salicylic acid absorption into human skin and the relationship between local salicylic acid concentration and its pharmacological activity. Lodén et al. (1995) utilized a tape-stripping method to evaluate the effect of skin absorption of salicylic acid on the binding forces within the stratum corneum. However, no relationship between these two parameters has been established. Studies with corticosteroids have indicated that the drug concentration in the stratum corneum can be correlated to the pharmacodynamic response, as determined by vasoconstrictor assay, and that the drug concentration may be used to estimate bioavailability of topical formulations (Pershing et al., 1992, 1994a). Similarly, differential uptake of topical miconazole and ketoconazole into the stratum corneum was correlated with their antifungal activities (Pershing et al., 1994b). Based on these findings, if we consider the stratum corneum as the target site for desquamating activity, salicylic acid concentration in the stratum corneum should provide information regarding its clinical efficacy.

The vehicle of topical formulations influences the drug uptake into the stratum corneum. This study was performed to evaluate the distribution of salicylic acid into healthy human stratum corneum in vivo using a tape-stripping technique. Six different topical formulations of salicylic acid were tested including cream, ointment and solution. The salicylic acid concentrations in those formulations ranged from 2% to 16.7%. The results may provide information about relative bioavailability of various salicylic acid formulations.

2. Materials and methods

2.1. Materials

Salicylic acid was purchased from Wako Pure Chemicals (Osaka, Japan). HPLC grade methanol and acetonitrile were purchased from Fisons Scientific Equipment (Loughborough, UK). Six different salicylic acid preparations (SA, Salic, TSSS, Duofilm, SAO and SAC) which were formulated with a variety of bases and salicylic acid concentrations were obtained from the National Cheng Kung University Hospital Pharmacy. Their formulations are listed in Table 1. Other chemicals were of reagent grade and obtained from Tokyo Chemical Industry Co. (Tokyo, Japan; tetramethylammonium hydroxide pentahydrate and 4-cyanophenol) and Merck KGaA (Darmstadt, Germany; glacial acetic acid).

2.2. Study design

Four young Chinese women, aged 22–25 years, with no known history of drug allergy or dermatological disease, were included in the study. Approximately 62.5 mg or 62.5 μl of each formulation was evenly spread onto a 2.5×2.5 cm^2 surface area on the ventral forearms to provide a 10 mg or 10 μl formulation/cm^2. A maximum of three sites, spaced 1–2 cm apart, was applied to each forearm of the subject. The order and site of formulations applied to the forearm were randomized. All experiment sites were left unoccluded for 2 h after the application.

Table 1
Topical salicylic acid formulations evaluated

Formulation	Active ingredients	Base	Manufacturer
TSSS	2% salicylic acid 2% sulfur, precipitated 2% pine tar	Absorption ointment	NCKU Hospital Pharmacy
Salic	2.5% salicylic acid	o/w cream	Standard Chem. & Pharm. Co. (Taiwan)
SA	5% salicylic acid	5% glycerin 95% alcohol	NCKU Hospital Pharmacy
SAO	10% salicylic acid	Simple ointment (ChP)	NCKU Hospital Pharmacy
SAC	10% salicylic acid	Flexible collodion (USP)	NCKU Hospital Pharmacy
Duofilm	16.7% salicylic acid 16.7% lactic acid	Flexible collodion (USP)	Stiefel Laboratories (Ireland)

The 2-h treatment period was arbitrarily selected to approximate the residence time of formulations on the skin surface under clinical conditions. During this period, the volunteers were instructed to carefully maintain the sites undisturbed.

The residual formulations were removed from the drug-treated sites at the end of the dosing interval (2 h) by gently wiping with three independent cotton balls followed by air-drying for 5 min except for the two formulations based on collodion (SAC and Duofilm). The white film formed on the skin surface from the two collodion formulations was first removed by gentle rubbing with an eraser and followed by the cotton-ball procedure presented above. Twenty individual 2×2 cm^2 squares of adhesive tape (Four Pillars Brand, Taiwan) were utilized to sequentially tape-strip the stratum corneum at the center of the 2.5×2.5 cm^2 drug-treated sites. Before and after skin tape-stripping, each of these squares was weighed on a Mettler balance (Model AT20, sensitivity of 2 μg) to quantitate the weight of stratum corneum removed. Each tape-strip was extracted with methanol and submitted to high-performance liquid chromatography (HPLC) for quantitation of salicylic acid content.

2.3. Analytical methods

Salicylic acid content in the various samples was identified chromatographically by HPLC (Waters Co., Milford, MA) on a C18 reverse phase column (Spherisorb® S5 ODS2 4.6 mm × 25.0 cm, Waters Co.) at 25°C, using a 1.5 ml/min flow rate of mobile phase of 0.0225% tetramethylammonium hydroxide pentahydrate in water–methanol–acetonitrile–glacial acetic acid (70:15:15:0.1, v/v). A UV detector with tunable wavelength (Waters model 486) set at 280 nm was used to detect salicylic acid in the tape-stripped stratum corneum. Drug concentration in the tape-stripped samples was determined from salicylic acid standard curves (0.5–80 μg/ml) generated with the pure compound. The retention times were about 15.3 min and 8.1 min for salicylic acid and the internal standard, 4-cyanophenol, respectively. The standard curves were log–linear for the peak height ratio of salicylic acid to 4-cyanophenol. The inter-run reproducibility of the HPLC assay for salicylic acid is shown in Table 2.

Each tape-strip was placed into a 15-ml pyrex tube and 2 ml of methanol was added to extract the drug. The tubes was capped, vortexed for 1

Table 2
Inter-run assay reproducibility of salicylic acid

Concentration (μg/ml)	Concentration determined		% C.V.	*n*
	Mean	S.D.		
0.5	0.51	0.05	9.06	12
1.0	0.97	0.06	5.92	16
5.0	4.97	0.31	6.22	10
10.0	10.11	0.63	6.19	16
20.0	20.74	0.98	4.75	10
40.0	39.83	2.17	5.46	10

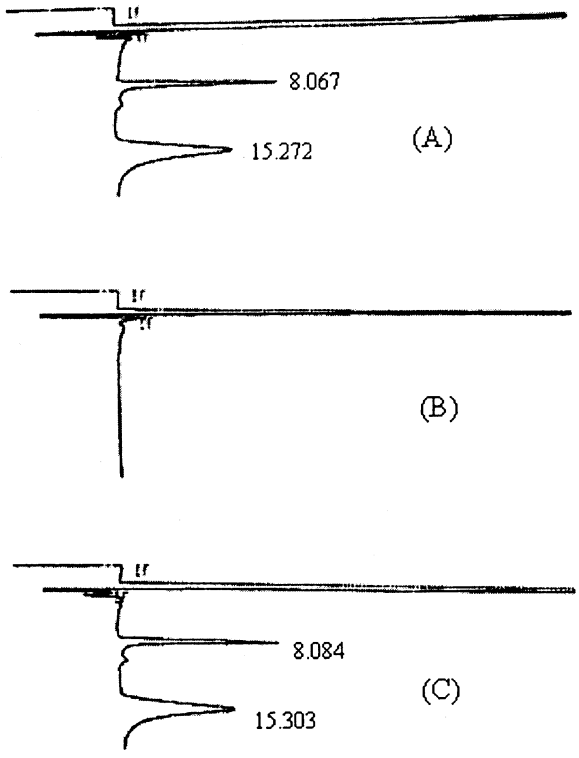

Fig. 1. Representative HPLC chromatogram of salicylic acid and 4-cyanophenol in pure standards and skin. (A) Salicylic acid (RT = 15.3 min) and 4-cyanophenol (RT = 8.1 min). (B) Methanol extract of skin + tape. (C) Methanol extract of skin + tape + salicylic acid formulations, spiked with 4-cyanophenol.

min at high speed, and left overnight. After centrifugation at 3000 rpm for 5 min, 1 ml of supernatant was transferred into a 3-ml glass tube and 100 μl of 4-cyanophenol solution (40 μg/ml in methanol) was added as internal standard; 50 μl of the solution were then injected onto the HPLC. The recovery of salicylic acid from a single extraction of the tape-strips containing drug and stratum corneum was approximately $83.1 \pm 6.3\%$, $88.5 \pm 1.3\%$, $96.9 \pm 2.4\%$ and $100.2 \pm 2.6\%$ ($n = 3$) for 1, 4, 20 and 80 μg salicylic acid per tape, respectively. Preliminary tests with the methanolic extracts of tape, tape + skin and salicylic acid free base from all the formulations did not reveal any substances interfering with salicylic acid in the chromatographic analysis (Fig. 1).

3. Results and discussion

3.1. The amount of stratum corneum removed by tape-stripping

Cumulative amounts of stratum corneum removed with the tape-stripping technique following 2 h application of the six salicylic acid formulations are presented in Fig. 2. The profile of stratum corneum removed was linear and almost identical with treatment of the three semisolid type formulations, TSSS, SAO and Salic, indicating that similar amounts of stratum corneum were removed with each tape-strip from these three formulations. For the three solution type formulations (SA, SAC and Duofilm) the cumulative amounts of stratum corneum removed from the tape-strippings increased more rapidly in the initial four to eight strips and gradually reached a linear profile for the latter strippings. The profiles were nearly identical in samples obtained following treatment with two collodion-based formulations (SAC and Duofilm), while a greater weight was removed from sites treated with SA after four strippings in comparison with sites treated with SAC and Duofilm. Among the six formulations, the total stratum corneum weights removed in the 20 tape-strips decreased in the order: SA > Duofilm, SAC > TSSS, SAO and Salic, where the differences between the treatment by SA and the three semisolid formulations (TSSS, SAO and Salic) were statistically significant ($P < 0.05$, one-way ANOVA) (Fig. 3).

3.2. The amount of salicylic acid in the tape-strips

Fig. 4 shows the cumulative amounts of salicylic acid in the tape-strips following 2 h application of the six formulations. The profile of cumulative amounts of salicylic acid in the tape-strips from sites treated with SAO and Salic paralleled each other with slightly greater amounts of salicylic acid in SAO-treated sites than in Salic-treated sites. Although the potency of salicylic acid contained in the two collodion-based formulations, SAC and Duofilm, differed by 67%, the profiles of cumulative amounts of salicylic acid in

the 20 tape-strips from sites treated with these two formulations were essentially the same. When the total amount of salicylic acid in the 20 tape-strips was compared among the six formulations, we found that the drug content in the stratum corneum decreased in the following order: SA (5%) > > SAC (10%), Duofilm (16.7%) > TSSS (2%) > SAO (10%), Salic (2.5%), as illustrated in Fig. 5. The 5% SA formulation delivered a significantly greater amount of salicylic acid into the stratum corneum than the other five formulations, with differences ranging from 2.6- to 6.7-fold. In addition, the amount of salicylic acid partitioned into the stratum corneum by the treatment of 10% collodion formulation (SAC) was more than 2-fold greater than with the 10% ointment formulation (SAO). Furthermore, treatment with the least potent TSSS formulation (2%) resulted in more amount of salicylic acid in the 20 tape-strips than treatment with the more potent SAO (10%) and Salic (2.5%) formulations ($P < 0.1$, one-way ANOVA). The cumulative amount of salicylic acid in the 20 tape-strips removed from sites treated with 10% SAO formulation was not statistically different from that in tape-strips removed from treatment by 2.5% Salic formulation ($P = 0.70$, one-way ANOVA). The degree of partitioning of salicylic acid into the stratum corneum appeared to be independent of the drug potency in the formulations, but rather dependent on formulation types.

Since variable amounts of salicylic acid were contained in the tape-strips from the treatment by different formulations, we have then calculated

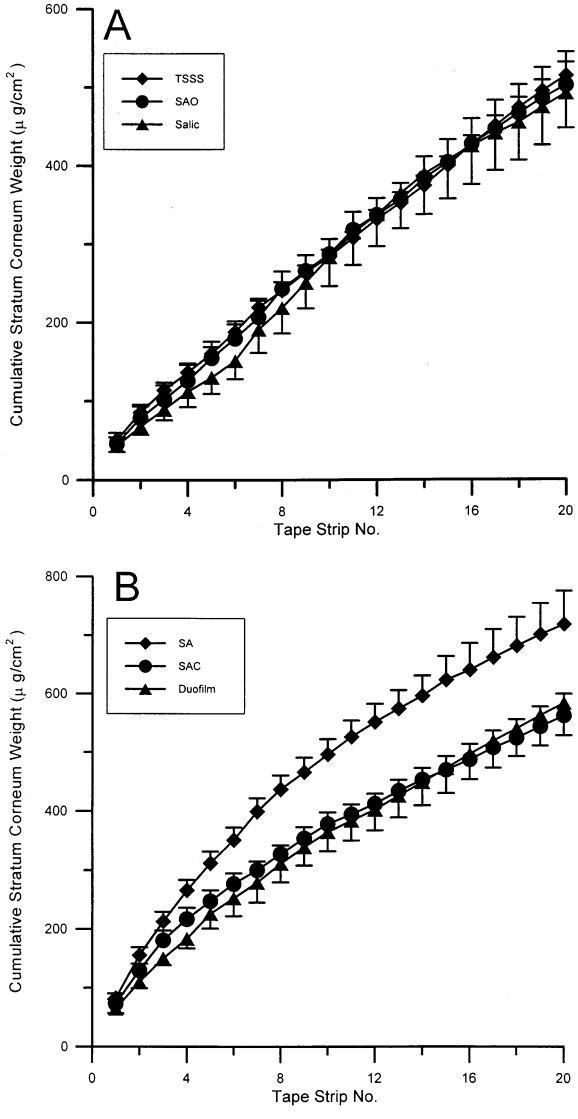

Fig. 2. Cumulative stratum corneum weight removed via tape-stripping of human ventral forearm skin following a 2-h application of salicylic acid formulations. Data represent the mean ± S.E.M. for the four subjects. (A) TSSS, SAO and Salic. (B) SA, SAC and Duofilm.

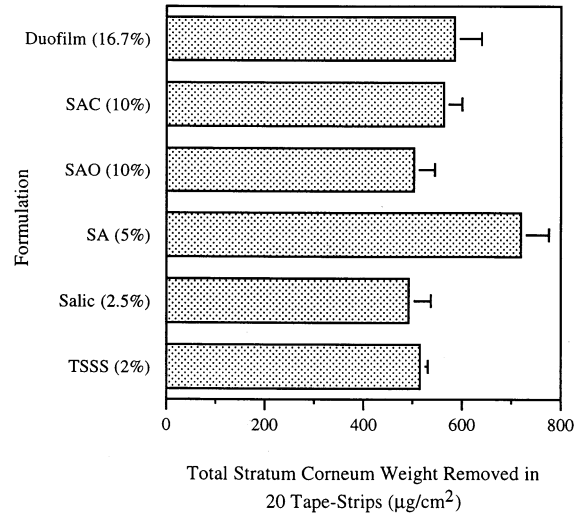

Fig. 3. Total stratum corneum weight removed in 20 tape-strips following a 2-h application of six salicylic acid formulations. Data represent the mean and S.E.M. for four subjects.

the net stratum corneum weight of each tape-strip by substracting the drug content from the crude stratum corneum weight. As shown in Fig. 6, the same rank order of formulations was concluded in the total net stratum corneum weight removed in the 20 tape-strips as the total crude stratum corneum weight. However, the differences in the total net stratum corneum weight between the treatment by the six formulations turned out not to be statistically significant ($P > 0.05$, one-way ANOVA), in contrast to the total crude stratum corneum weight (Fig. 6 versus Fig. 3).

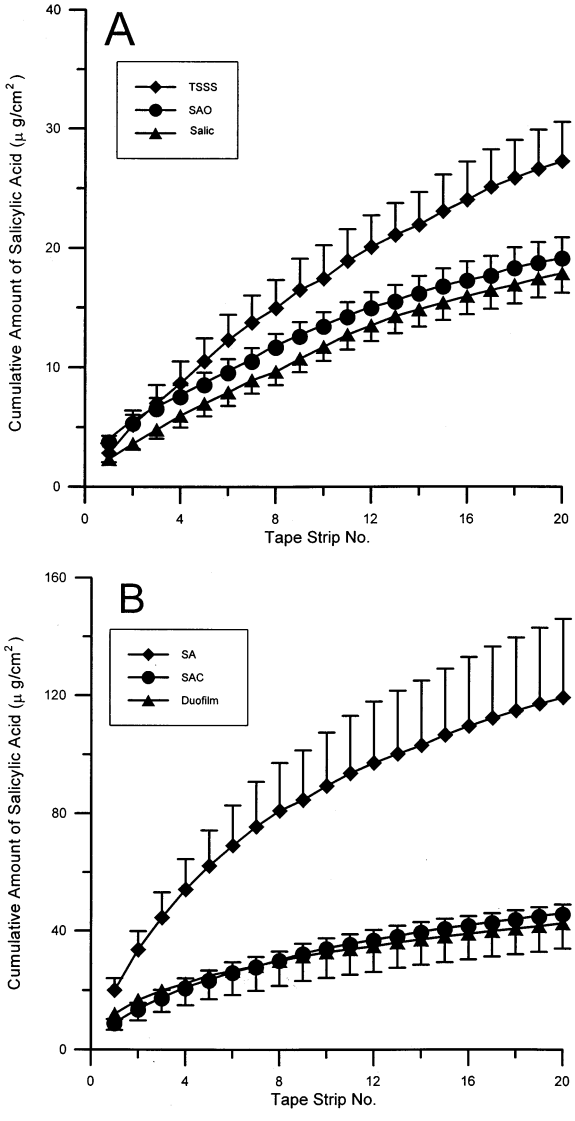

Fig. 4. Cumulative amount of salicylic acid within the tape-stripped stratum corneum following a 2-h application of six salicylic acid formulations. Data represent the mean ± S.E.M. for four subjects. (A) TSSS, SAO and Salic. (B) SA, SAC and Duofilm.

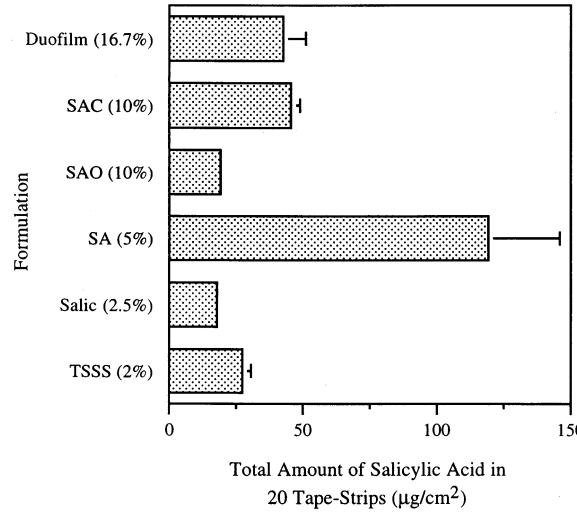

Fig. 5. Total amount of salicylic acid within the tape-stripped stratum corneum following the application of six salicylic acid formulations. Data represent the mean and S.E.M. for four subjects.

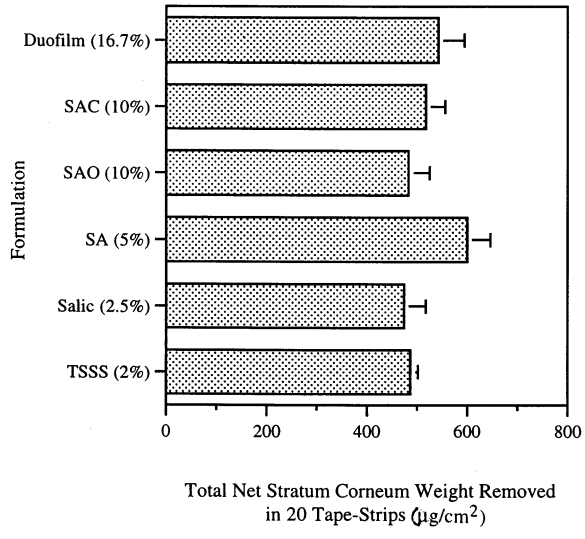

Fig. 6. Total net stratum corneum weight removed in 20 tape-strips following a 2-h application of six salicylic acid formulations. Data were calculated by removing salicylic acid content from the crude stratum corneum weight and represent the mean and S.E.M. for four subjects.

3.3. Effect of formulations

An in vivo quantitative tape-stripping method for assessing the drug content in human stratum corneum following topical application of 0.05% betamethasone dipropionate ointment and cream was proposed by Pershing et al. (1992). In this study, we used a similar tape-stripping method to evaluate the partitioning of salicylic acid into human stratum corneum in vivo from six topical formulations. Modifications to Pershing's method included use of unoccluded applications and different adhesive tapes. The formulation types tested included cream, ointment and solution. The salicylic acid content in those formulations ranged from 2% to 16.7%. Our results demonstrating that approximately the same amount of stratum corneum was removed with each tape-strip from the treated sites of the three semisolid-type formulations based on either ointment or cream (i.e. TSSS, Salic and SAO) was consistent with the findings of Pershing et al. (1992). However, when the same method was utilized to evaluate sites treated with the two collodion-based formulations (Duofilm and SAC), slightly greater amounts of stratum corneum (530 ± 86 μg/cm^2) were removed than the semisolid formulations (482 ± 66 μg/cm^2). These increased amounts were mainly due to increases in the first few strippings. Nonetheless, the differences are not statistically significant. Among the six test formulations, treatment with SA resulted in the greatest cumulative net amount of stratum corneum removal (600 ± 92 μg/cm^2). We speculated that glycerol, a non-volatile ingredient contained in the SA formulation may have added to the weights of stratum corneum removed from the tape-strippings since the weighing method did not distinguish residual formulation excipients from the stratum corneum. It is also possible that more corneocytes may have been removed by tape-stripping due to the effect of glycerol to facilitate desmosome digestion (Rawlings et al., 1995). Consequently, when comparing drug uptake into the stratum corneum from different types of formulations, it is important to note that variability may exist in the profile and total amounts of stratum corneum removed by tape-stripping method.

Since the cumulative stratum corneum weights removed with the tape-stripping technique varied from one formulation to another, it is possible that the observed differences in drug distribution in the stratum corneum might have been partially due to the collection of greater or lesser amounts of tissue. To assess this possibility, we analyzed the cumulative drug content as a function of the cumulative net amount of stratum corneum removed in the tape-strippings, as illustrated in Fig. 7. When a cumulative weight of approximately 480 μg/cm^2 of stratum corneum (representing the least weight among the six formulations) was used in the calculation for all formulations, the estimates for distribution of salicylic acid in the stratum corneum decreased for the treatment of SA, SAC and Duofilm as compared to the estimates of drug content in total stratum corneum weights removed in 20 tape-strips. However, the rank order of drug content in the skin from sites treated with the six formulations remained the same for both cases. The topical bioavailability of salicylic acid in the stratum corneum following a single 10 mg or 10 μl/cm^2 dose for 2 h from each formulation appeared in the order: SA ($23.9 \pm 10.7\%$) > TSSS ($13.6 \pm 3.3\%$) > Salic ($7.2 \pm 1.6\%$) > SAC ($4.6 \pm 0.7\%$) > Duofilm ($2.6 \pm 1.0\%$) > SAO ($1.9 \pm 0.4\%$). These results indicate that the topical bioavailability of salicylic acid in the stratum corneum varies substantially among the tested formulations, and the alcoholic solution containing glycerol (SA) has the greatest topical bioavailability. The use of an absorption ointment as base (TSSS) also enhanced the uptake of salicylic acid into the stratum corneum in comparison with formulations based on o/w cream (Salic) and simple ointment (SAO). This finding agreed with results recently published by Murakami et al. (1998) that the use of absorptive ointment increased the retention of salicylic acid in the rat stratum corneum. The partitioning of salicylic acid from collodion formulations (SAC and Duofilm) appeared to be concentration-independent.

In conclusion, the results of the present study substantiate the feasibility of measuring drug content in the stratum corneum by the tape-stripping method. For salicylic acid, measurement of the

drug content in tape-stripped stratum corneum provides information regarding its concentration at the target site, which may indicate the keratolytic and desquamation responses to treatment. Once the correlation is established between the salicylic acid concentration in the stratum corneum and its pharmacological effect, the technique can be routinely applied to evaluate the efficacy of various salicylic acid formulations.

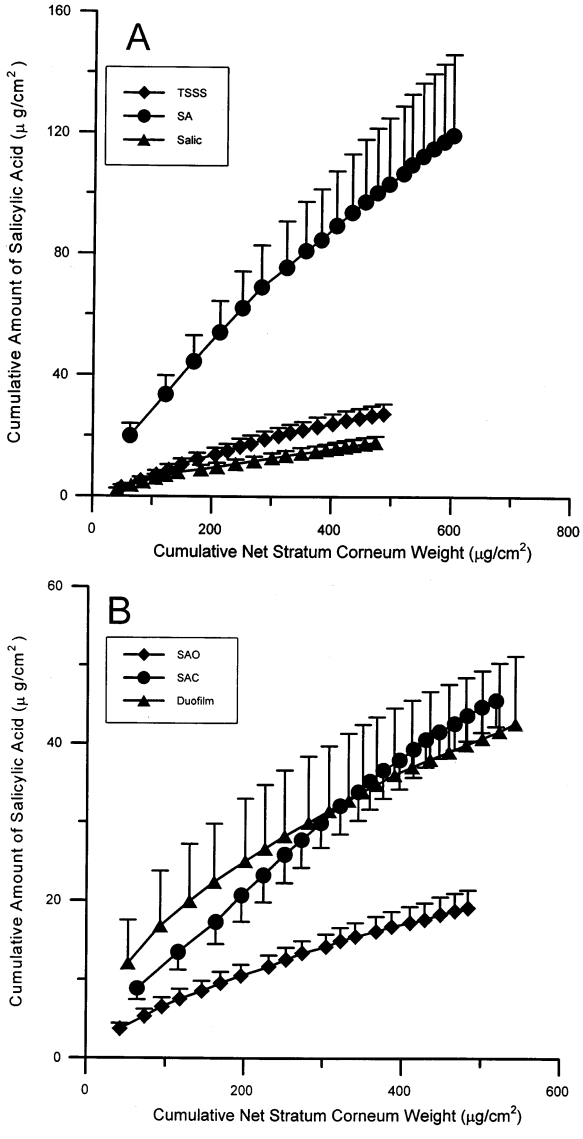

Fig. 7. Cumulative amount of salicylic acid within the tape-strips as a function of cumulative net stratum corneum weight removed following a 2-h application of six salicylic acid formulations. Data represent the mean ± S.E.M. for four subjects. (A) TSSS, SA and Salic. (B) SAO, SAC and Duofilm.

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